

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

205109Orig1s000

PHARMACOLOGY REVIEW(S)

ADDENDUM TO
PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 205109
Supporting document/s: 23
Applicant's letter date: 8/19/2013
CDER stamp date: 8/19/2013
Product: Velphoro (sucroferric oxyhydroxide) [name in original submission - (b) (4) (b) (4)]
Indication: The control of serum phosphorus levels in patients on dialysis
Applicant: Vifor Fresenius Medical Care Renal Pharma France
Review Division: Division of Cardiovascular and Renal Products
Reviewer: Baichun Yang, PhD, DABT
Supervisor/Team Leader: Thomas Papoian, PhD, DABT
Division Director: Norman L Stockbridge, MD, PhD
Project Manager: Anna Park

Background

In the agent's General Advice Letter signed on 7/5/2013, FDA commented under Pharmacology that -

In the label Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility, you states that

(b) (4)
 Treatment-related neoplastic adenocarcinomas were seen in mouse colon and cecum with all (b) (4) dose groups. There were also (b) (4) dose-related increases in incidence and/or severity of GI epithelial hyperplasia in mice and increased incidence of GI epithelial/mucosal hyperplasia in (b) (4) treated rats. Although the dog was negative for GI changes, this may be because of the reduced residence time resulting from chronic diarrhea seen in these animals.

As you know, chronic GI irritation, inflammation, and/or hyperplasia are known risk factors for development of GI cancer in humans. I (b) (4)

(b) (4) the label will reflect the rodent findings because of its relevance for humans treated chronically with (b) (4)

This submission

The sponsor responded to these comments along with an “Expert Evaluation of Preclinical Toxicity and Carcinogenicity Studies of Orally Administered PA21 (Dr. R. Maronpot, 17 July 2013)”. Dr. Maronpot re-examined the mouse large intestinal lesions in the carcinogenicity study, and re-classified the mouse intestinal lesions as shown in the table below -

PA21 Dose (mg Fe/kg/day)	Males				Females			
	Control	250	500	1,000	Control	250	500	1,000
Colon								
No. examined	57	60	57	60	59	58	57	60
Adenocarcinoma ⁽¹⁾	0 (1)	0 (3)	3 (5)	1 (9)	0	0	0	0 (3)
Mucosal diverticulum/ cysts/hyperplasia	2	1	3	6	0	0	2	4
Cecum								
No. examined	51	58	50	54	57	54	55	59
Adenocarcinoma ⁽¹⁾	0	0 (1)	0 (2)	0 (1)	0	0 (1)	0	0
Adenoma	0	0	0	1	0	0	0	0
Mucosal diverticulum/ cysts/hyperplasia	0	3	4	12 ⁽²⁾	0	0	0	3

1 Incidence values based on original classification shown in parentheses.

2 p<0.01

Cytological features of murine colonic and cecal adenocarcinomas in Dr. Maronpot’s classification included “increased mitoses, crowding of cells, loss of cellular polarity, anisokaryosis, hyperbasophilia, stromal invasion, and cellular atypia”. The morphological features of “diverticulum/cysts/epithelial hyperplasia” lesions with

submucosal cystic glands herniated through the outer muscular tunic (tunica muscularis) of the large intestine were still classified as diverticulum/cysts/epithelial hyperplasia. With information from studies in mice (large intestinal adenocarcinoma and epithelial hyperplasia), rats (large intestinal epithelial hyperplasia), and dogs (no findings), Dr. Maronpot concluded that [REDACTED] (b) (4)

The sponsor considered that [REDACTED] (b) (4)

[REDACTED] (b) (4)

[REDACTED] (b) (4)

Evaluation and Comments

The reviewer consulted and discussed with an FDA veterinary pathologist Dr. Luann Mckinney. The reviewer consider that cytological features of murine colonic and cecal adenocarcinomas in Dr. Maronpot's classification are consistent with literature descriptions of adenocarcinoma and are diagnostically straightforward, and agree with Dr. Maronpot's re-classification of the mouse intestinal lesions. Therefore, for the carcinogenicity study in mice, NOAEL and LOAEL levels for adenocarcinoma were 250, and 500 mg iron/kg/day, represent 5 and 10 times (on a body weight basis), respectively, the maximum recommended clinical dose of 3,000 mg/day.

However, there is no new evidence to support that large intestinal adenocarcinoma in mice is species-specific, since increased incidences in epithelial hyperplasia with or without submucosal inflammation in duodenum, cecum and colon were seen in rats, and a similar phosphate binding substance in another IND (b) (4) also caused large intestinal epithelial hyperplasia in monkeys. It is curious that neither drug showed GI hyperplasia in the dog, suggesting that the dog may be insensitive with respect to the - iron-starch-sucrose complex-induced epithelial hyperplasia, possibly because of increased diarrhea observed or intestinal transit rate.

For Section 13.1 of the USPI, the reviewer recommends following changes (bold for addition, strikethrough for deletion) based on the sponsor's original submission -

(b) (4)



(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/

BAICHUN YANG
10/07/2013

THOMAS PAPOIAN
10/08/2013
Concur.

**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: 205109
Supporting document/s: 1
Applicant's letter date: 1/31/2013
CDER stamp date: 2/1/2013
Product: PA21 [REDACTED] (b) (4)
Chewable Tablet, 500 mg
Indication: Control of serum phosphorus levels in patients
with end-stage renal disease (ESRD)
Applicant: Vifor Fresenius Medical Care Renal Pharma
France
Review Division: Division of Cardiovascular and Renal Products
Reviewer: Baichun Yang, PhD, DABT
Supervisor/Team Leader: Thomas Papoian, PhD, DABT
Division Director: Norman L Stockbridge, MD, PhD
Project Manager: Anna J Park, RPh.

Template Version: September 1, 2010 (Modified by DCRP: December 4, 2012)

Disclaimer

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1 Executive Summary

1.1 Introduction (and Clinical Rationale)

There is evidence that hyperphosphatemia contributes to the morbidity and mortality of patients with end stage renal disease (ESRD). PA21 is a mixture of polynuclear iron (III)-oxyhydroxide, starch and sucrose. The active component polynuclear iron (III)-oxyhydroxide is practically insoluble and not absorbed. The iron oxide hydroxide binds phosphate in the gastrointestinal (GI) tract through a direct ionic interaction between the negatively charged oxygen ions on the phosphate and the ferric ions in the ferric oxide, and prevents phosphorus absorption. Thus, PA21 acts as a phosphate binder, reducing the amount of dietary phosphorus absorbed from GI tract and lowering the serum phosphorus levels. PA21 is being developed for the control of serum phosphorus levels in patients with ESRD.

1.2 Brief Discussion of Nonclinical Findings

In vitro studies demonstrated efficient phosphate binding by PA21 under simulated GI tract conditions (pH range of 1.2 to 8.5), with a phosphate binding capacity at least equivalent to currently available phosphate binders. In rat models of chronic renal failure (CRF), PA21 was as effective as calcium carbonate, sevelamer carbonate and lanthanum carbonate in correcting the hyperphosphatemia and associated secondary hyperparathyroidism, was more effective than calcium carbonate and, to some extent, lanthanum carbonate in preventing vascular calcification in the thoracic aorta, and was effective in correcting elevated bone turnover observed in CRF rats. Because of the insoluble property of PA21 active component polynuclear iron (III)-oxyhydroxide, PA21 has advantages as an oral phosphate binder regarding systemic toxicological effects.

A complete set of preclinical second pharmacology, safety pharmacology, and toxicology studies was performed to assess safety profile of PA21. Several safety issues were identified. The most severe safety issue is the increased incidences of adenocarcinomas in colon and cecum in males at all dose levels (1250, 2500, and 5000 mg/kg/day) and in females at 5000 mg/kg/day. The lowest PA21 dose with adenocarcinomas in colon and cecum is only 5X the maximal human dose. There were also dose-related epithelial hyperplasia and mucosal diverticulum/cysts/hyperplasia in colon and cecum, adenoma and evidence of local irritation in non-glandular forestomach with increased epithelial hyperplasia and hyperkeratosis at 5000 mg/kg/day, dilated/cystic sinuses in enlarged mesenteric lymph nodes of males at all doses, and inflammatory cells in esophagus of females at the mid and high doses. The correlation between PA21-induced hyperplasia and the presence of adenocarcinomas in both colon and cecum, and the presence of adenoma and local irritation in non-glandular forestomach suggested that the neoplastic changes were part of a continuum that originated from chronic irritation, and subsequent proliferative response of the GI-tract to oral administered PA21. Similar chronic irritation and subsequent proliferative

(mucosal hyperplasia with or without submucosal inflammation/edema) were also seen at PA21 2500 mg/kg/day in the rat carcinogenicity study, the 4-week and 26-week repeat dose toxicity studies in rats. Therefore, although adenocarcinomas were seen only in mice, the PA21-associated epithelial hyperplasia in response to PA21-associated chronic irritation and/or inflammation in gastro-intestinal tract is not a mouse species-specific finding, but was seen in rats as well. It has been known for a long time that chronic irritation/inflammation to epithelium in humans is risk factor for cancer (1). Oral administration of PA21 in human may also be a risk factor for cancer in GI due to PA21-associated chronic irritation and/or inflammation.

The second safety issue is binding/interaction of drugs to/with PA21. In vitro studies simulating physiological conditions showed strong/complete adsorption or interaction of furosemide, losartan, atorvastatin, doxycycline, alendronate, levothyroxine and paricalcitol to/with PA21. Since patients with CKD are most likely treated with other oral drugs, the binding/interaction of the drugs to/with PA21 in GI tract will affect the effects of other drugs. According to the sponsor, PA21 did not alter the systemic exposure of a single dose of losartan, furosemide, digoxin, warfarin, or omeprazole in normal volunteers. Thus, drugs may bind to or interact with PA21 in GI include alendronate, doxycycline, levothyroxine, atorvastatin, and paricalcitol.

The third safety issue is the oral PA21-associated effects in embryo-fetuses and F1 animals. The reproductive and developmental studies demonstrated higher incidence of post implantation loss in rats at 4000 mg/kg/day, more fetuses with incomplete/unossified epiphyses and metacarpals/phalanges in rabbits at 1000 mg/kg/day, lower body weight gain in F1 male rats during age 5-13 weeks, and delayed neuromuscular function F1 male rats at the dose 4000 mg/kg/day. These findings may be attributed to maternal toxicity of PA21, i.e., reduced body weight gain in dams and one death of rabbit dam. Considering oral PA21 was not teratogenic in rats and rabbits, and did not affect rat fertility, the risk of PA21 treatment-related reproductive toxicity may be negligible if maternal health was not affected.

One of the degradation products of polynuclear iron (III)-oxyhydroxide, mononuclear iron (III)-hydroxide, is released depending on the pH, and is soluble at the pH < 4. The absorption of the limited soluble mononuclear iron (III)-hydroxide resulted in moderate higher plasma iron levels and moderate iron deposition in GI tract, liver, kidney, spleen, mesenteric and mandibular lymph nodes in chronic studies in rats, mice, and dogs. However, the iron accumulation was ≤ 2 times normal levels, and not associated with toxicological injury. The iron release from polynuclear iron (III)-oxyhydroxide in vitro under pH conditions in the stomach in the fed state was minimal (0-0.04%). Iron absorption in PA21 ADME studies was <3% in rats and negligible in dogs. The daily iron uptake from PA21 at maximal dose of 3000 mg iron/day may not be an issue to cause any concern in patients with ESRD, because patients with ESRD usually need more daily iron intake.

Lastly, there were exaggerated pharmacological effects of, or physiological response to, PA21-mediated phosphate depletion from the GI tract in normophosphatemic animals.

These effects included alterations in urine and, to some extent, plasma calcium and phosphorus, and increased bone turnover in repeat dose studies in mice, rats, rabbits, and dogs; higher incidence of urinary bladder calculi, associated with transitional epithelial hyperplasia in urinary bladder and dilated medulla tubules in the kidney of mice, slightly impaired renal function and a slightly increased incidence of benign thyroid c-cell adenoma in rats. In CKD patients with hyperphosphatemia, PA21 treatment will correct the higher plasma phosphate, but not lead to phosphate depletion. Therefore, the exaggerated pharmacological response or physiological response to phosphate depletion induced by PA21 treatment seen in normal animals is not applicable in CKD patients.

In conclusion, the drug interaction and local irritation/carcinogenicity findings in the GI tract identified in the preclinical studies suggest relevant risks in CKD patients on long-term treatment.

1.3 Recommendations

1.3.1 Approvability

Approvable

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Findings in the reproductive and developmental studies were ignored from the label. The interpretation for the mouse carcinogenicity study results and risk assessment are not acceptable. Revised wording (additions in bold; deletions in strikeout) are recommended in the following boxes:

(b) (4)



2 Drug Information

2.1 Drug

CAS Registry Number

β -iron (III)-oxyhydroxide: CAS No. 12134-57-5

Sucrose: CAS No. 57-50-1

Starch: CAS No. 9005-25-8

 (b) (4) 

Generic Name:  (b) (4)

Code Name: PA21

Chemical Name: The mixture of polynuclear iron (III)-oxyhydroxide (pn-FeOOH),
sucrose and starches

Molecular Formula/Molecular Weight: pn-FeOOH / N/A

Structure or Biochemical Description:



Pharmacologic Class: Phosphate binder

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 075610

2.3 Drug Formulation

PA21 was formulated as chewable tablets containing following ingredients:

Component	Function	Quantity per Tablet (mg)	Quantity per Tablet (%)
PA21 drug substance (iron(III)-oxyhydroxide, sucrose, starches)	Active ingredient		(b) (4)
Woodberry flavour ⁽²⁾	(b) (4)		
Neohesperidin dihydrochalcone			
Magnesium stearate ⁽⁴⁾			
Silicon dioxide, colloidal			
Total			

(1) Each tablet is standardized to contain 500 mg iron. The quantity of PA21 drug substance is based on an assumed 20% iron content.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population are patients with end-stage renal disease. Dosing regimen starts with one 2500 mg-tablet (500 mg iron) 3 times daily with meals (chewed and not swallowed whole) and is adjusted by 1 tablet per day as needed until an acceptable serum phosphorus level (≤ 5.5 mg/dL) is reached, with a maximal daily dose of 6 tablets (3000 mg iron).

2.7 Regulatory Background

None

3 STUDIES SUBMITTED

3.1 Studies Reviewed

See details in the TABLE OF CONTENT and Sections 4-9

3.2 Studies Not Reviewed

V8091, Efficacy of adsorber compounds on the binding of phosphate in the meal during transit through a dynamic model of the upper and the lower gastrointestinal tract (TIM-1 and TIM-2 system)

V9209, Evaluation of the phosphate binding capacity in the gastro-intestinal tract using an in vitro model of the stomach and the small intestine (TIM-1)

(b) (4)

Validation of a HPLC method with UV detection for the quantification of metoprolol, furosemide, enalapril and enalaprilate, losartan, nifedipine, digoxin, warfarin, ciprofloxacin, and omeprazole, atorvastatin, hydrochlorothiazide, metformin, pioglitazone, candesartan cilexetil, paricalcitol, bile acids cholyglycine, chenodeoxycholyglycine and deoxycholytaurine, and nifedipine (respectively to each study number) in aqueous solutions under GLP conditions

ANR-PA21DDI-A-001-001, Validation of an analytical method for the UPLC-FLR quantification of alendronate in water with alpha-amylase

ANR-PA21DDI-A-002-001 and ANR-PA21DDI-A-004-001, Validation of an analytical method for the UPLC-MS/MS quantification of cinacalcet, doxycycline, glipizide, quinidine, and levothyroxine in water with alpha-amylase

STU-PA21DDI-B201318-001 and STU-PA21DDI-B200829-001, Validation of an analytic method for the UPLC-MS/MS quantification of atorvastatin and paricalcitol in phosphate buffer with alpha-amylase

VFR074, PA21 – Preliminary toxicity and palatability study by dietary administration to CD rats for 2 weeks

VFR077, PA21 – Pilot toxicity study by oral capsule administration to beagle dogs

3.3 Previous Reviews Referenced

None

Figures and tables below were created by the reviewer or cited otherwise.

4 Pharmacology

Summary

The intended mechanism of action for PA21 is to bind dietary phosphate in the gastrointestinal (GI) tract, resulting in phosphate excretion with the feces and thereby preventing phosphate absorption. In vitro studies demonstrated efficient phosphate binding by PA21 under simulated GI tract conditions (pH range of 1.2 to 8.5), with a phosphate binding capacity at least equivalent to currently available phosphate binders. In vitro studies also indicated that iron phosphate was the chemical species formed under acidic conditions, whereas iron (III)-oxyhydroxide was the favored chemical

species over iron phosphate under alkaline conditions. In rat models of chronic renal failure (CRF), PA21 was as effective as calcium carbonate, sevelamer carbonate and lanthanum carbonate in correcting the hyperphosphatemia and associated secondary hyperparathyroidism observed in the model, was more effective than calcium carbonate and, to some extent of lanthanum carbonate in preventing vascular calcification in the thoracic aorta, and did not induce defective bone mineralization. The elevated bone turnover observed in CRF rats was corrected by PA21, with no evidence of iron deposition in the bone.

No adverse effects of PA21 on cardiovascular, central nervous, or respiratory systems were observed in safety pharmacology studies, and there was no biologically significant effect on GI motility in a charcoal propulsion study conducted in mice.

A short-term study in mice showed no influence of dietary components or drugs on absorption of iron from PA21. In vitro studies simulating physiological conditions demonstrated strong/complete adsorption or interaction of furosemide, losartan, atorvastatin, doxycycline, alendronate, levothyroxine and paricalcitol to/with PA21. The observed adsorption of levothyroxine, paricalcitol and atorvastatin was less pronounced in the presence of phosphate. No significant adsorption to or interaction with PA21 was found in the case of ciprofloxacin, digoxin, enalapril, metoprolol, nifedipine, warfarin, hydrochlorothiazide, metformin, quinidine, bile acids, water soluble B vitamins, amino acids, fluoride, or oxalate. Adsorption of pioglitazone to PA21 was moderate, and PA21 affected the solubility of omeprazole in vitro. Adsorption of cinacalcet, glipizide, candesartan cilexetil, or enalaprilate to PA21 was inconclusive due to inconsistent solubility at different pH levels or poor tablet dissolution. Furthermore, no influence of the macronutrient oxalate on phosphate binding capacity of PA21 was observed in in vitro interaction studies simulating GI tract conditions.

4.1 Primary Pharmacology

PA21 is mixture of iron (III)-oxyhydroxide (the active pharmaceutical ingredient, API), starch ((b) (4) in case of the original PA21 or (b) (4) in case of PA21-2) and sucrose. Iron content was (b) (4) in both PA21 and PA21-2. PA21 and PA21-2 acted similarly in the primary pharmacological studies, and were not all differentially identified in this review section.

4.1.1 In vitro phosphate adsorption of PA21 under various gastrointestinal conditions (SR-1330-01/E01, TC-1118/E01, REP000122TC-En03v.1, and REP000128TC-EN03v.1)

These in vitro studies were conducted at Vifor (International) Inc., issued during 2009-2012, and tested the phosphate adsorption of PA21 under various simulated gastrointestinal (GI) conditions.

Study #SR-1330-01/E01 assessed the in vitro phosphate adsorption of PA21 at pH levels similar to an empty or full stomach. PA21 (50 mg iron) was mixed with 10 ml phosphate buffer (1114 mg phosphorus/L) over the pH ranges 1.2 to 7.5 and 2.5 to 7.5 and incubated at 37°C (n=6 per condition). Phosphate in the aqueous solution was quantified photometrically. The phosphate adsorption was determined as difference between the added amount of phosphate and the non-adsorbed amount in the aqueous solution. At the lowest pH tested (i.e., pH 1.2), PA21 adsorbed 0.18 mg phosphorus (P)/mg iron (Fe), the amount adsorbed between pH 2.5 to 7.5 being slightly higher (0.20 to 0.21 mg P/mg Fe, Table 1). Thus, effective phosphate adsorption by PA21 was observed over the pH range 1.2 to 7.5.

Table 1. In vitro phosphate adsorption of P21 at 37°C (modified from the application)

	pH	Mean mg P/mg Fe	Coefficient of variance (%)
Test 1	1.2	0.184	1.2
	2.5	0.214	0.5
	4.5	0.212	0.6
	7	0.209	0.8
	7.5	0.204	0.6
Test 2	2.5	0.207	0.7
	4.5	0.205	1
	7	0.203	0.9
	7.5	0.201	0.7

Study #TC-1118/E01 compared in vitro phosphate adsorption of PA21 to commercially available phosphate binders. PA21 (100 mg iron), Renagel® (sevelamer hydrochloride), Fosrenol® (lanthanum carbonate), PhosLo® (calcium acetate) and calcium carbonate were suspended in 20 mL of 20 mM phosphate solution (619 mg phosphorus/L), incubated under three different pH conditions (3.0, 5.5 and 8.0) for 1 hour at room temperature (n=2 per condition). Phosphate in the aqueous solution was quantified photometrically. The phosphate adsorption was determined as difference between the added amount of phosphate and the non-adsorbed amount in the aqueous solution. Based on the results obtained, the quantity of active substance needed to bind 1,000 mg of phosphate (pill burden) was calculated for all the phosphate binders.

Phosphate adsorptions of PA21 and current clinical available phosphate binders Renagel, Fosrenol, PhosLo, and calcium carbonate were shown in Table 2. At pH 3, 5.5, and 8, phosphate adsorptions of PA21 were 37.9, 26.5, and 16.8 mg P/g active pharmaceutical ingredient (API) respectively; PA21 API needed to bind 1000 mg phosphate was 8.6, 12.3, and 19.5 g respectively, and pill burden was 3.7, 5.2, and 8.3 respectively. This study demonstrated that PA21 had a high affinity for phosphate over the entire pH range encountered in the GI tract, especially in the acidic environment of the stomach. In contrast, the phosphate binding capacity of Fosrenol, PhosLo and calcium carbonate was more dependent on the pH.

Table 2. Comparison of phosphate binders in vitro (modified from the application)

	pH	Adsorption (mg P/g API)	API (g) needed to bind 1000 mg phosphate	Pill burden
PA21	3	37.9	8.6	3.7
	5.5	26.5	12.3	5.2
	8	16.8	19.5	8.3
Renagel	3	46.2	7.1	8.9
	5.5	111.5	2.9	3.7
	8	81.2	4	5
Fosrenol	3	47.2	6.9	4.9
	5.5	4.8	91.5	64
	8	7.2	45.1	31.5
PhosLo	3	0.3	1096	1644
	5.5	94.7	3.4	5.2
	8	120	2.7	4.1
Calcium carbonate	3	2.3	149.9	
	5.5	108.2	3	
	8	4.3	78.1	

Study REP000122TC-EN03v.1 examined the in vitro phosphate binding capacity and iron release of PA21 over two different pH cycles, simulating the passage through the GI tract starting with low pH values in the stomach followed by higher pH values in the intestinal tract. PA21 chewable tablets were mixed in phosphate solution (800 mg phosphate /L = 25.8 mM phosphate) and incubated at various levels of pH. Phosphate binding and iron release of PA21 were determined.

- pH sequence A (initial pH)
 - pH 4.5 (15 minutes)
 - pH 2.5 (2 hours)
 - pH 8.5 (15 minutes)
 - pH 6.5 (3.5 hours)
 - pH 7.0 (22 hours)
- pH sequence B (initial pH values)
 - pH 6.5 (15 minutes)
 - pH 4.0 (30 minutes)
 - pH 1.5 (3 hours)
 - pH 8.5 (30 minutes)
 - pH 7.0 (30 minutes)
 - pH 4.0 (3 hours)

Phosphate binding capacities of PA21 during a simulated passage through the GI tract are shown in Figure 1. Phosphate adsorption was lowest at the beginning of the experiments, probably due to not enough time for equilibrium. Phosphate adsorption peaked at pH 2.6 in pH sequence A, and at pH 1.2 in pH sequence B. PA21 showed a high phosphate binding capacity especially at a low pH, and a robust phosphate binding capacity at higher pH values when the equilibrium had been reached. Phosphate concentrations in the incubation mixtures went down to ~25 mg/L in pH sequence A and 76-147 mg/L in pH sequence B. Iron release was negligible except at the lowest pH value of 1.2 (6.3%, Table 3), which represents the fasting state of the stomach.

Figure 1. In vitro PA21 binding capacity during a simulated passage through the GI tract (from the application)

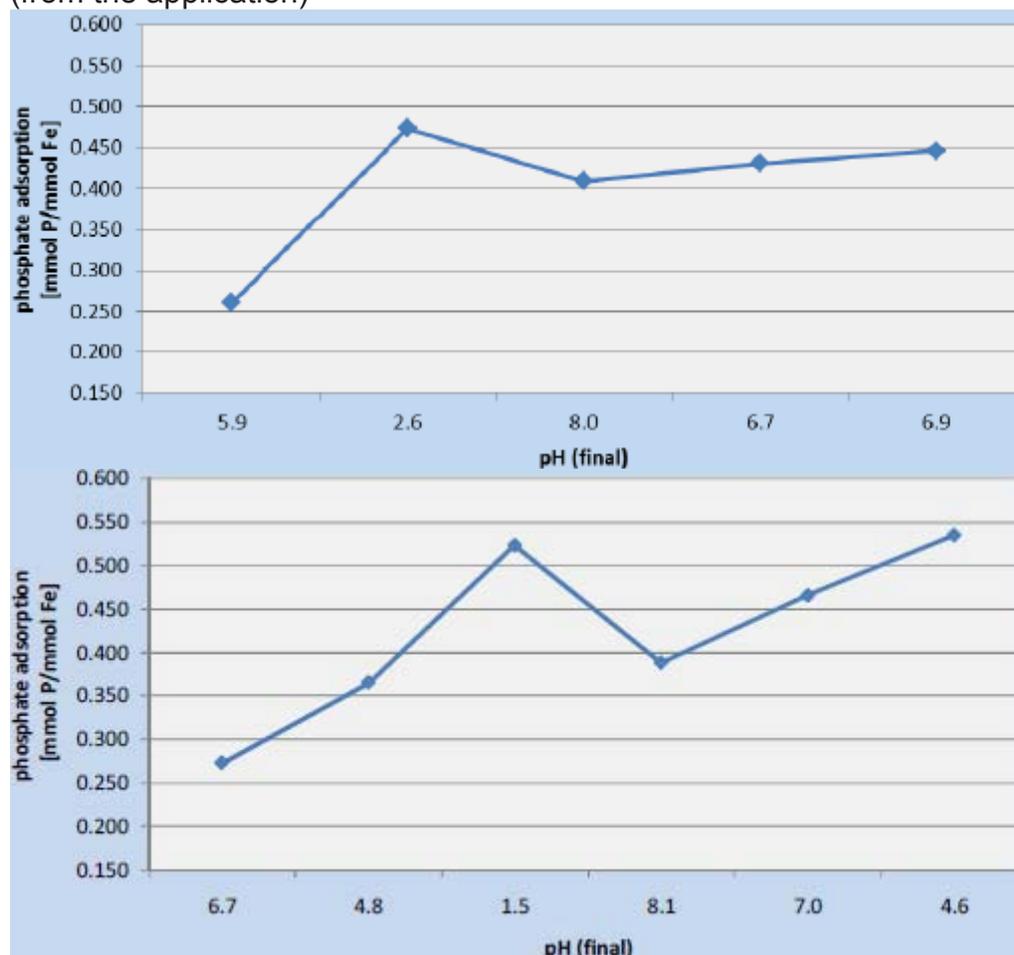


Table 3. Iron release of PA21 during a simulated passage through the GI tract (modified from the application)

pH sequence A				pH sequence B			
initial pH	weighed amount [g]	titrant [ml]	iron release % [m/m]	initial pH	weighed amount [g]	titrant [ml]	iron release % [m/m]
4.5	2.5666	0.1660	0.31	6.5	2.5741	1.1215	1.60
2.5	2.5889	0.1920	0.35	4.0	2.5630	0.3135	0.45
8.5	2.5870	0.1400	0.26	1.5	2.5813	4.4420	6.33
6.5	2.5879	0.1520	0.28	8.5	2.5721	1.6185	2.31
7.0	2.5884	0.1560	0.29	7.0	2.5868	0.7375	1.05
7.0	2.5855	0.1620	0.30	4.0	2.5941	0.1280	0.18

Study REP000128TC-EN03v.1 determined the chemical species that may be formed under physiological conditions via investigating the interactions between PA21 and phosphate over a range of pH values with various experimental methodologies. One gram of PA21 API was incubated with 40 mL of phosphate buffer (57 g phosphorus/L) at different pH levels (initial pH 1.0, 2.7, 5.5, and 8.1) for 2 hours at 37°C (final pH 1.2, 3.0, 5.6, and 8.2). Precipitates were washed and dried. Dried samples were then

analyzed for iron and phosphorus by inductively coupled plasma optical emission spectroscopy (ICP-OES), attenuated total reflectance Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy (XPS), as well as color analysis. Thermodynamic calculations and molar iron/phosphorus ratios were also employed in the analysis.

The quantitative and qualitative characteristics of dried precipitates were shown in Table 4. The phosphorus/Fe ratio was similar at final pH values of 3.0 and 5.6, whilst lower phosphate adsorption was observed at pH 8.2. The phosphorus/Fe ratio of 2.05 at pH 1.2 was unfeasibly high (maximum ratio 1.0), probably due to a very low iron resulting from iron lost during filtration. X-ray photoelectron spectroscopy measurements confirmed that under acidic conditions, iron was predominantly present as iron phosphate, whilst under alkaline conditions the amount of iron (III)-oxyhydroxide was significantly higher. The characteristics of the dried precipitates obtained from different pH levels indicate that under acidic conditions mainly iron phosphate forms, if PA21 is exposed to a phosphate solution. In slightly alkaline conditions, iron oxyhydroxide is favored over iron phosphate.

Table 4. Characteristics of dried precipitates (modified from the application)

samples Final pH	phosphorous concentration (mg/g PA21 API)	iron concentration (mg/g PA21 API)	phosphate adsorption (mg P/mg Fe)	phosphate adsorption (mmol P/mmol Fe)
1.2	9.32	8.19	1.14	2.05
3.0	95.87	221.66	0.43	0.78
5.6	91.38	213.29	0.43	0.77
8.2	51.33	264.43	0.19	0.35

Binding energies (BE) of P and Fe

Sample	pH	BE P 2p / eV	BE Fe 2p3 (of FePO ₄) / eV	BE Fe 2p3 (of PA21) / eV
PA21		-	-	710.4
PA21	1.2	132.6	712.1 (94%)	708.7 (6%)
	3.0			
	5.6	132.0	711.4 (89%)	710.4 (11%)
	8.2	131.7	711.4 (47%)	710.0 (53%)

Elemental ratios

Sample	pH	O / Fe ratio	P / Fe / O ratio
FePO ₄		11 : 1	1 : 0.4 : 4.0
PA21		6 : 1	
	1.2	20 : 1	1 : 0.3 : 5.5
	3.0	10 : 1	1 : 0.4 : 3.3
	5.6	9 : 1	1 : 0.4 : 3.7
	8.2		

Ratio of oxygen (PA21/(PA21 and iron phosphate))

Sample	pH	FeOOH / [FeOOH+PO ₄]
1.2		2.8%
3.0		2.2%
5.6		2.5%
8.2		15.2%

pFe derived from solubility products of FePO₄ and FeOOH

pH	pFe for FePO ₄	pFe for FeOOH
8.2	20.3	22.2
7.0	18.9	18.6
5.6	16.1	14.4
3.0	10.9	6.6
1.2	6.4	1.2

Color comparison

	PA21 API	FePO ₄ x2H ₂ O
Dried precipitate	1.2	
	3.0	
	5.6	
	8.2	

4.1.2 Effects of PA21 on vascular calcification in chronic renal failure rats and comparison among phosphate binders (Phan 2012a and Phan 2012b)

These in vivo studies were performed by [REDACTED] (b) (4) issued in Aug 2012, and sponsored by Vifor (international) Ltd.

In Study Phan 2012a, male Wistar rats (10 weeks old) were fed a high phosphorus diet (1.3% phosphorus, 1.06% calcium, 1000 IU/kg vitamin D3 and 23% protein) containing 0.75% adenine for four weeks to induce chronic renal failure (CRF). Then, adenine was withdrawn from the high phosphorus diet, and the CRF rats continued on high phosphorus diet only, or high phosphorus diet containing 0.5%, 1.5, or 5% PA21 (i.e., 0.1, 0.3, or 1% iron), or 3% calcium carbonate (i.e., 1.2% calcium) for 4 weeks (n=20, 9, 9, 20, and 20 respectively). Non-CRF rats were assigned to control, 3% calcium carbonate (CaCO₃), or 5% PA21 groups (treatment duration = 4 weeks, n=8/group). Rats were sacrificed at the end of the four-week treatment. Arterial blood pressure was determined 24 hours prior to sacrifice. Blood samples collected prior to and after the 4-week treatment and urine samples collected at sacrifice were analyzed for creatinine (Creat), calcium (Ca) and phosphorus (P). Blood samples were also determined for serum intact parathyroid hormone (iPTH), ferritin, and hematocrit (Ht). The proximal aorta was isolated for assessment of calcification using histomorphometry of aortic sections stained with Von Kossa's method.

There were no treatment-related deaths. There were no intergroup differences in blood pressure or heart rate. Four weeks of high phosphorus diet containing 0.75% adenine resulted in lower body weight and renal failure, indicated by marked elevations in serum creatinine and phosphorus (Table 5). In CRF rats, PA21 at the 5% level and calcium carbonate (3%) lowered serum phosphorus to levels observed in non-CRF rats, partly reversed the increase in serum iPTH levels, dramatically reduced phosphate in urine, but had no effect on the CRF-associated lower Ht (Table 5). PA21 at 5% in diet and calcium carbonate at 3% in diet also markedly reduced urine phosphate in non-CRF rats. As shown in Figure 2 and Table 6, chronic renal failure rats showed marked vascular calcification and PA21 at 5% in diet was associated with a significantly lower rate of calcification compared to CRF controls and calcium carbonate-treated CRF rats. Vascular calcification was also reduced by PA21 at the 1.5% dosage level.

Table 5. Serum/urine biochemistry and blood hematocrit (modified from the application)

	Group	N	Weight (g)	Serum		
				Creatinine ($\mu\text{mol/l}$)	Ca (mmol/l)	P (mmol/l)
Prior to treatment	Non-CRF control	8	254 \pm 7	67.1 \pm 3.2	2.45 \pm 0.04	2.68 \pm 0.18
	Non-CRF PA21 5%	8	285 \pm 6	66.2 \pm 0.5	2.48 \pm 0.03	3.05 \pm 0.97
	Non-CRF CaCO ₃ 3%	8	291 \pm 17	75.2 \pm 5.7	2.41 \pm 0.07	3.10 \pm 0.97
	CRF control	20	197 \pm 5 ^a	286 \pm 11.9 ^a	2.46 \pm 0.40	5.39 \pm 0.16 ^a
	CRF PA21 5%	20	206 \pm 5 ^a	260 \pm 15.3 ^a	2.41 \pm 0.41	4.90 \pm 0.21 ^a
	CRF PA21 1.5%	9	204 \pm 6 ^a	268 \pm 21.4 ^a	2.50 \pm 0.73	4.74 \pm 0.34 ^a
	CRF PA21 0.5%	9	200 \pm 3 ^a	254 \pm 30.5 ^a	2.52 \pm 0.17	4.98 \pm 0.62 ^a
	CRF CaCO ₃ 3%	20	202 \pm 5 ^a	289 \pm 8.7 ^a	2.37 \pm 0.03	5.28 \pm 0.31 ^a

Group	Weight g	Serum					Ht %	Urine	
		Creat $\mu\text{mol/l}$	Ca mmol/l	P mmol/l	iPTH pg/ml	Ferritin ng/ml		Ca/creat mmol/mmol	P/creat mmol/mmol
Non-CRF Control	410 \pm 7	44.6 \pm 2.1	2.40 \pm 0.03	2.15 \pm 0.11	105 \pm 14	176 \pm 28	45.8 \pm 1.0	0.31 \pm 0.06	5.15 \pm 1.64
Non-CRF PA21 5%	384 \pm 16	42.8 \pm 3.6	2.43 \pm 0.05	1.70 \pm 0.12	134 \pm 50	291 \pm 121	46.7 \pm 1.3	0.46 \pm 0.15	0.66 \pm 0.58
Non-CRF CaCO ₃ 3%	362 \pm 15	46.7 \pm 3.9	2.50 \pm 0.35	2.14 \pm 0.22	102 \pm 9	324 \pm 78	44.5 \pm 2.5	0.63 \pm 0.16	0.14 \pm 0.07
CRF Control	255 \pm 7 ^a	188 \pm 14 ^a	2.38 \pm 0.12	2.91 \pm 0.24	3261 \pm 397	381 \pm 51	27.4 \pm 1.4 ^a	1.15 \pm 0.19 [*]	13.9 \pm 1.6 ^{**}
CRF PA21 5%	256 \pm 8 ^a	180 \pm 15 ^a	2.41 \pm 0.03	2.21 \pm 0.09 ^b	1138 \pm 228 ^b	585 \pm 76	26.7 \pm 1.0 ^a	1.18 \pm 0.16	5.9 \pm 0.6 ^{##}
CRF PA21 1.5%	266 \pm 9 ^a	169 \pm 16 ^a	2.23 \pm 0.04	2.29 \pm 0.25 ^c	2727 \pm 695 ^d	632 \pm 104	27.3 \pm 2.2 ^a	0.63 \pm 0.13	9.5 \pm 1.3
CRF PA21 0.5%	250 \pm 9 ^a	188 \pm 18 ^a	2.39 \pm 0.07	2.74 \pm 0.37	4830 \pm 624 ^d	500 \pm 131	32.9 \pm 1.8 ^a	0.71 \pm 0.08	9.35 \pm 1.8
CRF CaCO ₃ 3%	245 \pm 8 ^a	191 \pm 15 ^a	2.39 \pm 0.06	2.06 \pm 0.11 ^b	1299 \pm 300 ^b	691 \pm 115 ^b	28.7 \pm 1.8 ^a	1.06 \pm 0.20	4.9 \pm 0.6 ^{##}

iPTH, intact parathyroid hormone; Ht, hematocrit. ^a $p < 0.001$ versus all non-CRF, ^b $p \leq 0.001$ versus CRF control, ^c $p < 0.05$ versus CRF control, ^d $p < 0.05$ versus CRF PA21 5% or CaCO₃ 3%. ^{*} $p < 0.05$ versus non-CRF control, ^{**} $p < 0.01$ versus non-CRF control, ^{##} $p < 0.01$ versus CRF control.

Figure 2. von Kossa stains of thoracic aortas for vascular calcification at the end of four weeks of treatment (from the application)

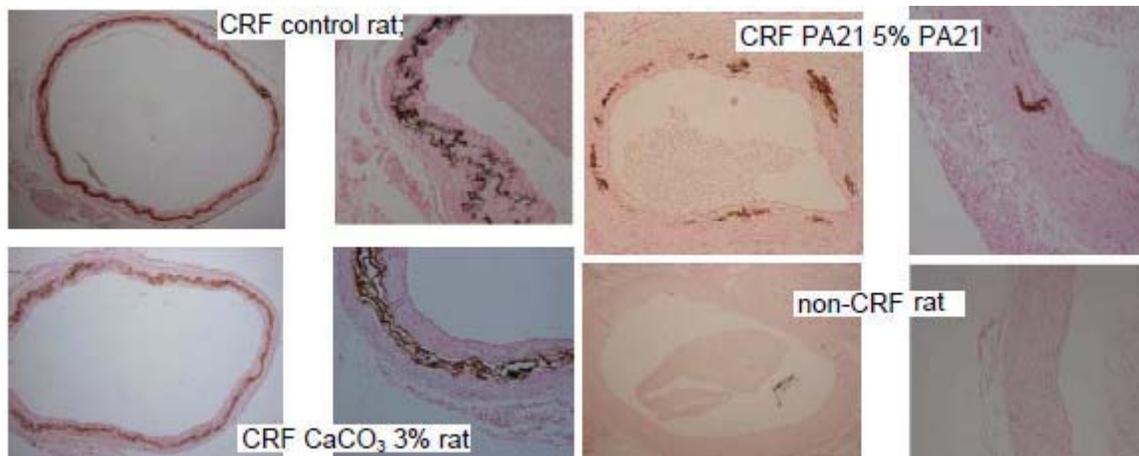


Table 6. Vascular calcification score after 4 weeks of phosphate binder treatment (modified from the application)

	CRF control	CRF PA21 5%	CRF PA21 1.5%	CRF PA21 0.5%	CRF CaCO ₃ 3%	Non-CRF control
N	17	18	9	7	19	7
Score, n (%)						
0	3 (17%)	13 (72%)	7 (78%)	1 (14%)	8 (42%)	6 (86%)
1	1 (6%)	1 (6%)	0	0	0	1 (14%)
2	5 (29%)	4 (22%)	1 (11%)	2 (29%)	7 (37%)	0
3	8 (47%)	0	1 (11%)	4 (57%)	4 (21%)	0
High calcification score, n (%) ^a	13 (76%)	4 (22%) ^{*#}	2 (22%) ^{**}	6 (86%)	11 (58%)	0

^a High calcification score was defined as score 2 and 3.

** p<0.01 versus CRF controls

p<0.05 compared to CaCO₃ 3%-treated CRF rats

In study Phan 2012b, effects of PA21 on phosphate homeostasis and vascular calcifications were compared to lanthanum carbonate and sevelamer carbonate in a rat model of CRF (model details in study Phan 2012a). CRF rats were on diet containing 5% PA21 (1% Fe), 2% lanthanum carbonate (1% lanthanum), 1.5% sevelamer carbonate (1% active substance), or diet alone for 4 weeks (n=20-22/group). A non-CRF control group of 6 rats was included. Rats were killed following the 4 week treatment period. Urine and feces were collected over the few days before sacrifice. At termination, blood samples were collected for determining hematocrit (Ht) and serum creatinine, Ca, P and alkaline phosphatase (ALP), and iPTH. The aorta, carotid and femoral arteries were assessed for calcification using histomorphometry of sections stained with Von Kossa's method. Femur samples were collected for examination in a secondary pharmacology study (b) (4).

Four weeks of high phosphorus diet containing 0.75% adenine resulted in renal failure, indicated by marked elevations in serum creatinine (251±17.7 µmol/L) and phosphorus (4.83±0.28 mmol/L). Eight out of 89 rats were euthanized one week after the end of adenine due to malnutrition associated with end stage renal disease (weight inferior to 160 g): 4/22 CRF-control, 1/20 CRF-PA21 and 3/20 CRF-lanthanum.

In the rat model of CRF, all 3 phosphate binders significantly reduced the elevated serum and urinary phosphorus levels, PA21 appearing to be the most effective (Table 7, Table 8, Table 9); all 3 drugs also reduced serum iPTH to a similar degree (Table 7). Phosphate excretion route differed between the untreated and treated CRF groups with a shift from urinary to fecal excretion following administration of the phosphate binders (Table 10). All 3 drugs caused reductions in the extent of calcification in the thoracic aorta. In the superior thoracic aorta, PA21 was significantly more effective than lanthanum carbonate in preventing calcification (Figure 3).

Table 7. Body weight, hematocrit, and serum biochemistry (modified from the application)

prior to treatment	N	Weight g	Creatinine $\mu\text{mol/l}$	Ca mmol/l	P mmol/l
	CRF control	22	214 \pm 5	251 \pm 17.7	2.27 \pm 0.40
CRF PA21	19	216 \pm 3	239 \pm 19.0	2.44 \pm 0.04	4.75 \pm 0.32
CRF Sevelamer	20	223 \pm 4 ^a	229 \pm 16.2	2.42 \pm 0.06	4.44 \pm 0.23
CRF Lanthanum	18	209 \pm 4	233 \pm 10.6	2.41 \pm 0.03	4.62 \pm 0.21

after 4 weeks of treatment, at the time of sacrifice

	N	Weight g	Creatinine $\mu\text{mol/l}$	Ca mmol/l	P mmol/l	iPTH pg/ml	ALP mmol/l	Ht %	Ferritin ng/ml
Non-CRF Control	4	299 \pm 11	48.3 \pm 3.05	2.46 \pm 0.05	2.09 \pm 0.05 ^d	275 \pm 38 ^d	292 \pm 21	42 \pm 0.8 ^d	2765 \pm 385
CRF Control	20	308 \pm 3.1	144 \pm 11 ^b	2.32 \pm 0.05	3.30 \pm 0.29	3567 \pm 593	430 \pm 26	26.7 \pm 0.5 ^e	2359 \pm 281
CRF PA21	19	306 \pm 5.1	141 \pm 10 ^b	2.45 \pm 0.03	2.06 \pm 0.06 ^d	1459 \pm 242 ^d	363 \pm 19	26.4 \pm 0.5 ^e	2185 \pm 173
CRF Sevelamer	20	313 \pm 3.9 ^a	147 \pm 11 ^b	2.48 \pm 0.03 ^c	2.51 \pm 0.12 ^d	1569 \pm 238 ^d	367 \pm 19	21.9 \pm 0.5 ^{d,e}	2188 \pm 134
CRF Lanthanum	18	299 \pm 11	140 \pm 7.9 ^b	2.52 \pm 0.03 ^c	2.24 \pm 0.07 ^d	1360 \pm 170 ^d	379 \pm 29	22.7 \pm 0.5 ^{d,e}	2361 \pm 184

^a p <0.05 versus CRF Lanthanum.^b p <0.01 versus non-CRF control,^c p <0.01 versus CRF control, ^d p <0.0001 versus CRF control, ^e p <0.0001 versus non-CRF control

Table 8. Urinalysis at sacrifice (modified from the application)

	N	Ca/creat U mmol/mmol	P/creat U mmol/mmol	Alb/creat mmol/mmol
Non-CRF Control	3	0.59 \pm 0.10	10.4 \pm 1.42	0.51 \pm 0.24
CRF Control	18	1.45 \pm 0.17	17.8 \pm 1.91	5.23 \pm 1.13
CRF PA21	16	1.12 \pm 0.12	4.31 \pm 0.61 ^a	2.70 \pm 0.69
CRF Sevelamer	20	1.44 \pm 0.12	10.1 \pm 0.73 ^{a,b}	5.08 \pm 1.15
CRF Lanthanum	17	1.45 \pm 0.12	7.89 \pm 0.61 ^a	4.32 \pm 0.83

^a p <0.001 versus CRF control, ^b p <0.05 versus CRF PA21

Table 9. 24 hours urinary values of Ca, P, and Creat for rats in metabolic cages (modified from the application)

	Ca U $\mu\text{mol}/24\text{h}$	P U $\mu\text{mol}/24\text{h}$	Creat U $\mu\text{mol}/24\text{h}$	Ca/creat U $\mu\text{mol}/\mu\text{mol}$	P/creat U $\mu\text{mol}/\mu\text{mol}$
CRF Control	62.7 \pm 13.8	1384 \pm 547	101 \pm 36.4	0.64 \pm 0.11	13.7 \pm 3.5
CRF PA21	41.6 \pm 11.5	142 \pm 176 ^a	107 \pm 16.3	0.40 \pm 0.15	1.19 \pm 1.36 ^a
CRF Sevelamer	98.7 \pm 56.7	482 \pm 295	71.7 \pm 15.4	1.55 \pm 1.20	7.63 \pm 6.34
CRF Lanthanum	83.0 \pm 30.3	119 \pm 39 ^a	83.1 \pm 10.8	1.02 \pm 0.39	1.49 \pm 0.71 ^a

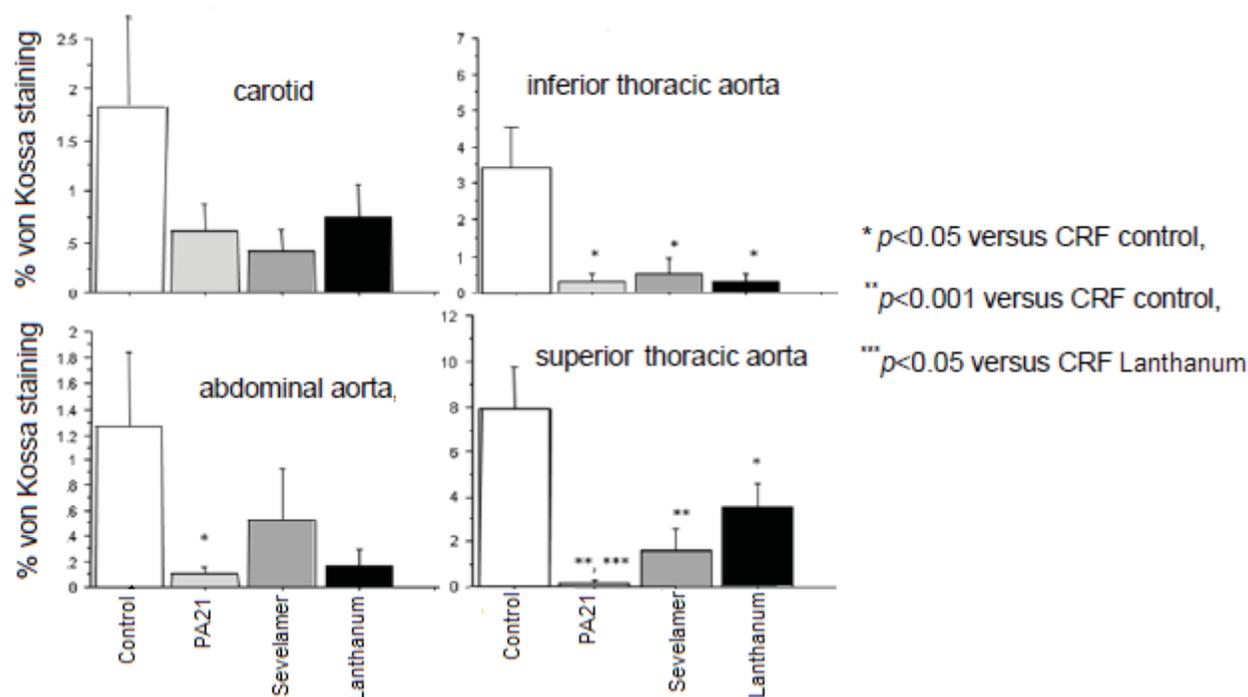
^a p <0.05 versus CRF control, n = 3/groups.

Table 10. Balance of phosphorus intake and excretion for rats in metabolic cages (modified from the application)

	Intake Diet and water mg P	Excretion		% Recovery P excretion /P intake %
		Urine mg P	Feces mg P	
CRF Control	201.8± 110.5	42.9± 16.9	89.6 ± 23.8	74 ± 22
CRF PA21	174.1± 97.6	4.4± 5.4 ^a	123.2 ± 52.2	76± 7
CRF Sevelamer	152.3± 59.0	14.9 ± 9.1	115.1 ± 47.9	84 ± 9
CRF Lanthanum	138.7± 13.2	3.7 ± 1.2 ^a	95.3 ± 7.5	72 ± 9

^a $p < 0.01$ versus CRF control, ^b $p < 0.001$ versus CRF control, $n = 3/\text{groups}$.

Figure 3. Effects of phosphate binders on vascular calcification (von Kossa staining) in CRF rats (modified from the application)



4.2 Secondary Pharmacology

4.2.1 Effects of PA21 compared to lanthanum carbonate and sevelamer carbonate on bone abnormalities in a rat model of chronic renal failure (2012)

The study report was signed by [redacted] on June 20, 2012. Rat femur samples were obtained from afore Study Phan 2012b. Bone samples were histologically processed, and stained with modified Masson-Goldner trichrome and Gomori stain (for iron). Static and dynamic parameters of trabecular bone structure, formation and resorption were measured at a standardized

site below the growth plate using a semi-automated method. Cortical thickness was also measured.

As shown in Table 11, there were no differences in trabecular bone volume/tissue volume, trabecular thickness, trabecular separation and cortical thickness between the study groups. Compared to sham controls (non-CRF), osteoid volume/bone volume, osteoid surface/bone surface and osteoid thickness were significantly increased in CRF rats. These increases were not influenced by treatment with PA21, lanthanum carbonate, or sevelamer carbonate. The numbers of osteoblasts/bone perimeter and osteoblast surface/bone surface ratios were higher in CRF controls than in sham controls, and treatment with PA21, sevelamer carbonate, or lanthanum carbonate lowered the numbers of osteoblasts. There was no effect of the various treatments on bone erosion parameters. Bone formation rate/bone surface was significantly higher in CRF controls than sham controls, and treatment with PA21 or lanthanum carbonate returned formation rates/bone surface to levels observed in sham controls. Analysis of the data revealed osteomalacia in all groups of CRF rats, irrespective of treatment, and was considered related to adenine-induced changes in renal function and associated tubular phosphate losses. Special stains did not reveal any iron deposition in the bone of treated rats. In conclusion, the increased bone turnover observed in CRF rats was corrected by administration of PA21 or lanthanum carbonate.

Table 11. Bone histomorphometric parameters at the end of treatment

	Sham control	CRF rats			
		Control	PA21	Sevelamer	Lanthanum
Parameters of Bone structure					
Trabecular Bone Volume/Tissue Volume - %	22.2 ± 4.9	22.8 ± 2.1	19.4 ± 2.2	23.2 ± 2.5	21.5 ± 2.7
Trabecular Thickness (Plate) - μm	61.7 ± 4.1	72.7 ± 6.1	63.7 ± 5.1	78.9 ± 8.4	69.0 ± 7.6
Trabecular Separation (Plate) - μm	269 ± 56	269 ± 26	297 ± 27	274 ± 19	272 ± 21
Cortical Thickness - μm	463 ± 43	462 ± 26	438 ± 15	432 ± 18	408 ± 19
Static Parameters of Bone Formation					
Osteoid Volume/Bone Volume - %	2.5 ± 1.0	17.3 ± 4.8*	25.7 ± 4.1*	25.7 ± 5.6*	26.9 ± 6.5*
Osteoid Surface/Bone Surface - %	13.4 ± 3.7	43.0 ± 9.3*	46.0 ± 5.6*	59.7 ± 12.4*	50.3 ± 9.0*
Osteoid Thickness - μm	5.1 ± 1.1	11.1 ± 1.7*	14.6 ± 1.7*	15.9 ± 2.3*	13.2 ± 1.8*
Osteoblast Number/Bone Perimeter - #/100mm	189 ± 78	788 ± 191*	128 ± 41 [^]	423 ± 114 [^]	221 ± 80 [^]
Osteoblast Surface/Bone Surface - %	2.5 ± 1.0	11.0 ± 2.5*	1.8 ± 0.6 [^]	6.2 ± 1.6 [^]	3.3 ± 1.2 [^]
Parameters of Bone Erosion					
Erosion Surface/Bone Surface - %	3.56 ± 1.10	4.67 ± 0.91	4.61 ± 0.83	5.52 ± 1.03	4.52 ± 1.21
Erosion Depth - μm	8.86 ± 0.80	11.4 ± 1.07	12.3 ± 1.05	11.9 ± 0.99	10.3 ± 0.86
Osteoclast Number/Bone Perimeter - #/100mm	79.6 ± 23.3	94.2 ± 19.3	97 ± 18.3	117 ± 21.5	101 ± 28
Osteoclast Surface/Bone Surface - %	2.86 ± 0.91	4.07 ± 0.78	3.91 ± 0.68	4.51 ± 0.85	3.89 ± 1.15
Dynamic Parameters					
Mineral Apposition Rate/Day - μm/Day	2.4 ± 0.33	2.88 ± 0.25	2.12 ± 0.16	2.32 ± 0.17	2.61 ± 0.25
Double Labels/Bone Surface - %	6.48 ± 1.24	11.8 ± 2.32	7.43 ± 1.26	9.2 ± 2.01	7.06 ± 1.19
Single Labels/Bone Surface - %	4.51 ± 0.56	6.01 ± 0.79	4.91 ± 0.70	3.23 ± 0.35	4.05 ± 0.60
Mineralized Surface/Bone Surface - %	8.73 ± 1.24	14.8 ± 2.32	9.89 ± 1.48	10.8 ± 2.08	9.09 ± 1.36
Bone Formation Rate/Bone Surface - mm ³ /cm ² /year	7.69 ± 1.68	18.7 ± 2.76*	8.53 ± 1.31 [^]	13.5 ± 2.37	9.90 ± 1.39 [^]
Mineralization Lag Time - Days	5.01 ± 2.32	70.4 ± 63.7	98.8 ± 58.9	42.5 ± 25.2	50.1 ± 26.2
Osteoid Maturation Time - Days	2.58 ± 0.39	6.33 ± 3.42	7.96 ± 1.35	6.07 ± 1.31	6.13 ± 1.21

* p<0.01 vs Sham control. [^] p<0.05 vs CRF control. Data in mean±SEM

4.2.2 Effects of various phosphate binders on mineral and bone metabolism

(b) (4) 2010)

The study report was signed by (b) (4) on Aug 13, 2010. Male Sprague Dawley rats (10-11 weeks old) were used. Chronic renal failure (CRF) in rats was induced with a high phosphate (0.73% phosphorus) diet containing adenine (0.75% in the first week, 0.50% thereafter for 3 weeks). Rats on the high phosphate diet alone served as sham control (non-CRF). One of the phosphate binders (PA21, aluminum hydroxide, calcium acetate, lanthanum carbonate, and sevelamer carbonate) was added at 600 mg/kg/day into the diet during the induction of CRF for 4 weeks (n=8/group). Urine samples were collected once per week and serum samples were obtained at the end of treatment, for the determination of renal function and calcium/phosphate metabolism markers (serum and urinary creatinine, calcium and phosphorus levels) as well as markers of bone metabolism [serum parathyroid hormone (PTH), calcitriol, and fibroblast growth factor 23 (FGF-23)]. Detailed histology and histomorphometry of femur samples, including static and dynamic parameters of bone structure, formation, and resorptions, were performed at the end of study.

Three animals died from distress (not eating) during the second week of the experiment (1/8 in the CRF control group, 1/8 in the CRF+Lanthanum group, and 1/8 from the CRF+Calcium acetate group). Body weight in the CRF control group were 6-10% lower than in sham group, which was not affected by treatment with any of the phosphate binders (Table 12).

Table 12. Body weight (mean \pm SEM, modified from the application)

	Sham	CRF					
	Control	Control	PA21	Al	CA	LaC	SevC
Baseline	341 \pm 7.1	341 \pm 6.5	345 \pm 7.7	348 \pm 5.4	359 \pm 7.5	341 \pm 6.4	356 \pm 7.5
Week 1	364 \pm 7.5	341 \pm 6.0	327 \pm 8.2	325 \pm 9.0	341 \pm 13.6	314 \pm 14.4	332 \pm 13.0
Week 2	385 \pm 9.5	347 \pm 4.3	345 \pm 8.4	341 \pm 7.1	358 \pm 10.8	336 \pm 9.4	353 \pm 8.1
Week 3	391 \pm 10.0	362 \pm 5.2	365 \pm 9.3	364 \pm 9.9	386 \pm 8.5	358 \pm 7.0	377 \pm 7.0
Week 4	403 \pm 10.5	376 \pm 5.4	380 \pm 10.5	378 \pm 6.9	397 \pm 7.1	369 \pm 6.9	399 \pm 6.0

CA: Calcium acetate. Al: Aluminum hydroxide. LaC: Lanthanum carbonate. SevC: Sevelamer carbonate.

Creatinine clearance was reduced and urinary phosphorus was increased significantly in all CRF rats, with no difference between control and treatment groups (Table 13, Table 14). Serum phosphorus and urinary and serum calcium levels (Table 15), serum calcitriol, FGF-23, and PTH levels (Table 16) were similar among all study groups.

Table 13. Renal function (modified from the application)

	Sham Control	CRF						
		Control	PA21	Al	CA	LaC	SevC	
Urinary Creatinine (mg/dl)	Baseline	87.0±10.0	93.2±6.9	67.4±9.9	88.7±11.3	88.5±14.0	72.4±11.2	70.0±9.9
	Week 1	142±12.3	50.9±4.0	51.3±5.3	63.2±5.6	60.0±8.0	61.4±3.5	84.1±14.5
	Week 2	106±10.0	49.9±4.3	36.8±6.2	50.2±6.5	45.0±7.5	40.0±4.3	44.6±7.6
	Week 3	85.5±12.3	51.2±6.5	37.2±5.1	45.8±7.9	68.3±25.9	44.4±5.9	46.8±7.5
	Week 4	104±14.7	57.7±2.9	46.6±8.2	57.1±7.7	61.3±8.6	51.6±8.7	53.8±11.5
Serum Creatinine (mg/dl)	0.53±0.3	0.59±0.5	0.68±0.6	0.74±0.5	0.61±0.5	0.69±0.3	0.65±0.05	
Creatinine Clearance (ml/min)	1.79±0.25	0.94±0.12	0.67±0.13	0.74±0.12	0.92±0.12	0.71±0.15	0.82±0.23	

CA: Calcium acetate . Al: Aluminum hydroxide . LaC: Lanthanum carbonate . SevC: Sevelamer carbonate .

Table 14. Urinary and serum phosphorus levels (modified from the application)

	Sham Control	CRF						
		Control	PA21	Al	CA	LaC	SevC	
Urinary Phosphorus (mg/24 hrs)	Baseline	7.50±1.56	3.39±0.89	11.6±2.51	6.56±1.18	5.99±1.39	11.4±1.17	11.6±1.56
	Week 1	17.3±3.01	40.1±4.03	39.9±4.28	32.4±2.36	32.8±4.37	30.8±3.35	23.9±3.66
	Week 2	23.9±2.94	33.6±3.03	49.7±7.36	42.6±6.36	42.8±3.77	40.4±4.44	42.2±7.05
	Week 3	32.3±4.72	44.4±4.79	58.7±7.52	53.4±5.77	49.0±9.06	48.6±3.97	51.9±6.77
	Week 4	29.2±5.53	53.0±9.06	54.8±6.17	42.1±6.68	49.3±3.86	49.3±6.51	58.9±8.72
Serum Phosphorus (mg/dl)	9.04±0.37	8.29±0.29	7.99±0.52	8.34±0.45	8.83±0.86	9.01±0.77	9.79±0.64	

CA: Calcium acetate . Al: Aluminum hydroxide . LaC: Lanthanum carbonate . SevC: Sevelamer carbonate .

Table 15. Calcium levels (modified from the application)

	Sham Control	CRF						
		Control	PA21	Al	CA	LaC	SevC	
Urinary Calcium (mg/dl)	Baseline	12.0±0.86	13.5±1.70	11.7±1.66	13.4±1.25	14.4±1.04	10.6±0.90	11.8±0.64
	Week 1	8.53±0.89	6.17±0.39	5.98±0.36	6.48±0.52	9.24±1.49	6.02±0.21	7.06±0.97
	Week 2	6.85±0.61	5.17±0.20	5.76±0.46	5.13±0.33	5.21±0.44	5.21±0.21	5.13±0.32
	Week 3	5.58±0.74	4.91±0.35	5.29±0.55	5.80±0.71	7.39±1.67	4.54±0.23	6.03±0.66
	Week 4	7.73±1.45	7.95±1.18	8.63±1.29	6.04±0.59	6.85±0.69	7.61±0.79	8.80±1.08
Serum Calcium (mg/dl)	10.1±0.24	9.11±0.68	9.48±0.65	9.96±0.17	9.66±0.81	10.3±0.30	10.1±0.41	

CA: Calcium acetate . Al: Aluminum hydroxide . LaC: Lanthanum carbonate . SevC: Sevelamer carbonate .

Table 16. Levels of bone metabolism markers at the end of treatment (modified from the application)

	Sham	CRF					
	Control	Control	PA21	Al	CA	LaC	SevC
VITD 125	148±18.6	163±12.1	125±17.5	117±16.6	108±18.4	150±19.8	132±14.7
FGF-23	18.2±3.44	13.4±1.72	19.4±5.83	23.3±4.75	22.8±5.74	19.1±4.24	25.4±5.48
PTH	85.7±16.4	123±38.5	94.8±29.6	123±35.7	73.68±22.8	143±36.0	157±47.3

CA: Calcium acetate. Al: Aluminum hydroxide. LaC: Lanthanum carbonate. SevC: Sevelamer carbonate.
VITD 125: Calcitriol (pg/ml). FGF-23: Fibroblast Growth Factor-23 (pg/ml). PTH: Parathyroid Hormone (pg/ml).

There were no marked differences in parameters of bone structure, formation, resorption, and dynamics between sham controls and CRF control, although rats treated with PA21 showed highest bone volume and trabecular thickness, and exhibited the highest rate of bone formation per osteoblast, which were not statistically significant (Table 17). None of the phosphate binders induced osteomalacia.

In conclusion, preventatively administration of PA21 and other phosphate binders (each at 600 mg/kg/day) to rats with renal insufficiency did not show any effects on renal function or mineral and bone metabolism.

Table 17. Bone histomorphometric parameters at the end of treatment (modified from the application)

	Sham	CRF					
	Control	Control	PA21	Al	CA	LaC	SevC
Bone Structure							
Bone Volume/ Total Volume (%)	12.0±1.01	14.6±3.23	18.3±3.2	16.3±1.64	15.7±2.6	14.9±2.0	14.8±2.6
Trabecular Thickness (micron)	42.1±2.41	50.0±5.71	54.5±3.1	48.5±2.64	49.6±3.72	45.8±4.7	44.0±4.3
Trabecular Separation (Plate) (micron)	322±33.8	356±69.4	281.5±39.1	270±32.5	306±50.9	278±27.6	290±39.3
Growth plate width (micron)	93.0±4.72	101±3.92	96.3±4.94	98.5±3.00	98.3±4.37	98.9±8.75	99.6±5.42
Cortical thickness (micron)	345±54.9	295±55.4	314±52.9	294±45.5	372±62.2	301±50.4	354±40.3
Bone Formation							
Osteoid Volume/ Bone Volume (%)	1.16±0.21	1.15±0.35	0.42±0.10	0.59±0.20	0.48±0.21	0.89±0.27	0.36±0.11
Osteoid Surface/ Bone Surface (%)	7.35±1.54	7.26±1.25	3.51±0.65	3.43±0.83	4.18±1.15	5.01±0.84	3.25±0.86
Osteoid Thickness (micron)	3.40±0.24	3.48±0.75	2.89±0.50	3.84±0.57	2.37±0.45	3.53±0.59	2.49±0.67
Osteoblast Number/ Bone Length (per 100 mm)	45.1±5.58	113±54.1	39.2±10.0	35.4±11.6	74.1±40.9	81.2±22.1	41.2±11.8
Bone Resorption							
Erosion Surface/ Bone Surface (%)	7.89±1.62	10.5±2.73	7.24±1.28	5.75±1.09	5.94±1.46	6.32±1.75	8.65±1.39
Erosion Depth (micron)	8.61±0.56	9.69±0.85	8.76±0.42	9.31±0.94	7.99±1.04	7.99±0.46	8.29±0.70
Osteoclast Number/ Bone Length (per 100 mm)	220±47.0	315±93.0	206±30.1	166±30.1	206±57.0	169±46.8	311±48.8
Bone Dynamics							
Mineral Apposition Rate/Day (micron/Day)	2.62±0.25	3.22±0.38	2.98±0.18	2.24±0.30	2.66±0.40	3.21±0.39	2.79±0.29
Mineralized Surface / Bone Surface (%)	14.1±1.87	16.9±2.83	12.6±2.55	14.0±2.16	14.5±3.23	15.0±3.32	15.8±1.70
Bone Formation Rate/ Bone Surface (mm ³ /cm ² /y)	13.7±2.11	19.1±3.29	13.8±2.75	12.2±2.62	16.5±4.14	18.8±6.23	16.7±3.28
Bone Formation Rate/ Osteoblast (mm ² /Ob/yr)	335±59.0	309±107	434±95.0	330±74.2	281±100	251±52.9	358±55.0
Mineralization Lag Time (days)	0.96±0.33	0.81±0.24	0.40±0.12	0.73±0.26	0.67±0.34	0.93±0.46	0.22±0.08
Osteoid Maturation Time (days)	1.64±0.15	1.38±0.28	1.17±0.20	2.65±0.77	1.21±0.33	1.56±0.42	1.21±0.39

CA: Calcium acetate. Al: Aluminum hydroxide. LaC: Lanthanum carbonate. SevC: Sevelamer carbonate.

4.3 Safety Pharmacology

4.3.1 Evaluation of respiratory parameters in the conscious rat using whole body bias flow plethysmography (VFR081/052332)

This GLP study (VFR 081) was conducted in (b) (4) (sponsored by Vifor) in February 2005.

Male CD rats (~6 weeks old, fasted, 8/group) were orally gavaged with vehicle 1% w/w methylcellulose (10 ml/kg) or a single dose of PA 21 at 1250, 2500, or 5000 mg/kg. Rats orally dosed with 200 mg/kg morphine sulphate served as positive controls. All rats were assessed for respiration rate, tidal volume, and minute volume prior to dosing, at ~30, 90, 150 and 300 minutes post-dose using whole body bias flow plethysmography.

Oral morphine sulphate at 200 mg/kg resulted in statistically significant decreases in respiration rate and minute volume at 30 minutes post-dose and a significant decrease in respiration rate at 90 minutes post-dose when compared to vehicle controls. A single oral dose of PA21 up to 5,000 mg/kg did not affect respiration rate, tidal volume, or minute volume during any post-dose time points (Table 18).

Table 18. Respiratory parameters

Treatment	Dose (mg/kg)	Pre-dosing	Post-dose (min)			
			30	90	150	300
Respiration rate (br/min)						
Vehicle		335 ± 22	229 ± 30	161 ± 32	171 ± 70	147 ± 35
PA 21	1250	332 ± 22	217 ± 46	181 ± 26	149 ± 51	137 ± 37
	2500	333 ± 30	203 ± 35	179 ± 54	161 ± 28	183 ± 34
	5000	338 ± 30	209 ± 66	171 ± 37	139 ± 33	154 ± 43
Morphine sulphate	200	331 ± 28	123 ± 20**	131 ± 14*	137 ± 20	145 ± 55
Tidal volume (ml)						
Vehicle		1.38 ± 0.16	1.44 ± 0.07	1.71 ± 0.23	1.72 ± 0.31	1.98 ± 0.38
PA 21	1250	1.51 ± 0.10	1.57 ± 0.08	1.68 ± 0.18	1.86 ± 0.21	2.13 ± 0.26
	2500	1.49 ± 0.12	1.65 ± 0.25	1.60 ± 0.17	1.63 ± 0.21	1.92 ± 0.36
	5000	1.50 ± 0.10	1.65 ± 0.34	1.74 ± 0.21	1.83 ± 0.26	2.12 ± 0.36
Morphine sulphate	200	1.48 ± 0.13	1.61 ± 0.24	1.73 ± 0.21	1.86 ± 0.27	1.83 ± 0.39
Minute volume (ml)						
Vehicle		433 ± 69	288 ± 34	240 ± 39	257 ± 84	266 ± 39
PA 21	1250	465 ± 53	297 ± 68	264 ± 29	243 ± 67	266 ± 54
	2500	461 ± 62	285 ± 45	248 ± 57	234 ± 30	309 ± 40
	5000	467 ± 27	283 ± 53	256 ± 32	234 ± 41	285 ± 34
Morphine sulphate	200	451 ± 54	198 ± 52**	226 ± 39	251 ± 50	253 ± 107

* p<0.05 vs Vehicle, ** p<0.01 vs Vehicle

4.3.2 Irwin dose range in rats, including body temperature and locomotor assessment (VFR082/052395)

This GLP study (VFR 082) was conducted in (b) (4) (sponsored by Vifor) during February-March, 2005.

Male CD rats (fasted, 6-7 weeks old, 4/group) were orally gavaged with vehicle (1% w/w methylcellulose, 10 ml/kg) or a single dose PA 21 at dose 1250, 2500, or 5000 mg/kg. General behavior, body temperature, and spontaneous locomotor activity (Table 19) were assessed pre-dose, at 30, 90, 150, and 300 minutes after dosing, and again on Day 2. The animals were then observed daily for mortalities and for the appearance of delayed effects of toxicity up to Day 7.

Table 19. Observations for spontaneous locomotor activity

Lethality		Startle response		Aggressiveness
Restlessness		Loss of righting reflex		Body tone
Apathy		Abnormal body carriage	Type	Grip strength
Writhing			Score	Cutaneous blood flow
Fighting		Abnormal gait	Type	Cyanosis
Stereotyped behaviour	Type		Score	Ptosis
	Score	Straub tail		Lacrimation
Tremor		Piloerection		Salivation
Twitches		Pupil diameter		Pain response
Convulsions	Type	Touch response		Paralysis
	Score	Fearfulness		Grooming
Exophthalmos		Pinna reflex		Diarrhoea
Respiration	Type	Corneal reflex		Vocalisation
	Score	Catalepsy		Increased urination
Alertness		Passivity		Miscellaneous observations

There was no death during the study. There were no PA21-related effects on behavior, body temperature, or spontaneous locomotor activity.

4.3.3 Charcoal propulsion test in rats (VFR085/052415)

This GLP study (VFR085) was conducted in (b) (4) (sponsored by Vifor) during February-March, 2005.

Male CD rats (fasted, 6-7 weeks old, 10/group) were orally gavaged with vehicle (1% w/w methylcellulose, 10 ml/kg), a single dose PA 21 at dose 1250, 2500, or 5000 mg/kg, 50 mg/kg morphine sulphate, or carbohydrate (30% w/w saccharose, 30% w/w starch in 1% w/w methylcellulose). Forty-five minutes after oral administration of test articles, each animal received 1.0 ml of a 5% (w/v) medicinal charcoal suspension in water by gavage. Rats were killed 30 minutes after charcoal administration and the distance the charcoal meal had travelled from the pyloric sphincter was determined, as a measure of gastrointestinal (GI) motility.

PA21 at 1,250 mg/kg had no effect on intestinal motility. At 2,500 and 5,000 mg/kg, slight increases in travel distance of the charcoal meal were observed, but the increases were small and not dosage-related (Table 20). Therefore, these changes were unlikely to be treatment-related or of biological significance. Positive control rats with oral morphine sulphate 50 mg/kg showed the expected significant reduction in GI motility.

Table 20. Effects of oral PA 21 on GI motility in rats (modified from the application)

Oral treatment	Dose (mg/kg)	distance travelled by charcoal % of total gut length	% change from vehicle-treated animals
Vehicle (1% w/w methylcellulose)	-	51.1 ±5.80	-
PA 21	1250	48.5 ±4.78	-4.97
PA 21	2500	58.9 ±6.38*††	15.27
PA 21	5000	56.2 ±6.02*††	10.00
Morphine sulphate	50	38.5 ±10.82**	-24.67
Carbohydrate control	-	44.5 ±7.29*	-12.94

Compared with vehicle-treated group: * $p < 0.05$, ** $p < 0.01$

Compared with carbohydrate control group: †† $p < 0.01$

4.3.4 Evaluation of cardiovascular effects in conscious telemetered beagle dogs (VFR083/043639)

This GLP study (VFR083) was conducted in (sponsored by Vifor) during Oct-Nov, 2004.

(b) (4)

Two male and two female drug-naïve beagle dogs were implanted with telemetry transmitters. PA21 (250, 500, or 1000 mg/kg) or vehicle (empty gelatin capsules) was orally administered to dogs at once weekly intervals to allow at least 6 days washout between treatments, according to the randomized Latin square design shown in Table 21. Animals were observed for behavior and clinical signs. Arterial blood pressure, heart rate and electrocardiograph (ECG, lead II) were continuously recorded 30 minutes prior to dosing until 12 hours after dosing. Records of blood pressure and heart rate were obtained at 10-minute intervals prior to dosing then for 5 minute intervals up to 60 minutes after dosing and thereafter at 10 minute intervals between 1 and 3 hours post-dose, finally at 15 minute intervals from 3 to 12 hours post-dose. "Snapshot" records of ECG (lead II) were assessed RR, PR, QRS, and QT intervals at just prior to dosing, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 hours post-dose.

Table 21. Dosing regimen (M-male; f-female)

Test session number	Treatment and dose (mg/kg) of PA21 for dog number:			
	77m	78f	79m	80f
1	Vehicle (placebo)	1000	500	250
2	500	250	Vehicle (placebo)	1000
3	1000	500	250	Vehicle (placebo)
4	250	Vehicle (placebo)	1000	500

A single oral dose of PA21 up to 1,000 mg/kg to dogs showed no adverse effects on arterial blood pressure (Figure 4) or heart rate (Figure 5). There were no effects of PA21 on electrocardiographic rhythm, waveform morphology, or waveform intervals (including QT interval, Table 22). Clinical findings in the test animals were restricted to a dose-dependent increase in the incidence of dark colored feces on the day after PA21 administration, attributable to the excretion of PA21 and/or its iron content, this finding not being considered adverse.

Figure 4. Arterial blood pressure in dogs (modified from the application)

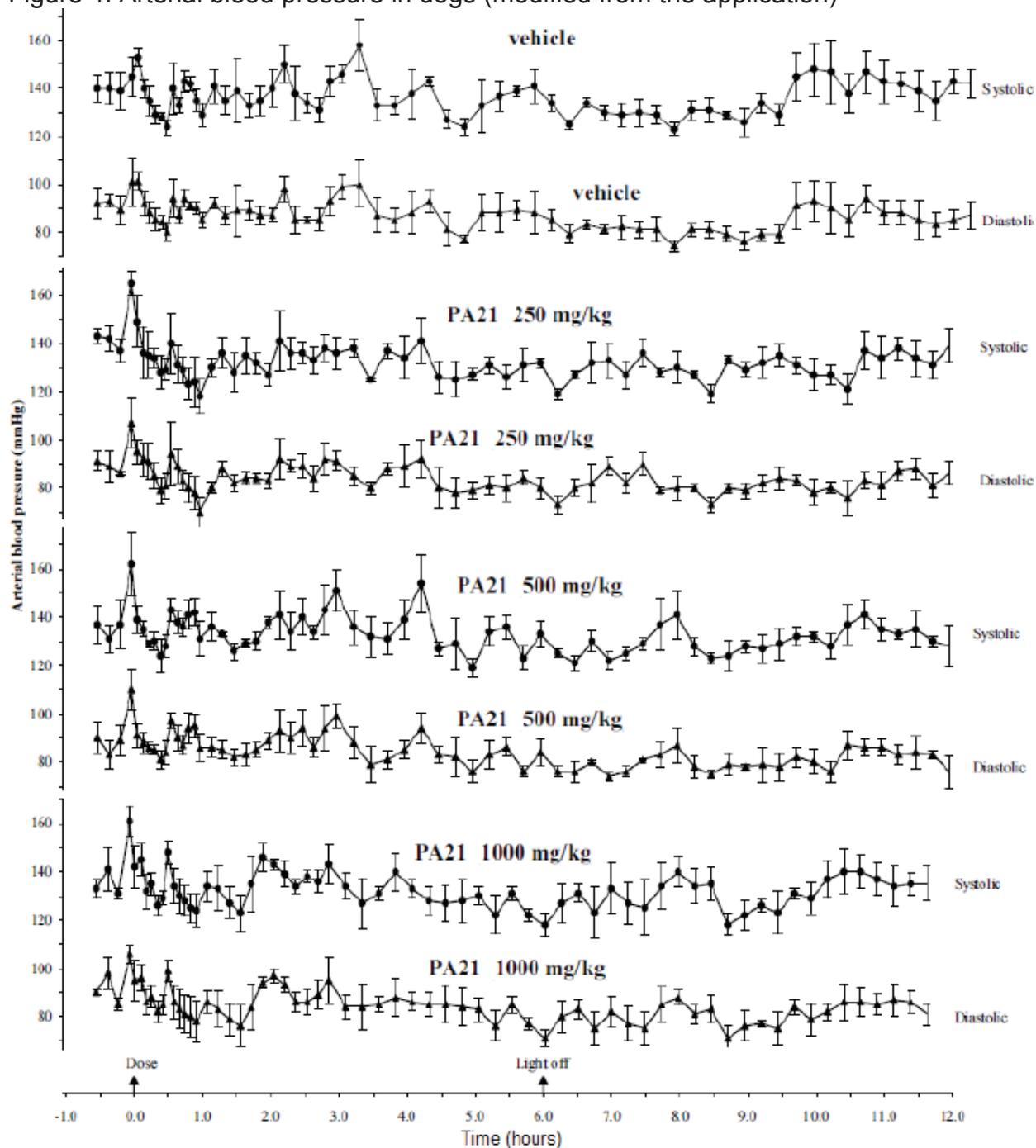


Figure 5. No effect of PA21 on heart rate in dogs (modified from the application)

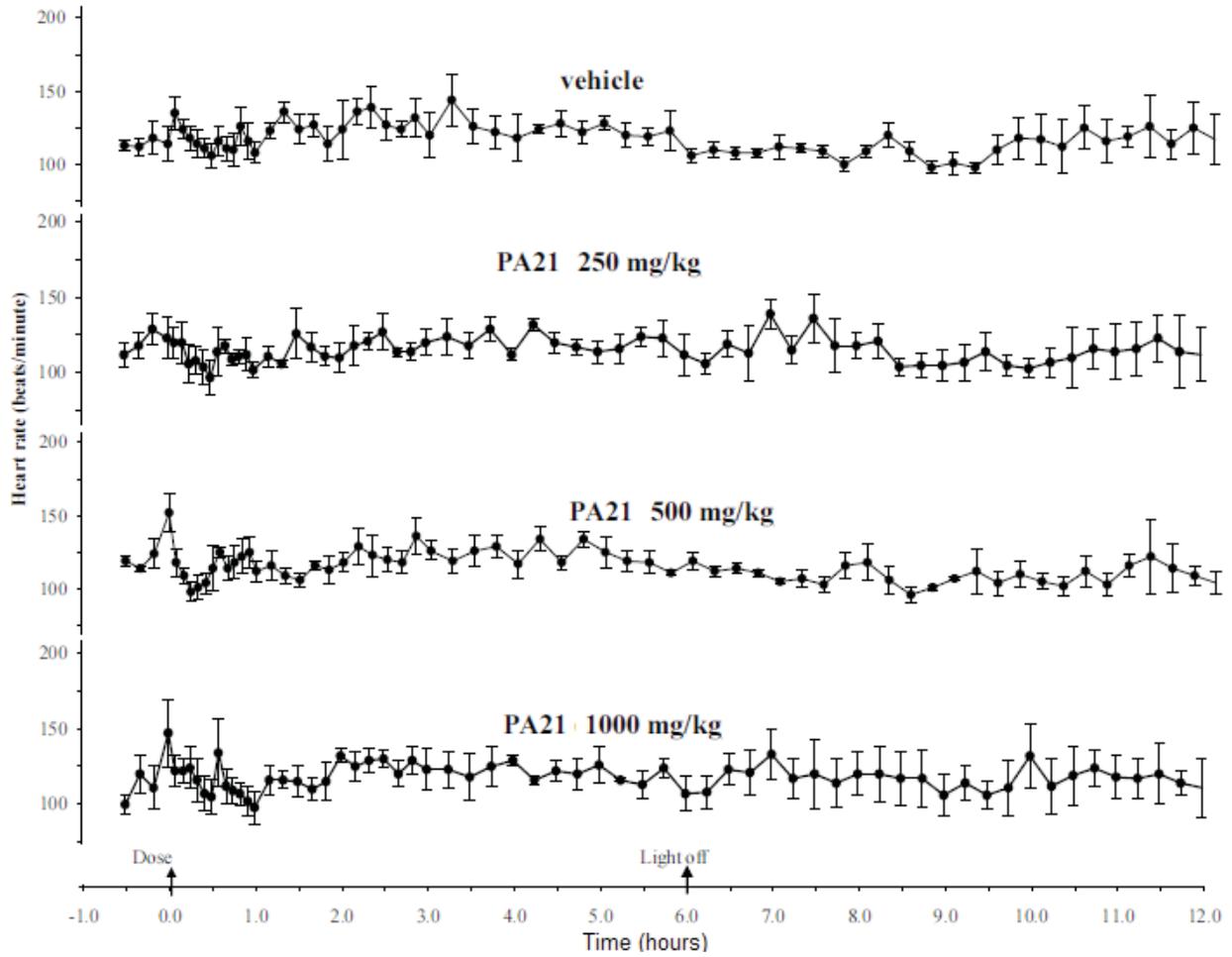


Table 22. ECG (lead II) interval data prior to and following oral dosing (modified from the application)

Time point (hr)	Heart rate (derived from)		ECG intervals (ms)											
			RR		PR		QRS		QT		QTcR			
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.		
vehicle	0	116	7.2	524	31.3	111	7.9	42	2.3	207	9.0	203	7.1	
	0.5	107	7.7	569	41.4	114	8.4	44	3.0	215	1.7	205	5.2	
	1	116	11.5	533	55.3	111	7.9	44	3.3	210	5.2	204	4.1	
	2	117	10.1	527	51.1	109	5.3	45	2.2	208	5.6	202	4.0	
	3	118	9.3	522	49.7	108	7.8	41	1.8	198	4.0	194	6.0	
	4	113	7.0	538	37.7	103	4.8	41	2.3	196	3.5	190	4.2	
	6	108	6.7	563	34.5	103	4.6	42	3.8	207	4.6	198	3.0	
	8	103	2.8	586	15.6	104	4.1	41	3.2	208	4.7	197	5.8	
	10	123	17.1	518	73.4	97	2.9	41	3.7	196	8.4	191	9.4	
	12	107	8.3	572	40.6	101	5.3	44	4.0	207	5.4	197	6.6	
	PA21 250 mg/kg	0	116	6.9	525	32.7	103	4.8	45	3.0	213	5.2	209	2.1
		0.5	106	13.0	595	74.1	108	6.2	44	2.0	215	15.6	200	9.4
1		100	5.6	603	34.7	112	2.4	45	3.0	218	5.6	204	7.6	
2		109	7.4	558	36.8	106	2.7	43	2.1	207	6.2	198	4.9	
3		116	3.7	518	17.2	106	5.1	44	2.8	199	6.9	196	7.3	
4		122	10.1	501	37.9	104	5.5	42	2.2	194	6.2	192	6.6	
6		114	7.6	533	36.7	104	1.3	41	4.3	201	7.4	197	3.8	
8		107	9.6	574	52.1	106	4.6	43	2.3	212	6.4	202	2.3	
10		100	5.9	608	37.5	105	2.4	43	3.6	208	7.0	193	4.5	
12		106	17.4	605	80.3	104	4.5	43	1.8	206	11.5	191	3.9	
PA21 500 mg/kg		0	102	8.5	599	47.9	119	7.3	44	3.0	218	6.7	205	10.8
		0.5	100	7.3	611	44.2	117	7.1	44	2.3	223	6.5	209	8.6
	1	121	7.2	503	32.1	107	5.5	42	2.3	203	0.6	201	4.0	
	2	115	8.0	530	39.5	107	7.1	43	2.8	204	4.4	199	7.0	
	3	119	7.9	512	35.4	104	5.4	43	2.8	203	8.5	201	6.6	
	4	113	8.2	540	34.1	103	5.6	42	3.2	198	8.3	192	6.0	
	6	110	3.1	547	14.8	103	4.4	42	3.3	199	2.8	192	2.9	
	8	101	1.4	593	8.1	101	4.4	44	4.0	212	3.8	199	4.2	
	10	106	8.1	575	40.2	99	6.1	41	1.5	206	5.0	195	6.0	
	12	111	8.5	549	44.6	100	5.8	43	2.2	207	3.4	199	8.8	
	PA21 1000 mg/kg	0	102	8.9	606	65.1	114	3.3	45	2.9	220	7.8	206	5.7
		0.5	102	12.2	614	78.8	115	3.1	44	3.8	217	10.0	202	2.7
1		106	11.0	591	73.2	110	3.2	46	2.9	221	8.3	209	1.4	
2		122	12.3	507	52.0	106	1.9	45	2.4	203	8.1	201	3.0	
3		116	11.8	534	57.3	105	5.5	44	3.6	199	9.6	194	6.6	
4		121	16.1	522	62.9	102	4.2	40	2.7	196	15.0	192	7.4	
6		109	11.5	570	64.3	106	6.4	42	4.2	205	9.9	195	5.6	
8		116	21.8	563	81.6	100	6.2	42	4.7	202	13.7	192	5.3	
10		116	14.3	542	63.8	100	0.9	41	0.9	200	10.0	194	4.3	
12		109	14.3	579	69.2	98	1.5	42	1.0	207	8.0	196	5.8	

4.4 Pharmacodynamic Drug Interactions

4.4.1 Absorption of ^{59}Fe from a new iron containing phosphate binder with different foodstuffs and different pharmaceutical products in mice (SR-1039/E01)

This non-GLP study (SR-1039/E01) was conducted in Vifor Pharma (Signed in Aug, 1998). Male NMRI mice (body weight 22 ± 1 g) were orally dosed with ^{59}Fe -PA21 1140 mg /kg/day (~10 times intended human dose) along with or without dietary components or drugs that may be co-administered in CKD patients listed below for 5 consecutive days (n=3/group). Excretion of the administered radioactivity in urine and feces was determined. On day 16 (11 days post-dose), mice were killed for determination of radioactivity concentrations (^{59}Fe) in the blood, liver, spleen, kidneys, stomach, intestine, and bone.

Supplement	Dosage human (mg / 60 kg bw/d)	Dosage mouse (x Factor 10) (mg / g bw/d)
A) Caffeine (4 cups)	400	0.0667
B) Ascorbic acid	70	0.0117
C) Calcium gluconate	1200	0.2000
D) Magnesium aspartate	400	0.0667
E) Phytic acid	1293	0.2156
F) Lasix	1500	0.2500
G) Reniten	40	0.0067
H) Lopirin (Captopril)	150	0.0250
I) Control		0
J) Control		0

As shown in Figure 6, excretion of the administered ^{59}Fe in urine and feces was almost complete 48 hours post the final administration. Eleven days after the final dose, less than 1% of the administered ^{59}Fe existed in the blood and tissues (Table 23). There were no influences of caffeine, ascorbic acid, calcium gluconate, magnesium aspartate, phytic acid, lasix, reniten, or captopril on absorption of iron from PA21 in the GI.

Figure 6. Iron excretion in urine and feces as % of total administered iron (modified from the application)

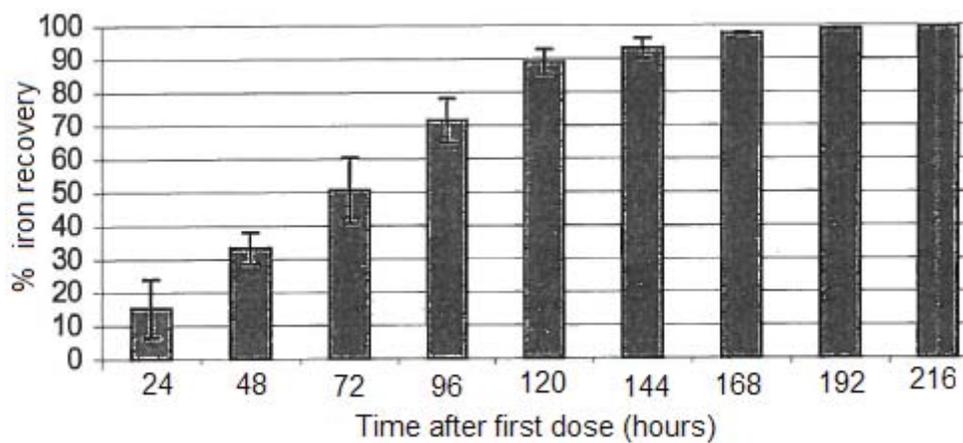


Table 23. Radioactive iron residual on post dosing day 11

Supplement	Body weight (BW, g)	%of total administered radioactive iron							
		Blood (7% of BW)	Liver	Spleen	Kidney	Stomach	Intestine	Skeleton (Femur x 5)	Sum
caffeine	32±0	0.29±0.06	0.13±0.06	0.01±0.00	0.01±0.00	0.01±0.00	0.04±0.02	0.01±0.00	0.49±0.14
ascorbic acid	32±6	0.43±0.13	0.29±0.21	0.02±0.02	0.03±0.03	0.02±0.02	0.03±0.01	0.01±0.00	0.83±0.27
calcium gluconate	32±2	0.35±0.07	0.12±0.06	0.01±0.00	0.01±0.00	0.01±0.00	0.03±0.01	0.01±0.01	0.54±0.16
magnesium aspartate	35±3	0.34±0.04	0.10±0.06	0.01±0.00	0.01±0.00	0.00±0.00	0.02±0.00	0.00±0.00	0.49±0.04
phytic acid	27±7	0.30±0.06	0.08±0.01	0.01±0.00	0.01±0.00	0.01±0.00	0.05±0.03	0.00±0.00	0.46±0.10
lasix	32±1	0.39±0.05	0.12±0.02	0.01±0.00	0.01±0.05	0.01±0.00	0.04±0.01	0.01±0.00	0.59±0.08
reniten	34±2	0.39±0.04	0.20±0.14	0.01±0.01	0.01±0.00	0.00±0.00	0.02±0.01	0.01±0.00	0.65±0.20
captopril	32±2	0.34±0.13	0.14±0.09	0.01±0.00	0.02±0.01	0.01±0.01	0.04±0.03	0.00±0.00	0.56±0.21
Control 1	31±3	0.32±0.06	0.12±0.03	0.00±0.00	0.01±0.00	0.01±0.00	0.03±0.01	0.00±0.00	0.49±0.02
Control 2	32±3	0.40±0.03	0.01±0.01	0.01±0.01	0.03±0.02	0.01±0.00	0.02±0.00	0.00±0.00	0.62±0.11

4.4.2 Determination of possible interactions of ciprofloxacin, digoxin, enalapril, furosemide, losartan, metoprolol, nifedipine, omeprazole, or warfarin with PA21 in vitro (VIT01/ (b) (4))

This GLP study (VIT01) was conducted in (b) (4) (sponsored by Vifor Pharma) during Dec 2006-Nov 2008. This study assessed the possible interactions of ciprofloxacin, digoxin, enalapril and its metabolite enalaprilate, furosemide, losartan, metoprolol, nifedipine, omeprazole, or warfarin with PA21 in vitro via measuring the concentrations of these drugs in aqueous samples obtained after incubation with PA21 by HPLC with UV detection.

To each of the following solutions (1L per test, set at 37±1°C)

- Aqueous solution containing 0.01% α-Amylase, pH 3.0 (blank)
- Aqueous solution containing 0.01% α-Amylase, pH 5.5 (blank)
- Aqueous solution containing 0.01% α-Amylase, pH 8.0 (blank)
- Aqueous solution containing 0.01% α-Amylase, pH 3.0, 1.25 g of PA21 (PA21)
- Aqueous solution containing 0.01% α-Amylase, pH 5.5, 1.25 g of PA21 (PA21)
- Aqueous solution containing 0.01% α-Amylase, pH 8.0, 1.25 g of PA21 (PA21)
- Aqueous solution containing 0.01% α-Amylase, pH 3.0, 1.25 g of placebo (placebo)
- Aqueous solution containing 0.01% α-Amylase, pH 5.5, 1.25 g of placebo (placebo)
- Aqueous solution containing 0.01% α-Amylase, pH 8.0, 1.25 g of placebo (placebo)

a tablet (only 1 tablet of 1 formulation per test) of Ciproxine® 750, Digoxin-Sandoz 0.250 mg, Reniten, Lasix® 500, Cosaar™ forte, Loprésor® 100, Adalat® CR, Antramups 40, or Coumadin 5 mg was added. The mixtures were kept at 37±1°C, and shaken immediately after adding the tablets. The pH values of all mixtures were checked and adjusted to the desired levels during the incubation. After 2, 4, and 6 hours, samples were taken from each mixture in duplicate, centrifuged at rcf 19940,

4°C, for at least 5 min. The supernatant of each sample was analyzed for ciprofloxacin, digoxin, enalapril and its metabolite enalaprilate, furosemide, losartan, metoprolol, nifedipine, omeprazole, or warfarin by the HPLC system. The concentrations of the analytes in the aqueous part of samples were back calculated from the calibration curves.

An adsorption of the analytes onto PA21 placebo [1] was calculated as the difference between the analyte concentrations in the mixtures without PA21 and PA21 placebo and the mixtures containing PA21 placebo. An adsorption of the analytes onto PA21 [2] was calculated as the difference between the analyte concentrations in the mixtures without PA21 and PA21 placebo and the mixtures containing PA21. An adsorption of the analytes onto PA21 active portion [3] was calculated as the difference between [2] and [1]. Calculations below $\pm 10\%$ were considered to be no adsorption.

Mean concentrations of analytes were shown in Table 24. There were slight to marked adsorption of furosemide (22-60%) to PA21 at pH 3 and 5.5, and slight adsorption of losartan (~25%) to PA21 at pH 3. PA21 slightly to moderately enhanced solubility of furosemide at pH 8. There were no or only marginal adsorptions of ciprofloxacin, digoxin, enalapril, metoprolol, nifedipine, omeprazole, or warfarin to PA21 or PA21 placebo at any pH level. Enalaprilate could not be analyzed during the study.

Table 24

Mean concentration of analytes in the aqueous part of the mixtures and the effect of PA21. There were 2 analyses in part 1 and 6 analyses in part 2. blq = below limit of quantification. The concentrations of ciprofloxacin, digoxin, enalapril, metoprolol, nifedipine, and warfarin were not or only slightly affected by PA21 at pH 3, 5.5, or 8. Incubation of furosemide with PA21 resulted in markedly lower furosemide concentrations at pH 3, moderately lower furosemide concentrations at pH 5.5, and moderately higher furosemide concentrations at pH 8. Incubation of losartan with PA21 resulted in markedly lower losartan concentrations at pH 3. Omeprazole was not stable (degraded, blq) at pH 3.

Analyte	pH	Time (h)	Aqueous solution, µg/ml		with PA21 placebo, µg/ml		with PA21, µg/ml		Effect of PA21 placebo, %		Effect of PA21, %		Effect of PA21 active part, %	
			Part 1	Part 2	Part 1	Part 2	Part 1	Part 2	Part 1	Part 2	Part 1	Part 2	Part 1	Part 2
Ciprofloxacin	3	2	791	759	787	748	724	730	-0.5	-1.4	-8.5	-3.8	-8.0	-2.4
		4	766	753	781	756	705	733	2.0	0.4	-8.0	-2.7	-9.7	-3.0
		6	748	753	755	749	730	737	0.9	-0.5	-2.4	-2.1	-3.3	-1.6
		2	653	732	651	718	556	685	-0.3	-1.9	-14.9	-6.4	-14.6	-4.6
		4	629	743	643	741	538	688	2.2	-0.3	-14.5	-7.4	-16.3	-7.2
		6	656	734	648	733	524	686	-1.2	-0.1	-20.1	-6.5	-19.1	-6.4
	5.5	2	156	166	335	187	222	193	114.7	12.7	42.3	16.3	33.7	3.2
		4	121	157	123	166	136	157	1.7	5.7	12.4	0.0	-10.6	-5.4
		6	125	172	124	182	115	173	-0.8	5.8	-8.0	0.6	-7.3	-4.9
		2	224	254	258	267	221	253	15.2	5.1	-1.3	-0.4	-14.3	-5.2
		4	250	242	289	244	222	224	15.6	0.8	-11.2	-7.4	-23.2	-8.2
		6	245	232	263	228	214	204	7.3	-1.7	-12.7	-12.1	-18.6	-10.5
Digoxin	3	2	259	273	296	290	255	257	14.3	6.2	-1.5	-5.9	-13.9	-11.4
		4	249	259	291	274	248	238	16.9	5.8	-0.4	-8.1	-14.8	-13.1
		6	260	267	294	272	246	233	13.1	1.9	-5.4	-12.7	-16.3	-14.3
		2	273	260	293	249	273	248	7.3	-4.2	0.0	-4.6	-6.8	-0.4
		4	276	270	290	253	265	246	5.1	-6.3	-4.0	-8.9	-8.6	-2.8
		6	252	272	288	260	262	252	14.3	-4.4	4.0	-7.4	-9.0	-3.1
	5.5	2	16.5	14.5	15.2	14.5	14.8	13.6	-7.9	0.0	-10.3	-6.2	-2.6	-6.2
		4	16.6	14.3	15.1	14.4	14.4	13.6	-9.0	0.7	-13.3	-4.9	-4.6	-5.6
		6	16.5	14.3	15.2	14.3	14.2	13.6	-7.9	0.0	-13.9	-4.9	-6.6	-4.9
		2	16.3	14.8	14.8	15.2	14.5	14.5	-9.2	2.7	-11.0	-2.0	-2.0	-4.6
		4	16.4	16.3	14.7	15.7	14.2	15.2	-10.4	-3.7	-13.4	-6.7	-3.4	-3.2
		6	16.2	16.7	14.6	14.2	14	13.3	-9.9	-15.0	-13.6	-20.4	-4.1	-6.3
8	2	15.8	14.3	15.5	14	15.4	14.2	-1.9	-2.1	-2.5	-0.7	-0.6	1.4	
	4	15.7	14.3	15.3	14	15.2	14.1	-2.5	-2.1	-3.2	-1.4	-0.7	0.7	
	6	15.8	14.1	15.1	14	15	14.1	-4.4	-0.7	-5.1	0.0	-0.7	0.7	
	2	12.6	11.8	12.8	12.1	4.79	4.73	1.6	2.5	-62.0	-59.9	-62.6	-60.9	
	4	13.7	12.7	14.2	12.8	7.31	6.44	3.6	0.8	-46.6	-49.3	-48.5	-49.7	
	6	14.3	13.4	14.5	13.2	9.1	8.42	1.4	-1.5	-36.4	-37.2	-37.2	-36.2	
Furosemide	3	2	142	114	152	141	152	88.5	7.0	23.7	7.0	-22.4	0.0	-37.2
		4	175	161	197	186	191	124	12.6	15.5	9.1	-23.0	-3.0	-33.3
		6	201	166	225	222	217	159	11.9	33.7	8.0	-4.2	-3.6	-28.4
		2	248	176	283	217	418	263	14.1	23.3	68.5	49.4	47.7	21.2
		4	348	253	350	277	518	320	0.6	9.5	48.9	26.5	48.0	15.5
		6	388	280	391	317	566	349	0.8	13.2	45.9	24.6	44.8	10.1
	5.5	2	42.3	92	42.5	93.4	20.4	72.4	0.5	1.5	-51.8	-21.3	-52.0	-22.5
		4	42.9	93.6	42.3	92.4	17.8	69.9	-1.4	-1.3	-58.5	-25.3	-57.9	-24.4
		6	44.5	90.7	44.6	90.8	17.2	66.3	0.2	0.1	-61.3	-26.9	-61.4	-27.0
		2	93.4	88.9	88.1	89.5	85.3	83	-5.7	0.7	-8.7	-6.6	-3.2	-7.3
		4	92.3	88.9	87.7	90.7	81	81.2	-5.0	2.0	-12.2	-8.7	-7.6	-10.5
		6	94.3	88.9	90.2	90.4	85.2	80.4	-4.3	1.7	-9.7	-9.6	-5.5	-11.1
8	2	93.5	91.9	111	90.3	105	92.1	18.7	-1.7	12.3	0.2	-5.4	2.0	
	4	92.5	92.8	113	90.2	105	90.2	22.2	-2.8	13.5	-2.8	-7.1	0.0	
	6	93.6	91.4	111	88.6	104	88.6	18.6	-3.1	11.1	-3.1	-6.3	0.0	
	2	73.7		81.6		80.5		10.7		9.2		-1.3		
	4	74.2		81.2		80.6		9.4		8.6		-0.7		
	6	76.1		80.7		78.3		6.0		2.9		-3.0		
5.5	2	72.9		73.7		78.1		1.1		7.1		6.0		
	4	72.7		75.9		75.6		4.4		4.0		-0.4		
	6	74		70.9		75.8		-4.2		2.4		6.9		
	2	73.7		74.5		72.5		1.1		-1.6		-2.7		
	4	71.6		74.5		67.1		4.1		-6.3		-9.9		
	6	73.9		74.9		65.3		1.4		-11.6		-12.8		
Losartan	3	2	blq		blq		blq		N/A		N/A		N/A	
		4	4.19		4.24		3.83		1.2		-8.6		-9.7	
		6	5.91		5.96		5.49		0.8		-7.1		-7.9	
		2	blq		blq		blq		N/A		N/A		N/A	
		4	2.89		3.09		2.76		6.9		-4.5		-10.7	
		6	4.69		5.14		4.61		9.6		-1.7		-10.3	
	5.5	2	blq		blq		blq		N/A		N/A		N/A	
		4	5		4.94		5.4		-1.2		8.0		9.3	
		6	7.39		7.38		7.47		-0.1		1.1		1.2	
		2	blq		blq		blq		N/A		N/A		N/A	
		4	blq		blq		blq		N/A		N/A		N/A	
		6	blq		blq		blq		N/A		N/A		N/A	
Omeprazole	3	2	0.638	0.9	3.2	5.08	2.87	5.11	401.6	464.4	349.8	467.8	-10.3	0.6
		4	15.1	18.6	16.3	16	20	18.4	7.9	-14.0	32.5	-1.1	22.7	15.0
		6	11.5	9.29	9.39	6.82	10.2	7.81	-18.3	-26.6	-11.3	-15.9	8.6	14.5
		2	7.91	9.98	6.12	7.62	23.5	11.5	-22.6	-23.6	197.1	15.2	284.0	50.9
		4	34.8	34.7	33.6	34.9	36.7	34.7	-3.4	0.6	5.5	0.0	9.2	-0.6
		6	34.2	34.2	33.8	34	36	34.1	-1.2	-0.6	5.3	-0.3	6.5	0.3
	5.5	2	3.46		3.37		3.24		-2.6		-6.4		-3.9	
		4	3.53		3.39		3.24		-4.0		-8.2		-4.4	
		6	3.53		3.4		3.23		-3.7		-8.5		-5.0	
		2	4.44		4.36		4.2		-1.8		-5.4		-3.7	
		4	4.44		4.34		4.16		-2.3		-6.3		-4.1	
		6	4.41		4.31		4.11		-2.3		-6.8		-4.6	
8	2	4.53		4.54		4.54		0.2		0.2		0.0		
	4	4.45		4.53		4.55		1.8		2.2		0.4		
	6	4.54		4.52		4.5		-0.4		-0.9		-0.4		

4.4.3 Determination of possible interactions of atorvastatin, hydrochlorothiazide, metformin, pioglitazone, candesartan cilexetil, ciprofloxacin, enalapril, metoprolol, nifedipine, paricalcitol, or bile acids in aqueous solution with PA21 by HPLC UV detection under GLP conditions (b) (4)

These in vitro GLP studies were conducted in (b) (4) (sponsored by Vifor Pharma) during 2011. With similar methods/techniques used in study VIT01/ (b) (4) these studies assessed potential bindings of atorvastatin, hydrochlorothiazide, metformin, pioglitazone, candesartan cilexetil, ciprofloxacin, enalapril, metoprolol, nifedipine, or paricalcitol to PA21 at higher dosages (5.0 g PA21/L) than used previously (1.25 g PA21/L), so as to reflect clinical conditions more closely, under simulated physiological conditions that may be encountered in the GI tract. To each reaction solutions, a tablet (only 1 tablet of 1 formulation per test) of Sortis (80 mg Atorvastatin), Esidrex (100 mg Hydrochlorothiazide), Metfin 1000 (1000 mg Metformin hydrochloride corresponding to 780 mg Metformin), Actos 45 mg (45 mg Pioglitazone), Atacand (32 mg candesartan cilexetil), Ciproxin 750 (750 mg ciprofloxacin), Reniten (20 mg enalapril maleate, corresponding to 15.28 mg enalapril), Lopresor 100 (100 mg metoprolol tartrate), or Adalat CR (60 mg nifedipine), or 20 ml of Paricalcitol work solution (final concentration 32 ng/ml), or bile acids (sodium glycocholate hydrate, sodium glycochenodeoxycholate and sodium taurodeoxycholate hydrate) was added. For bile acid reaction mixtures, final concentrations were 6.43 mM for cholyglycine and chenodeoxycholyglycine and 2.14 mM deoxycholytaurine (i.e. 15 mM for total bile acids).

The mean concentrations of analytes in the reaction mixtures are shown in Table 25. Virtually complete adsorptions of atorvastatin and paricalcitol to PA21 were seen at pH 3.0 and pH 5.5. At pH 8.0, paricalcitol was still mostly adsorbed to PA21, but Atorvastatin was not.

There were marginal adsorption of metoprolol (15%) to PA21 at pH 8.0, slight adsorption of ciprofloxacin (25%) to PA21 at the pH level of 5.5, marginal-slight adsorption of nifedipine (10-30%) to PA21 at pH 8.0, marginal to slight adsorption of Pioglitazone (10-30%) to PA21 at pH 3.0 and pH 5.5, and marginal adsorption of bile acid chenodeoxycholyglycine (11-15%) to PA21 at pH 5.5 and 8.0. At pH 8.0, pioglitazone showed a slightly to moderately increased solubility in the presence of PA21. These are considered as maximum interactions between PA21 and metoprolol/ciprofloxacin/nifedipine/pioglitazone as the studies were done in the absence of phosphate or other nutritional components that under *in vivo* conditions compete for adsorption to PA21.

There were no adsorptions of hydrochlorothiazide, metformin, enalapril, and bile acids cholyglycine and deoxycholytaurine on PA21 or PA21 placebo at any pH level.

No conclusion could be made for candesartan cilexetil since all samples were below the limit of quantitation (blq) due to poor dissolution of the tablets.

Table 25. Mean concentrations of analytes in the reaction mixtures

Analyte	pH	Time (h)	Mean concentration of test drug (µg/ml)			Effect, %			Analyte	pH	Time (h)	Mean concentration of test drug (µg/ml)			Effect, %		
			Blank	Placebo	PA21	placebo	PA21	PA21 active part				Blank	Placebo	PA21	placebo	PA21	PA21 active part
Atorvastatin	3	2	28.9	26.9	blq	-6.9	~-100	~-100	Ciprofloxacin	3	2	662	688	619	3.9	-6.5	-10.0
		4	26.5	19	blq	-28.3	~-100	~-100			4	655	688	621	5.0	-5.2	-9.7
		6	26.3	23.7	blq	-9.9	~-100	~-100			6	650	689	611	6.0	-6.0	-11.3
	5.5	2	50.6	49.5	1.87	-2.2	-96.3	-96.2		2	669	667	503	-0.3	-24.8	-24.6	
		4	50.4	49.1	1.09	-2.6	-97.8	-97.8		4	670	667	497	-0.4	-25.8	-25.5	
		6	49.7	47.8	0.8	-3.8	-98.4	-98.3		6	667	660	490	-1.0	-26.5	-25.8	
	8	2	64.9	73.1	79.9	12.6	23.1	9.3		2	191	207	183	8.4	-4.2	-11.6	
		4	66.4	73.4	81.5	10.5	22.7	11.0		4	174	176	165	1.1	-5.2	-6.3	
		6	67.3	72.9	51.5	8.3	-23.5	-29.4		6	183	184	164	0.5	-10.4	-10.9	
Hydrochlorothiazide	3	2	95.8	91	89.7	-5.0	-6.4	-1.4	Enalapril	3	2	15.7	15.8	15.2	0.6	-3.2	-3.8
		4	96.9	96	94.8	-0.9	-2.2	-1.3			4	15.9	15.5	14.6	-2.5	-8.2	-5.8
		6	97.4	96.8	96.6	-0.6	-0.8	-0.2			6	15.6	15.4	14.1	-1.3	-9.6	-8.4
	5.5	2	98.6	97.1	96.7	-1.5	-1.9	-0.4		2	15.5	16.6	15	7.1	-3.2	-9.6	
		4	97.3	97.2	96.3	-0.1	-1.0	-0.9		4	15.5	16.6	14.4	7.1	-7.1	-13.3	
		6	97.3	96.1	95.5	-1.2	-1.8	-0.6		6	15.4	16.5	13.9	7.1	-9.7	-15.8	
	8	2	95.9	95.7	96.6	-0.2	0.7	0.9		2	16.8	16.7	16.3	-0.6	-3.0	-2.4	
		4	93.3	93.8	94.5	0.5	1.3	0.7		4	16.6	16.5	16.2	-0.6	-2.4	-1.8	
		6	93.1	92.7	93.5	-0.4	0.4	0.9		6	16.5	16.4	16.2	-0.6	-1.8	-1.2	
Metformin	3	2	794	781	784	-1.6	-1.3	0.4	Metoprolol	3	2	97.9	97	94.3	-0.9	-3.7	-2.8
		4	788	779	784	-1.1	-0.5	0.6			4	97.7	98.3	98.4	0.6	0.7	0.1
		6	786	775	776	-1.4	-1.3	0.1			6	97.3	98	99.1	0.7	1.8	1.1
	5.5	2	760	789	769	3.8	1.2	-2.5		2	98.4	98.4	98	0.0	-0.4	-0.4	
		4	763	791	771	3.7	1.0	-2.5		4	96.6	97.1	96.1	0.5	-0.5	-1.0	
		6	761	785	770	3.2	1.2	-1.9		6	97.8	96.5	97.1	-1.3	-0.7	0.6	
	8	2	765	765	754	0.0	-1.4	-1.4		2	109	107	94.1	-1.8	-13.7	-12.1	
		4	761	755	746	-0.8	-2.0	-1.2		4	107	106	92.6	-0.9	-13.5	-12.6	
		6	760	759	743	-0.1	-2.2	-2.1		6	108	105	90.9	-2.8	-15.8	-13.4	
Pioglitazone	3	2	40.5	35.5	35.7	-12.3	-11.9	0.6	Nifedipine	3	2	blq	blq	blq	N/A	N/A	N/A
		4	41.1	35.4	37.9	-13.9	-7.8	7.1			4	2.41	2.07	2.21	-14.1	-8.3	6.8
		6	41.2	35.5	37.3	-13.8	-9.5	5.1			6	4.28	3.6	3.83	-15.9	-10.5	6.4
	5.5	2	22	17	15.2	-22.7	-30.9	-10.6		2	blq	blq	blq	N/A	N/A	N/A	
		4	21.5	17.8	14.1	-17.2	-34.4	-20.8		4	3.23	3.23	3.09	0.0	-4.3	-4.3	
		6	28.7	19.9	22	-30.7	-23.3	10.6		6	4.83	4.4	4.11	-8.9	-14.9	-6.6	
	8	2	24	24.6	32.2	2.5	34.2	30.9		2	blq	blq	blq	N/A	N/A	N/A	
		4	23.7	23.8	33	0.4	39.2	38.7		4	2.84	1.99	1.95	-29.9	-31.3	-2.0	
		6	24.8	20.4	29.2	-17.7	17.7	43.1		6	5.27	4.17	4.24	-20.9	-19.5	1.7	
Candesartan cilexetil	3	2	blq	blq	blq	N/A	N/A	N/A	Paricalcitol*	3	2	13.2	3.44	blq	-74.0	~-100	~-100
		4	blq	blq	blq	N/A	N/A	N/A			4	9.32	3.64	blq	-60.9	~-100	~-100
		6	blq	blq	blq	N/A	N/A	N/A			6	11.1	2.81	blq	-74.6	~-100	~-100
	5.5	2	blq	blq	blq	N/A	N/A	N/A		2	15.6	8.17	blq	-47.5	~-100	~-100	
		4	blq	blq	blq	N/A	N/A	N/A		4	15.5	10.42	1.02	-32.6	-93.4	-90.2	
		6	blq	blq	blq	N/A	N/A	N/A		6	16.4	8.13	0.74	-50.5	-95.5	-90.9	
	8	2	blq	blq	blq	N/A	N/A	N/A		2	9.76	5.58	2.11	-42.8	-78.4	-62.2	
		4	blq	blq	blq	N/A	N/A	N/A		4	19.2	6.08	6.05	-68.3	-68.4	-0.5	
		6	blq	blq	blq	N/A	N/A	N/A		6	20.8	6.68	5.12	-67.9	-75.4	-23.4	
Cholyglycine	5.5	2	3107	3129	2971	0.7	-4.4	-5.0	Deoxy-cholytaurine	5.5	2	1048	1054	1013	0.6	-3.3	-3.9
		4	3130	3145	2971	0.5	-5.1	-5.5			4	1065	1069	1018	0.4	-4.4	-4.8
		6	3165	3171	2997	0.2	-5.3	-5.5			6	1085	1087	1037	0.2	-4.4	-4.6
	8	2	3116	3100	3026	-0.5	-2.9	-2.4		2	1043	1048	1022	0.5	-2.0	-2.5	
		4	3111	3104	3014	-0.2	-3.1	-2.9		4	1037	1043	1014	0.6	-2.2	-2.8	
		6	3120	3111	3024	-0.3	-3.1	-2.8		6	1040	1043	1018	0.3	-2.1	-2.4	
Chenodeoxy-cholyglycine	5.5	2	2967	2954	2565	-0.4	-13.5	-13.2	* Concentraion in ng/ml. Blank: Reaction solution without PA21 or PA21 placebo. blq: below limit of quantification. N/A: not applicable.	5.5	2	2938	2908	2607	-1.0	-11.3	-10.4
		4	2924	2899	2567	-0.9	-12.2	-11.5			4	3006	2983	2561	-0.8	-14.8	-14.1
		6	3048	3012	2581	-1.2	-15.3	-14.3			6	3048	3012	2581	-1.2	-15.3	-14.3
	8	2	2938	2908	2607	-1.0	-11.3	-10.4		2	2938	2908	2607	-1.0	-11.3	-10.4	
		4	2924	2899	2567	-0.9	-12.2	-11.5		4	2924	2899	2567	-0.9	-12.2	-11.5	
		6	2931	2893	2563	-1.3	-12.6	-11.4		6	2931	2893	2563	-1.3	-12.6	-11.4	

4.4.4 Investigation of possible interactions of doxycycline, alendronate, levothyroxine, quinidine, cinacalcet, glipizide, paricalcitol, or atorvastatin in aqueous α -amylase solution with PA21 by UPLC-MS/MS UV detection (STU-PA21DDI-B101527-001, STU-PA21DDI-B101617-001, STU-PA21DDI-B101529-001, STU-PA21DDI-B101616-001, STU-PA21DDI-B101528-001, STU-PA21DDI-B101615-001, STU-PA21DDI-B200713-001, STU-PA21DDI-B200830-001, STU-PA21DDI-B201319-001)

These in vitro GLP studies, conducted in (b) (4) (sponsored by Vifor Pharma) during 2011, investigated potential binding of tested articles to PA21 under simulated physiological conditions that may be encountered in the GI tract. In a 1 L aqueous solution containing 0.1 mg/mL α -amylase, 5 g PA21 (2 tablets of formulated product) or the placebo was co-incubated with 200 mg doxycycline, 70 mg alendronate, 300 μ g levothyroxine, 600 mg quinidine sulphate, 180 mg cinacalcet, 15 mg glipizide, 32 μ g paricalcitol, or 80 mg atorvastatin for up to 6 hours at 37°C, at pH values of 3.0, 5.5 or 8.0. The reaction mixtures for paricalcitol, atorvastatin, and 1 of the 2 levothyroxine preparations contained 400 mg phosphorus/L. Samples were taken after 2, 4 and 6 hours of incubation, and assayed for analyte concentrations (soluble part) using a validated UPLC-MS/MS method.

The mean concentrations of analytes in the reaction mixtures are shown in Table 26. Extensive-complete adsorptions of doxycycline, alendronate, and levothyroxine to PA21 were seen at all pH levels. In the presence of phosphorus, the adsorptions of levothyroxine, paricalcitol, and atorvastatin to PA21 were markedly reduced compared to reactions without phosphorus.

There was no adsorption of quinidine to PA21 at all pH values. Cinacalcet was not adsorbed to PA21 at pH 3.0, but had marginal adsorption to PA21 at pH 5.5 (Table 26). Because of poor solubility for glipizide at all pH levels and cinacalcet at pH 8.0, relevant results were inconclusive.

Table 26. Mean concentrations of analytes in the reaction mixtures

Analyte	pH	Time (h)	Mean concentration of test drug (µg/ml)			Effect, %			Analyte	pH	Time (h)	Mean concentration of test drug (µg/ml)			Effect, %				
			Blank	Placebo	PA21	placebo	PA21	PA21 active part				Blank	Placebo	PA21	placebo	PA21	PA21 active part		
Doxycycline	3	2	209	209	112	0.0	-46.4	-46.4	Alendronate	3	2	74.7	68	1.64	-9.0	-97.8	-97.6		
		4	212	209	115	-1.4	-45.8	-45.0			4	76.1	68.9	0.915	-9.5	-98.8	-98.7		
		6	202	201	108	-0.5	-46.5	-46.3			6	75.1	66.6	0.751	-11.3	-99.0	-98.9		
	5.5	2	208	207	6.08	-0.5	-97.1	-97.1		2	62.8	63.2	blq	0.6	~-100	~-100			
		4	209	209	2.26	0.0	-98.9	-98.9		4	63.9	60.7	blq	-5.0	~-100	~-100			
		6	198	210	blq	6.1	~-100	~-100		6	59.3	54.1	blq	-8.8	~-100	~-100			
	8	2	122	190	2.78	55.7	-97.7	-98.5		2	66.4	62.4	blq	-6.0	~-100	~-100			
		4	144	185	blq	28.5	~-100	~-100		4	59.7	59.4	blq	-0.5	~-100	~-100			
		6	160	188	blq	17.5	~-100	~-100		6	50.5	56.4	blq	11.7	~-100	~-100			
	Levothyroxine*	3	2	122	139	20.7	13.9	-83.0		-85.1	Levothyroxine**	3	2	118	147	151	24.6	28.0	2.7
			4	134	155	19.7	15.7	-85.3		-87.3			4	175	185	154	5.7	-12.0	-16.8
			6	136	156	20.5	14.7	-84.9		-86.9			6	187	206	164	10.2	-12.3	-20.4
5.5		2	137	131	8.2	-4.4	-94.0	-93.7	2	139		144	94.5	3.6	-32.0	-34.4			
		4	150	152	6.26	1.3	-95.8	-95.9	4	181		123	179	-32.0	-1.1	45.5			
		6	132	130	4.93	-1.5	-96.3	-96.2	6	182		192	127	5.5	-30.2	-33.9			
8		2	239	176	81.2	-26.4	-66.0	-53.9	2	262		149	253	-43.1	-3.4	69.8			
		4	277	230	91.8	-17.0	-66.9	-60.1	4	319		208	292	-34.8	-8.5	40.4			
		6	282	258	93.4	-8.5	-66.9	-63.8	6	323		239	309	-26.0	-4.3	29.3			
Quinidine		3	2	204	208	215	2.0	5.4	3.4	Cinacalcet		3	2	219	218	218	-0.5	-0.5	0.0
			4	277	281	282	1.4	1.8	0.4				4	222	222	221	0.0	-0.5	-0.5
			6	326	322	329	-1.2	0.9	2.2				6	215	213	214	-0.9	-0.5	0.5
	5.5	2	215	204	216	-5.1	0.5	5.9	2		203	167	178	-17.7	-12.3	6.6			
		4	280	279	282	-0.4	0.7	1.1	4		208	171	184	-17.8	-11.5	7.6			
		6	324	326	327	0.6	0.9	0.3	6		204	164	178	-19.6	-12.7	8.5			
	8	2	209	204	201	-2.4	-3.8	-1.5	2		26.5	38.6	9.36	45.7	-64.7	-75.8			
		4	279	278	270	-0.4	-3.2	-2.9	4		15.2	25.3	8.61	66.4	-43.4	-66.0			
		6	322	324	313	0.6	-2.8	-3.4	6		9.27	10	5.75	7.9	-38.0	-42.5			
	Glipizide	3	2	1.69	1.66	1.53	-1.8	-9.5	-7.8		Paricalcitol**	3	2	6.3	2.83	1.78	-55.1	-71.7	-37.1
			4	1.71	1.75	1.49	2.3	-12.9	-14.9				4	7.32	2.76	1.64	-62.3	-77.6	-40.6
			6	1.36	1.68	1.71	23.5	25.7	1.8				6	6.14	1.91	1.8	-68.9	-70.7	-5.8
5.5		2	3.89	3.73	4.75	-4.1	22.1	27.3	2	4.7		2	1.79	-57.4	-61.9	-10.5			
		4	9.15	3.62	4.55	-60.4	-50.3	25.7	4	2.55		1.33	0.8	-47.8	-68.6	-39.8			
		6	3.33	3.68	4.84	10.5	45.3	31.5	6	2.09		1.26	0.74	-39.7	-64.6	-41.3			
8		2	13.6	13.4	13.7	-1.5	0.7	2.2	2	4.47		1.1	11.5	-75.4	157.3	945.5			
		4	13.4	13	14.2	-3.0	6.0	9.2	4	4.56		1.62	9.71	-64.5	112.9	499.4			
		6	13.1	13.4	14.2	2.3	8.4	6.0	6	4.62		1.28	8.17	-72.3	76.8	538.3			
Atorvastatin#		3	2	43.8	38.8	27.8	-11.4	-36.5	-28.4	# Contain 400 mg phosphorus/L. * Concentration in ng/ml. Blank: Reaction solution without PA21 or PA21 placebo. blq: below limit of quantification. N/A: not applicable.		3	2	43.8	38.8	27.8	-11.4	-36.5	-28.4
			4	24	28.8	22.6	20.0	-5.8	-21.5				4	24	28.8	22.6	20.0	-5.8	-21.5
			6	17.2	18.5	17.6	7.6	2.3	-4.9				6	17.2	18.5	17.6	7.6	2.3	-4.9
	5.5	2	46.3	61	62	31.7	33.9	1.6	2		46.3	61	62	31.7	33.9	1.6			
		4	61.3	60.5	64.4	-1.3	5.1	6.4	4		61.3	60.5	64.4	-1.3	5.1	6.4			
		6	52	60.1	59.1	15.6	13.7	-1.7	6		52	60.1	59.1	15.6	13.7	-1.7			
	8	2	74.3	78.1	74	5.1	-0.4	-5.2	2		74.3	78.1	74	5.1	-0.4	-5.2			
		4	78.3	69.8	74.8	-10.9	-4.5	7.2	4		78.3	69.8	74.8	-10.9	-4.5	7.2			
		6	73.8	71.6	71	-3.0	-3.8	-0.8	6		73.8	71.6	71	-3.0	-3.8	-0.8			

4.4.5 Interaction studies on PA21 API with vitamins, amino acids, fluoride, sulfate and oxalate (REP000075SR-EN03v.3)

This in vitro study was conducted in Vifor Pharma (issued in June 2012), investigated degradation and adsorption of water-soluble B-complex vitamins (folate, B9, B11), niacin (B3), pantothenic acid (B5), biotin (B7), pyridoxine (B6), amino acids (tryptophan, methionine) and fluoride as well as sulfate and oxalate to PA21. The influence of oxalate on the phosphate binding of PA21 API was also investigated.

Considering a dose of PA21 (5 g) and a low daily uptake of 800 mg phosphorus in 1 L gastric fluid, 50 mg PA21 in 10 ml water or phosphate buffer (containing 8 mg phosphorus, adjusted pH with HCl and/or NaOH to 3, 5.5, or 8) were mixed with the amount of vitamins, amino acids or fluoride described in Table 27, and incubated for 2

hours at 37°C. The supernatants were quantized for the five B-complex vitamins (folate, niacin, pantothenic acid, biotin, pyridoxine) and two amino acids (tryptophan, methionine) using HPLC with diode array and/or mass spectrometry (MS) detection, and for fluoride, sulfate, oxalate, and phosphate using ion chromatography with conductivity detection.

Table 27. Test concentrations of water-soluble vitamins, amino acids, fluoride, sulfate and oxalate (modified from the application)

water-soluble vitamins	daily doses	recommended intake	adequate intake	concentration to be tested** [µg/10 ml]
folate (B9, B11)	400*	x		4
niacin (B3)	16000*	x		160
pantothenic acid (B5)	5000*		x	50
biotin (B7, H)	30*		x	0.3
pyridoxine (B6)	1300*	x		13
amino acids				
methionine	700000*	x		7000
tryptophan	210000*	x		2100
anion				
fluoride	4000* µg/day		x	40
sulfate	4400 mg/day		x	44000
oxalate	100 mg/day		x	1000

* values taken for males 31 - 50 years old. ** taking into account substance to be diluted in 1 l of gastric juice

No biologically relevant adsorption or degradation of B-complex vitamins (folate, niacin, pantothenic acid, biotin, and pyridoxine), fluoride and amino acids (tryptophan, methionine) by PA21 took place under physiologically relevant conditions (Table 28). No influence of macronutrients (sulfate, oxalate) on the PA21 binding capacity of phosphate was detectable (Table 29).

Table 28. Adsorption of water-soluble vitamins, amino acids, fluoride, sulfate and oxalate to PA21 in vitro

vitamin, amino acid or fluoride	pH-value	Incubation at individual pH preparation				Incubation simulating GI passage with phosphate	
		without phosphate		with phosphate		recovery [%]	rsd [%]
		recovery [%]	rsd [%]	recovery [%]	rsd [%]		
folate (B9, B11)	3.0	< 14.0 (LOD)	-	71.9	9.1	95.5	< 1.9
	5.5	< 7.2 (LOD)	-	104.4	1.0		
	8.0	12.7	15.0	100.7	1.1		
niacin (B3)	3.0	45.2	1.0	94.1	0.4		
	5.5	76.4	4.3	96.9	0.8		
	8.0	97.9	0.7				
pantothenic acid (B5)	3.0	16.1	15.4	97.0	2.8		
	5.5	49.5	11.0	97.8	2.4		
	8.0	91.9	4.6				
biotin (B7, H)	3.0	17.1	13.2	107.1	2.9		
	5.5	65.6	3.0	107.5	4.9		
	8.0	93.7	2.1				
pyridoxine (B6)	3.0	71.2	4.2	95.9	2.4	95.8	1.1
	5.5	12.4	10.2	76.6	2.5		
	8.0	13.3	3.9	75.5	4.7		
methionine	3.0	96.5	0.7				
	5.5	95.1	0.8				
	8.0	95.3	0.4				
tryptophan	3.0	92.4	2.9				
	5.5	99.1	1.3				
	8.0	95.6	1.0				
fluoride	3.0	5.0	12.9	23.0	11.5	93.6	< 3.6
	5.5	26.8	21.2	92.0	3.2		
	8.0	83.7	1.9				
sulfate	3.0	91.0	3.6				
	5.5	94.1	5.1				
	8.0	100.4	0.3				
oxalate	3.0	< 3.0 (LOD)	-	45.9	2.9	79.4	1.5
	5.5	< 1.0 (LOD)	-	77.8	2.7		
	8.0	69.6	2.3	102.1	0.4		

Table 29. Oxalate did not affect PA21 binding capacity of phosphate in vitro simulating gastro-intestinal passage (from application)

incubation	phosphorous [mg P/50 mg PA21-	rsd, assay [%]	mass balance (Σ ICP-OES, IC) [% recovery, % rsd]
with oxalate	2.19	7.8	97.8 (2.4)
without oxalate	2.08	7.9	95.7 (4.1)

5 Pharmacokinetics/ADME/Toxicokinetics

Summary

Pharmacokinetic studies were conducted in mice, rats and dogs with oral ⁵⁹Fe-PA21 at clinically relevant dosages. After a single oral administration of ⁵⁹Fe-labeled PA21 in SD

rats, < 3% of the administered radioactivity was recovered from sampled tissues of iron storage or utilization (blood cells, liver, spleen, and bone marrow) with small amounts (< 0.5%) in the walls of small and large intestines at 24 hours post-dose. A major portion of the administered dose was recovered from feces during the first 48 hours postdose. No radioactivity was recovered from urine, and there was neither biliary excretion nor entero-hepatic circulation of ⁵⁹Fe. Studies in pigmented (Lister Hooded) rats showed similar finding to SD rats, indicating a lack of binding or interactions of PA21 with melanin. In dogs, a low level of absorption was observed in only 1 of 3 animals studied. Excretion studies showed that virtually the total iron dose was excreted in the feces of all species within 48 hours post PA21oral administration.

In vitro studies conducted under simulated GI tract conditions showed highest iron release from PA21 (~6%) under low pH conditions as present in the lumen of the stomach in the fasting state, and minimal release of iron under pH conditions in the stomach in the fed state as well as in the duodenum, jejunum and colon. PA21 was degraded to simple endogenous and/or innocuous molecules in vitro similar to mixtures of its components, and the carbohydrates stabilized the iron (III)-oxyhydroxide core.

A study in mice showed that iron uptake from PA21 was not influenced by a number of common foodstuffs or pharmaceutical products. Oral daily PA21 treatment for 14 days in rats did not affect the amounts or activities of cytochrome P450 (CYP) isoenzymes in liver and small intestines, indicating low potential for drug-drug interactions at the metabolic level.

5.1 PK/ADME

5.1.1 ⁵⁹Fe-PA21 - Pharmacokinetic, absorption, distribution and excretion studies in rats after single oral dosing (VFR076/052010)

This GLP study (VFR076) was conducted in (b) (4)
(Sponsored by Vifor Pharma) during Sept-Dec, 2004.

Single oral doses of ⁵⁹Fe-PA21 250 mg/kg were administered to male and female Sprague Dawley rats (body weight 225-296 g) and male Lister-Hooded rats, appropriate samples were collected as detailed below in Table 30 for determining radioactivity. For the assessment of the total amounts of radioactivity contained in tissues including plasma, whole blood, blood cells, and bone marrow were assumed to be 4, 7, 3, and 0.35%, respectively, of animal body weight (BW) at sacrifice.

Table 30. Summary of methods/procedures

Study phase	Group	M/F	Procedures	Determination
PK, SD rats, ⁵⁹ Fe-PA21 250 mg/kg (1.2 x 10 ⁶ cpm), oral	Subgroup 1	3/3	Bleeding at 15 min, 4 and 72 hours	Radioactivity in whole blood, plasma, and blood cells at each time point (A)
	Subgroup 2	3/3	Bleeding at 30 min, 6 and 96 hours	
	Subgroup 3	3/3	Bleeding at 1, 8 and 120 hours	
	Subgroup 4	3/3	Bleeding at 2, 24 and 144 hours	
	Subgroup 5	3/3	Bleeding at 3, 48 and 168 hours	
Tissue distribution, SD rats, ⁵⁹ Fe-PA21 350 mg/kg (1.7 x 10 ⁶ cpm), oral	Subgroup 6	3M	Bleeding and killed at 2 hours post-dose	A + Radioactivity in Adrenals, Aorta, Bone, Bone marrow, Brain, Eyes, Fat (peri-renal), Fat (brown), Harderian glands, Heart, Kidneys, Lacrimal glands, Liver, Lungs, Lymph nodes (mesenteric), Muscle (skeletal), Pancreas, Pituitary, Prostate, Salivary glands, Skin, Spinal cord, Spleen, Testes, Thymus, Thyroid, Urinary bladder, Vena cava, Stomach, Small intestine, Large intestine.
	Subgroup 7	3M	Bleeding and killed at 6 hours post-dose	
	Subgroup 8	3M	Bleeding and killed at 24 hours post-dose	
	Subgroup 9	3M	Bleeding and killed at 96 hours post-dose	
	Subgroup 10	3M	Bleeding and killed at 168 hours post-dose	
Tissue distribution, Lister Hooded rats, ⁵⁹ Fe-PA21 350 mg/kg (1.7 x 10 ⁶ cpm), oral		6M	Bleeding and killed at 3, 24, 48, 168, 336 or 504 hours post-dose (1 animal/time point)	A + Radioactivity in Eyes, Kidneys, Liver, Skin (pigmented), Skin (non-pigmented) and Spleen
Excretion balance, SD rats, ⁵⁹ Fe-PA21 250 mg/kg (1.2 x 10 ⁶ cpm), oral, housed individually for separated collection of urine and feces		3/3	Collecting urine and feces at 0-6, 6-24 hours and subsequently at 24-hour intervals up to 168 hours post-dose.	Radioactivity in all urine and feces samples and the carcasses at termination (168 hours post-dose)
Biliary excretion, bile duct cannulated SD rats, ⁵⁹ Fe-PA21 350 mg/kg (1.7 x 10 ⁶ cpm), oral, housed individually for separated collection of bile, urine and feces		5/5	Bile collection: 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-24 and 24-48 hours after dosing. Urine collection: 0-6, 6-24 and 24-48 hours Feces collection: 0-24 and 24-48 hours post-dose	Radioactivity in all samples, GI tract contents and washings (combined), liver, GI tract walls, plasma and remaining carcasses at termination (48 hours post-dose)

Following the oral administration of ⁵⁹Fe-PA21 to the SD rats, mean whole-blood radioactivity levels were detected mainly between 24 and 168 hours after dosing, with maximal levels detected at 120 hours in males and 96 hours in females. In plasma, measurable concentrations of radioactivity were only transiently detected in the first 8 hours after dosing. Amounts of radioactivity in the whole-blood and blood cells reached 1.3-1.4% of the dose in males and 0.7-0.8% of the dose in females at 96 and/or 120 hours post-dose. Values were somewhat lower in females (Table 31). Greatest mean concentrations of radioactivity were observed in the separated sections of the GI tract wall at 2 and 6 hours post-dose, where >80% of the dose was recovered (Table 32). Of the remaining tissues sampled, concentrations of radioactivity were detected in the liver, spleen and bone marrow (up to a maximum of 0.1781, 0.0198 and 0.0817% dose respectively) during the experimental regime. At 24 hours post-dose, <3% of the dose was determined in all sampled tissues, with residual amounts of radioactivity within the walls of the small and large intestine at this time (Table 32, Table 33).

Table 31. Radioactivity levels in blood following an oral ⁵⁹Fe-PA21 dose in SD rats

Time after dosing (hours)		male			female		
		Whole-blood	Plasma	Blood cell	Whole-blood	Plasma	Blood cell
15 minutes	μg equivalents iron/g	-	-	-	-	-	-
30 minutes		-	-	-	-	-	-
1		-	-	-	-	-	-
2		-	1.948 ± 1.696	-	-	-	-
3		-	-	-	-	-	-
4		-	-	-	-	-	-
6		2.829 ± 0.794	1.858 ± 1.619	2.127 ± 1.937	-	-	-
8		3.120 ± 0.291	1.460 ± 1.267	4.070 ± 0.326	-	-	-
24		5.313 ± 0.709	-	10.36 ± 1.06	2.464 ± 0.441	-	4.029 ± 0.915
48		3.981 ± 2.287	-	8.148 ± 4.333	2.361 ± 2.060	-	5.140 ± 2.685
72		5.364 ± 1.947	-	10.96 ± 3.57	3.182 ± 1.052	-	6.861 ± 2.492
96		9.187 ± 2.670	-	21.82 ± 5.61	5.194 ± 0.309	-	12.85 ± 2.07
120		9.868 ± 0.229	-	19.90 ± 0.34	4.479 ± 1.152	-	9.812 ± 2.305
144		6.076 ± 0.871	-	11.15 ± 2.32	3.779 ± 0.726	-	7.530 ± 1.005
168		2.949 ± 2.955	-	6.288 ± 3.362	2.370 ± 2.071	-	4.858 ± 2.212
15 minutes		% dose	-	-	-	-	-
30 minutes	-		-	-	-	-	-
1	-		-	-	-	-	-
2	-		0.153 ± 0.133	-	-	-	-
3	-		-	-	-	-	-
4	-		-	-	-	-	-
6	0.393 ± 0.107		0.147 ± 0.128	0.126 ± 0.114	-	-	-
8	0.438 ± 0.035		0.116 ± 0.101	0.245 ± 0.018	-	-	-
24	0.736 ± 0.097		-	0.615 ± 0.060	0.342 ± 0.064	-	0.240 ± 0.056
48	0.554 ± 0.309		-	0.486 ± 0.251	0.329 ± 0.287	-	0.307 ± 0.160
72	0.754 ± 0.275		-	0.660 ± 0.216	0.436 ± 0.143	-	0.403 ± 0.145
96	1.278 ± 0.360		-	1.302 ± 0.324	0.731 ± 0.042	-	0.775 ± 0.126
120	1.388 ± 0.015		-	1.200 ± 0.018	0.632 ± 0.169	-	0.593 ± 0.146
144	0.842 ± 0.120		-	0.662 ± 0.135	0.525 ± 0.105	-	0.448 ± 0.063
168	0.409 ± 0.407		-	0.375 ± 0.194	0.330 ± 0.289	-	0.290 ± 0.133

- Not calculated (at least two of the three individual samples Not detected)

Table 32. Radioactivity levels (μg equivalents iron/g) in tissues following an oral ^{59}Fe -PA21 dose in SD rats

Tissue	2 hours	6 hours	24 hours	96 hours	168 hours
Plasma	2.449 \pm 0.025	2.036 \pm 0.364	-	-	-
Whole-blood	1.834 \pm 0.081	2.156 \pm 0.500	4.118 \pm 0.200	6.207 \pm 1.810	7.139 \pm 1.497
Blood cells	1.186 \pm 0.030	1.606 \pm 0.754	5.094 \pm 0.872	11.88 \pm 3.60	12.06 \pm 2.83
Aorta	-	-	-	-	-
Vena cava	-	-	-	-	-
Brain	-	-	-	-	-
Spinal cord	-	-	-	-	-
Eyes	-	-	-	-	-
Heart	-	-	-	-	-
Kidney	-	-	-	-	-
Urinary bladder	-	-	-	-	-
Liver	-	0.713 \pm 0.628	2.041 \pm 0.782	1.382 \pm 0.231	2.330 \pm 1.031
Lungs	-	-	-	-	-
Pancreas	-	-	-	-	-
Spleen	-	4.647 \pm 1.654	6.459 \pm 1.866	-	-
Adrenal glands	-	-	-	-	-
Harderian glands	-	-	-	-	-
Lacrimal glands	-	-	-	-	-
Lymph nodes (mesenteric)	-	-	-	-	-
Pituitary gland	-	-	-	-	-
Salivary gland	-	-	-	-	-
Thymus	-	-	-	-	-
Thyroid	-	-	-	-	-
Prostate	-	-	-	-	-
Testes	-	-	-	-	-
Bone	-	-	-	-	-
Bone marrow	-	16.59 \pm 3.69	12.10 \pm 10.58	-	-
Fat (brown)	-	-	-	-	-
Fat (peri-renal)	-	-	-	-	-
Muscle (skeletal)	-	-	-	-	-
Skin	-	-	-	-	-
Stomach wall	140.8 \pm 78.2	62.15 \pm 35.28	-	-	-
Small intestine wall	58.67 \pm 35.3	19.77 \pm 0.45	1.635 \pm 0.216	-	-
Large intestine wall	2.760 \pm 2.88	98.09 \pm 7.47	7.524 \pm 0.925	-	-

- Not calculated (not detected in at least two out of three individual samples)

Table 33. Tissue radioactivity levels (% dose and blood/tissue ratio) following an oral ^{59}Fe -PA21 dose in SD rats

Tissue		2 hours		6 hours		24 hours		96 hours		168 hours		
		Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	
Plasma	% dose	0.1376	0.0007	0.1148	0.0217							
Whole-blood		0.1804	0.0062	0.2129	0.0515	0.4117	0.0213	0.6879	0.2093	0.8536	0.1779	
Blood cells		0.0499	0.0014	0.0682	0.0326	0.2184	0.0387	0.5639	0.1765	0.6210	0.1678	
Liver				0.0445	0.0394	0.1336	0.0422	0.1008	0.0135	0.1781	0.0961	
Spleen				0.0170	0.0076	0.0198	0.0049					
Bone marrow				0.0817	0.0193	0.0604	0.0530					
Stomach contents		34.28	14.10	2.975	0.434							
Stomach wall		1.027	0.598	0.4417	0.2250							
Small intestine contents		44.96	14.13	9.150	3.563	0.0616	0.0567					
Small intestine wall		1.483	1.062	0.5292	0.1382	0.0331	0.0036					
Large intestine contents		0.1039	0.0335	69.07	3.87	1.581	0.451					
Large intestine wall		0.0296	0.0294	1.012	0.015	0.0842	0.0184					
Whole-blood		whole-blood tissue ratio	1.00		1.00		1.00		1.00		1.00	
Plasma			1.34	0.05	0.95	0.05						
Blood cells	0.65		0.02	0.72	0.21	1.25	0.27	1.91	0.07	1.69	0.17	
Liver				0.45	0.01	0.49	0.17	0.23	0.04	0.32	0.10	
Spleen				2.11	0.3	1.56	0.38					
Bone marrow				7.71	0.7	4.53	0.68					
Stomach wall	77.98		46.78	31.30	23.67							
Small intestine wall	31.52		17.84	9.52	2.33	0.40	0.03					
Large intestine wall	2.22	1.25	47.57	13.51	1.83	0.15						

Following oral administration of ^{59}Fe -PA21 to partially pigmented Lister Hooded rats, plasma radioactivity was only detected at 3 hours after dosing, whilst highest whole blood and blood cell radioactivity concentrations were observed at 504 hours after dosing, the final sampling point. Measured radioactivity concentrations in plasma, whole blood and blood cells were <1% of the dose at all time points studied. Tissue radioactivity was detectable only in liver at 24 hours post-dose (Table 34). From the available data, there were no notable differences in the uptake of radioactivity into blood cells between albino and pigmented rats.

Table 34. Radioactivity levels following an oral ^{59}Fe -PA21 dose in Lister Hooded rats

Samples		3 hours	24 hours	48 hours	168 hours	336 hours	504 hours
Plasma	µg equivalents iron/g	1.357	ND	ND	ND	ND	ND
Whole-blood		1.381	3.167	2.646	2.524	3.243	4.538
Blood cells		0.922	5.739	5.219	5.256	7.681	8.962
Eyes		ND	ND	ND	ND	ND	ND
Kidney		ND	ND	ND	ND	ND	ND
Liver		ND	1.085	ND	ND	ND	ND
Spleen		ND	ND	ND	ND	ND	ND
Skin (non-pigmented)		ND	ND	ND	ND	ND	ND
Skin (pigmented)		ND	ND	ND	ND	ND	ND
Plasma	% dose	0.0766					
Whole-blood		0.1364	0.3077	0.2759	0.2969	0.4338	0.6398
Blood cells		0.0390	0.2390	0.2332	0.2650	0.4403	0.5415
Liver			0.0804				

ND Not detected

Following oral ^{59}Fe -PA21 to SD rats, the vast proportion of the dose was recovered in the feces during the first 24 hours post-dose in both sexes, with a smaller amount recovered during the next 24 hour sampling period (Table 35). Data from the bile-duct cannulated rats demonstrated that this excretion was not a result of entero-hepatic circulation of the compound (Table 36). There was no detectable urinary excretion of radioactivity at any time-point during the study for either sex of animal, and no or minimal radioactivity was detected in the cage washings performed during the experimental regime (Table 35, Table 36).

Table 35. Recovery of radioactivity in excreta of SD rats following an oral ⁵⁹Fe-PA21 dose (% of dose)

Sample	Time hours after dosing	Males				Females			
		Recovery		Cumulative recovery		Recovery		Cumulative recovery	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd
Urine	0 - 6	-	-	-	-	-	-	-	-
	6 - 24	-	-	-	-	-	-	-	-
	24 - 48	-	-	-	-	-	-	-	-
	48 - 72	-	-	-	-	-	-	-	-
	72 - 96	-	-	-	-	-	-	-	-
	96 - 120	-	-	-	-	-	-	-	-
	120 - 144	-	-	-	-	-	-	-	-
	144 - 168	-	-	-	-	-	-	-	-
	Subtotal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Faeces	0 - 24	85.71	19.62	85.71	19.62	64.37	18.95	64.37	18.95
	24 - 48	15.30	11.72	101.01	11.32	18.41	5.74	82.78	13.39
	48 - 72	1.29	1.36	102.30	10.43	2.34	1.74	85.12	11.72
	72 - 96	0.35	0.30	102.65	10.46	0.18	0.17	85.30	11.56
	96 - 120	0.05	0.09	102.70	10.39	0.05	0.09	85.35	11.52
	120 - 144	0.03	0.05	102.73	10.35	-	-	85.35	11.52
	144 - 168	0.00	0.00	102.73	10.35	0.16	0.28	85.52	11.42
	Subtotal	102.73	10.35	102.73	10.35	85.52	11.42	85.52	11.42
Cage wash	24	0.26	0.45	0.26	0.45	-	-	-	-
	48	-	-	0.26	0.45	-	-	-	-
	72	-	-	0.26	0.45	-	-	-	-
	96	-	-	0.26	0.45	-	-	-	-
	120	-	-	0.26	0.45	-	-	-	-
	144	-	-	0.26	0.45	-	-	-	-
	168	-	-	0.26	0.45	-	-	-	-
	Subtotal	0.26	0.45	0.26	0.45	0.00	0.00	0.00	0.00
Carcass	168	1.14	1.97	1.14	1.97	-	-	-	-
	Total	104.13	9.35	104.13	9.35	85.52	11.42	85.52	11.42

- Not detected.

Table 36. Recovery of radioactivity in excreta of bile-duct cannulated SD rats following an oral ⁵⁹Fe-PA21 dose (% of dose)

Sample	Time (hours after dosing)	Males				Females			
		Recovery		Cumulative recovery		Recovery		Cumulative recovery	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd
Bile	0 - 1	-	-	-	-	-	-	-	-
	1 - 2	-	-	-	-	-	-	-	-
	2 - 3	-	-	-	-	-	-	-	-
	3 - 4	-	-	-	-	-	-	-	-
	4 - 5	-	-	-	-	-	-	-	-
	5 - 6	-	-	-	-	-	-	-	-
	6 - 24	-	-	-	-	-	-	-	-
	24 - 48	-	-	-	-	-	-	-	-
	Subtotal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Urine	0 - 6	-	-	-	-	-	-	-	-
	6 - 24	-	-	-	-	-	-	-	-
	24 - 48	-	-	-	-	-	-	-	-
		Subtotal	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Faeces	0 - 24	76.84	13.41	76.84	13.41	79.24	7.85	79.24	7.85
	24 - 48	13.69	9.75	90.53	7.80	8.12	6.62	87.36	3.32
		Subtotal	90.53	4.80	90.53	4.80	87.36	3.32	87.36
Cage wash	24	-	-	-	-	-	-	-	-
	48	-	-	-	-	-	-	-	-
		Subtotal	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Carcass Plasma Liver GI Tract contents GI Tract wall	48	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		0.14	0.06	0.14	0.06	0.04	0.04	0.04	0.04
		1.35	1.80	1.35	1.80	1.15	1.46	1.15	1.46
		0.10	0.08	0.10	0.08	0.14	0.20	0.14	0.20
		Subtotal	1.59	1.91	1.59	1.91	1.33	1.61	1.33
Total		92.12	4.05	92.12	4.05	88.69	2.12	88.69	2.12

- Not detected.

In summary, following the oral administration of ⁵⁹Fe-PA21 to SD rats at nominal doses of 250 mg or 350 mg PA21/kg, radioactivity was excreted exclusively via the fecal route. Low levels of radioactivity (1.3-1.4% of the dose) were detected in the blood (primary in blood cells). During the first 6 hours post-dose, majority of the radioactivity was recoverable from the sections of the GI tract. Radioactivity was transiently detectable in the liver, spleen and bone marrow tissues during 6-24 hours post-dose. Excretion of radioactivity was effectively complete within 48 hours post-dose. There was absorption of ≤3% of the administered radioactivity from the GI tract.

5.1.2 ⁵⁹Fe-PA21 - Pharmacokinetic, absorption, distribution and excretion studies in dogs after single oral dosing (VFR079/043552)

This GLP study (VFR079) was conducted in [REDACTED] (b) (4)
(Sponsored by Vifor Pharma) during Aug-Nov, 2004.

Single oral doses of ^{59}Fe -PA21 250 mg/kg were administered to 3 male Beagle dogs (~10 months old). Blood samples were collected at 15, 30 and 45 minutes and then at 1, 2, 3, 4, 6, 8, 24, 48, 72, 96, 120, 144, and 168 hours post-dose for determining radioactivity levels in whole blood, plasma, and blood cells. Urine was collected separately from each animal at 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours post-dose. Feces was collected separately at 24-hour intervals during 168 hours post-dose. At the end of 168-hour sample collection period, dogs were sacrificed, and adrenal glands, bone, bone marrow, brain, epididymis, heart, kidneys, liver, lungs, mesenteric lymph nodes, skeletal muscle, pituitary gland, spleen, testes, thymus, and GI tract were sampled. Radioactivity in each sample was then determined.

Following an oral dose of 250 mg ^{59}Fe -PA21/kg, only one dog showed radioactivity in blood cells (<1% of the dose) between 96 and 168 hours post-dose and in the spleen (0.14% of the dose) at 168 hours post-dose (Table 37). There were no detectable radioactivity in any other sampled tissues and blood in the other 2 dogs. The vast proportion of the ^{59}Fe -PA21 dose was recovered in the feces during the first 48 hours post-dose (Table 38). There was no detectable urinary excretion of radioactivity at any time point during the study (Table 38). Thus, following an oral dose of 250 mg ^{59}Fe -PA21/kg to male Beagle dogs, radioactivity was excreted exclusively via the fecal route within 48 hours; and in 1 of 3 dogs. GI absorption of ^{59}Fe -PA21 was about 1%.

Table 37. Blood and tissue radioactivity of 1/3 dogs following an oral ⁵⁹Fe-PA21 dose

Time after dosing (unless otherwise specified hours)	Blood cells		Tissue	2M ug iron/g	2M % dose
	2M µg iron/g	2M % dose†			
15 minutes	ND	ND	Whole blood	ND	ND
30 minutes	ND	ND	Plasma	ND	ND
45 minutes	ND	ND	Blood cells †	10.91	0.8161
1	ND	ND	Brain	ND	ND
2	ND	ND	Heart	ND	ND
3	ND	ND	Kidney	ND	ND
4	ND	ND	Liver	ND	ND
6	ND	ND	Lungs	ND	ND
8	ND	ND	Spleen	10.73	0.1383
24	ND	ND	Adrenal glands	ND	ND
48	ND	ND	Lymph nodes (mesenteric)	ND	ND
72	ND	ND	Pituitary gland	ND	ND
96	8.150	0.6096	Thymus	ND	ND
120	9.493	0.7102	Epididymis	ND	ND
144	10.97	0.8208	Testes	ND	ND
168	10.91	0.8161	Bone	ND	ND
			Bone marrow	ND	ND
			Muscle (skeletal)	ND	ND
			Stomach contents	ND	ND
			Stomach wall	ND	ND
			Small intestine contents	ND	ND
			Small intestine wall	ND	ND
			Large intestine contents	ND	ND
			Large intestine wall	ND	ND

ND Not detected

† Blood cells were assumed to be 4%
of terminal body weight

Table 38. Recovery of radioactivity in excreta of dogs following an oral ⁵⁹Fe-PA21 dose (% of dose)

Sample	Time (hours after dosing)	Recovery		Cumulative recovery	
		Mean	sd	Mean	sd
Urine	0 - 6	-	-	-	-
	6 - 24	-	-	-	-
	24 - 48	-	-	-	-
	48 - 72	-	-	-	-
	72 - 96	-	-	-	-
	96 - 120	-	-	-	-
	120 - 144	-	-	-	-
	144 - 168	-	-	-	-
	Subtotal	0.00	-	0.00	-
Faeces	0 - 24	93.13	9.51	93.13	9.51
	24 - 48	13.48	8.77	106.60	1.17
	48 - 72	0.60	1.05	107.21	0.43
	72 - 96	-	-	107.21	0.43
	96 - 120	-	-	107.21	0.43
	120 - 144	-	-	107.21	0.43
	144 - 168	-	-	107.21	0.43
		Subtotal	107.21	0.43	107.21
Cage wash	24	0.57	0.08	0.57	0.08
	48	-	-	0.57	0.08
	72	-	-	0.57	0.08
	96	-	-	0.57	0.08
	120	-	-	0.57	0.08
	144	-	-	0.57	0.08
	168	-	-	0.57	0.08
		Subtotal	0.57	0.08	0.57
Total		107.78	0.50	107.78	0.50

- Not calculated (no sample or not detected in all three samples)

5.1.3 Comparability of the properties of PA21 and its components (REP000150TC-EN03v.1)

This non-GLP study (REP000150TC-EN03v.1) was conducted in Vifor Pharma (issued in Aug 2012), investigating whether PA21 is degraded in a similar way as its individual components, iron (III)-oxyhydroxide, starch ((b) (4) in case of the original PA21 or (b) (4) in case of PA21-2) and sucrose.

PA21 and starch were incubated with α -amylase and the resulting maltose was quantified. Degradation of starch was only partial; the amounts of maltose found for PA21 active pharmaceutical ingredients (API) were significantly higher than those for

(b) (4) at each time-point (Figure 7). Addition of iron (III)-oxyhydroxide to (b) (4) increased the amount of maltose found (Figure 7), although the variation was large and test number was only 2. However, in the case of PA21-2, enzymatic degradations by α -amylase were similar between PA21 and the mixture of (b) (4) and (b) (4) (Table 39).

Figure 7. Enzymatic degradation by α -amylase - maltose found in the reactions (modified from the application)

(b) (4)

Table 39. Enzymatic degradation by α -amylase (modified from the application)

(b) (4)

PA21-2 and various mixtures of iron (III)-oxyhydroxide, starch and/or sucrose were exposed to porcine pancreatin and rat intestinal mucosa, and analyzed for glucose release (Figure 8). The percentage of formed glucose from PA21 concurred with the values expected from its individual components (Table 40), suggesting similar behavior of PA21 under incubation with porcine pancreatin and rat intestinal mucosa to a mixture of iron (III)-oxyhydroxide, sucrose, and starch.

Figure 8. In vitro enzymatic degradation by pork pancreatin and rat intestinal mucosa (modified from the application)

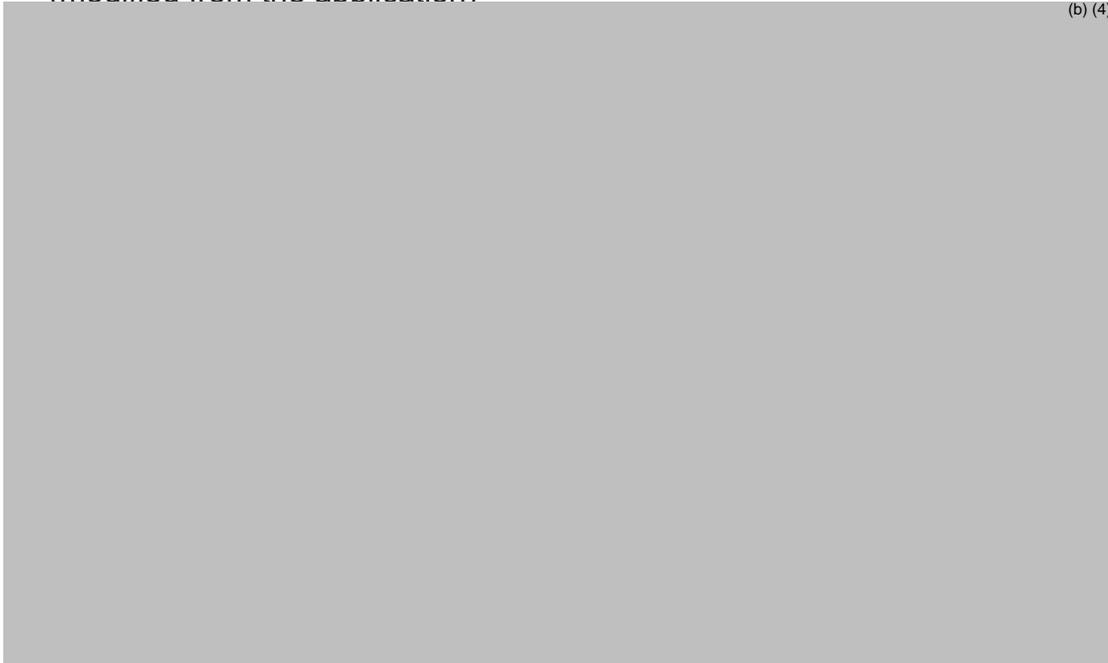
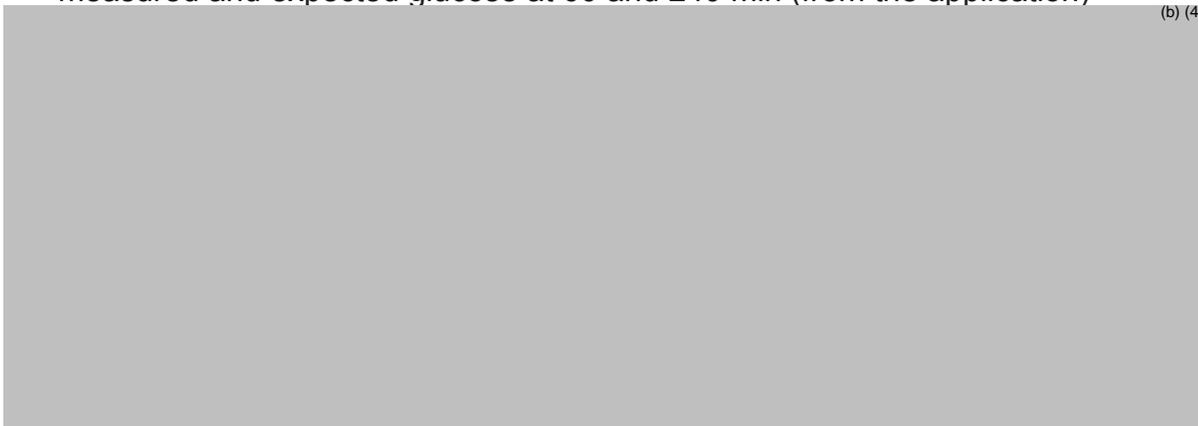


Table 40. Enzymatic degradation by pork pancreatin and rat intestinal mucosa – measured and expected glucose at 60 and 240 min (from the application)



Incubation of sucrose and PA21 with invertase (sucrase) resulted in complete degradation of sucrose into glucose and fructose (Table 41). Finally, the iron release from the iron (III)-oxyhydroxide suspension and PA21 was determined at pH 3, 37°C. Iron release from PA21 was significantly lower (1.4%) than from iron (III)-oxyhydroxide (4.6%) (Table 42).

Table 41. Sucrose degradation with invertase (from the application)

sample	time [h]	glucose % [m/m]	fructose % [m/m]	sum of glucose and fructose % [m/m]	mean % [m/m]
sucrose	0.25	52.0	50.6	102.5	102.6
	0.25	51.2	49.7	100.9	
	1	51.9	50.6	102.5	
	1	52.0	50.7	102.7	
	5	52.0	51.5	103.5	
PA21 API	5	52.0	51.7	103.7	96.4
	0.25	51.1	44.7	95.9	
	0.25	51.9	45.3	97.3	
	1	51.3	45.1	96.4	
	1	51.4	45.1	96.5	
	5	51.6	44.6	96.3	
	5	51.6	44.8	96.3	

Table 42. Comparison of iron release from the iron (III)-oxyhydroxide used for the production of PA21 API lot no. 160807J1 with that from 13 batches of PA21 API (from the application)

PA21 API		iron(III)-oxyhydroxide
batch no.	released iron % [m/m]	released iron % [m/m]
210807J1	0.9	
220807J1	1.1	
090807J1	1.0	
130807J1	1.1	
130807J2	1.6	
140807J1	1.9	
150807J1	1.4	
160807J1	1.2	4.6
200807J1	1.5	
200807J2	1.2	
230807J1	2.0	
270807J1	1.2	
270807J2	1.5	

In summary, the data of this study indicated that PA21 behaved like a mixture of its components and that the carbohydrates stabilized the iron (III)-oxyhydroxide core.

5.1.4 An in vitro study to investigate potential effects of PA21 on intestinal and liver cytochrome P-450 (CYP) in rats (b) (4)

This non-GLP study (b) (4) was conducted in (b) (4) and (b) (4) (sponsored by Vifor Pharma) during March-April, 2008. This study investigated the potential effects of PA21 on CYP expression/activities in the liver and small intestine of male CrI:CD (SD) IGS BR rats.

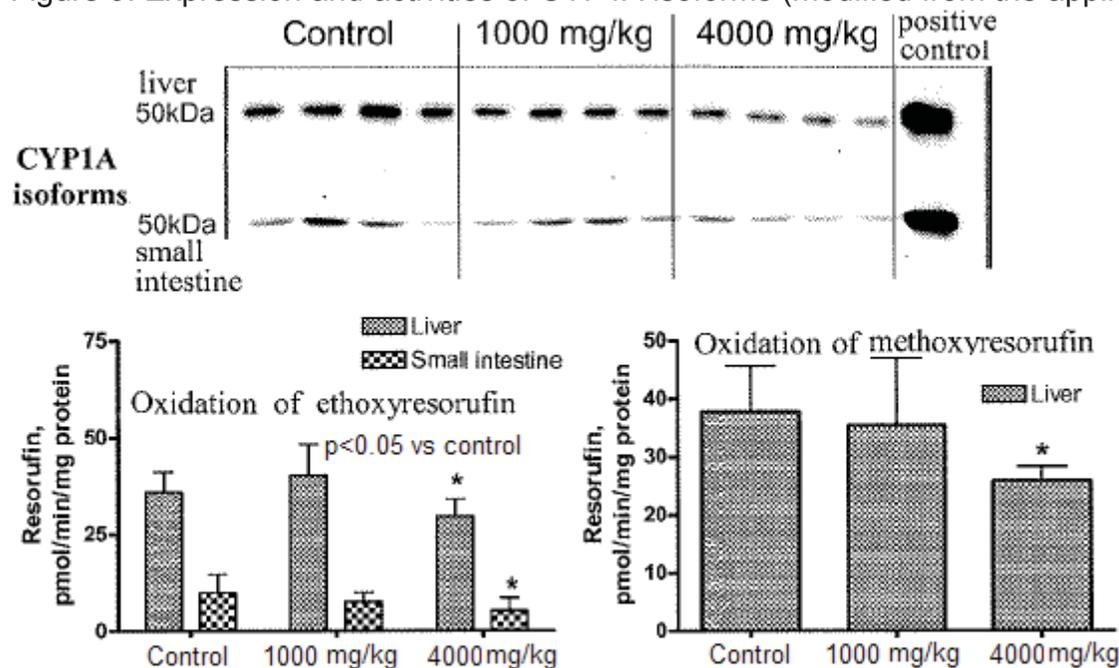
Male SD rats were on diet containing PA21 at doses 0, 1000, or 4000 mg/kg/day for 14 days (n=7-8/group). On day 15, microsomes were prepared from liver and small intestine, and analyzed for CYP protein expression (Western immunoblotting) and CYP

isoform-specific activities in oxidation of testosterone, midazolam, ethoxyresorufin, methoxyresorufin, or pentoxyresorufin.

PA21-treated animals were more vocal on handling. Rats given 4000 mg/kg/day had much darker and more pungent feces.

Treatment with PA21 for 14 days showed no effects on CYP2B and CYP3A protein level in liver and small intestine, no effects on liver CYP2B activity in oxidation of pentoxyresorufin, and no effects on liver and small intestinal CYP3A activities in testosterone 6 β -hydroxylation and midazolam 1'-hydroxylation. At 4000 mg/kg/day, PA21 treatment resulted in small decreases in liver and intestinal CYP1A protein levels, associated with decreases in CYP1A activities (in liver, ~80% of control for ethoxyresorufin oxidation, ~68% of control for methoxyresorufin *O*-dealkylation; in intestine, ~54% of control for ethoxyresorufin *O*-dealkylation) (Figure 9). These slight changes in CYP1A were not of biologically significant.

Figure 9. Expression and activities of CYP1A isoforms (modified from the application)



5.1.5 Iron release from PA21 API under gastrointestinal conditions (TC-1026/E01)

This non-GLP in vitro study was conducted in Vifor Pharma in 2007. The release of iron from PA21 was tested under simulated GI tract conditions.

Artificial gastric fluid (i.e., without phosphate, pH 1.2) and simulated intestinal fluid (pH 7.5) were prepared and mixed to obtain solutions with pH values of 1.7, 2.1, 2.5, 4.5 and 7.0. PA21 was added into the solutions at concentration of 5.0 mg Fe/mL, and incubated at 37°C for between 1 and 2.5 hours. Supernatants were analyzed for released iron using inductively coupled plasma mass spectrometry.

At nominal pH 1.2 (measured pH of 2.1, due to buffering action of PA21), 66.9% of the iron was released, which is unreasonably high and was not repeated in subsequent assays. At higher pH values (nominal pH range: 1.7 to 7.5, measured pH range of 2.5 to 7.5), very low or no release of iron (0.0 to 1.3%) was observed (Table 43).

Table 43. Summary of iron release from PA21 under gastrointestinal conditions (from the application)

pH set	pH found	time exposure [h]	iron released [%]	sd [%]
VARIATION 1				
1.2	2.1	1.0	66.9	3.1
2.5	6.0	1.0	0.0	0.0
4.5	6.7	1.5	0.1	0.0
7.0	7.2	2.0	0.4	0.1
7.5	7.4	2.5	0.4	0.0
VARIATION 2				
2.5	6.6	1.0	0.0	0.0
4.5	7.1	1.5	0.2	0.0
7.0	7.4	2.0	0.3	0.0
7.5	7.5	2.5	0.2	0.0
ADDITIONAL EXPERIMENTS				
1.7	2.5	1.0	1.3	0.1
2.1	5.6	1.0	0.0	0.0

5.1.6 Iron release from phosphate adsorbers under gastrointestinal conditions at pH 1.2 (TC-1094/E01)

This non-GLP in vitro study was conducted in Vifor Pharma in 2009. The release of iron from PA21 was tested under worst-case conditions at pH 1.2.

PA21 samples were incubated with inorganic phosphate solution (approximately 1111 mg phosphorus/L) at pH 1.2 for 1 hour at 37°C, at a concentration of 5 mg Fe/mL. The pH of the reaction solutions was adjusted to 1.2 after addition of PA21. Supernatants were analyzed for released iron using inductively coupled plasma optical emission spectrometry. Analysis of 6 replicate samples of PA21 and PA21-2 at pH 1.2 showed iron release of $5.39 \pm 0.50\%$ and $6.24 \pm 0.59\%$ respectively. Thus, under worst-case pH conditions that may be encountered in the GI tract, i.e., empty stomach, in the presence of phosphate, low iron release from PA21 and PA21-2 was observed.

5.2 Toxicokinetics

Systemic exposure was not assessed since PA21 is practically insoluble and already known to be not absorbed.

6 General Toxicology

6.1 Single-Dose Toxicity

6.1.1 PA21 – Acute oral toxicity study in the rats (VFR073/043299)

This GLP study (VFR073) was conducted in [REDACTED] (b) (4) (Sponsored by Vifor Pharma) during 2004-2005, to determine the highest non-lethal or lowest lethal dose of PA21 following a single oral dose by gavage to CrI:CD® (SD)IGS BR rats.

Five male and 5 female CrI:CD (SD) rats received a single oral dose of PA21 at a dosage of 5,000 mg/kg (Batch # 423000) in methylcellulose (1% w/w in water). This dosage was based on a preliminary phase group comprising one male and one female received a single dose of 5000 mg/kg, and represented about 40 times the maximum likely human clinical dose of 7500 mg/day (=125 mg/kg, when based on a 60 kg individual). Animals were checked for mortality and clinical condition twice daily for 14 days. Body weights were recorded prior to dose, on day 1, and weekly then after. All surviving animals were killed on day 15 and examined macroscopically.

No deaths or abnormal signs were observed. There were no PA21-related findings following the 14-day observation period. The lowest lethal oral dose of PA21 in CD rats was greater than 5,000 mg/kg.

6.2 Repeat-Dose Toxicity

6.2.1 PA21 – Toxicity study by dietary administration to CD rats for 26 weeks followed by a 6-week recovery period

Study no.:	VFR0096
Study report location:	[REDACTED] (b) (4)
Conducting laboratory and location:	[REDACTED]
Date of study initiation:	Sept 22, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PA21; 429000/95.4%, 423000/95.6%
Deviation from study protocol:	No impact to the results

Key Study Findings

Body weight gain was lower in males during treatment at dose 2500 mg/kg/day and was transiently and slightly lower in males at dose 750 mg/kg/day.

Thickened and darkened cecum and/or colon were observed in both sexes at doses 750 and/or 2500 mg/kg/day at the end of treatment. Mucosal hyperplasia with or without

submucosal inflammation/edema was seen in the cecum, colon, and/or rectum of one male given 750 mg/kg/day and of 3 males given 2500 mg/kg/day. After 6 weeks of recovery, mucosal hyperplasia was seen in the colon of one male given 2500 mg/kg/day

At doses 750 and/or 2500 mg/kg/day, both males and/or females showed higher plasma levels of alkaline phosphatase (ALP), urea, and phosphorus; lower urine volume and phosphorus, and higher urine pH at weeks 13 and/or 26. At 2500 mg/kg/day, two males had thickened urinary bladder containing calculi and ureter distension; there were kidney pelvic dilatation in 3 males, bladder transitional cell hyperplasia in 3 males, and ureter mucosal hyperplasia in 2 males.

A range of other findings were attributed to reduced phosphate uptake as a result of the action of PA21, the coloration of the test material (due to its high iron content), the uptake of iron from GI, or to metabolic changes related to the phosphate-depleting effects of PA21. The findings included higher serum and tissue levels of iron, increased bone resorption, and increased degree of hemosiderosis and positive Perl's staining in the spleens. These changes were minimal to moderate and not considered to be toxicologically significant.

NOAEL was 200 mg/kg/day.

Methods

CrI:CD (SD) rats, initial age of 6-7 weeks (211-263 g for males & 161-200 g for females), were on diet containing PA21 at 0, 200, 750 and 2500 mg/kg/day for 26 weeks (n = 21/sex for each control and high dose groups, n=15/sex for each low and mid dose groups). Six/sex from each control and high dose groups continued without treatment for a 6-week recovery period. Five/sex of age-matched rats were orally gavaged with cyclophosphamide (20 mg/kg/time) 72 and 48 hours prior to blood sampling during week 24, and served as positive control for micronucleus test. Doses selected in this study were based on a 4- and a 13-week toxicity studies in rats performed in the same facility (VFR0075 and VFR0087). PA21 doses in diet were 500, 1000, 2500, and 4000 mg/kg/day for the 4-week study (VFR0075) and 300, 1000, and 3000 mg/kg/day for the 13-week study (VFR0087). In the 4-week study, body weight gain was ~15 and ~30% less at doses 2500 and 4000 mg/kg/day, respectively; mucosal hyperplasia occurred in the rectum at 4000 mg/kg/day. In the 13-week study, PA21 3000 mg/kg/day resulted in reduced weight gain (33% in males and 19% in females), decreased kidney function, mucosal hyperplasia in the cecum and rectum, and transitional epithelial hyperplasia in the urinary bladder. Other findings in these two studies (VFR0075 and VFR0087) were attributed to the pharmacological activity of PA21 or to its high iron content. Thus, the dose 2500 mg/kg/day was selected as the high dose in the current study. The low dose 200 mg/kg/day was close to the highest likely human clinical dose. Samples of the drug-diet formulations were analyzed at weeks 1, 13, 25 of treatment to confirm the concentrations of PA21 in the treated diets and the absence in the control diet.

During the study, rats were inspected visually at least twice daily for clinical signs and morbidity/death. Physical examination was weekly performed on each animal. Body weight was recorded at week -1, study initiation, weekly thereafter, and before necropsy. Mean weekly food consumption per animal was assessed at week -1 and weekly thereafter. Ophthalmoscopy was performed prior to treatment on all animals, during week 26 on all survival animals of control and high dose groups. Blood samples were collected from all surviving animals during week 13, 26, and recovery week 6 after overnight fast, for determining hematology, plasma chemistry (Table 44), and serum osteocalcin level (bone turnover marker). Serum levels of vitamins A, D (25-hydroxy vitamin D, and 1,25-dihydroxy vitamin D), E, and K were determined at study termination on week 26 and recovery week 6 from all surviving animals. Overnight urine samples were collected from all animal during weeks 13, 16, and recovery week 6 for urinalysis (Table 45) and for determining deoxypyridinoline (bone turnover marker) and creatinine (for normalization of deoxypyridinoline values). During weeks 4 and 24, blood samples from 5/sex/group were collected without fast for micronucleus test (via flow cytometric analysis).

Table 44. Parameters for hematology and plasma chemistry

Hematology parameters	Plasma chemistry
Hematocrit (Hct)	Alkaline phosphatase (ALP)
Hemoglobin concentration (Hb)	Alanine aminotransferase (ALT)
Erythrocyte count (RBC)	Aspartate aminotransferase (AST)
Reticulocyte count (Retic)	Total bilirubin (Bili)
Mean cell hemoglobin (MCH)	Lipase (LIP) Urea
Mean cell hemoglobin concentration (MCHC)	Creatinine (Creat)
Mean cell volume (MCV)	Glucose (Gluc)
Total white cell count (WBC)	Cholesterol [Total (Chol), HDL, LDL]
Differential WBC count	Triglycerides (Trig)
Neutrophils (N)	Phospholipids (PLIP)
Lymphocytes (L)	Sodium (Na)
Eosinophils (E)	Potassium (K)
Basophils (B)	Chloride (Cl)
Monocytes (M)	Calcium (Ca)
Large unstained cells (LUC)	Inorganic phosphorus (Phos)
Platelet count (Plt)	Iron (Fe)
Morphology flags	Total protein (Total Prot)
Prothrombin time (PT)	Albumin(Alb)
Activated partial thromboplastin time (APTT)	α 1, α 2, β , and γ globulin

Table 45. Parameters of urinalysis

Qualitative	Quantative
Appearance (App)	Volume (Vol)
	pH
Glucose (Gluc)	Specific gravity (SG)
Ketones (Keto)	Protein (Prot)
Bile pigments (Bili)	Sodium (U-Na)
Bllod pigments (Ublod)	Potassium (U-K)
	Chloride (U-Cl)
Microscopic examination of urine sediment	Calcium (U-Ca)
	Inorganic phosphorus (U-IP)

On completion of 26 weeks of treatment and 6 weeks off treatment, all main and recovery animals were killed and subjected to a detailed necropsy. Organs/tissues listed in Table 46 were weighed, collected, histologically processed, and microscopically examined. Samples from liver (residue of left lobe), kidney (poles from left kidney), and spleen (poles) from all animals were weighed, frozen in liquid nitrogen for determination of iron content. Samples of any macroscopic abnormal tissues were also retained and processed for histopathological examination. No peer review was mentioned in this report.

Table 46. List of organs

	Weigh (1)	Fixation	Histological processed	Perl's stain for iron content	Light microscopy (2)		
					Week 26	Recovery	
Adrenals	√	10% neutral buffered formalin	√		√		
Aorta - thoracic			√		√		
Brain	√		√		√		
Cecum			√	√	√*	√	
Colon			√	√	√*	√	
Duodenum			√	√	√*	√	
Epididymides	√			√		√	
Eyes		D	√		√		
Femurs+		10% neutral buffered formalin	√		√		
Harderian glands			√		√		
Head							
Heart	√			√		√	
Ileum				√	√	√*	√
Jejunum				√	√	√*	√
Kidney	√			√	√	√*	√
Lachrymal glands				√		√	
Larynx				√		√	
Liver	√			√	√	√*	√
Lumbar vertebra (lumbar 6)				√		√	
Lungs	√			√		√	
Lymph node -mandibular -mesenteric				√	√	√*	√
				√	√	√*	√
Mammary area - caudal				√		√	
Esophagus				√		√	
Optic nerves				√		√	
Ovaries	√			√		√	
Pancreas				√		√	
Pituitary	√			√		√	
Prostate	√			√		√	
Rectum				√	√	√*	√
Salivary glands (submandibular parotid, sublingual)+	√			√		√	
Sciatic nerves+				√		√	
Seminal vesicle	√			√		√	
Skeletal muscle - thigh+				√		√	
Skin				√		√	
Spinal cord				√		√	
Spleen	√			√	√	√*	√
Sternum				√		√	
Stomach				√	√	√	
Testes	√			√		√	
Thymus	√			√		√	
Thyroid with parathyroids	√		√		√		
Tibia (right)+			√		√		
Tongue			√		√		
Trachea			√		√		
Ureters			√		√		
Urinary bladder			√		√ ^m	√	
Uterus and cervix	√		√		√		
Vagina			√		√		
Any abnormal tissue			√		√		

(1) Bilateral organs were weighed together. + Only one processed for examination. D - Davidson's fluid

(2) From control and high dose groups. * from all groups. ^m included males in low and mid dose groups.

Results

The overall achieved doses at 200, 750, and 2500 mg/kg/day were 199, 751, and 2532 mg/kg/day for males and 199, 759, 2595 mg/kg/day for females, respectively.

One female given 2500 mg/kg/day died in week 15. Necropsy revealed a large liver (35 g/body weight of 305 g), an enlarged and swollen spleen, congested lymph nodes, and red fluid in the nasal turbinates. The cause of death was determined to be lymphocytic/lymphoblastic lymphoma, and not PA21-related.

There were dark feces and brown staining of tails at mid and high dose groups, which were due to the brown color of PA21 and/or its iron content. Body weight gain was lower in males during treatment at dose 2500 mg/kg/day and was transiently and slightly lower in males at dose 750 mg/kg/day (Table 47). There were no treatment-related effects on food consumption and ophthalmoscopy.

Table 47. Body weight gain during the study

Sex	Group/ PA21 dose	Body weight (g)	Body weight gain (g)				
		Week 0	Weeks 0-1	Weeks 1-4	Weeks 4-26	Weeks 0-26	Recovery 0-6
Male	Control	237 ± 13	58 ± 7	115 ± 15	249 ± 48	421 ± 55	37 ± 19
		n=21	n=21	n=21	n=21	n=21	n=6
	200 mg/kg/day	236 ± 14	54 ± 8	107 ± 16	249 ± 38	410 ± 54	
		n=15	n=15	n=15	n=15	n=15	
	750 mg/kg/day	237 ± 14	52 ± 6*	115 ± 19	255 ± 48	422 ± 61	
		n=15	n=15	n=15	n=15	n=15	
	2500 mg/kg/day	233 ± 12	40 ± 6**	92 ± 21**	229 ± 51	360 ± 69**	55 ± 28
		n=21	n=21	n=21	n=21	n=21	n=6
Female	Control	172 ± 5	25 ± 4	41 ± 6	82 ± 20	147 ± 23	25 ± 16
		n=21	n=21	n=21	n=21	n=21	n=6
	200 mg/kg/day	172 ± 4	27 ± 5	39 ± 7	87 ± 16	152 ± 22	
		n=15	n=15	n=15	n=15	n=15	
	750 mg/kg/day	174 ± 10	25 ± 4	40 ± 7	90 ± 27	156 ± 28	
		n=15	n=15	n=15	n=15	n=15	
	2500 mg/kg/day	175 ± 9	21 ± 5	39 ± 7	74 ± 14	133 ± 17	31 ± 16
		n=21	n=21	n=21	n=20	n=20	n=6

* p<0.05 vs control; ** p<0.01 vs control

Plasma chemistry showed higher levels of iron in both sexes at weeks 13 and/or 26 at doses 750 and/or 2500 mg/kg/day. There were also higher plasma levels of ALP, urea, phosphorus in males and/or females at weeks 13 and/or 26, at doses 750 and/or 2500 mg/kg/day. Difference in plasma calcium levels were minor and within the physiological variation (Table 48). At doses 750 and/or 2500 mg/kg/day, both males and/or females showed lower urine volume and phosphorus, and higher pH and urine calcium at weeks 13 and/or 26 (Table 49), higher serum osteocalcin and urine deoxypyridinoline at weeks 13 and 26 (Table 50), and higher serum vitamin D levels at week 25 (Table 51). At week 26, iron levels in liver, kidney, and spleen were higher in males and/females at doses 750 and/or 2500 mg/kg/day (Table 52). These changes described here indicated moderate iron absorption from GI PA21, slightly increased bone resorption, and slightly

decreased kidney function, among which, only the decreased kidney function in both sex at 2500 mg/kg/day was of toxicological significance. Changes in hematology were limited to minor increases in red blood cell parameter (Table 53), which were within the physiological variation and may be related to increased iron uptake. The hematological changes and other changes in serum vitamins were either minimal and within the physiological variation or not consistent, and were of toxicologically insignificant.

Table 48. Findings of plasma chemistry in the 26-week rat study

Sex	Group/PA21 dose (mg/kg/day)	ALP (U/L)	Urea (mM)	Creat (μ M)	Ca (mM)	Phos (mM)	Fe (μ M)	A bumin/ Globulin ratio
Week 13 (n=14-15)								
Male	Control	81 \pm 12.8	5.2 \pm 0.9	36 \pm 4.0	2.62 \pm 0.05	2.15 \pm 0.10	42 \pm 9.0	0.86 \pm 0.06
	200	80 \pm 13.7	5.4 \pm 0.9	42 \pm 13.9	2.64 \pm 0.06	2.13 \pm 0.11	42 \pm 9.5	0.81 \pm 0.07
	750	87 \pm 11.0	5.9 \pm 1.3	42 \pm 17.5	2.60 \pm 0.06	2.31 \pm 0.16*	47 \pm 7.8	0.80 \pm 0.09
	2500	94 \pm 21.0*	6.7 \pm 1.1**	37 \pm 2.9	2.67 \pm 0.08*	2.78 \pm 0.35**	67 \pm 8.4**	0.76 \pm 0.09**
Female	Control	62 \pm 18.6	4.9 \pm 0.6	38 \pm 3.2	2.69 \pm 0.05	1.78 \pm 0.20	70 \pm 18.4	1.07 \pm 0.08
	200	69 \pm 12.6	5.6 \pm 0.8*	40 \pm 3.4	2.63 \pm 0.10	1.62 \pm 0.14	81 \pm 12.0	1.08 \pm 0.15
	750	69 \pm 15.4	5.3 \pm 0.6*	42 \pm 3.0*	2.67 \pm 0.08	1.66 \pm 0.17	82 \pm 12.4*	1.01 \pm 0.07
	2500	90 \pm 16.7**	5.9 \pm 0.7**	43 \pm 4.3**	2.63 \pm 0.08	2.09 \pm 0.26**	95 \pm 14.5**	1.06 \pm 0.10
Week 26 (n=14-21)								
Male	Control	64 \pm 12.6	5.1 \pm 0.9	38 \pm 3.5	2.69 \pm 0.05	1.93 \pm 0.13	40 \pm 9.6	0.81 \pm 0.06
	200	62 \pm 18.9	5.0 \pm 0.7	38 \pm 3.9	2.68 \pm 0.06	1.92 \pm 0.16	37 \pm 6.3	0.83 \pm 0.08
	750	62 \pm 6.3	5.1 \pm 0.7	41 \pm 5.2	2.64 \pm 0.06	1.93 \pm 0.15	39 \pm 6.2	0.79 \pm 0.07
	2500	73 \pm 34.4	6.7 \pm 0.9**	40 \pm 4.4	2.79 \pm 0.14*	2.07 \pm 0.28	66 \pm 16.8**	0.78 \pm 0.11
Female	Control	29 \pm 8.8	5.9 \pm 1.1	43 \pm 5.9	2.80 \pm 0.12	1.60 \pm 0.20	75 \pm 17.3	1.16 \pm 0.09
	200	31 \pm 9.2	6.2 \pm 1.1	43 \pm 3.7	2.78 \pm 0.08	1.44 \pm 0.16	73 \pm 16.3	1.08 \pm 0.14
	750	27 \pm 8.1	5.6 \pm 0.7	43 \pm 3.9	2.82 \pm 0.09	1.45 \pm 0.18	87 \pm 14.1*	1.15 \pm 0.11
	2500	37 \pm 12.0*	6.5 \pm 1.2	44 \pm 4.5	2.81 \pm 0.08	1.57 \pm 0.23	96 \pm 12.0**	1.10 \pm 0.14
Recovery week 6 (n=6)								
Male	Control	64 \pm 17.6	4.6 \pm 0.8	33 \pm 5.1	2.60 \pm 0.02	1.64 \pm 0.10	35 \pm 2.4	0.79 \pm 0.09
	2500	57 \pm 9.7	4.9 \pm 1.4	39 \pm 14.3	2.65 \pm 0.06*	1.75 \pm 0.14	41 \pm 6.8*	0.72 \pm 0.09
Female	Control	40 \pm 11.5	5.8 \pm 1.3	41 \pm 3.4	2.72 \pm 0.05	1.48 \pm 0.23	83 \pm 16.0	0.98 \pm 0.10
	2500	42 \pm 24.4	5.6 \pm 1.0	38 \pm 3.8	2.66 \pm 0.09	1.38 \pm 0.20	70 \pm 11.4	1.08 \pm 0.20

* p<0.05 vs Control; ** p<0.01 vs control.

Table 49. Findings of urinary analysis in the 26-week rat study

Sex	Group/PA21 dose (mg/kg/day)	Vol (ml)	pH	SG (g/L)	U-ca (mmol/L)	U-IP (μ mol/L)
Week 13 (n=15-21)						
Male	Control	7.7 \pm 2.3	7.7 \pm 0.51	1036 \pm 7.3	2.08 \pm 1.23	20 \pm 14.5
	200	8.1 \pm 2.9	8.0 \pm 0.40	1039 \pm 11.5	3.35 \pm 1.91	10.2 \pm 9.7
	750	8.1 \pm 1.7	8.4 \pm 0.32**	1039 \pm 3.7	6.68 \pm 4.44**	1.9 \pm 2.8**
	2500	5.1 \pm 1.7*	8.6 \pm 0.21**	1048 \pm 9.4**	22.2 \pm 6.6**	0.5 \pm 0.2**
Female	Control	4.5 \pm 1.8	6.3 \pm 0.30	1041 \pm 12.5	8.62 \pm 3.31	57.6 \pm 22.3
	200	5.2 \pm 2.2	6.7 \pm 0.60	1039 \pm 12.9	8.75 \pm 4.07	38.7 \pm 26.4
	750	5.3 \pm 1.7	7.3 \pm 0.72**	1038 \pm 12.5	8.22 \pm 4.40	19.5 \pm 18.8**
	2500	3.8 \pm 1.1	8.2 \pm 0.41**	1042 \pm 9.5	20.8 \pm 5.3**	0.4 \pm 0.3**
Week 26 (n=13-21)						
Male	Control	7.8 \pm 3.7	8.1 \pm 0.37	1034 \pm 10.2	2.08 \pm 1.23	14.3 \pm 10.9
	200	8.0 \pm 2.9	8.0 \pm 0.42	1033 \pm 7.6	1.44 \pm 0.93	31.4 \pm 15**
	750	7.4 \pm 2.1	8.1 \pm 0.39	1034 \pm 7.2	1.05 \pm 0.51	13.7 \pm 8.5
	2500	4.8 \pm 1.7**	8.5 \pm 0.51**	1044 \pm 9.5**	15.95 \pm 7.34**	0.4 \pm 0.4**
Female	Control	3.4 \pm 1.6	6.9 \pm 0.84	1037 \pm 7.1	7.82 \pm 3.5	54.6 \pm 23
	200	3.8 \pm 1.8	6.5 \pm 0.42	1035 \pm 5.8	6.31 \pm 2.6	62.5 \pm 24.7
	750	4.3 \pm 2.0	6.9 \pm 0.80	1036 \pm 8.4	5.79 \pm 3.1	37.8 \pm 35.3
	2500	3.4 \pm 1.6	8.1 \pm 0.76**	1039 \pm 9.4	12.1 \pm 5.2**	5.5 \pm 13.3**
Recovery week 6 (n=6)						
Male	Control	9.9 \pm 2.3	7.5 \pm 0.42	1030 \pm 5.6	1.13 \pm 0.66	35.7 \pm 7.8
	2500	10.4 \pm 3.3	7.0 \pm 0.83	1033 \pm 8.8	3.13 \pm 3.49	36.1 \pm 10.4
Female	Control	5.3 \pm 2.3	7.0 \pm 0.68	1038 \pm 16.2	5.11 \pm 2.03	64.0 \pm 42.2
	2500	5.3 \pm 3.1	6.3 \pm 0.29*	1036 \pm 7.4	6.43 \pm 3.97	68.8 \pm 16.7

* p<0.05 vs Control; ** p<0.01 vs control.

Table 50. Bone turnover markers in the 26-week rat study

Sex	Group/PA 21 dose (mg/kg/day)	Osteocalcin (ng/ml)			Deoxypyridinoline/creatinine (nM/mM)		
		Week 13	Week 26	Recovery	Week 13	Week 26	Recovery
Male	Control	15.85 \pm 4.10	13.36 \pm 4.20	18.66 \pm 10.26	89.7 \pm 25.0	40.9 \pm 11.3	23.5 \pm 9.6
	200	14.01 \pm 4.67	13.03 \pm 6.01		101.1 \pm 33.0	49.7 \pm 15.9	
	750	11.91 \pm 4.29*	10.54 \pm 3.01		128.4 \pm 32.3*	41.5 \pm 11.4	
	2500	20.25 \pm 3.26*	16.62 \pm 3.68*	15.95 \pm 6.25	198.4 \pm 57.0*	96.7 \pm 29.5*	31.1 \pm 7.5
Female	Control	16.31 \pm 3.17	12.11 \pm 2.96	15.07 \pm 3.94	53.0 \pm 14.0	17.8 \pm 6.7	11.2 \pm 4.7
	200	17.01 \pm 2.34	12.43 \pm 2.70		58.5 \pm 12.1	19.2 \pm 5.4	
	750	18.67 \pm 2.17	13.28 \pm 2.21		56.6 \pm 14.8	16.9 \pm 4.5	
	2500	21.52 \pm 3.38*	14.85 \pm 3.6*	12.03 \pm 4.62	92.3 \pm 34.5	30.4 \pm 9.4*	15.7 \pm 3.8

* p<0.05 vs Control.

Table 51. Serum vitamin levels in rats of the 26-week study*

PA21 Dose (g/kg/day)	Vitamin A (µmol/L)				Vitamin E (µmol/L)				Vitamin K1 (ng/L)			
	Week 26		Week R6		Week 26		Week R6		Week 26		Week R6	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
0	1.14 ±0.24	0.33 ±0.12	0.63 ±0.31	0.38 ±0.09	15.82 ±4.10	25.59 ±6.98	11.42 ±5.75	20.51 ±6.68	598 ±301	526 ±283	1124 ±341	543 ±89
0.2	1.26 ±0.41	0.32 ±0.11			15.32 ±4.87	22.20 ±5.03			589 ±358	384 ±163		
0.75	1.16 ±0.16	0.40 ±0.15			15.82 ±3.56	27.43 ±6.95			427 ±218	418 ±303		
2.5	1.36 ±0.35	0.39 ±0.16	1.06 ^a ±0.21	0.42 ±0.12	17.23 ±5.95	22.75 ±9.72	19.47 ^a ±2.72	23.15 ±5.11	288 ^b ±191	124 ^b ±102	893 ±340	495 ±336
PA21 Dose (g/kg/day)	25-hydroxyvitamin D (nmol/L)				1, 25-dihydroxyvitamin D (pmol/L)				R6 Recovery Week 6			
	Week 26		Week R6		Week 26		Week R6		a - p<0.05; b - p<0.01 vs control (0)			
	Males	Females	Males	Females	Males	Females	Males	Females	* Study validations (Study number WLY0013) indicated that these assays with the exception of 25-hydroxyvitamin D failed to meet acceptance criteria set for precision and accuracy. Thus, data here were just for reference			
0	92 ±31	67 ±31	27 ±8	27 ±11	16 ±12	10 ±5	15 ±14	8 ±5				
0.2	90 ±32	83 ±35			18 ±13	9 ±7						
0.75	95 ±33	85 ±37			43 ±44	11 ±7						
2.5	73 ±24	126 ^b ±42	22 ±12	23 ±9	359 ^b ±117	117 ^b ±74	11 ±9	7 ±6				

Table 52. Tissue iron content in the 26-week rat study

Sex	Group/PA 21 dose (mg/kg/day)	Liver (mg/kg)		Kidney (mg/kg)		Spleen (mg/kg)	
		Week 26	Recovery	Week 26	Recovery	Week 26	Recovery
Male	Control	174 ± 44	182 ± 39	132 ± 76	88 ± 14	1902±445	1962±642
	200	210 ± 44*		148 ± 97		2354±392*	
	750	268 ± 61**		132 ± 57		2698±775**	
	2500	477 ± 106**	415 ± 146**	132 ± 41	149 ± 78*	5937±1425**	5328±2056**
Female	Control	438 ± 93	429 ± 128	120 ± 35	118 ± 33	3186±898	2567±698
	200	515 ± 113*		117 ± 26		3248±630	
	750	610 ± 112**		143 ± 27*		3880±896	
	2500	781 ± 213**	565 ± 85	169 ± 25**	137 ± 20	4365±1216**	4391±1056**

* p<0.05 vs Control; ** p<0.01 vs control.

Table 53. Hematological findings in the 26-week rat study

Sex	Group/dose (mg/kg/day)	Hct (L/L)	Hb (g/dL)	RBC (x10 ¹² /L)	Retic (%)	MCH (pg)	MCHC (g/dL)	MCV (fL)	PT (sec)	APTT (sec)
Week 13 (n=14-15)										
Male	Control	0.441 ± 0.016	15.3 ± 0.7	8.63 ± 0.20	2.43 ± 0.43	17.7 ± 0.7	34.7 ± 0.66	51.1 ± 1.81	15.7±0.5	15.8±2.9
	200	0.438 ± 0.016	15.3 ± 0.6	8.41 ± 0.35	2.47 ± 0.43	18.3 ± 0.6*	35.1 ± 0.36*	52.1 ± 1.43	15.7±0.8	16.9±2.8
	750	0.438 ± 0.016	15.4 ± 0.5	8.39 ± 0.40	2.38 ± 0.61	18.4 ± 0.7**	35.1 ± 0.44*	52.3 ± 1.63*	15.9±0.8	17.3±2.6
	2500	0.468 ± 0.015**	16.4 ± 0.6**	8.61 ± 0.34	2.26 ± 0.67	19.1 ± 0.5**	35.1 ± 0.39*	54.3 ± 1.12**	16.6±0.5**	18.6±1.1*
Female	Control	0.409 ± 0.013	14.7 ± 0.5	7.67 ± 0.32	1.85 ± 0.42	19.2 ± 0.6	35.9 ± 0.5	53.4 ± 1.34	15.0±0.6	16.4±3.3
	200	0.419 ± 0.015*	15.0 ± 0.6	7.90 ± 0.31	1.64 ± 0.39	19.0 ± 0.4	35.7 ± 0.5	53.1 ± 0.80	15.8±0.5**	15.8±1.7
	750	0.422 ± 0.05*	14.9 ± 0.6	7.81 ± 0.26	1.84 ± 0.51	19.1 ± 0.5	35.4 ± 0.7*	54.1 ± 1.06*	16.0±0.5**	15.1±1.4
	2500	0.426 ± 0.013**	15.1 ± 0.5	7.91 ± 0.27*	1.65 ± 0.58	19.1 ± 0.5	35.4 ± 0.5*	53.9 ± 1.54	16.2±0.48**	15.5±2.1
Week 26 (n=14-21)										
Male	Control	0.424 ± 0.031	14.7 ± 1.2	8.87 ± 0.66	2.56 ± 0.70	16.6 ± 0.88	34.7 ± 0.75	47.9 ± 2.11	15.0±1.1	17.2±2.8
	200	0.423 ± 0.020	14.7 ± 0.6	8.58 ± 0.44	2.42 ± 0.45	17.2 ± 0.63*	34.8 ± 0.75	49.3 ± 2.03*	14.8±1.1	18.4±2.5
	750	0.438 ± 0.013	15.0 ± 0.4	8.81 ± 0.36	2.33 ± 0.63	17.1 ± 0.63*	34.3 ± 0.42	49.7 ± 1.41**	14.9±0.9	19.2±2.9*
	2500	0.460 ± 0.020**	15.8 ± 0.8**	8.92 ± 0.43	2.22 ± 0.61	17.7 ± 0.56**	34.3 ± 0.54	51.6 ± 1.42**	15.7±0.9*	19.1±1.9*
Female	Control	0.390 ± 0.014	14.2 ± 0.6	7.73 ± 0.33	1.74 ± 0.43	18.3 ± 0.60	36.3 ± 0.67	50.5 ± 1.31	15.5±0.5	17.6±1.4
	200	0.399 ± 0.021	14.2 ± 0.7	7.94 ± 0.40	1.57 ± 0.39	17.9 ± 0.34	35.6 ± 0.55*	50.2 ± 0.76	15.3±0.8	15.1±2.1**
	750	0.399 ± 0.014	14.2 ± 0.4	7.79 ± 0.34	1.53 ± 0.34	18.2 ± 0.50	35.6 ± 0.47*	51.3 ± 1.25	15.2±0.8	14.5±2.7**
	2500	0.421 ± 0.015**	14.9 ± 0.5**	8.25 ± 0.32**	1.51 ± 0.35	18.1 ± 0.63	35.4 ± 0.44*	51.1 ± 1.69	15.6±0.6	17.1±1.7
Recovery week 6 (n=6)										
Male	Control	0.448 ± 0.013	15.6 ± 0.4	9.05 ± 0.12	1.50 ± 0.16	17.2 ± 0.57	34.7 ± 0.21	49.5 ± 1.63	15.0±1.2	18.6±2.0
	2500	0.450 ± 0.025	15.8 ± 0.8	8.77 ± 0.57	1.87 ± 0.34	18.1 ± 0.50*	35.2 ± 0.47	51.3 ± 0.91*	15.3±1.1	17.7±2.0
Female	Control	0.421 ± 0.019	14.9 ± 0.7	7.98 ± 0.43	1.32 ± 0.21	18.7 ± 0.45	35.3 ± 0.23	52.8 ± 1.35	15.3±0.5	19.4±3.0
	2500	0.433 ± 0.019	15.3 ± 0.6	8.23 ± 0.38	1.33 ± 0.44	18.6 ± 0.69	35.3 ± 0.26	52.6 ± 1.85	15.1±0.5	19.6±2.9

* p<0.05 vs Control; ** p<0.01 vs control.

PA21 treatment did not cause any increases in the number of micronucleated reticulocytes or any decreases in the proportion of reticulocytes in week 4 or week 24. Thus, the peripheral blood micronucleus test was negative (see details under section 7).

Necropsy at the end of PA21 treatment revealed dark contents in the GI tract at all doses and brown staining of the tail at doses 750 and 2500 mg/kg/day. At 2500 mg/kg/day, two males had thickened urinary bladder containing calculi and ureter distension. Thickened and darkened cecum and/or colon were observed in both sexes at doses 750 and/or 2500 mg/kg/day at the end of treatment. After 6 weeks of recovery, stained tail and dark coloration of the colon were still present in half of males at high dose. Weights of kidney, thymus, and thyroids in males at 2500 mg/kg/day were slightly higher than controls at the end of treatment, which disappeared at the end of recovery, were not associated with histological changes, and were not of toxicological significance. Differences in liver and lung weight among groups were minimal and not of toxicological significance (Table 54).

Table 54. Findings in organ weights in the 26-week rat study

Sex	Group/PA21 dose (mg/kg/day)	Body weight (BW, g)	Kidneys (%BW)	Liver (%BW)	Thymus (%BW)	Thyroids + Paras (%BW)	Lung + Brochi (%BW)
Week 26 (n=14-15)							
Male	Control	661 ± 60.1	0.51±0.03	3.08 ± 0.31	0.021±0.007	0.0030±0.0004	0.27 ± 0.02
	200	645 ± 62.7	0.52±0.06	3.00 ± 0.39	0.024±0.007	0.0030±0.0007	0.28 ± 0.03
	750	658 ± 62.8	0.54±0.04*	2.79 ± 0.33*	0.022±0.005	0.0032±0.0006	0.28 ± 0.02
	2500	578 ± 72.6**	0.61±0.10**	2.94 ± 0.38	0.028±0.009*	0.0038±0.0009**	0.30 ± 0.02**
Female	Control	322 ± 28.5	0.60 ± 0.04	3.01 ± 0.22	0.049±0.015	0.0050±0.0008	0.36 ± 0.03
	200	326 ± 20.4	0.60 ± 0.04	2.97 ± 0.24	0.051±0.009	0.0052±0.0013	0.39 ± 0.03**
	750	330 ± 32.4	0.60 ± 0.05	3.01 ± 0.31	0.059±0.021	0.0051±0.0008	0.38 ± 0.03
	2500	310 ± 23.6	0.55 ± 0.06*	2.77 ± 0.14**	0.054±0.012	0.0049±0.0009	0.40 ± 0.03**
Recovery week 6 (n=5-6)							
Male	Control	680 ± 88.5	0.54 ± 0.08	2.91 ± 0.37	0.018±0.004	0.0038±0.0011	0.26 ± 0.03
	2500	681 ± 92.0	0.54 ± 0.07	2.93 ± 0.39	0.018±0.004	0.0035±0.0013	0.28 ± 0.03
Female	Control	345 ± 16.1	0.56 ± 0.03	3.11 ± 0.29	0.033 ± 0.015	0.0040±0.0004	0.37 ± 0.02
	2500	337 ± 38.3	0.56 ± 0.05	2.98 ± 0.27	0.043 ± 0.005	0.0047±0.0014	0.38 ± 0.04

* p<0.05 vs Control; ** p<0.01 vs control.

Histopathological changes related to PA21 treatment occurred in the colon, cecum, rectum, kidneys, urinary bladder, ureters, and spleen (Table 55). Mucosal hyperplasia with or without submucosal inflammation/edema was seen in the cecum, colon, and/or rectum of one male given 750 mg/kg/day and of 3 males given 2500 mg/kg/day. Dose-dependent increases in incidence and/or degree of positive Perl's staining (for iron) and/or pigmented macrophages were seen in the gastrointestinal tract, liver, mesenteric lymph nodes of both males and females. At 2500 mg/kg/day, there were kidney pelvic dilatation in 3 males (also 3 males at 200 mg/kg/day), bladder transitional cell hyperplasia in 3 males, and ureter mucosal hyperplasia in 2 males. There were increased degree of hemosiderosis and positive Perl's staining in the spleens of both sexes given 2500 mg/kg/day (Table 55).

After 6 weeks of recovery, there were mucosal hyperplasia in the colon of one male, and pigmented macrophages and positive Perl's staining in the colon of 5 males and 5 females previously given 2500 mg/kg/day. There were also increased degree of spleen hemosiderosis and positive Perl's staining, and increased GI tract positive Perl's staining in all animals previously given 2500 mg/kg/day (Table 55).

Table 55. Microscopic findings in the 26-week rat study

Organ	Findings	Male (PA21 dose: mg/kg/day)				Female (PA21 dose: mg/kg/day)			
		0	200	750	2500	0	200	750	2500
Week 26 (n=13-15)									
Duodenum	Positive Perl's stain	1	1	12**	15**	6	10	10	14**
Jejunum	Positive Perl's stain	0	1	0	12**	2	1	3	6
Ileum	Positive Perl's stain	0	0	1	2	0	0	0	1
Cecum	Mucosal hyperplasia, Submucosal inflammation/edema	0	0	0	3	0	0	0	0
	Positive Perl's stain	0	0	0	7**	0	0	2	6**
Colon	Mucosal hyperplasia	0	0	1	1	0	0	0	0
	Pigmented macrophages	0	1	3	15**	0	0	8**	12**
	Positive Perl's stain	0	11**	14**	15**	2	9*	15**	14**
Rectum	Mucosal hyperplasia	0	0	0	1	0	0	0	0
	Positive Perl's stain	0	0	0	15**	0	1	4*	13**
Liver	Portal inflammatory cells	2	3	3	6	3	3	4	6
	Positive Perl's stain	7	13*	14*	15**	8	9	13	14**
Mesenteric lymph node	Sinus erythrocytosis /erythrophagocytosis	2	2	2	3	0	0	0	1
	Positive Perl's stain	10	12	14	15*	13	14	14	14
Mandibular lymph node	Positive Perl's stain	14	15	14	15	15	15	15	13
Spleen	Hemosiderosis, minimal-slight	15	15	14	8	13	15	14	8
	Hemosiderosis, moderate-marked	0	0	0	7	2	0	1	6
	Positive Perl's stain, minimal-slight	15	12	10	4	13	7	3	5
	Positive Perl's stain, moderate-marked	0	3	5	11	2	8	12	10
Kidney	Interstitial lymphoid cells	1	3	1	2	1	3	3	4
	Pelvic dilatation	0	3	0	3	0	0	0	0
	Positive Perl's stain	5	8	6	7	5	5	4	9
Urinary Bladder	Refluxed seminal colloid plug	0	0	0	2	0	-	-	0
	Transitional cell hyperplasia	0	0	0	3	0	-	-	0
Ureters	Mucosal hyperplasia	0	-	-	2	0	-	-	0
Pancreas	Lymphoid infiltration	0	-	-	4*	1	-	-	2
Prostate	Inflammation	0	-	0	2	-	-	-	-
	Lymphoid aggregates	1	-	0	3	-	-	-	-
Recovery week 6 (n=6)									
Spleen	Hemosiderosis, minimal-slight	6	-	-	4	6	-	-	4
	Hemosiderosis, moderate	0	-	-	2	0	-	-	2
	Positive Perl's stain, minimal-slight	6	-	-	2	3	-	-	2
	Positive Perl's stain, moderate-marked	0	-	-	4	3	-	-	4
Liver	Portal inflammatory cells	2	-	-	1	0	-	-	2
	Positive Perl's stain	3	-	-	6	5	-	-	5
Mesenteric lymph node	Positive Perl's stain	2	-	-	6*	6	-	-	6
Mandibular lymph node	Positive Perl's stain	6	-	-	6	6	-	-	6
Mammary	Glandular pigment	0	-	-	0	0	-	-	2
Duodenum	Positive Perl's stain	0	-	-	6*	1	-	-	6*
Jejunum	Positive Perl's stain	0	-	-	2	0	-	-	0
Ileum	Positive Perl's stain	0	-	-	0	0	-	-	1
Colon	Mucosal hyperplasia	0	-	-	1	0	-	-	0
	Pigmented macrophages	0	-	-	5**	0	-	-	5**
	Positive Perl's stain	0	-	-	5**	0	-	-	4**
Rectum	Positive Perl's stain	0	-	-	3	0	-	-	3
Kidneys	Positive Perl's stain	1	-	-	2	4	-	-	6

* p<0.05 vs control. ** p<0.01 vs control. - Not examined.

6.2.2 PA21 and PA21-2 - Comparative toxicity study by dietary administration to CD rats for 4 weeks

Study no.: VFR 0117
Study report location: (b) (4)
Conducting laboratory and location: (b) (4)
Date of study initiation: March 11, 2008
GLP compliance: Yes
QA statement: Yes
Drug/lot #/% purity: PA21/ 010307J1/95.8 (b) (4)
Deviation from study protocol: No impact to the results

Key Study Findings

This study compared a mixture of iron (III)-oxyhydroxide, (b) (4) and sucrose (PA21) to a mixture of iron (III)-oxyhydroxide, (b) (4) and sucrose (PA21-2). There were no deaths. Dark feces, brown staining of the tail and other body parts, dark contents in the GI tract, and darkened rectum were observed in all treated groups with higher incidences in the groups of 4000 mg/kg/day PA21 or PA21-2. These findings were considered to be due the color of the test materials.

Body weight gains were significantly less in males receiving either PA21 or PA21-2 at 4000 mg/kg/day, and in females receiving PA21-2 at 4000 mg/kg/day, associated with slight reduction (3-7%) of food consumption.

Comparing with the controls, small changes in clinical pathology included slightly higher levels of plasma alkaline phosphatase activities, urea, and calcium, slightly lower plasma potassium; darker urine color, higher urine ketones, lower urinary volumes associated with higher specific gravity, higher urinary pH, and slightly higher levels of urine calcium, sodium and potassium, and negligible levels of urine phosphorus in males and females receiving 4000 mg/kg/day of either test material. These findings indicated a slightly elevated bone turnover and renal function impairment. Urinary phosphorus excretion was also decreased in the low dose groups of PA21 and PA21-2.

There were higher plasma iron levels, higher iron contents in liver and spleen (trended high in kidney), and higher incidences of positive Perls' staining (iron pigment) in the liver, mesenteric lymph node, and in the small and large intestines in high dose groups of PA21 and PA21-2. Similar changes at lower incidence or low extent were also seen at the dose 1000 mg/kg/day. These findings were attributed to GI iron uptake from the test materials.

At PA21 and PA21-2 4000 mg/kg/day, epithelial hyperplasia of the large intestine was seen mostly in male groups (one incidence in PA21-treated females).

There were no apparent difference between PA21 and PA21-2. NOAELs were 1000 mg/kg/day for both PA21 and PA21-2 in this study.

Methods

Crl:CD (SD) rats, initial age of ~5 weeks (118-145 g for males & 108-135 g for females), were on diet containing PA21 at 0, 1000 or 4000 mg/kg/day or PA21-2 at 1000 or 4000 mg/kg/day (group 1, 2, 3, 4, and 5, respectively) for 4 weeks (n = 10/sex/group). Doses selected in this study were based on a 4-week toxicity study in rats performed in the same facility (VFR0075). In the 4-week study (VFR0075), PA21 doses at 500, 1000, 2500, and 4000 mg/kg/day were well tolerated and did not result in any mortality. The PA21-related findings included less body weight gain (~15 and ~30% at doses 2500 and 4000 mg/kg/day, respectively) and mucosal hyperplasia in the rectum at 4000 mg/kg/day. Thus, the dose 4000 mg/kg/day was selected as the high dose in the current study. Samples of the drug-diet formulations were analyzed at weeks 1 and 4 of treatment to confirm the concentrations of PA21 and PA21-2 in the treated diets and the absence in the control diet.

During the study, rats were inspected visually at least twice daily for clinical signs and morbidity/death. Physical examination was weekly performed on each animal. Body weight was recorded at week -1, study initiation, weekly thereafter, and before necropsy. Mean weekly food consumption per animal was assessed at week -1 and weekly thereafter. A binocular ophthalmoscopy was performed on all animals prior to treatment, and on control and high dose animals during week 4. Blood samples were collected from all surviving animals during week 4 after overnight fast, for determining hematology, plasma chemistry (Table 45), and serum levels of vitamins A, D (25-hydroxy vitamin D, and 1,25-dihydroxy vitamin D), E, and K. Overnight urine samples were collected from all animals during week 4 for urinalysis (Table 46).

On completion of the 4 weeks treatment, all animals were killed and subjected to a detailed necropsy. Organs/tissues listed in Table 56 were weighed, collected, histologically processed, and microscopically examined. Samples from liver (residue of left lobe), kidney (poles from left kidney), and spleen (poles) from all animals were weighed, snap-frozen in liquid nitrogen for determination of iron content. Samples of any macroscopic abnormal tissues were also retained and processed for histopathological examination. No peer review was mentioned in this report.

Table 56. List of organs and processes for the 4-week rat study

Organs/tissues	Organ weight	Histologically processed	Light Microscopy ^b	Organs/tissues	Organ weight	Histologically processed	Light Microscopy ^b
Adrenals	√	√	√	Pancreas		√	√
Aorta - thoracic		√	√	Peyer's patches		√	√
Brain	√	√	√	Pituitary	√	√	√
Caecum		√	√ ^a	Prostate	√	√	√
Colon		√	√ ^a	Rectum		√	√ ^a
Duodenum		√	√ ^a	Salivary glands			
Epididymides	√	√	√	- submandibular	√ ^{**}	√+	√
Eyes		√	√	- parotid		√+	√
Femurs+		√+	√	- sublingual	√ ^{**}	√+	√
Harderian glands		√	√	Sciatic nerves		√+	√
Head#		√#		Seminal vesicles	√	√	√
Heart	√	√	√	Skeletal muscle		√+	√
Ileum		√	√ ^a	Skin		√	√
Jejunum		√	√ ^a	Spinal cord		√	√
Kidneys	√	√	√ ^a	Spleen	√	√	√ ^a
Lachrymal glands		√	√	Sternum		√	√
Larynx		√	√	Stomach		√	√ ^a
Liver	√	√	√ ^a	Testes	√	√	√
Lungs	√	√	√	Thymus	√	√	√
Lymph nodes				Thyroid with parathyroids	√ [*]	√	√
- mandibular		√	√ ^a	Tongue		√	√
- mesenteric		√	√ ^a	Trachea		√	√
- left axillary		√	√	Urinary bladder		√	√ ^a
Mammary area - caudal		√	√	Ureters		√	√
Oesophagus		√	√	Uterus and cervix	√	√	√
Optic nerves		√	√	Vagina		√	√
Ovaries		√	√				

+ Only processed for examination. # Not processed for examination. * Weighed after partial fixation. ** Weighed together as salivary glands. √^a Examined in all animals. ^b Examined in control and high dose groups except √^a

Results

The overall achieved doses at 1000 and 4000 mg/kg/day of PA21 were 1005 and 4143 mg/kg/day for males and 1079 and 4263 mg/kg/day for females, respectively. For PA21-2 the overall achieved doses at 1000 and 4000 mg/kg/day were 1009 and 4064 mg/kg/day for males and 1048 and 4200 mg/kg/day for females, respectively.

There were no deaths. There were no treatment-related ophthalmic findings. Signs related to treatment included dark feces, brown staining of the tail and the upper dorsal thorax in males and females treated with PA21 and PA21-2 with higher incidences at the high doses (Table 57). The brown staining was due to the brown color of the test material and/or its iron content, and was similar between PA21 and PA21-2 treatment. Body weight gains over the treatment period were significantly less in males receiving either PA21 or PA21-2 at 4000 mg/kg/day, and in females receiving PA21-2 at 4000

mg/kg/day (Figure 10). Food consumption was slightly reduced (3-7%) in males and females receiving 4000 mg/kg/day of each test material (Table 58).

Table 57. Summary of clinical signs during the 4-week rat study

Category	Observation	Number of animals affected																			
		Group/sex: 1M		2M		3M		4M		5M		1F		2F		3F		4F		5F	
		Number in group: 10																			
Behaviour	Irritable			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Coat	Hair loss, Forelimbs			0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	
	Hair loss, Hindlimb (Right)			0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Skin	Encrustation, Upper Ventral Thorax			0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	
Staining	Abnormal Colour, Brown, Forelimbs			0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
	Abnormal Colour, Brown, Head			0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	
	Abnormal Colour, Brown, Tail			0	0	9	10	7	0	0	0	7	0	10	0	0	0	0	0	0	
	Abnormal Colour, Brown, Upper Dorsal Thorax			0	1	0	0	2	1	2	6	2	3	0	0	0	0	0	0	0	

Groups 1, 2, 3, 4, and 5 were control, PA21 1000, PA21 4000, PA21-1 1000, and PA21-2 4000 mg/kg/day, respectively.

Figure 10. Rat body weight during the 4-week rat study

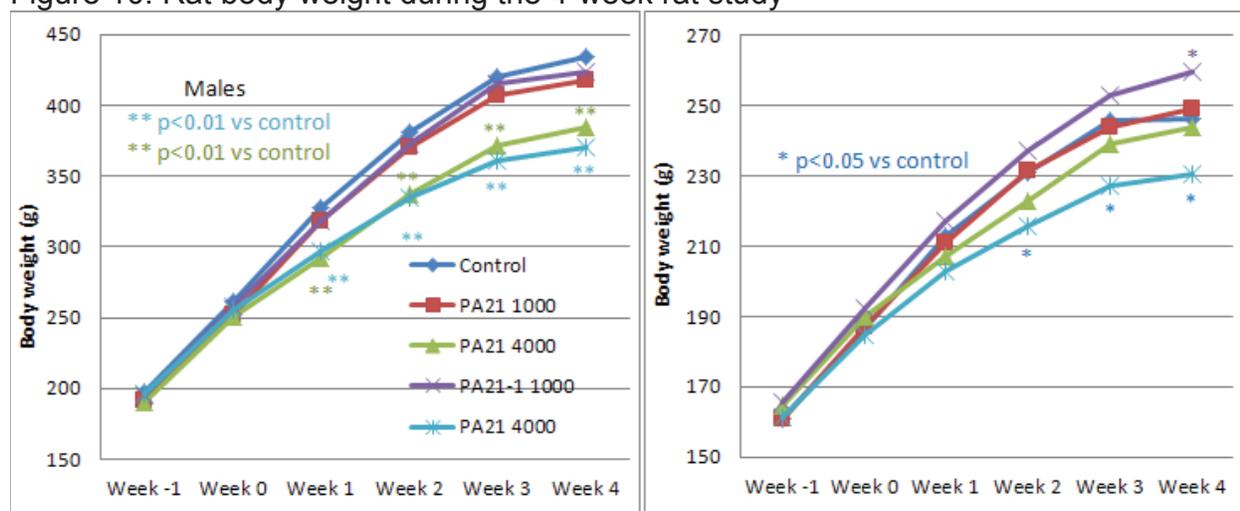


Table 58. Food consumption during the 4-week rat study

Group mg/kg/day	Sex		Week -1	Week 1	Week 2	Week 3	Week 4	Weekly Mean	As % of Control
Control	Male	Mean	212	222	227	226	185	215	-
		SD	6.4	3.6	7.1	14.2	5.8		
PA21 1000		Mean	205	225	222	221	181	212	99
		SD	4.1	3.7	5.7	0.6	0.1		
PA21 4000		Mean	195	207	218	221	191	209	97
		SD	2.8	3.5	3.7	8.1	3.8		
PA21-2 1000		Mean	208	222	226	223	187	215	100
		SD	2.7		9.0	3.3	1.1		
PA21-2 4000		Mean	198	206	204	210	186	202	94
		SD	5.5	11.9	15.9	7.4	3.7		
Control	Female	Mean	138	141	142	172	147	151	-
		SD	2.9	10.5	1.7	12.2	19.2		
PA21 1000		Mean	143	146	144	167	142	150	99
		SD	8.7	6.0	2.0	0.2	14.7		
PA21 4000		Mean	138	141	149	159	135	146	97
		SD	2.4	2.4	1.0	1.4	0.1		
PA21-2 1000		Mean	141	144	139	168	137	147	97
		SD	6.7	2.2	5.2	0.0	5.1		
PA21-2 4000		Mean	147	140	137	154	130	140	93
		SD	4.5	8.7	6.6	2.4	1.1		

There were minor differences of some hematological parameters between the control and treatment groups (Table 59). However, the differences did not occur in both sexes, were within the physiological ranges, and are of no toxicological significance. Comparing with the controls, plasma alkaline phosphatase activities and urea were slightly higher in males and females receiving 4000 mg/kg/day of either test material. Plasma iron levels in males receiving PA21 4000 mg/kg/day or PA21-2 4000 mg/kg/day were higher than control. Compared with the controls, small differences in plasma electrolyte levels included lower plasma potassium and higher plasma calcium in males receiving 4000 mg/kg/day of either PA21 or PA21-2. The magnitude of the changes in plasma chemistry was similar for both test materials. All other changes in plasma chemistry and serum vitamins were either minor, or lacked dose-relationship, or not consistent, and were not of toxicological or biological importance (Table 60).

Table 59. Hematology findings in the 4-week rat study

Group mg/kg/day	Sex		Hct L/L	Hb g/dL	RBC $\times 10^{12}/L$	Retic %	MCHC g/dL	MCV fL	WBC $\times 10^9/L$	N $\times 10^9/L$	L $\times 10^9/L$	B $\times 10^9/L$	M $\times 10^9/L$	PT sec	APTT sec
Control		Mean	0.439	15.6	7.65	2.52	35.5	57.3	10.94	1.16	9.13	0.05	0.37	15.1	17.2
		SD	0.0222	0.84	0.419	0.563	0.61	1.26	1.415	0.362	1.304	0.016	0.097	0.69	1.20
PA21 1000		Mean	0.438	15.5	7.72	3.00	35.4	56.9	11.99	1.22	10.09	0.07	0.35	15.1	16.1
		SD	0.0202	0.79	0.552	0.713	0.79	1.66	6.667	0.473	6.065	0.089	0.105	0.76	2.71
PA21 4000	Male	Mean	0.451	16.0	7.87	2.51	35.5	57.4	15.89 b	2.27 a	12.68 b	0.09 a	0.56 a	15.7 a	16.9
		SD	0.0158	0.57	0.292	0.375	0.74	0.92	4.436	1.569	3.394	0.044	0.204	0.48	2.48
PA21-2 1000		Mean	0.436	15.5	7.73	3.01	35.5	56.5	13.79 a	1.59	11.46 a	0.09	0.39	15.4	16.0
		SD	0.0110	0.45	0.319	0.663	0.68	1.46	3.524	0.797	2.944	0.068	0.149	0.81	1.96
PA21-2 4000		Mean	0.457 a	16.5 a	8.06 a	2.44	36.1 a	56.7	14.26 b	1.43	12.02 b	0.08	0.45	16.4 b	16.6
		SD	0.0165	0.65	0.409	0.470	0.51	1.47	3.100	0.582	2.703	0.045	0.149	0.42	1.43
Control		Mean	0.412	14.8	7.56	2.65	35.9	54.5	12.25	1.41	10.16	0.06	0.38	15.2	14.8
		SD	0.0192	0.76	0.412	0.680	0.44	1.08	1.424	1.006	1.645	0.022	0.133	0.57	1.85
PA21 1000		Mean	0.418	15.0	7.80	2.39	35.9	53.5 a	10.40	0.82	8.98	0.04	0.35	14.8	14.1
		SD	0.0161	0.55	0.256	0.629	0.46	0.66	2.799	0.301	2.646	0.021	0.118	0.45	2.27
PA21 4000	Female	Mean	0.410	14.9	7.60	2.12 a	36.3	54.0	10.85	1.33	8.95	0.03 b	0.29	15.3	18.2 a
		SD	0.0153	0.54	0.306	0.281	0.70	1.41	2.093	1.153	1.936	0.009	0.113	0.70	3.62
PA21-2 1000		Mean	0.408	14.8	7.58	2.61	36.2	53.8	10.91	0.90	9.51	0.04	0.25 a	14.7	17.6 b
		SD	0.0152	0.59	0.272	0.527	0.66	0.99	3.830	0.193	3.612	0.029	0.134	1.52	1.24
PA21-2 4000		Mean	0.412	15.1	7.64	2.02 a	36.7 b	54.0	10.44	0.99	9.01	0.05	0.18 b	15.2	17.3 b
		SD	0.0254	0.93	0.390	0.609	0.44	1.87	3.547	0.365	3.373	0.047	0.146	0.84	1.85

a p<0.05 vs control; b p<0.01 vs control

Table 60. Plasma chemistry findings in the 4-week rat study

Group mg/kg/day	Sex		ALP U/L	Bili $\mu\text{mol/L}$	Urea mmol/L	Creat $\mu\text{mol/L}$	Chol	Trig	K	Ca	Phos	Fe	FeBC
							mmol/L					$\mu\text{mol/L}$	
Control		Mean	159	1	4.52	30	1.86	0.87	4.5	2.73	2.42	35	97.3
		SD	34.7	0.5	0.502	1.8	0.386	0.173	0.42	0.099	0.114	10.0	10.21
PA21 1000		Mean	175	2 a	5.01	30	1.74	0.79	4.4	2.68	2.83 b	35	93.0
		SD	28.4	0.3	0.451	2.3	0.353	0.359	0.43	0.097	0.154	7.9	10.23
PA21 4000	Male	Mean	232 b	2 a	6.24 b	32 b	1.96	0.74	4.1 b	2.92 b	2.44	65 b	86.8
		SD	42.3	0.5	1.029	2.7	0.237	0.135	0.23	0.070	0.193	8.4	10.57
PA21-2 1000		Mean	181	2	5.19 a	31	1.84	0.92	4.6	2.70	2.77 b	34	110.0 a
		SD	38.7	0.5	0.732	2.3	0.311	0.258	0.46	0.057	0.198	5.9	10.35
PA21-2 4000		Mean	263 b	2 b	5.43 b	30	2.21 a	1.12 a	3.9 b	3.00 b	2.26 a	68 b	105.4 a
		SD	66.5	0.6	0.687	2.6	0.276	0.384	0.29	0.136	0.177	9.8	17.87
Control		Mean	108	2	6.02	35	2.07	0.56	4.2	2.77	1.97	67	96.3
		SD	15.4	0.8	1.127	3.4	0.577	0.170	0.24	0.076	0.159	10.1	7.87
PA21 1000		Mean	126	2	6.22	35	1.93	0.51	4.0	2.73	2.11	72	100.4
		SD	33.8	0.5	1.099	3.1	0.209	0.126	0.29	0.062	0.176	12.5	12.66
PA21 4000	Female	Mean	158 b	2	6.42	34	1.95	0.59	4.0	2.83	2.03	77 a	88.7
		SD	48.5	0.6	0.856	4.2	0.363	0.171	0.18	0.064	0.242	5.0	8.06
PA21-2 1000		Mean	135	2	6.14	36	2.12	0.62	4.2	2.70	2.15	75	96.6
		SD	35.2	0.3	0.753	3.5	0.381	0.168	0.48	0.069	0.217	11.2	11.95
PA21-2 4000		Mean	152 b	2	7.17 a	44 b	1.99	0.63	4.0	2.75 a	1.96	71	92.6
		SD	37.0	0.0	1.937	21.6	0.318	0.329	0.39	0.112	0.353	7.1	12.42

a p<0.05 vs control; b p<0.01 vs control

Table 61. Serum vitamins in rats at the end of 4-week study*

Dose (g/kg/day)	Vitamin A (µmol/L)		Vitamin E (µmol/L)		Vitamin K1 (ng/L)	
	Males	Females	Males	Females	Males	Females
0	2.34 ±0.89	0.84 ±0.18	18.75 ±4.66	23.38 ±6.81	552 ±244	262 ±201
1 (PA21)	2.34 ±0.69	1.01 ±0.24	19.41 ±5.22	25.46 ±3.78	636 ±288	189 ±90
1 (PA21-2)	1.83 ±0.61	0.79 ±0.14	15.82 ±2.67	22.13 ±3.22	763 ±365	287 ±212
4 (PA21)	1.87 ±0.79	0.78 ±0.12	15.02 ±4.56	19.78 ±2.87	713 ±191	82 ^a ±83
4 (PA21-2)	1.56 ^a ±0.26	0.68 ^a ±0.08	15.78 ±2.46	21.16 ±3.57	482 ±279	311 ±304

Dose (g/kg/day)	25-hydroxyvitamin D (nmol/L)		1, 25-dihydroxyvitamin D (pmol/L)	
	Males	Females	Males	Females
0	64 ±21	80 ±26	246 ±97	139 ±67
1 (PA21)	64 ±11	68 ±18	244 ±45	121 ±21
1 (PA21-2)	57 ±12	72 ±18	279 ±69	132 ±49
4 (PA21)	40 ^b ±9	60 ^b ±6	620 ^b ±145	402 ^b ±134
4 (PA21-2)	41 ^b ±7	59 ^a ±18	975 ^b ±215	400 ^b ±114

a - p<0.05; b - p<0.01 vs control (0)

* Study validations (Study number WLY0013) indicated that these assays with the exception of 25-hydroxyvitamin D failed to meet acceptance criteria set for precision and accuracy. Thus, data here were just for reference

The urine color from 3 males receiving PA21 4000 mg/kg/day and 3 males receiving PA21-2 4000 mg/kg/day appeared deeper than controls. Ketones were more prevalent in samples obtained from high dose males than in the controls (Table 62). Compared to the controls, lower urinary volumes associated with higher specific gravity occurred in all treated males and females with the exception of females receiving 1000 mg/kg/day PA21 (Table 63). Urinary pH was higher than controls in all groups of treated animals except males receiving PA21-2 at 1000 mg/kg/day. Urinary phosphorus excretion was decreased in all treated groups and was reduced to negligible levels in animals receiving 4000 mg/kg/day of either test material. Urinary calcium was increased in all treated animals, and was markedly high in those receiving the highest dose of either PA21 or PA21-2. There were slightly higher urinary sodium and potassium excretion and slight lower urinary chloride excretion mostly in males receiving PA21 or PA21-2 4000 mg/kg/day. The urinary protein was reduced slightly in all treated female groups except those receiving PA21-2 at 1000 mg/kg/day, and was not of toxicological significance. These findings in urinalysis were mostly due to a slightly elevated bone turnover in normal animals by PA21 so as to maintain serum phosphorus levels while GI phosphate uptake was reduced. In general, there was no difference in the extent of these responses in animals given either PA21 or PA21-2 (Table 63).

Table 62. Urine ketones in rats at the end of 4-week study

Group/sex	1M	2M	3M	4M	5M
	PA21		PA21-2		
Dose (mg/kg/day)	0	1000	4000	1000	4000
No. of animals examined	10	10	10	10	10
Trace	4	1	0	1	2
1+	5	8	5	9	5
2+	0	1	5	0	3

Table 63. Findings in urinalysis at the end of the 4-week rat study

Group mg/kg/day	Sex		Vol mL	pH	SG g/L	Prot g/L	U-Na mmol/L	U-K mmol/L	U-Cl mmol/L	U-Ca mmol/L	U-IP mmol/L
Control	Male	Mean	9.1	7.8	1028	0.69	52.7	190.7	61.3	1.01	42.34
		SD	2.01	0.65	4.5	0.241	12.53	37.61	16.35	0.758	18.285
PA21 1000		Mean	6.1 b	8.3 a	1034	0.64	52.9	223.2	59.2	3.10 b	13.35 b
		SD	1.69	0.42	5.8	0.243	11.33	63.86	17.13	2.397	8.809
PA21 4000		Mean	3.7 b	8.6 b	1053 b	0.27 b	98.8 b	259.4 a	35.0 b	26.38 b	0.35 b
		SD	1.45	0.23	14.3	0.053	35.78	75.24	8.97	11.186	0.227
PA21-2 1000		Mean	5.9 b	8.1	1035	0.72	53.3	233.1	58.3	1.99	28.25 a
		SD	1.65	0.50	6.0	0.207	22.37	50.46	19.68	1.714	21.032
PA21-2 4000		Mean	3.4 b	8.4 a	1049 b	0.29 b	69.8 a	252.4	41.1 a	32.73 b	0.37 b
		SD	1.48	0.38	18.8	0.074	23.21	97.85	24.61	9.327	0.226
Control	Female	Mean	4.4	6.7	1040	0.17	68.6	211.9	62.4	1.58	73.23
		SD	1.25	0.30	10.0	0.021	29.11	53.59	23.54	0.520	26.989
PA21 1000		Mean	4.8	7.5 b	1033	0.13 b	53.8	183.5	59.8	2.58	39.75 a
		SD	1.46	0.71	4.8	0.034	17.91	32.94	20.72	2.452	19.780
PA21 4000		Mean	2.7 b	7.8 b	1051	0.13 b	79.7	192.0	50.6	16.86 b	0.96 b
		SD	1.36	0.75	17.2	0.029	25.47	37.37	23.58	10.555	0.927
PA21-2 1000		Mean	3.1 a	7.2 a	1044	0.15	77.4	207.1	62.6	1.62	50.04
		SD	1.12	0.61	13.0	0.033	33.85	32.36	20.29	0.933	12.357
PA21-2 4000		Mean	2.5 b	8.7 b	1041	0.11 b	78.4	187.3	36.3 a	17.75 b	0.61 b
		SD	0.89	0.27	4.9	0.020	36.41	61.72	13.50	8.420	0.668

a p<0.05 vs control; b p<0.01 vs control

Significantly higher iron content of the livers was observed in both sexes receiving 4000 mg/kg/day of either test material (Table 64). Levels of iron in the spleen were elevated in all treated male groups and in females receiving 4000 mg/kg/day of PA21. The level of iron in the kidneys was also slightly increased in treated female groups. The extent of the increases in tissue iron content was similar for both test materials at equivalent dosages (Table 64).

Table 64. Tissue iron content (mg/kg) in rats at the end of 4-week study

Dose mg/ kg/ day	Male			Female		
	Liver	Kidney	Spleen	Liver	Kidney	Spleen
Control	134 ± 31	74 ± 16	441 ± 89	392 ± 83	75 ± 15	914 ± 270
PA21 1000	155 ± 42	98 ± 46	761 ± 272**	462 ± 127	87 ± 15	1026 ± 304
PA21 4000	296 ± 78**	86 ± 25	887 ± 239**	883 ± 239**	99 ± 11**	1378 ± 284**
PA21-2 1000	155 ± 26	65 ± 8	752 ± 372**	500 ± 152	92 ± 25*	1101 ± 280
PA21-2 4000	331 ± 81**	123 ± 98	1283 ± 384**	850 ± 368**	96 ± 16*	1107 ± 283

* p<0.05 vs control; ** p<0.01 vs control

Macroscopic examination revealed dark contents in the gastro-intestinal tract at all doses in males and females given PA21 or PA21-2. Darkened colon was reported in all treated groups and darkened rectum was mostly seen in animals given 4000 mg/kg/day of either PA21 or PA21-2. These findings were considered to be due the color of the test materials. At 4000 mg/kg/day, PA21 and/or PA21-2 treatment were associated with slightly higher organ weights of spleen, liver, and salivary glands in males only (Table 65). These slight changes in organ weights were not associated with histological abnormality and were not of toxicologically significant.

Table 65. Findings in organ weight (g, adjusted with body weight) in the 4-week rat study

Dose mg/ kg/ day	Sex	Body weight		Liver		Salivary glands		Spleen	
		Mean	SD	raw	adjusted	raw	adjusted	raw	adjusted
Control	Male	433	27	16.2	14.9	0.67	0.64	0.68	0.62
PA21 1000		417	24	15.8	15.2	0.7	0.69	0.69	0.67
PA21 4000		384**	21	14.5	15.5	0.7	0.72*	0.74	0.79**
PA21-2 1000		423	10	15.8	14.9	0.71	0.69	0.74	0.71
PA21-2 4000		370**	32	14.5	16.2*	0.66	0.71	0.67	0.74*
Control	Female	249	11	7.8	7.8	0.45	0.45	0.55	0.55
PA21 1000		253	13	8.6	8.4	0.45	0.44	0.55	0.54
PA21 4000		246	12	7.7	7.8	0.44	0.45	0.54	0.55
PA21-2 1000		263	11	8.4	7.7	0.45	0.43	0.57	0.52
PA21-2 4000		232**	18	7.5	8.3	0.44	0.46	0.5	0.57

* p<0.05 vs control; ** p<0.01 vs control

Histopathological changes related to treatment with either PA21 or PA21-2 occurred in the liver, mesenteric lymph node, and in the small and large intestines. The findings were epithelial hyperplasia of the large intestine at 4000 mg/kg/day and higher incidences of positive Perls' staining (iron pigment) in the liver, mesenteric lymph node, and in the small and large intestines (Table 66). In the majority of cases, there were no clear differences of incidence or severity between PA21 and PA21-2 treated groups. Other changes were sparse and not dose-related, and were not of toxicologically significant.

Table 66. Histological findings in the 4-week rat study

Tissue and Finding	Male groups (n=10/group)					Female groups (n=10/group)				
	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Liver Perls										
Positive Staining of Kupffer Cells/Macrophages	1	2	8**	3	10**	2	7*	9**	8*	10**
Positive Staining of Periportal Hepatocyte	0	0	4*	0	6**	1	8**	10**	6*	10**
Kidney Perls										
Positive Staining of Tubules	0	1	2	0	2	0	0	0	0	0
Spleen Perls										
Positive Staining of Macrophages (Red Pulp)	10	10	10	10	10	10	10	10	10	10
Positive Staining of Macrophages in White Pulp	10	10	10	10	10	10	10	10	10	10
Mesenteric lymph node Perls										
Positive Staining of Macrophages in Follicles	1	0	2	0	1	1	2	5	1	2
Positive Staining of Medulla	2	4	6	1	5	3	6	8*	2	5
Positive Staining of Paracortex	0	2	7**	4*	10**	0	8**	5*	6**	9**
Duodenum Perls										
Positive Staining of Luminal Contents	0	2	3	2	3	0	2	3	5*	4*
Positive Staining of Macrophages in Lamina Propria Mucosa	2	8*	9**	7*	10**	1	8**	10**	9**	8**
Ileum Perls										
Positive Staining of Luminal Contents	0	1	0	1	7**	0	0	5*	2	8**
Colon										
Pigments in Lamina Propria Mucosa	0	6**	10**	4*	10**	0	4*	10**	4*	10**
Colon Perls										
Positive Staining of Epithelium	0	10**	10**	10**	10**	0	10**	10**	9**	10**
Positive Staining of Luminal Contents	0	3	7**	2	7**	0	2	6**	3	5*
Positive Staining of Macrophages in Lamina Propria Mucosa	1	10**	10**	10**	10**	0	10**	10**	9**	10**
Jejunum Perls										
Positive Staining of Luminal Contents	0	0	1	0	1	0	0	3	0	2
Positive Staining of Macrophages in Lamina Propria Mucosa	0	2	4*	0	2	0	0	3	0	2
Caecum										
Epithelial Hyperplasia	0	0	1	0	4*	0	0	0	0	0
Mucosal Inflammation	1	0	0	0	2	0	1	0	0	0
Positive Perls Staining of Epithelium	0	0	9**	0	10**	0	0	7**	0	10**
Positive Perls Staining of Luminal Contents	0	4*	9**	6**	9**	0	2	6**	5*	6**
Rectum										
Epithelial Hyperplasia	0	0	2	0	0	0	0	1	0	0
Positive Perls Staining of Epithelium	0	0	10**	0	10**	0	3	10**	0	10**
Positive Perls Staining of Luminal Contents	0	1	9**	0	7**	0	1	4*	0	5*
Positive Perls Staining of Macrophages in Lamina Propria Mucosa	0	1	9**	0	10**	0	2	10**	0	9**
Prostate										
Inflammation	0	3	7**	4*	4*					

1M and 1F: control. 2M and 2F: PA21 1000 mg/kg/day. 3M and 3F: PA21 4000 mg/kg/day. 4M and 4F: PA21-2 1000 mg/kg/day. 5M and 5F: PA21-2 4000 mg/kg/day. * p<0.05 vs Control; ** p<0.01 vs Control

6.2.3 PA21 – Toxicity study by oral capsule administration to beagle dogs for 26 or 39 weeks followed by a 6-week recovery period

Study no.:	VFR097
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Sept 28, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug; lot # / % purity:	PA21; 429000/95.3%, 423000/95.6%, 426000/94.7%
Deviation from study protocol:	No impact to the results

Key Study Findings

Compared to control animals, high incidence of liquid feces occurred in all treated male groups and in females receiving 0.1 or 1.0 g/kg b.i.d. There were slightly higher plasma phosphorus levels in males at dose 1 g/kg b.i.d. and dose-related reduction in urine phosphorus in both sexes during the treatment period, and dose-related elevation of iron contents in liver, kidney, and spleen of males and/or females at week 39. Higher incidences of pigmented macrophages and/or positive Perl's stain (for iron) in liver, spleen, GI tract, mesenteric and mandibular lymph nodes of both males and females at doses 0.1, 0.3, or 1 g/kg b.i.d. at both interim and terminal sacrifice, which were still present after a 6-week drug-off recovery period. These findings were either attributed to action of PA21, i.e., reduced phosphate uptake and consequential metabolic changes, or due to iron uptake follow PA21 degradation. All these findings were minimal, and were not of toxicological significance. NOAEL was 1 g/kg b.i.d. (= 2 g/kg/day) in this study.

Methods

Beagle dogs, initial age of 26-40 weeks (9.3-12.7 kg for males and 7.1-10.7 kg for females), received oral PA21 capsules or empty capsules for 26 weeks (3/sex/group at 0, 0.3, and 1.0 g/kg, b.i.d.) or 39 weeks (6/sex/group at 0 and 1.0 g/kg b.i.d.; 4/sex/group at 0.1 and 0.3 g/kg b.i.d.). Two dogs/sex from each 0 and 1.0 g/kg b.i.d. group on the 39-week treatment continued on a 6-week recovery period. The two doses were given 2 hours apart. The doses selected for the study were based on previous 4- and 13-week toxicity studies (GLP) in dogs with PA21 (VFR084 and VFR088 respectively) conducted in the same laboratory. In these studies, dogs were orally dosed with PA21 (in capsules) at 0, 0.25, 0.5, and 1 g/kg, b.i.d. (2 hours apart), for 4 weeks (VFR084, 3/sex/dose, initiated on Oct 8, 2004) or 13 weeks (VFR088, 4/sex/dose, initiated on April 27, 2006). Oral PA21 0.25-1 g/kg b.i.d. resulted in slightly higher plasma phosphorus level, reduction in urine phosphorus, trend to minimal elevation of iron contents in liver, kidney, and spleen, and higher incidences of iron deposition in liver, spleen, GI tract, mesenteric and mandibular lymph nodes. All these findings were minimal and pharmacology action of PA21 or secondary to the iron uptake

from PA21, and were not of toxicological significance. The maximum practical dosage of 2 g/kg/day (1 g/kg b.i.d.) was considered to be the NOAEL for these two studies, and was selected to be the high dose in the current study. The low dose 0.2 g/kg/day (0.1 g/kg b.i.d.) in the current study was close to the maximum likely clinical dose.

During the study, dogs were inspected visually at least twice daily for clinical signs and morbidity/death. Physical examination was weekly performed on each animal. Body weight was recorded weekly. Food consumption for each animal was recorded daily and weekly food consumption was then calculated. Ophthalmic examination was performed prior to treatment on all animals, in week 13 on the interim phase (26-week) animals, and during weeks 26, 39, and recovery week 6 on all survival animals.

Electrocardiographic tracing (ECG; leads I, II, III, aVR, aVL, and aVF) was recorded from all animals at pre-dose and 2 hours after the 2nd daily dose during the pre-treatment period, weeks 13, 26, and 39, and recovery week 6. The ECG traces were examined for any visual abnormalities and heart rate. Wave intervals of P, PR, QRS, ST, QT, and QTc were calculated. Direct blood pressure (a needle inserted into the intermediate auricular artery) was recorded at same frequency and time as electrocardiography. Systolic, diastolic, and mean arterial pressure and pulse rate were determined. Blood samples were collected from all available animals during weeks 0, 13/14, 26, 39 (before dosing on each occasion) and recovery week 6 for determining hematology, plasma chemistry (Table 67), serum osteocalcin and crosslinked C-terminal telopeptide of type-1 collagen (CTX) levels (bone turnover marker), and serum levels of vitamins A, E, K, and D (25-hydroxy vitamin D, and 1,25-dihydroxy vitamin D). Overnight urine samples were collected from all animals during weeks 0, 13, 26, and 39, and recovery week 6 for urinalysis (Table 45) and for determining deoxypyridinoline (bone turnover marker, except for week 39) and creatinine (for normalization of deoxypyridinoline values).

Table 67. Parameters for hematology and plasma chemistry

Hematology parameters	Plasma chemistry
Hematocrit (Hct)	Alkaline phosphatase (ALP) and isoenzymes (Bone BAP, Intestinal IAP, and Liver LAP)*
Hemoglobin concentration (Hb)	
Erythrocyte count (RBC)	Alanine aminotransferase (ALT)
Reticulocyte count (Retic)	Aspartate aminotransferase (AST)
Mean cell hemoglobin (MCH)	Lipase (LIP)
Mean cell hemoglobin concentration (MCHC)	Total bilirubin (Bili)
Mean cell volume (MCV)	Creatinine (Creat) , Urea
Total white cell count (WBC)	Glucose (Gluc)
Differential WBC count	Total cholesterol (Chol)
Neutrophils (N)	Triglycerides (Trig)
Lymphocytes (L)	Phospholipids (PLIP)
Eosinophils (E)	Sodium (Na)
Basophils (B)	Potassium (K)
Monocytes (M)	Chloride (Cl)
Large unstained cells (LUC)	Calcium (Ca)
Platelet count (Plt)	Inorganic phosphorus (Phos)
Morphology flags	Iron (Fe)
Prothrombin time (PT)	Total protein (Total Prot)
Activated partial thromboplastin time (APTT)	Albumin(Alb), α 1, α 2, β , and γ globulin

* Data ascribing error for pre-treatment alkaline phosphatase isoenzyme analysis, not included.

On completion of 26 or 39 weeks of treatment and 6 weeks off treatment, all interim, main, and recovery animals were killed and subjected to a detailed necropsy. Organs/tissues listed in Table 68 were weighed, collected, and histologically processed. Samples from liver (residue of left lobe), kidney (poles from left kidney), and spleen (poles) from all animals were weighed, and snap-frozen in liquid nitrogen for determination of iron content. A complete histopathological examination was performed on all animals from all groups (Table 68). Additional sections from the cecum, colon, duodenum, ileum, jejunum, kidney, liver, mandibular and mesenteric lymph nodes, spleen, stomach and rectum were stained for iron using Perl's stain. A scoring system was used to quantify iron-positive staining in these tissues. Samples of any macroscopic abnormal tissues were also retained and processed for histopathological examination. A reviewing pathologist undertook a peer review of the microscopic findings.

Table 68. Organ/tissue list for 26- and 39-week dog study

	Weigh ¹	Fixation	Histological processed	Perl's stain for iron content	Light microscopy
Adrenals	√		√		√
Aorta - thoracic		10%	√		√
Brain	√	neutral	√		√
Cecum		buffered	√	√	√
Colon		formalin	√	√	√
Duodenum			√	√	√
Epididymides	√	B	√		√
Eyes		D	√		√
Femurs with Joint			√		√
Gall bladder			√		√
Heart	√		√		√
Ileum with Peyer's patch			√	√	√
Jejunum			√	√	√
Kidney	√		√	√	√
Larynx			√		√
Liver	√		√	√	√
Lumbar vertebra			√		√
Lungs and bronchi	√		√		√
Lymph node -mandibular -mesenteric			√	√	√
Mammary area - caudal		10%	√		√
Nictitans glands		neutral	√		√
Esophagus		buffered	√		√
Optic nerves		formalin	√		√
Ovaries	√		√		√
Pancreas			√	√	√
Pituitary	√		√		√
Prostate	√		√		√
Rectum			√	√	√
Salivary glands (submandibular parotid, sublingual)	√		√		√
Sciatic nerves+			√		√
Skeletal muscle - thigh+			√		√
Skin			√		√
Spinal cord			√		√
Spleen	√		√	√	√
Sternum			√		√
Stomach			√	√	√
Testes	√	B	√		√
Thymus	√		√		√
Thyroid with parathyroids	√		√		√
Tibia bone			√		√
Tongue		10%	√		√
Trachea		neutral	√		√
Ureters		buffered	√		√
Urinary bladder		formalin	√		√
Uterus and cervix	√		√		√
Vagina			√		√
Any abnormal tissue			√		√

¹ - Bilateral organs were weighed together. + Only one processed for examination

B - Fixed in Bouin's solution prior to transfer to 70% industrial methylated spirit

D - Fixed in Davidson's fluid prior to transfer to 70% industrial methylated spirit

Results

Dark feces were observed in all treated animals from week 1 that was attributed to the PA21 itself, but was absent during recovery period. Compared to control animals, high incidence of liquid feces occurred in all treated male groups and in females receiving 0.1 or 1.0 g/kg b.i.d.. There were no PA21-related effects on morbidity/death, body weight, food consumption, ophthalmic examination, ECG, blood pressure, heart rate, or hematology.

The only findings in plasma chemistry and urinalysis were slightly higher plasma phosphorus in males at dose 1 g/kg b.i.d. (Table 69) and dose-related reduction in urine phosphorus in both sexes (Table 70) during the treatment period. PA21 resulted in dose- and time-related elevation of iron contents in liver, kidney, and spleen of males and/or females (Table 71), but did not affect the bone turnover markers (Table 72) and serum levels of vitamins A, E, K, and D (Assay validations under Study WLY0013 indicated that the vitamin assays with the exception of 25-hydroxyvitamin D assay failed to meet acceptance criteria set for precision and accuracy. Thus, data here were just for reference). All these changes were minimal or attributed to the pharmacological effects of PA21, and were not of toxicological significance.

Table 69. Findings in plasma chemistry

Parameter	Time	Male (PA21 dose: g/kg, b.i.d.)				Female (PA21 dose: g/kg, b.i.d.)			
		0	0.1	0.3	1	0	0.1	0.3	1
Plasma calcium (mmol/L)	Week 0	2.71±0.08	2.71±0.08	2.69±0.08	2.64±0.30	2.68±0.08	2.71±0.09	2.72±0.15	2.60±0.06
	Week 13/14	2.67±0.08	2.67±0.05	2.68±0.04	2.67±0.03	2.67±0.08	2.74±0.09	2.71±0.06	2.64±0.06
	Week 26	2.67±0.09	2.67±0.09	2.68±0.06	2.71±0.06	2.66±0.06	2.66±0.11	2.67±0.09	2.67±0.03
	Week 39	2.69±0.08	2.70±0.09	2.74±0.06	2.81±0.07*	2.69±0.06	2.71±0.05	2.68±0.15	2.67±0.09
	End of recovery	2.55±0.01			2.60±0	2.70±0.08			2.70±0.13
Plasma phosphorus (mmol/L)	Week 0	1.76±0.33	1.81±0.13	1.90±0.23	1.88±0.36	1.71±0.34	1.73±0.22	1.75±0.28	1.68±0.15
	Week 13/14	1.40±0.14	1.50±0.24	1.57±0.19	1.70±0.20**	1.38±0.18	1.46±0.15	1.37±0.22	1.44±0.16
	Week 26	1.16±0.12	1.17±0.10	1.23±0.16	1.44±0.14**	1.15±0.09	0.92±0.25	1.09±0.34	1.29±0.19
	Week 39	1.14±0.14	1.11±0.17	1.30±0.20	1.43±0.18*	0.99±0.29	0.84±0.39	0.93±0.34	1.08±0.12
	End of recovery	0.82±0.25			1.08±0.02	1.25±0.53			1.27±0.27
Plasma iron (µmol/L)	Week 0	24±5	18±3	20±4	20±3	28±9	23±10	22±7	19±4
	Week 13/14	30±8	21±3	26±6	25±9	37±9	30±13	32±10	29±7
	Week 26	37±12	32±8	29±8	29±10	36±7	34±3	34±8	34±11
	Week 39	36±12	33±6	35±8	31±8	43±4	39±12	48±15	31±14
	End of recovery	46±5			29±4	50±19			34±16
Plasma urea (mmol/L)	Week 0	3.97±0.56	5.48±1.21	4.36±0.50	4.29±0.68	3.53±0.52	3.35±0.40	3.82±0.80	3.73±0.68
	Week 13/14	4.30±0.54	5.40±1.17	4.60±0.63	4.40±0.67	4.74±0.44	4.78±0.71	4.98±1.10	4.78±0.89
	Week 26	4.75±0.72	5.52±1.16	4.87±0.59	4.74±0.81	4.72±0.65	5.26±0.98	5.14±1.22	5.23±1.05
	Week 39	4.67±0.52	5.75±0.71	4.98±0.80	4.58±0.52	4.66±0.77	5.05±1.35	5.15±1.15	5.03±0.59
	End of recovery	4.74±0.74			4.80±0.33	4.62±0.52			4.52±0.97
Plasma creatinine (µmol/L)	Week 0	62±9	68±11	61±6	61±10	61±8	65±7	60±9	62±6
	Week 13/14	73±7	74±5	78±6	78±9	71±6	78±10	74±8	76±7
	Week 26	68±8	67±9	72±7	74±11	68±4	73±6	73±9	74±6
	Week 39	69±4	73±5	73±7	75±10	66±4	77±10	71±9	77±5*
	End of recovery	66±10			65±6	61±4			56±2

* p<0.05 vs control; ** p<0.01 vs control.

Table 70. Findings in urinalysis

Parameter	Time	Male (PA21 dose: g/kg, b.i.d.)				Female (PA21 dose: g/kg, b.i.d.)			
		0	0.1	0.3	1	0	0.1	0.3	1
Urine volume (ml)	Week 0	139±74	202±98	201±60	169±87	187±67	156±10	175±66	169±68
	Week 13/14	99±29	160±62	108±65	96±45	141±43	125±76	133±23	103±49
	Week 26	109±61	136±104	146±137	76±45	130±77	132±25	138±89	137±56
	Week 39	94±40	139±45	111±79	84±42	105±41	135±65	109±29	65±32
	End of recovery	173±173			165±92	151±1			155±137
Urine calcium (mmol/L)	Week 0	2.31±0.71	2.52±0.60	3.00±1.16	2.83±0.87	3.29±2.67	2.53±0.69	3.03±1.42	2.55±0.85
	Week 13/14	3.23±1.15	3.33±1.77	2.84±0.85	4.10±2.21	2.79±0.53	2.06±0.75	2.92±0.75	3.52±1.24
	Week 26	3.05±0.97	2.94±0.66	3.22±1.11	3.11±0.73	3.09±1.01	2.60±0.56	2.76±0.97	3.47±2.70
	Week 39	3.40±1.03	3.58±0.74	2.70±0.31	3.00±1.37	2.70±0.77	2.48±0.61	2.46±0.77	3.22±1.08
	End of recovery	1.89±0.04			3.12±2.30	3.06±1.60			2.52±0.05
Urine phosphorus (mmol/L)	Week 0	149±24	111±15	120±22	119±45	111±36	117±29	122±20	125±28
	Week 13/14	129±52	133±9	85±11	29±16**	135±36	124±7	128±21	38±30**
	Week 26	136±70	120±30	98±33	53±43**	132±37	107±13	90±24*	65±38**
	Week 39	132±28	89±23*	81±18**	35±30**	112±30	86±42	90±49	36±41**
	End of recovery	169±89			136±1	138±37			120±22

* p<0.05 vs control; ** p<0.01 vs control.

Table 71. Tissue iron levels (mg/kg)

Tissue	Time	Male (PA21 dose: g/kg, b.i.d.)				Female (PA21 dose: g/kg, b.i.d.)			
		0	0.1	0.3	1	0	0.1	0.3	1
Liver	Week 26	390±105		703±364	563±280	370±85		480±145	683±222
	Week 39	364±102	509±171	813±270*	778±250*	428±83	575±177	693±195	1045±357**
	End of recovery	325±64			1140±636	570±325			1040±594
Kidney	Week 26	48±2		58±12	60±2	43±2		84±49	82±13
	Week 39	51±10	59±16	70±20	76±4*	53±5	68±20	105±52	115±58
	End of recovery	65±10			100±0	54±8			91±9
Spleen	Week 26	790±121		840±66	1023±454	650±20		860±171	1063±320
	Week 39	770±29	860±50	953±226	1108±504	780±243	908±168	970±205	1090±112*
	End of recovery	735±177			1360±509	645±35			915±332

* p<0.05 vs control; ** p<0.01 vs control.

Table 72. Bone turnover markers

Parameter	Time	Male (PA21 dose: g/kg, b.i.d.)				Female (PA21 dose: g/kg, b.i.d.)			
		0	0.1	0.3	1	0	0.1	0.3	1
Urine deoxypyridinoline /creatinine (nM/mM)	Week 0	9.68±1.79	11.85±3.02	11.2±2.19	10.47±2.55	9.05±1.78	8.39±1.19	10.30±2.79	9.72±3.09
	Week 13	4.90±0.71	6.10±1.76	5.60±1.03	6.02±0.86	6.26±1.95	5.42±0.96	4.81±1.57	4.41±0.69*
	Week 26	4.15±1.77	3.99±1.25	3.63±0.26	4.14±1.08	4.33±0.70	3.78±0.36	4.01±0.92	3.65±0.42
	Week 39	No samples							
	End of recovery	4.06±7.45			4.90±3.07	6.24±0.46			3.59±0.74
Serum Osteocalcin (ng/ml)	Week 0	15.0±2.5	16.8±2.3	16.4±3.3	16.2±3.4	14.1±2.1	23.5±6.5*	21.1±4.3*	18.6±1.6*
	Week 13	13.4±4.0	13.1±1.8	14.5±3.4	13.7±4.1	9.9±3.0	10.2±1.5	13.5±3.3*	11.7±1.9
	Week 26	10.6±5.0	7.7±2.3	8.3±1.7	9.5±2.6	7.3±1.5	8.3±1.6	9.3±2.7	8.2±2.6
	Week 39	12.2±8.8	7.9±1.8	7.6±2.5	10.9±5.3	7.2±1.0	11.0±2.7*	12.1±1.6*	8.7±3.6
	End of recovery	22.2±15			8.5±2.9	7.2±0.9			8.2±4.5
Serum Crosslinked C-telopeptide of type 1 collagen (CTx, nM)	Week 0	6.8±1.2	7.0±0.7	5.9±0.9	5.5±0.6	6.4±1.3	6.9±2.2	5.8±1.2	7.0±1.2
	Week 13	7.7±2.1	8.3±1.2	7.2±1.0	8.2±1.5	6.5±1.7	6.2±1.4	5.7±1.6	7.5±1.0
	Week 26	7.1±2.0	7.2±1.1	6.5±1.5	6.9±1.5	5.8±1.6	6.5±1.1	6.4±1.1	6.6±1.6
	Week 39	6.0±2.3	6.7±1.5	6.3±1.0	7.5±2.1	5.0±1.0	6.2±1.4	4.7±1.4	6.8±1.4
	End of recovery	6.7±3.9			6.7±1.5	17.4±20.6			5.0±2.4

* p<0.05 vs control; ** p<0.01.

Necropsy revealed an increased incidence of dark contents in large intestines of all treated males and females, which was attributed to PA21 itself. Spleen weight of females at doses 0.3 and 1 g/kg b.i.d. was higher than control at week 26 (Table 73),

which was moderate without histological findings, not dose-dependent, not seen in males, and not of toxicological significance. The PA21-related histological changes were higher incidences of pigmented macrophages and/or positive Perl's stain (for iron) in liver, spleen, GI tract, mesenteric and mandibular lymph nodes of both males and females at doses 0.1, 0.3, or 1 g/kg b.i.d at both interim and terminal sacrifice. The changes were still present after a 6-week drug-off recovery period. The iron pigment deposition in the tissues was secondary to the iron uptake from GI PA21, and was not of toxicological significance.

Table 73. Findings in organ weight

	Time	Male (PA21 dose: g/kg, b.i.d.)				Female (PA21 dose: g/kg, b.i.d.)			
		0	0.1	0.3	1	0	0.1	0.3	1
Body weight (kg)	Week 26	14.3±0.6		12.9±1.0	12±1.7	10±1.4		10.6±0.8	10±1.8
	Week 39	13.4±1.0	13.3±0.8	13.4±0.9	13.4±1.2	10.6±1.3	11.2±1.3	9.7±0.6	10.1±0.6
	End of rec	12.8±1.8			14.6±1.9	11.8±0.6			11.5±0.6
Spleen weight (g)	Week 26	95.7±11.6		97.3±16.0	104.5±22.9	56.1±4.8		97.3±13.2**	75.6±4.4**
	Week 39	113.8±14.8	126±64.5	96.1±11.5	107.3±11.2	72.6±29.1	103.9±65.6	84.7±8.9	75.7±17.7
	End of rec	70.6±31			75.9±5.5	73.7±14.3			68.5±4.0

** p<0.01 vs control

Table 74. Number of animals with histological findings

Organ	Findings	Male (PA21 dose: g/kg, b.i.d.)				Female (PA21 dose: g/kg, b.i.d.)			
		0	0.1	0.3	1	0	0.1	0.3	1
Week 26 (n=3)									
Mesenteric lymph node	Sinus erythrocytosis /erythrophagocytosis	2		3	3	1		2	3
	Positive Perl's stain	1		2	2	1		0	3
Spleen	Pigmented macrophages	1		2	1	1		2	3
	Positive Perl's stain	3		3	3	3		3	3
Liver	Pigmented Kupffer cells/ macrophages	0		1	2	0		1	1
	Positive Perl's stain	2		3	3	3		2	3
Kidney	Cortex - inflammatory cells	0		0	2	0		1	0
Mandibular lymph node	Pigmented macrophages/Tattoo pigment	0		2	2	1		2	1
	Positive Perl's stain	2		3	3	2		2	3
Stomach	Positive Perl's stain	2		3	3	0		3	3
Duodenum	Positive Perl's stain	0		0	1	0		0	0
Jejunum	Positive Perl's stain	0		1	0	0		0	0
Cecum	Positive Perl's stain	1		3	3	1		2	3
Ileum	Positive Perl's stain	0		3	3	0		1	0
Colon	Positive Perl's stain	0		3	3	0		1	2
Rectum	Positive Perl's stain	1		2	3	1		2	2
Week 39 (n=4)									
Mesenteric lymph node	Sinus erythrocytosis /erythrophagocytosis	1	3	3	3	2	1	1	2
	Positive Perl's stain	1	3	4	4	2	3	3	4
Spleen	Pigmented macrophages	0	0	0	2	0	0	3	1
	Positive Perl's stain	4	4	4	4	4	4	4	4
Liver	Pigmented Kupffer cells/ macrophages	0	0	3	3	1	1	2	3
	Pigmented macrophages	3	4	4	4	3	4	4	4
Mandibular lymph node	Positive Perl's stain	2	4	4	4	1	3	3	3
Stomach	Positive Perl's stain	1	2	3	4	1	2	3	4
Duodenum	Positive Perl's stain	0	0	1	2	1	0	1	2
Jejunum	Positive Perl's stain	0	0	0	1	0	1	1	2
Cecum	Positive Perl's stain	3	4	4	4	2	3	4	4
Ileum	Positive Perl's stain	2	2	4	4	1	2	3	4
Colon	Positive Perl's stain	1	1	4	4	1	3	4	4
Rectum	Positive Perl's stain	2	2	4	4	2	4	4	4
Recovery week 6 (n=2)									
Spleen	Pigmented macrophages	0			2	1			1
	Positive Perl's stain	2			2	2			2
Liver	Pigmented Kupffer cells/ macrophages	0			1	0			2
	Positive Perl's stain	2			2	1			2
Mesenteric lymph node	Positive Perl's stain	0			2	0			2
Mandibular lymph node	Pigmented macrophages/Tattoo pigment	0			0	0			1
	Positive Perl's stain	2			2	2			2
Mammary	Glandular pigment	0			0	0			2
Stomach	Positive Perl's stain	1			2	0			2
Duodenum	Positive Perl's stain	0			1	0			0
Cecum	Positive Perl's stain	2			2	1			2
Ileum	Positive Perl's stain	0			0	0			2
Rectum	Positive Perl's stain	1			2	0			2

6.2.4 PA21 - Bridging toxicity study by oral capsule administration to fasted beagle dogs for 4 Weeks

Study no.:	VFR109
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Aug 6, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PA21, lot # 426000, purity 94.7%
Deviation from study protocol:	No impact to the results

Key Study Findings

Oral administration of PA21 to fasted beagle dogs for 4 weeks at the maximum practical dosage of 1.0 g/kg b.i.d. increased emesis/irritation, enhanced iron uptake into the mucosa of the gastro-intestinal tract, and resulted in a pharmacodynamic effect on urinary phosphate (decreased). Thus, the only differences compared to previous studies in which PA21 was administered immediately prior to feeding related to locally-mediated effects, i.e., emesis/irritation. There was no difference in apparent systemic toxicity of PA21 between fed and fasted animals at the maximum feasible dose of 2 g/kg/day.

Methods

Beagle dogs (3/sex/group), initial age of 21-24 weeks (7.8-10.6 kg for males and 7.8-10 kg for females), received oral PA21 capsules or empty capsules (at 1.0 or 0 g/kg, b.i.d., approximately two hours apart) before feeding for 4 weeks. This study assessed the effects of PA21 when administered to dogs under fasting condition, compared with effects produced when PA21 was administered with food, as in a previous 4-week toxicity study (VFR084) and longer-term toxicity studies (VFR097) performed at the same laboratories.

During the study, dogs were inspected visually at least twice daily for clinical signs and morbidity/death. Physical examination was weekly performed on each animal. Body weight was recorded weekly. Food consumption for each animal was recorded daily and weekly food consumption was then calculated. Ophthalmic examination was performed prior to treatment and in week 4 on all animals. Electrocardiographic tracing (ECG; leads I, II, III, aVR, aVL, and aVF) was recorded from all animals during the pre-treatment period, pre-dose and 2 hours after the 2nd daily dose on day 1 and in week 4. The ECG traces were examined for any visual abnormalities and heart rate. Wave intervals of P, PR, QRS, ST, QT, and QTc were calculated. Blood samples were collected from all animals prior to treatment and during week 4 before dosing for determining hematology, plasma chemistry (Table 67), and serum levels of vitamins A, E, and D (25-hydroxy vitamin D, and 1,25-dihydroxy vitamin D). Overnight urine samples were collected from all animals prior to treatment and during week 4 for urinalysis (Table 45).

At the end of 4 weeks treatment, all animals were killed and subjected to a detailed necropsy. Organs/tissues listed in Table 68 were weighed, collected, and histologically processed. Samples from liver (residue of left lobe), kidney (poles from left kidney), and spleen (poles) from all animals were weighed, and snap-frozen in liquid nitrogen for determination of iron content. A complete histopathological examination was performed on all animals from all groups (Table 68). Additional sections from the cecum, colon, duodenum, ileum, jejunum, kidney, liver, mandibular and mesenteric lymph nodes, spleen, stomach and rectum were stained for iron using Perl's stain. A scoring system was used to quantify iron-positive staining in these tissues. Samples of any macroscopic abnormal tissues were also retained and processed for histopathological examination. A reviewing pathologist undertook a peer review of the microscopic findings.

Results

There were no unscheduled deaths. Dark feces were observed in all animals receiving PA21. Emesis was observed in all males and females receiving 1.0 g/kg b.i.d. intermittently throughout the treatment period, with most animals being affected from Day 1 and one animal was affected from Day 6. Emesis occurred most frequently at approximately three hours after administration of the second dose (i.e. just prior to feeding). There were also isolated incidences of emesis in two control animals, which occurred occasionally when capsules were administered and was attributed to the dose administration procedure.

There were no PA 21-related effects on bodyweight gain, food consumption, ocular changes, ECG (Table 75), hematological and blood chemistry parameters (Table 76), and serum levels of vitamins (Assay validations under Study WLY0013 indicated that the vitamin assays with the exception of 25-hydroxyvitamin D assay failed to meet acceptance criteria set for precision and accuracy. Thus, data here were just for reference). In week 4, there was a marked reduction of urinary phosphorus in females receiving PA21 1.0 g/kg b.i.d. (Table 76), which related to the pharmacological effect of PA21 and was of no toxicological significance. After 4 weeks of PA21 treatment, there was no clear evidence of any increase in iron levels in the liver, kidney and spleen (Table 77).

Table 75. Heart rate and ECG intervals (ms)

	Sex, PA21 dose	Heart rate, beat/min	P	PR	QRS	ST	QT	QTcV
Before study start	M, Control	132±12	37±3	88±6	37±3	134±3	171±2	218±2
	M, 2.0 g/kg	150±13	31±3	85±6	36±3	131±6	167±5	219±7
	F, Control	160±8	37±2	84±5	37±3	129±6	166±9	220±9
	F, 2.0 g/kg	141±18	34±2	87±9	40±2	133±4	173±6	222±5
Day 1, Pre-dose	M, Control	132±7	31±3	84±12	40±3	135±6	175±3	222±5
	M, 2.0 g/kg	143±1	31±1	92±6	35±4	129±5	165±1*	215±4
	F, Control	133±11	33±4	84±10	37±6	136±3	173±3	220±2
Day 1, 2 hours post-dose	F, 2.0 g/kg	133±22	33±3	91±8	41±2	135±4	176±2	223±4
	M, Control	129±18	30±4	84±10	42±3	133±3	175±2	221±3
	M, 2.0 g/kg	133±14	35±5	91±9	37±2	140±9	177±8	225±4
Week 4, Pre-dose	F, Control	136±13	30±2	85±8	38±3	133±2	171±1	220±4
	F, 2.0 g/kg	131±6	31±4	90±7	39±8	140±4	179±6	226±4
	M, Control	140±18	31±1	83±8	35±6	137±6	172±5	221±1
Week 4, 2 hours post-dose	M, 2.0 g/kg	148±11	32±4	95±10	37±3	129±8	166±6	217±8
	F, Control	134±17	35±6	85±13	38±5	141±6	179±12	226±7
	F, 2.0 g/kg	140±8	32±0	98±6	39±3	135±6	174±9	224±9
Week 4, 2 hours post-dose	M, Control	111±6	33±3	87±8	41±5	144±9	185±5	225±6
	M, 2.0 g/kg	118±20	37±2	93±6	39±6	149±13	188±9	230±9
	F, Control	125±12	37±4	89±8	39±6	139±1	178±7	223±9
	F, 2.0 g/kg	117±9	34±2	93±5	44±4	145±8	189±8	231±6

* p<0.05 vs control

Table 76. Findings in relevant parameters of plasma chemistry and urinalysis

Group	Plasma chemistry						Urinalysis						
	Before treatment			During treatment week 4			Before treatment			During treatment week 4			
	Ca mmol/L	Phos mmol/L	Fe µmol/L	Ca mmol/L	Phos mmol/L	Fe µmol/L	U-Cr mmol/L	UCa mmol/L	UIP mmol/L	U-Cr mmol/L	UCa mmol/L	UIP mmol/L	
Male Control	Mean	2.83	2.29	20	2.73	2.12	28	144.7	3.09	56.77	167.3	4.09	57.82
	SD	0.074	0.179	2.8	0.04	0.19	5.7	8.02	0.262	0.90	19.63	0.860	15.850
Male PA21 1 g/kg BID	Mean	2.84	2.25	16	2.72	2.20	23	183.3	2.27	65.77	209.0*	2.52	65.89
	SD	0.074	0.414	4.4	0.02	0.35	0.6	31.75	0.515	13.02	14.93	0.781	16.460
Female Control	Mean	2.89	2.35	20	74.0	2.77	2.10	143.0	3.52	50.10	154.7	4.03	58.80
	SD	0.029	0.265	7.2	0.00	0.06	0.243	29.51	1.317	8.19	37.29	2.033	2.327
Female PA21 1 g/kg BID	Mean	2.89	2.36	18	77.3	2.77	2.17	150.0	3.15	61.23	201.7	5.63	19.74**
	SD	0.096	0.176	7.5	2.52	0.03	0.130	49.79	1.042	3.21	7.64	2.366	3.186

* p<0.05 vs control, ** p<0.01 vs control

Table 77. Tissue iron contents after 4 weeks of PA21 treatment in dogs

Group		Male			Female		
		Liver	Kidney	Spleen	Liver	Kidney	Spleen
Control	Mean	292	41	722	303	41	687
	SD	20.2	2.6	20.2	32.1	6.0	110.2
PA21 1.0 mg/kg BID	Mean	257	42	567	343	48	643
	SD	126.6	11.1	207.4	89.6	4.0	100.2

Organ weights were not affected by PA21 treatment. The macroscopic examination at the end of the 4-week treatment revealed dark contents in the GI tract in PA21-treated dogs, due to the properties of PA21 itself. Treatment-related histological findings were seen in the gastro-intestinal tract. Positive Perls' staining was seen in the contents of the intestines in all treated dogs, representing the presence of iron in the lumen of the gastro-intestinal tract. The presence of positive Perls' stain was seen in the mucosa of the stomach, duodenum, jejunum, ileum and rectum in treated dogs, indicative of the presence of iron in the mucosa of the gastro-intestinal tract, though the finding was not present in all regions of the gastro-intestinal tract in each animal (Table 78).

Table 78. Histological findings in the dog bridging study

Tissue and Finding	Group/Sex:	1M	2M	1F	2F
	Number:	3	3	3	3
Stomach Perls					
Positive Staining in Mucosa		0	3	0	3
Duodenum Perls					
Positive Staining in GI Contents		0	1	0	0
Positive Staining in Mucosa		0	1	0	1
Jejunum Perls					
Positive Staining in GI Contents		0	1	0	1
Positive Staining in Mucosa		0	0	0	1
Caecum Perls					
Positive Staining in GALT Associated Tissue		0	1	0	2
Positive Staining in GI Contents		0	1	0	1
Positive Staining in Mucosa		1	1	0	1
Ileum Perls					
Positive Staining in Mucosa		0	1	0	0
Colon Perls					
Positive Staining in GALT Associated Tissue		0	1	1	0
Positive Staining in GI Contents		0	2	0	3
Positive Staining in Mucosa		0	0	1	1
Rectum Perls					
	Number Examined:	3	2	3	3
Positive Staining in GALT Associated Tissue		1	1	0	1
Positive Staining in GI Contents		0	2	0	3
Positive Staining in Mucosa		0	1	0	2
GALT - gut-associated lymphoid tissue					

1M: Male control. 2M: Male PA21 1 g/kg/day, b.i.d.

1F: Female control. 2F: Female PA21 1 g/kg/day, b.i.d.

7 Genetic Toxicology

7.1 In vitro

7.1.1 PA21 – Bacterial reverse mutation test

Study no.:	VFR 078/043254
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Aug 6, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PA21, 423000, 97%

Key Study Findings

No evidence of mutagenic activity was seen at PA 21 concentrations 5- 5000 µg/plate in the Ames test.

Methods

Four strains of histidine auxotrophs *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and a tryptophan dependent *Escherichia coli* tester strain WP2 uvrA (pKM101) were used. For all assays, bacteria were cultured at 37°C for 10 hours prior to the commencement of experiments. The first test was a standard plate incorporation assay with PA21 at 5, 15, 50, 150, 500, 1500, or 5000 µg/plate (vehicle 1% methylcellulose) in the absence or presence of metabolic activator Aroclor 1254-induced rat liver post-mitochondrial fraction (S9). After mixing and plating, triplicate plates were incubated at 37°C for 72 hours. The second test included a pre-incubation stage, i.e., tubes containing mixtures of bacteria, buffer or S9 mix and PA21 (50, 150, 500, 1500, or 5000 µg/plate) were incubated at 37°C for 30 minutes with shaking before plating. Negative (vehicle) and positive controls were also included (Table 79). The appearance of the background bacterial lawn was examined and revertant colonies were counted using an automated colony counter (Perceptive Instruments Domino). A reproducible increase in revertant colony numbers of at least twice (three times in the case of strains TA1535 and TA1537) the concurrent vehicle controls, with evidence of a positive dose-response relationship, was considered as evidence of mutagenic activity in this test system.

Table 79. List of positive controls for the Ames assay

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1537	Rat	Benzo[a]pyrene (B[a]P)	5
TA100, TA1535		2-aminoanthracene (2AA)	5
WP2 <i>uvrA</i>			10
TA98	None	2-nitrofluorene (2-NF)	2
TA100, TA1535		sodium azide (NaAz)	2.0
TA1537		9-aminoacridine (9-Aa)	50
WP2 <i>uvrA</i>		4-Nitroquinoline-1-oxide (4NQO)	2.0

Results

No background lawn toxicity was observed. Precipitation occurred at PA21 5000 µg/plate. The mean revertant colony counts for the vehicle controls were within the 99% confidence limits of the historical control range of the laboratory except on one occasion (TA1537, test 1, in the absence of S9 mix). Positive controls showed marked increases of revertant colonies in all the tested strains with or without S9. There were apparent increases in revertant colony numbers in test 1 strain WP2 *uvrA* (pKM101) with S9 at concentrations 5-150 µg/plate. However, these increases were neither concentration-related nor reproducible, and were, therefore, considered not significant. There was no evidence of increased revertant colonies with PA21 up to 5000 µg/plate in other strains (Table 80). Thus, no evidence of mutagenic activity was seen at any concentration of PA 21 in either mutation test.

Table 80. Numbers of revertant colonies in the Ames test

PA21 ($\mu\text{g}/\text{plate}$)	Test	Without S9 activation					With S9 activation				
		TA98	TA100	TA1535	TA1537	WP2uvrA	TA98	TA100	TA1535	TA1537	WP2uvrA
Untreated	1	48 \pm 10	172 \pm 20	28 \pm 5	37 \pm 2	137 \pm 11	51 \pm 4	159 \pm 23	15 \pm 6	38 \pm 4	117 \pm 11
Vehicle		55 \pm 6	167 \pm 15	21 \pm 2	43 \pm 12	143 \pm 18	54 \pm 5	185 \pm 16	24 \pm 7	44 \pm 8	104 \pm 5
5		53 \pm 4	151 \pm 18	21 \pm 10	51 \pm 9	152 \pm 23	65 \pm 2	150 \pm 23	22 \pm 2	43 \pm 4	256 \pm 18
15		55 \pm 8	137 \pm 13	18 \pm 4	41 \pm 6	158 \pm 4	57 \pm 1	166 \pm 10	17 \pm 6	44 \pm 8	230 \pm 9
50		50 \pm 7	146 \pm 12	21 \pm 5	41 \pm 6	157 \pm 11	46 \pm 9	136 \pm 7	17 \pm 6	35 \pm 4	233 \pm 6
150		54 \pm 2	152 \pm 6	22 \pm 6	40 \pm 2	163 \pm 16	49 \pm 8	155 \pm 5	21 \pm 1	35 \pm 4	231 \pm 8
500		50 \pm 1	150 \pm 10	23 \pm 2	33 \pm 3	149 \pm 18	51 \pm 7	144 \pm 27	12 \pm 3	26 \pm 3	178 \pm 9
1500		26 \pm 11	149 \pm 12	18 \pm 4	29 \pm 6	132 \pm 9	58 \pm 7	162 \pm 26	15 \pm 7	22 \pm 6	176 \pm 9
5000		34 \pm 4	167 \pm 14	16 \pm 2	22 \pm 6	125 \pm 11	34 \pm 11	163 \pm 10	9 \pm 4	25 \pm 10	158 \pm 15
2-NF (2)		326 \pm 14									
NaN ₃ (2)			1008 \pm 22	1053 \pm 51							
9-Aa (50)					1302 \pm 464						
4NQO (20)						3201 \pm 370					
B[a]P (5)							154 \pm 14			141 \pm 2	
2AA (5 or 10)								1066 \pm 219	361 \pm 65		282 \pm 22
Untreated	2	38 \pm 1	174 \pm 14	17 \pm 3	26 \pm 3	113 \pm 14	48 \pm 7	189 \pm 15	22 \pm 1	44 \pm 5	160 \pm 13
Vehicle		46 \pm 3	162 \pm 11	20 \pm 3	22 \pm 3	115 \pm 10	53 \pm 11	187 \pm 20	20 \pm 2	47 \pm 3	144 \pm 12
50		47 \pm 11	160 \pm 14	15 \pm 4	28 \pm 2	108 \pm 14	41 \pm 4	168 \pm 26	21 \pm 2	40 \pm 9	184 \pm 41
150		37 \pm 11	181 \pm 16	18 \pm 1	38 \pm 10	111 \pm 10	39 \pm 7	169 \pm 18	19 \pm 2	46 \pm 6	173 \pm 27
500		49 \pm 5	176 \pm 15	18 \pm 6	26 \pm 2	116 \pm 9	38 \pm 4	194 \pm 29	22 \pm 3	48 \pm 11	145 \pm 40
1500		46 \pm 6	151 \pm 19	18 \pm 2	29 \pm 2	110 \pm 25	51 \pm 6	167 \pm 25	16 \pm 2	48 \pm 5	177 \pm 6
5000		41 \pm 6	160 \pm 13	15 \pm 2	30 \pm 1	121 \pm 16	39 \pm 4	177 \pm 18	18 \pm 3	42 \pm 4	160 \pm 9
2-NF (2)		420 \pm 72									
NaN ₃ (2)			1067 \pm 56	945 \pm 65							
9-Aa (50)					816 \pm 106						
4NQO (20)						1686 \pm 286					
B[a]P (5)							244 \pm 12			179 \pm 35	
2AA (5 or 10)								3011 \pm 107	486 \pm 67		412 \pm 42

7.1.2 PA21-2 – Bacterial reverse mutation test

Study no.: VFR0119
 Study report location: (b) (4)
 Conducting laboratory and location: (b) (4)
 Date of study initiation: April 14, 2008
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: PA21-2, 280807J1, 95.8% (b) (4)

Key Study Findings

No evidence of mutagenic activity was seen at PA 21-2 concentrations 5- 5000 $\mu\text{g}/\text{plate}$ in the Ames test.

Methods

Experimental procedures were the same as those described under section “7.1.1 PA21 – Bacterial reverse mutation test” except the test article was PA21-2.

Results

No evidence of toxicity was obtained following exposure to PA21-2. Particles were observed on all plates containing PA21-2 at 5000 µg/plate. The concurrent positive controls demonstrated the sensitivity of the assay and the metabolizing activity of the liver preparations. No substantial increases in revertant colony numbers over control counts were obtained with any of the tester strains following exposure to PA21-2 at any concentration up to 5000 µg/plate in either the presence or absence of S9 mix (Table 81). Thus, no evidence of mutagenic activity was seen at PA 21-2 concentrations 5-5000 µg/plate in the Ames test.

Table 81. Numbers of revertant colonies in the Ames test

Concentration per plate	Without metabolic activation					With metabolic activation				
	TA98	TA100	TA1535	TA1537	WP2 uvrA	TA98	TA100	TA1535	TA1537	WP2 uvrA
Test 1										
Vehicle	40.3 ± 4.9	158.7 ± 15.0	24.0 ± 1.7	13.3 ± 2.5	158.7 ± 2.5	59.7 ± 2.5	208.0 ± 24.8	24.7 ± 4.0	29.0 ± 3.0	194.3 ± 5.5
Untreated	41.3 ± 5.5	178.3 ± 16.6	25.0 ± 1.7	14.0 ± 1.7	165.3 ± 12.9	57.7 ± 2.9	195.7 ± 11.7	26.0 ± 1.7	31.3 ± 4.6	204.0 ± 10.5
PA21-2 5 µg	42.0 ± 2.6	177.3 ± 21.4	25.0 ± 4.6	16.7 ± 2.9	153.7 ± 8.1	55.0 ± 6.2	212.3 ± 30.2	19.0 ± 2.6	30.3 ± 4.2	152.7 ± 16.3
15 µg	42.7 ± 5.0	167.3 ± 13.6	23.0 ± 1.0	12.3 ± 2.3	110.3 ± 11.1	49.7 ± 4.0	175.7 ± 6.1	20.0 ± 1.7	28.3 ± 2.5	179.0 ± 8.5
50 µg	38.3 ± 1.5	186.3 ± 8.5	25.0 ± 2.6	14.7 ± 2.9	135.7 ± 6.8	53.3 ± 2.5	196.3 ± 14.7	26.3 ± 4.5	27.0 ± 3.5	194.7 ± 13.0
150 µg	37.3 ± 3.8	166.7 ± 12.7	27.3 ± 4.2	11.7 ± 5.7	164.0 ± 17.1	59.7 ± 8.5	204.0 ± 15.7	23.7 ± 4.9	28.0 ± 1.7	211.7 ± 8.7
500 µg	40.0 ± 5.0	187.0 ± 3.5	33.3 ± 1.5	9.0 ± 4.0	141.7 ± 6.0	54.0 ± 8.5	219.3 ± 33.3	29.3 ± 2.9	22.0 ± 4.6	224.0 ± 14.1
1500 µg	37.7 ± 2.5	158.0 ± 8.5	32.3 ± 4.2	11.7 ± 3.1	145.0 ± 6.1	55.7 ± 1.5	224.3 ± 10.1	23.3 ± 1.2	19.7 ± 1.5	197.0 ± 28.0
5000 µg	41.7 ± 7.2	163.3 ± 12.9	32.7 ± 1.2	9.3 ± 0.6	161.3 ± 30.0	51.7 ± 2.9	193.7 ± 15.1	23.7 ± 2.5	19.7 ± 1.5	204.0 ± 14.9
2-NF 2 µg	268.0 ± 23.4									
NaN3 2 µg	881 ± 12.3		1417.7 ± 154.2							
9-Aa 50 µg	430.7 ± 58.0									
4-NQO 2 µg	2663.3 ± 370.9									
B[a]P 5 µg						324.7 ± 39.9		153.0 ± 23.5		
2-AAN 5 µg						4562 ± 226.6		239.3 ± 50.4		802.0 ± 87.2
Test 2										
Vehicle	26.0 ± 2.6	146.7 ± 6.4	16.0 ± 5.2	5.3 ± 1.5	124.3 ± 11.0	37.3 ± 6.0	176.7 ± 16.0	16.3 ± 0.6	27.0 ± 5.3	164.3 ± 21.8
Untreated	25.0 ± 1.7	144.7 ± 7.6	16.3 ± 4.5	8.0 ± 3.5	135.7 ± 10.0	40.0 ± 6.2	150.7 ± 11.9	17.7 ± 2.1	21.7 ± 1.2	154.3 ± 13.7
PA21-2 50 µg	25.7 ± 4.2	146.0 ± 13.1	19.3 ± 3.1	10.3 ± 2.3	120.0 ± 19.1	34.3 ± 7.1	186.3 ± 31.1	17.7 ± 2.5	25.0 ± 2.6	168.7 ± 17.6
150 µg	24.0 ± 0.0	140.7 ± 20.5	17.7 ± 5.9	9.0 ± 2.0	135.0 ± 7.5	45.0 ± 4.4	196.7 ± 25.0	20.0 ± 3.5	21.3 ± 5.7	132.7 ± 26.0
500 µg	21.7 ± 4.7	130.0 ± 8.5	14.7 ± 4.0	7.7 ± 3.1	137.3 ± 4.5	42.7 ± 3.1	177.0 ± 25.9	17.0 ± 2.6	25.7 ± 2.3	155.3 ± 11.5
1500 µg	22.0 ± 2.0	130.3 ± 6.7	16.7 ± 1.2	7.0 ± 2.0	95.7 ± 8.4	40.3 ± 1.5	156.7 ± 10.7	17.7 ± 4.5	25.0 ± 3.5	144.3 ± 19.0
5000 µg	25.7 ± 3.2	134.0 ± 9.6	13.0 ± 5.2	7.0 ± 0.0	104.0 ± 5.6	31.0 ± 5.3	165.7 ± 17.0	15.3 ± 2.1	21.7 ± 2.1	146.7 ± 19.6
2-NF 2 µg	272.3 ± 22.8									
NaN3 2 µg	635.7 ± 162.3		954.7 ± 32.9							
9-Aa 50 µg	431.0 ± 14.1									
4-NQO 2 µg	1254 ± 306									
B[a]P 5 µg						226.0 ± 65.0		113.3 ± 6.7		
2-AAN 5 µg						3271 ± 736		381 ± 36		889.7 ± 53.2

7.1.3 PA21 – In vitro mammalian chromosome aberration test in Chinese Hamster Lung (CHL) cells

Study no.:	VFR 080/043356
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Aug 10, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PA21, 423000, 97%

Key Study Findings

PA 21 312.5-5000 µg/ml did not show conclusive evidence of clastogenic activity in this in vitro cytogenetic test system under the experimental conditions described.

Methods

CHL cells grown and subcultured in tissue culture medium at 37°C were incubated with PA21 in both the absence and presence of S9 mix derived from rat livers. Vehicle 1% methylcellulose, untreated, and positive control cultures were also prepared. Two hours before the end of the incubation period, cell division was arrested using Colcemid®. The cells were then harvested and slides were prepared for examinations under light microscope. All cultures were duplicates.

- First test: With and without S9 mix, 3 hours treatment, 12 hours recovery, PA21 final concentrations 39.06, 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/ml.
- Second test: With and without S9 mix, 3 hours treatment, 12 hours recovery, PA21 final concentrations 1250, 2500, 3500, 4500 and 5000 µg/ml.
- Positive controls: Mitomycin C 0.1 and 0.2 µg /ml for without S9, Cyclophosphamide 5 and 10 µg /ml for with S9.

The proportion of mitotic cells per 1000 cells in all cultures was recorded. The cell count and the mitotic index were calculated to assess the toxicity of PA 21 to cultured CHL cells. The dose level causing ~ 50% reduction in mitotic index or, if there was no decrease, the maximum achievable concentration was used as the highest dose level for the metaphase analysis.

Metaphase cells were identified using a low power objective and examined at a magnification of x1000 using an oil immersion objective. One hundred metaphase figures were examined, where possible, from each culture. Chromosome aberrations were scored. Only cells with 23 - 27 chromosomes were analyzed. Polyploid and endoreduplicated cells were noted when seen. The vernier readings of all aberrant metaphase figures were recorded. The number of aberrant metaphase cells in each treatment group was compared with the vehicle control value using the one-tailed Fisher exact test.

Results

Precipitation was observed in all cultures containing PA21. PA 21 at various concentrations reduced cell counts up to 66% (the lowest) of the control value in test 1 with or without S9 mix, and in test 2 with S9 mix, which were not concentration-dependent (Table 82). In the presence of S9 mix, PA 21 reduced mitotic index to 48% and 62% of control value at 5000 µg/ml in test 1 and test 2, respectively. Without S9 mix, PA 21 reduced mitotic index to 63% and 87% of control value at 2500 µg/ml in test 1 and at 5000 µg/ml in test 2, respectively (Table 82).

Table 82. Cell count and mitotic index data

Test	Treatment, ug/ml	-S9, 3 h treatment + 12 h recovery			+S9, 3 h treatment + 12 h recovery		
		Relative cell count (%)	Mitotic Index		Relative cell count (%)	Mitotic Index	
			(Mean, %)	Relative, %		(Mean, %)	Relative, %
1	Untreated	-	10.7	-	-	11.2	-
	Vehicle	100	11.7	100	100	12.3	100
	PA21 39.06*	88	9.6	82	94	10.5	85
	PA21 78.13*	77	11.5	98	106	10.9	89
	PA21 156.25*	66	11.8	101	93	9.8	80
	PA21 312.5*	78	10.7	91	82	10.1	82
	PA21 625*	85	11.2	96	68	11.0	89
	PA21 1250*	83	8.9	76	74	9.0	73
	Pa21 2500*	80	7.4	63	70	6.7	54
	PA21 5000*	82	8.2	70	93	5.9	48
	Mitomycin C0.1	68	12.7	109			
	Mitomycin C0.2	67	7.7	66			
	Cydophosphamide 5				86	4.7	38
	Cydophosphamide 10				80	2.7	22
2	Untreated	-	10.1	-	-	9.9	-
	Vehicle	100	9.2	100	100	10.9	100
	PA21 1250*	113	9.7	105	82	10.9	100
	PA21 2500*	107	9	98	97	9.7	89
	PA21 3500*	97	9.1	99	84	8.4	77
	PA21 4500*	106	8.6	93	97	7.6	70
	PA21 5000*	102	8	87	79	6.8	62
	Mitomycin C0.1	85	9.3	101			
	Mitomycin C0.2	80	5.2	57			
	Cydophosphamide 5				64	6.6	61
	Cydophosphamide 10				60	3.7	34

On the basis of the cell toxicity data, PA21 concentrations 312.5, 1250 and 5000 µg/ml in test 1, 3500, 4500 and 5000 µg/ml in test 2 without S9 mix, and 2500, 3500 and 5000 µg/ml in test 2 with S9 mix were selected for metaphase analysis.

In the first test, PA 21 with or without S9 mix caused significant increases in the proportion of cells with chromosomal aberrations at 5000 µg/ml (both including and excluding gap-type aberrations; P<0.001 vs control) (Table 83, Table 84). In the second test, a significant increase in the proportion of cells with chromosomal aberrations was only seen with PA 21 5000 µg/ml without S9 mix (including gap-type aberrations only; P<0.01 vs control) (Table 85, Table 86). All positive control compounds caused large, statistically significant increases in the proportion of aberrant cells (Table 83 - Table 86), demonstrating the sensitivity of the test system and the efficacy of the S9 mix.

The reviewer agrees with the investigator's interpretation that the chromosomal aberrations noted at the high level treatment (5000 µg/ml) were not conclusive because of precipitation in the cultures, no concentration-dependent effects on cell toxicity and chromosome aberration, and not completely repeated in test 2. Thus, PA 21 312.5-5000 µg/ml did not show conclusive evidence of clastogenic activity in this in vitro cytogenetic test system, under the experimental conditions described.

Table 83. Metaphase analysis data in test 1 without S9 mix (from the application)

Nominal concentration of PA 21 (µg/ml)	No. cells examined	Aberrations						No. of aberrant cells				Relative Cell Count %	
		Chromatid type		Chromosome type		Others	Gaps		Exc. gaps	Mean %	Inc. gaps		Mean %
		ctb	cte	csb	cse		ctg	csg					
0 (Untreated)	100	2	1					3	3.0	3	4.0	-	
	100	2		1			2	3		5			
0 (1% methylcellulose solution)	100	1					3	1	1.0	4	3.0	100	
	100	1					1 1	1		2			
312.5	100	5		1				6	5.0	6	5.0	78	
	100	5	1				1	4		4			
1250	100	3			1		1	3	4.0	4	5.0	83	
	100	4			1		1	5		6			
5000	100	16		1		2	2	17	15.5	19	17.5	82	
	100	15		1	1		6	14	***	16	***		
0.1 Mitomycin C	100	18	7				2	22	19.0	23	20.0	68	
	100	10	8	3	1	1	1	16	***	17	***		

ctb - Chromatid break

cte - Chromatid exchange

csb - Chromosome break

cse - Chromosome exchange

ctg - Chromatid gap

csg - Chromosome gap

others - Cells with greater than 8 aberrations, pulverised cells and pulverised chromosomes

*** P<0.001

Table 84. Metaphase analysis data in test 1 with S9 mix (from the application)

Nominal concentration of PA 21 (µg/ml)	No. cells examined	Aberrations						No. of aberrant cells				Relative Cell Count %	
		Chromatid		Chromosome		Others	Gaps		Exc. gaps	Mean %	Inc. gaps		Mean %
		ctb	cte	csb	cse		ctg	csg					
0 (Untreated)	100	4					1		4	3.5	5	4.5	-
	100			2		1			3		3		
0 (1% methylcellulose solution)	100	2							2	2.0	2	2.5	100
	100	1		2			1		2		3		
312.5	100	4					1		4	4.0	5	4.5	82
	100	4		1				1	4		4		
1250	100	1					3		1	3.5	4	6.5	74
	100	5		1			3		6		9		
5000	100	8		1		1	3		9	8.0	12	11.0	93
	100	4		1	2		4	1	7	**	10	***	
5.0 Cyclophosphamide	100	24	14	1				2	25	25.5	26	26.0	86
	100	27	16	3			6		26	***	26	***	

*** P<0.001; ** P<0.01

Table 85. Metaphase analysis data in test 2 without S9 mix (from the application)

Nominal concentration of PA 21 (µg/ml)	No. cells examined	Aberrations						No. of aberrant cells				Relative Cell Count %	
		Chromatid		Chromosome		Others	Gaps		Exc. gaps	Mean %	Inc. gaps		Mean %
		ctb	cte	csb	cse		ctg	csg					
0 (Untreated)	100						1		0	0.0	1	1.0	-
	100						1		0		1		
0 (1% methylcellulose solution)	100	3		1			2		4	3.0	6	4.5	100
	100	1	1				1		2		3		
3500	100 ^a	5					2	2	4	5.5	8	8.5	97
	100 ^a	7					2	1	7		9		
4500	100 ^a	5				1	2		5	4.5	7	7.5	106
	100 ^a	4					5		4		8		
5000	100 ^a	6		1			3	1	6	6.5	10	11.5	102
	100 ^a	7	1	1			6	2	7		13	**	
0.1 Mitomycin C	100	27	4				5		22	19.0	25	22.5	85
	100	10	10	2			6	1	16	***	20	***	

*** P<0.001; ** P<0.01; ^a Some metaphases unscorable due to precipitate obscuring the chromosomes.

Table 86. Metaphase analysis data in test 2 with S9 mix (from the application)

Nominal concentration of PA 21 (µg/ml)	No. cells examined	Aberrations						No. of aberrant cells				Relative Cell Count %	
		Chromatid		Chromosome		Others	Gaps		Exc. gaps	Mean %	Inc. gaps		Mean %
		ctb	cte	csb	cse		ctg	csg					
0 (Untreated)	100	1		3			2		2	2.0	4	4.0	-
	100	2					2		2		4		
0 (1% methylcellulose solution)	100	3		6			1		3	2.0	4	3.5	100
	100	1					4		1		3		
2500	100 ^a	4		1		1	3		5	5.0	7	8.0	97
	100 ^a	4		2			5		5		9		
3500	100 ^a	5		1			6		3	3.5	7	8.0	84
	100 ^a	4				1	6		4		9		
5000	100 ^a	7					3		5	4.5	8	8.0	79
	100 ^a	4					5		4		8		
5 Cyclophosphamide	100	29	7	3		1	6	1	21	20.5	25	23.5	64
	100	30	2		1	1	3		20	***	22	***	

*** P<0.001. ^a Some metaphases unscorable due to precipitate obscuring the chromosomes.

7.1.4 PA21-2 – In vitro mammalian chromosome aberration test in CHL cells

Study no.: VFR0121
 Study report location: (b) (4)
 Conducting laboratory and location: (b) (4)
 Date of study initiation: June 4, 2008
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: PA21-2, 280807J1, 95.8% (b) (4)

Key Study Findings

PA21-2 showed no increase in structural aberrations and only a small increase in numerical aberrations, considered of little or no biological relevance, in this in vitro cytogenetic test system, under the conditions described.

Methods

Experimental procedures were the same as those described under section 7.2.3 “PA21 – In vitro mammalian chromosome aberration test in Chinese Hamster Lung (CHL) cells”, except test article PA21-2 and 15 hours treatment time in test 2 without S9 mix.

Results

Precipitation was observed in all cultures containing PA21-2. PA 21-2 at 5000 µg/ml reduced cell counts to 71-75% of the control value in test 1 without S9 mix and in test 2 with or without S9 mix (Table 87). Mitotic index was not reported.

Table 87. Relative cell counts (% of vehicle control. ^b precipitation)

Concentration of PA21-2 (µg/mL)	Relative cell count (%)			
	Test 1		Test 2	
	-S9	+S9	-S9	+S9
0	100	100	100	100
(1% v/v Methylcellulose)				
39.06 ^b	66	123		
78.13 ^b	98	88		
156.25 ^b	100	112		
312.5 ^b	99	116		
625 ^b	102	103		
1250 ^b	99	102	76	101
2500 ^b	95	102	78	95
3500 ^b			78	82
4500 ^b			69	75
5000 ^b	74	101	76	71
0.1 (Mitomycin C)	86		61	
0.2 (Mitomycin C)	77		80	
5 (Cyclophosphamide)		87		74
10 (Cyclophosphamide)		79		69

PA21-2 concentrations 1250, 2500, and 5000 µg/ml in test 1, 3500, 4500 and 5000 µg/ml in test 2 were selected for metaphase analysis. All positive control compounds caused statistically significant increases in the proportion of aberrant cells, demonstrating the sensitivity of the test system and the efficacy of the S9 mix.

PA21-2 without S9 caused a statistically significant increase in the proportion of cells with chromosomal aberrations at 5000 µg/mL (both including and excluding gap-type aberrations; $p < 0.001$ vs vehicle control) in the first test, but not in test 2. In the presence of S9 mix, PA21-2 did not increase in the proportion of cells with chromosomal aberrations at any dose level in either test (Table 88). The chromosomal aberrations at PA21-2 5000 µg/mL in the absence of S9 mix for 3 hour treatment was considered to be of questionable biological relevance since there were precipitations in the cultures, and not repeated in the second test.

In test 2, 15 hours continuous treatment with PA21-2 5000 µg/ml in the absence of S9 mix resulted in small increases in numerical aberrations in the form of polyploidy in this in vitro cytogenetic test system (Table 88). These increases were small, not

concentration-related, observed at highly precipitating concentrations, and were therefore considered of little or no biological relevance.

Table 88. Summary of cell numbers with chromosomal aberrations (from the application)

Exposure period (hours)	S9 mix (v/v)	Nominal concentration of PA21-2 (µg/mL)	Cells with aberrations excluding gaps			Cells with aberrations including gaps			Relative cell count (%)	Polyploidy frequency /200 cells		
			Individual values (%)	Mean (%)		Individual values (%)	Mean (%)					
First main test	3	-	0 (1% v/v Methylcellulose)	1.0	2.0	1.5	3.0	2.0	2.5	100	1	
			1250	5.0	5.0	5.0	9.0	6.0	7.5	99	1	
			2500	3.0	3.0	3.0	5.0	7.0	6.0	95	3	
			5000	7.0	5.0	6.0**	9.0	7.0	8.0**	74	3	
			0.1 (Mitomycin C)	28.0	22.0	25.0***	29.0	22.0	25.5***	86	1	
	3	+	(2%)	0 (1% v/v Methylcellulose)	2.0	1.0	1.5	2.0	1.0	1.5	100	1
				1250	3.0	4.0	3.5	4.0	7.0	5.5	102	4
				2500	4.0	2.0	3.0	5.0	4.0	4.5	102	1
				5000	2.0	5.0	3.5	2.0	5.0	3.5	101	1
				5 (Cyclophosphamide)	35.0	33.0	34.0***	38.0	34.0	36.0***	87	1
Second main test	15	-	(1% v/v Methylcellulose)	1.0	2.0	1.5	2.0	3.0	2.5	100	2	
			3500	2.0	3.0	2.5	6.0	4.0	5.0	78	12**	
			4500	2.4	2.5	2.4	7.1	3.7	4.9	69	8**	
			5000	5.0	2.0	3.5	8.0	7.0	7.5	76	13**	
			0.1 (Mitomycin C)	76.0	59.0	67.5***	80.0	61.0	70.5***	61	4	
	3	+	(2%)	(1% v/v Methylcellulose)	4.0	2.0	3.0	4.0	4.0	4.0	100	3
				2500	6.0	5.0	5.5	6.0	7.0	6.5	95	7
				3500	5.0	3.0	4.0	8.0	6.0	7.0	82	5
				5000	4.0	7.0	5.5	6.0	8.0	7.0	71	6
				5 (Cyclophosphamide)	41.0	39.0	40.0***	41.0	40.0	40.5***	74	6

One-tailed Fisher's exact test *** $p < 0.001$ ** $p < 0.01$

7.2 In vivo assays

7.2.1 PA21 - Rat peripheral blood micronucleus test using flowcytometric analysis (part of the 26-week repeat dose toxicity study in rats, VFR0096)

Study no.: VFR0096
 Study report location: (b) (4)
 Conducting laboratory and location: (b) (4)
 Date of study initiation: Sept 22, 2006
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: PA21; 429000/95.4%, 423000/95.6%
 Deviation from study protocol: No impact to the results

Key Study Findings

PA21 at 200, 750 or 2500 mg/kg/day for 4 or 24 weeks in rats did not cause any statistically significant increases in the number of micronucleated reticulocytes or any statistically significant decreases in the proportion of reticulocytes, and was consequently negative in this test.

Methods

Peripheral blood samples (5/sex/group) were collected from both male and female CD1 Sprague Dawley rats at weeks 4 and 24 in a dietary toxicology study with PA21 at 200, 750 or 2500 mg/kg/day (see details in VFR0096). Samples from the positive control group were collected in week 24 approximately 48 hours after the second dose of cyclophosphamide (20 mg/kg/day). The heparinized blood samples were fixed in pre-chilled methanol at -80°C, and stained with anti-CD71-FITC conjugate. RNA content in the samples was eliminated with ribonuclease A. The FITC labeled cells were then added with ice cold propidium iodide solution, and immediately analyzed using flow cytometer.

For the flow cytometer analysis, the bivariate graphs for rat samples were generated by the analysis of fixed rat erythrocytes infected with malaria (*P. berghei*). The frequencies of each cell population were automatically calculated by the computer software upon collection of the total number of reticulocytes. The dual labeling methodology with anti-CD71-FITC and Propidium Iodide allows the resolution of 5 cell types in whole peripheral blood. Instrument settings and fluorescent compensations were adjusted accordingly to optimize the RET (reticulocytes), MNRET (micronucleated reticulocytes), NCE (normochromatic erythrocytes), MNNCE (micronucleated normochromatic erythrocytes), and nucleated cell populations. A higher green fluorescent threshold was used for rat samples so that MN analysis was restricted to the youngest fraction of RETS. Flow cytometric measurements were restricted to the youngest fraction of reticulocytes based on transferrin receptor (CD71) staining.

The following criteria were applied for assessment of assay acceptability:

1. Each treated and control group should include at least 5 analyzable animals.
2. Diet control values for micronucleated reticulocytes must be consistent.
3. Positive controls must show clear unequivocal positive responses.
4. The proportion of reticulocytes among total erythrocytes in treated groups is not less than 20% of the control value.

Results

All criteria were met for the micronucleus test. The positive control compound cyclophosphamide (20 mg/kg, two doses) produced significant decreases in the proportion of reticulocytes and increases in the frequency of micronucleated reticulocytes (Table 89). Treatment with PA21 at 200, 750 or 2500 mg/kg/day for 4 or 24 weeks in rats did not cause any changes in either the proportion of reticulocytes or the

frequency of micronucleated reticulocytes treated, compared to diet control values (Table 89).

Table 89. Summary of reticulocyte proportion and micronucleated reticulocytes

Treatment	Dose mg/ kg/ day	Week	Proportion of RET (%)		Incidence MNRET (%)	
			Male	Female	Male	Female
Control diet	-	4	1.77 ± 0.24	1.07 ± 0.26	0.19 ± 0.10	0.21 ± 0.09
PA21	200		2.30 ± 0.60	1.07 ± 0.31	0.21 ± 0.08	0.31 ± 0.20
PA21	750		1.81 ± 0.50	1.31 ± 0.64	0.24 ± 0.10	0.19 ± 0.11
PA 21	2500		2.25 ± 0.51	1.13 ± 0.33	0.21 ± 0.09	0.17 ± 0.13
Control diet	-	24	1.58 ± 0.18	0.95 ± 0.23	0.30 ± 0.11	0.25 ± 0.14
PA21	200		1.53 ± 0.33	0.12 ± 0.09	0.29 ± 0.09	0.18 ± 0.05
PA21	750		1.31 ± 0.27	1.15 ± 0.30	0.26 ± 0.15	0.23 ± 0.07
PA 21	2500		1.43 ± 0.34	0.99 ± 0.21	0.26 ± 0.08	0.20 ± 0.06
Cydophosphamide	20#		0.04 ± 0.02**	0.04 ± 0.01**	4.53 ± 3.05**	3.99 ± 2.46**

RET - Reticulocytes. MNRET - Micronucleated reticulocytes. ** p<0.01 vs diet control.

- Dosed twice at approximately 48 and 72 hours prior to termination

7.2.2 PA21 - Detection of DNA damage in the stomach, duodenum, and colon of treated rats using the Comet assay

Study no.: 225/4; 225/6
 Study report location: (b) (4)
 Conducting laboratory and location: (b) (4)
 Date of study initiation: Jan 22, 2007; April 25, 2007
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: PA21, 230106J1, and 96%

Key Study Findings

Under the conditions of this Comet assay, PA21 up to 4000 mg/kg/day did not induce DNA damage in the stomach, duodenum, and colon of rats, when analyzed 3 hours after the second dose administration.

Methods

Groups of six male Sprague Dawley Crl:CD® (SD) rats (7-8 weeks old, body weight 263-322 g) were orally administered PA21 at 1000 and 4000 mg/kg/day, or vehicle 1% methylcellulose (10 ml/kg) once daily for two consecutive days, or a single dose the positive control ethyl methanesulfonate (EMS, dissolved in purified water) 250 mg/kg 3 hours prior to sampling. Animals were sacrificed 3 hours after the second administration, and stomach, duodenum, and colon samples were analyzed for DNA damage using the single cell gel electrophoresis assay (i.e. the Comet assay, 100

cells/sample were scored). Doses selected in this study were based on previous toxicity studies in rats.

Results

All test article formulations were found to be within 90-110% of nominal concentrations. No adverse effects or clinical signs were observed following dosing.

The mean tail moment and tail intensity for the vehicle control groups fell within the laboratory's historical vehicle control range. Mean tail moment and tail intensity values for the positive control groups exhibited a clear increase over concurrent vehicle controls. The studies were therefore accepted as valid (Table 90).

The supplementary cytotoxicity data ('cloud' assessment and diffusion slide analysis) for stomach, duodenum, and colon tissues from animals treated with PA21 were comparable to the concurrent vehicle control animals. Comet analysis of stomach, duodenum, and colon provided tail moment and tail intensities values that were considered consistent with the concurrent vehicle control group (Table 90). There was no evidence of any cross-linking or DNA damage effects.

Table 90. Summary data of the Comet assay

Treatment group (mg/kg/day)	Tail Moment	Tail Intensity % DNA in the tail	% clouds	% Diffused cells
Stomach				
Vehicle (0)	0.33 ± 0.04	2.68 ± 0.15	15.0 ± 0.06	14.8 ± 0.13
PA21 (1000)	0.27 ± 0.03	2.61 ± 0.28	15.3 ± 0.04	13.7 ± 0.08
PA21 (4000)	0.28 ± 0.02	2.73 ± 0.15	14.5 ± 0.05	13.7 ± 0.20
Ethyl methanesulfonate (250)	2.42 ± 0.21	9.73 ± 1.01	17.8 ± 0.04	18.5 ± 0.10
Duodenum				
Vehicle (0)	0.45 ± 0.07	2.87 ± 0.37	10.5 ± 0.08	12.7 ± 0.14
PA21 (1000)	0.27 ± 0.04	2.05 ± 0.28	11.5 ± 0.04	13.8 ± 0.09
PA21 (4000)	0.25 ± 0.02	2.04 ± 0.14	10.8 ± 0.06	12.7 ± 0.08
Ethyl methanesulfonate (250)	2.35 ± 0.22	10.04 ± 0.70	12.3 ± 0.07	10.3 ± 0.02
Colon				
Vehicle	1.75 ± 0.29	0.28 ± 0.05	24.0 ± 0.17	20.33 ± 0.08
PA21 (1000)	2.06 ± 0.29	0.37 ± 0.08	23.5 ± 0.10	26.67 ± 0.10
PA21 (4000)	2.22 ± 0.38	0.37 ± 0.07	21.8 ± 0.13	26.20 ± 0.13
Ethyl methanesulfonate (250)	7.72 ± 0.41	1.99 ± 0.15	24.3 ± 0.04	23.67 ± 0.11

In conclusion, under the conditions of this Comet assay, PA21 up to 4000 mg/kg/day for two days in rats did not induce DNA damage in the stomach, duodenum, and colon, when tissues were sampled and analyzed 3 hours after the second dose administration.

8 Carcinogenicity

8.1 PA21 - Carcinogenicity study by dietary administration to CD rats for 104 weeks

Study no.:	VFR0104	
Study report location:		(b) (4)
Conducting laboratory and location:		(b) (4)
Date of study initiation:	Feb 1, 2007	
GLP compliance:	Yes	
QA statement:	Yes	
Drug, lot #, and % purity:	PA21, 423000/95.6%, 426000/94.7%, 010307J1/95.8%, 020307J1/96%	
CAC concurrence:	No	
Deviation from study protocol:	No impact to the results	

Key Study Findings

There was a slightly increased incidence of benign thyroid c-cell adenoma in males at the dose 2500 mg/kg/day, which was not dose-dependent nor statistically significant. At doses 750 and/or 2500 mg/kg/day, PA21 resulted in minor lower body weight gain, markedly higher plasma and tissue iron level, slightly depressed kidney function (higher plasma urea and phosphorus, lower urine volume and urine phosphorus), and slightly elevated bone turnover due to reduced GI phosphate uptake (slightly higher levels of plasma calcium, urine calcium, serum 1,25-dihydroxyvitamin D, osteocalcin and deoxypyridinoline). Treatment-related non-neoplastic histological findings included higher level of pigmented macrophages (iron content) in gastro-intestinal tract, liver, kidney, spleen, pancreas, adrenal gland, mesenteric, mandibular, and axillary lymph nodes at doses 750 and 2500 mg/kg/day; and epithelial hyperplasia in gastro-intestinal tract, lymphoid aggregation, inflammation, and cyst in various organs/tissues at 2500 mg/kg/day.

Methods

CrI:CD (SD) rats, initial age of 5-6 weeks, were on diet containing PA21 at 0, 200, 750 and 2500 mg/kg/day (n = 65/sex/group). The highest dose 2500 mg/kg/day was based on the 13- and 26-week toxicity studies in rats (VFR087/063191 and VFR096/072989). At 3000 mg/kg/day in the 13-week study, there was reduced weight gain (33% in males and 19% in females) and one possible treatment-related death. A dosage of 2500 mg/kg/day was tolerated in the 26-week study, with slight reduction in weight gain, limited iron deposition in some tissues, and hyperplasia in large intestine and urinary bladder mucosa/epithelia. The low dose 200 mg/kg/day is close to the highest likely human clinical dose. The study was designed to continue for 104 weeks. However, the study with female rats was terminated at week 100/101 because of poor survival in both control and intermediate dose groups. Samples of the drug-diet formulations were

analyzed at weeks 1, 13, 26, 39, 52, 65, 78, 91, 100/103 of treatment to confirm the concentrations of PA21 in the treated diets and the absence in the control diet

During the study, rats were inspected visually at least twice daily for clinical signs and morbidity/death. Physical examination, including palpation, was weekly performed on each animal. Body weight was recorded at study initiation, weekly for first 16 weeks, every 2 weeks from Week 16 to 28, every four weeks thereafter, and at the end of the final week of treatment. Mean weekly food consumption per animal was assessed at week -1, each week during weeks 1-16, once every two week during weeks 17-28, once every 4 weeks during weeks 29-104. Ophthalmoscopy was performed prior to treatment on all animals, at week 52 and before study termination on 20 animals/sex of control and high dose groups. Blood samples were collected from all surviving animals during week 52 and before study termination after overnight fast, and from animals killed prematurely when possible, for determining hematology, plasma chemistry (n=20/sex/group, Table 91), and serum osteocalcin level (bone turnover marker, n=20/sex/group). Serum levels of vitamin A, 25-hydroxy vitamin D, and 1,25-dihydroxy vitamin D were determined at study termination from 10 rats/sex/group. Overnight urine samples were collected from 20 rats/sex/group during week 52 and at the end of treatment period, for urinalysis (Table 45) and for determining deoxypyridinoline (bone turnover marker) and creatinine (for normalization of deoxypyridinoline values).

Table 91. Parameters of hematology and plasma chemistry

Hematology parameters	Plasma chemistry
Haematocrit (Hct)	Alkaline phosphatase (ALP)
Haemoglobin concentration (Hb)	Alanine aminotransferase (ALT)
Erythrocyte count (RBC)	Aspartate aminotransferase (AST)
Reticulocyte count (Retic)	Total bilirubin (Bili)
Mean cell haemoglobin (MCH)	Urea
Mean cell haemoglobin concentration (MCHC)	Creatinine (Creat)
Mean cell volume (MCV)	Glucose (Gluc)
Total white cell count (WBC)	Total cholesterol (Chol)
Differential WBC count	Triglycerides (Trig)
Neutrophils (N)	Phospholipids (PLIP)
Lymphocytes (L)	Sodium (Na)
Eosinophils (E)	Potassium (K)
Basophils (B)	Chloride (Cl)
Monocytes (M)	Calcium (Ca)
Large unstained cells (LUC)	Inorganic phosphorus (Phos)
Platelet count (Plt)	Iron (Fe)
Morphology flags	Total protein (Total Prot)
Prothrombin time (PT)	albumin (Alb)
Activated partial thromboplastin time (APTT)	α 1, α 2, β and γ globulin

A detailed necropsy was performed on each animal killed either prematurely or at the end of scheduled treatment period. Tissue samples from liver (residue of left lobe), kidney (poles from left kidney), and spleen (poles) of 20 males and 10 females/group were weighed, and snap-frozen in liquid nitrogen for determination of iron content. Lumbar vertebra 6 and right tibia of 20 males and 10 females/group were collected for

histomorphometry analysis. Organs/tissues listed in Table 92 were sampled from all necropsied animals, and histologically processed. A complete histopathological examination was performed on all animals from all groups found dead, killed moribund, or sacrificed during or at the end of the experiment. Samples of any macroscopic abnormal tissues were also retained and processed for histopathological examination.

Table 92. Pathology procedures (modified from the application)

Tissue and regions to be examined	Necropsy		Histology	Pathology
	Weigh	Fix		Light microscopy
Abnormalities		*	*	*
Adrenals		*	*	*
Aorta - thoracic		*	*	*
Brain (cerebellum, cerebrum, midbrain)		*	*	*
Caecum		*	*	*
Colon		*	*	*
Duodenum		*	*	*
Epididymides		*	*	*
Eyes		*	*	*
Femur (longitudinal section through joint)		b)	*	*
Harderian glands		*	*	*
Head		a)	#	#
Heart (including auricular and ventricular regions)		*	*	*
Pleum		*	*	*
Jejunum		*	*	*
Kidneys	*d)	*	*	*
Lachrymal glands		*	*	*
Larynx		*	*	*
Liver (section from all main lobes)	*d)	*	*	*
Lungs (section from two major lobes including bronchi)		*	*	*
Lymph nodes – mandibular		*	*	*
- mesenteric		*	*	*
- left axillary		*	*	*
Oesophagus		*	*	*
Optic nerves		*	*	*
Ovaries (with oviduct)		*	*	*
Pancreas		*	*	*
Peyer's patches		*	*	*
Pituitary		*	*	*
Preputial/clitoral gland		*	*	*
Prostate		*	*	*
Rectum		*	*	*
Salivary glands – submandibular		*	†	†
- parotid		*	#	#
- sublingual		*	#	#
Sciatic nerves		*	†	†
Seminal vesicles		*	*	*
Skeletal muscle		*	†	†
Skin with mammary glands (inguinal area)		*	*	*
Spinal cord (transverse and longitudinal sections at the cervical, thoracic and lumbar levels)		*	*	*
Spleen	*d)	*	*	*
Sternum		*	*	*
Stomach		*	*	*
Testes		*	*	*
Thymus		*	*	*
Thyroid with parathyroids		*	*	*
Tongue		*	*	*
Trachea		*	*	*
Ureters		*	*	*
Urinary bladder		*	*	*
Uterus with cervix		*	*	*
Vagina		*	*	*
Zymbals gland with external ear		*	#	#

a) Including nasal cavity, paranasal sinuses and nasopharynx. b) Both hindlimbs retained, one sectioned where appropriate.

d) Additional samples weighed and snap frozen in liquid nitrogen for iron analysis.

* Organs weighed, samples fixed or sections examined microscopically.

Examined if effects suspected during the study. † Only one examined.

Results

The overall achieved doses at 200, 750, and 2500 mg/kg/day were 206, 761, and 2542 mg/kg/day for males and 205, 765, 2547 mg/kg/day for females, respectively.

There was no treatment-related effect on mortality (Table 93). Feces in the treated group were darker than in control group due to the brown color of PA21. There were increased incidences of tails with brown staining and/or scabs at doses ≥ 750 mg/kg/day (Table 101). Body weight gain was lower in males during first week at doses ≥ 750 mg/kg/day and over weeks 0-103 at the dose 2500 mg/kg/day, and trended lower in females over weeks 0-88 at 2500 mg/kg/day (Figure 11, Table 94). There were no treatment-related effects on food consumption and ophthalmoscopy.

Table 93. Mortality over study period in control and PA21 treatment groups

Group Week	Control		200 mg/kg/day		750 mg/kg/day		2500 mg/kg/day	
	No. of Death	Cum. %						
Males								
0 - 52	2	3.08	2	3.08	1	1.54	4	6.15
53 - 78	10	18.46	15	26.15	10	16.92	9	20.00
79 - 91	10	33.85	13	46.15	17	43.08	16	44.62
92 - 103	22	67.69	11	63.08	7	53.85	11	61.54
Ter. Sac.	21	32.31	24	36.92	30	46.15	25	38.46

Total	N=65		N=65		N=65		N=65	
Females								
0 - 52	3	4.62	2	3.08	1	1.54	5	7.69
53 - 78	21	36.92	13	23.08	16	26.15	12	26.15
79 - 91	11	53.85	15	46.15	21	58.46	17	52.31
92 - 99	11	70.77	8	58.46	7	69.23	5	60.00
Ter. Sac.	19	29.23	27	41.54	20	30.77	26	40.00

Total	N=65		N=65		N=65		N=65	

Figure 11. Mean body weight during experiment (modified from the application)

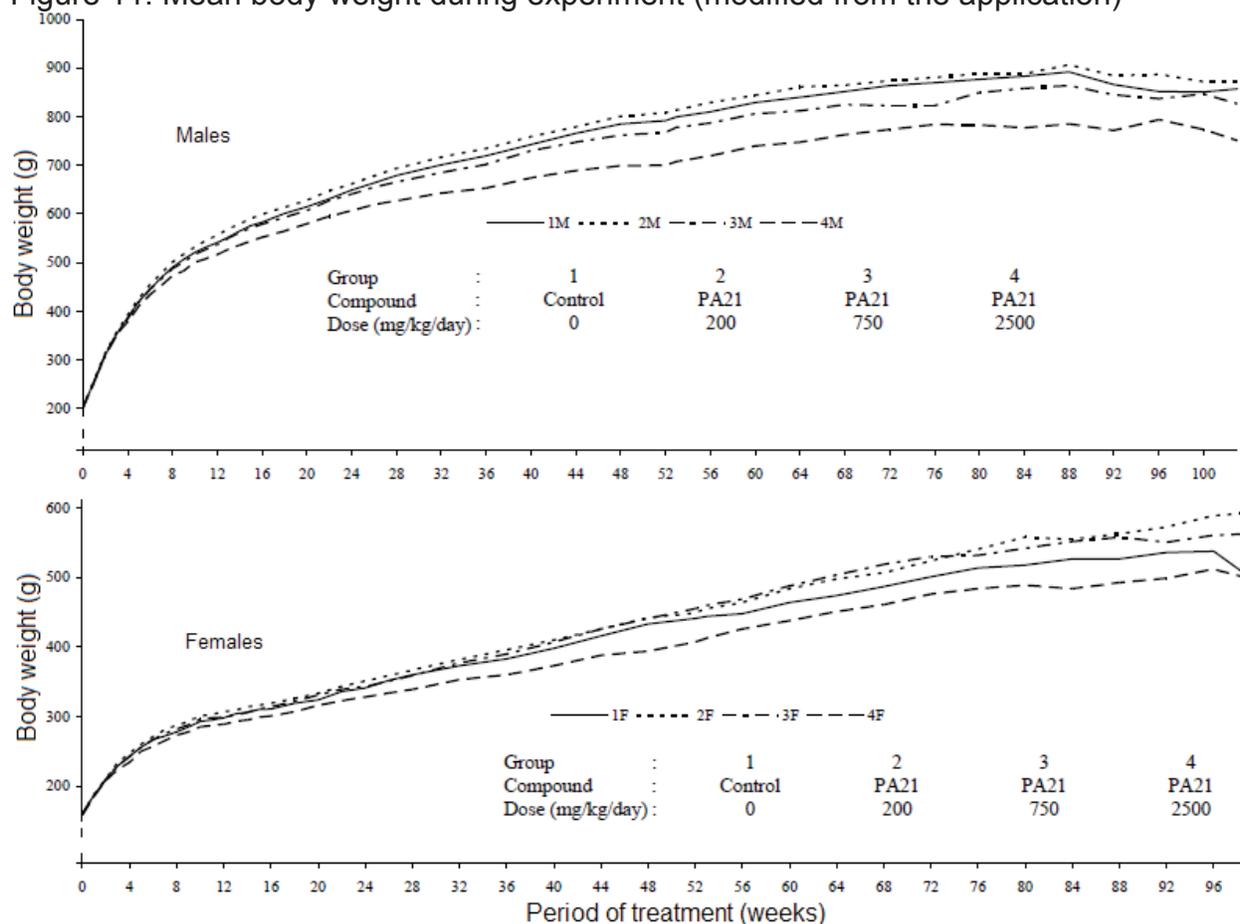


Table 94. Body weight gain (g) during the study (Mean ± SD)

Sex	Group/dose	Week 0-1	Weeks 0-16	Weeks 0-53	Weeks 0-88	Weeks 0-103/99 [§]
Male	Control	57 ± 7.7	384 ± 57.4	600 ± 93.1	694 ± 135.3	663 ± 108
		n=65	n=64	n=63	n=47	n=19
	200 mg/kg/day	57 ± 9.6	400 ± 54.7	615 ± 103.1	709 ± 122.2	675 ± 111.5
		n=65	n=65	n=63	n=42	n=24
	750 mg/kg/day	54 ± 6.5**	380 ± 57.7	579 ± 95.6	665 ± 115.1	631 ± 128.3
		n=65	n=66	n=64	n=43	n=30
	2500 mg/kg/day	49 ± 9.8**	350 ± 59.9**	506 ± 85.5**	586 ± 121.3**	550 ± 98.8**
		n=65	n=64	n=61	n=40	n=25
Female	Control	27 ± 6.1	150 ± 24.9	283 ± 65.6	369 ± 105	346 ± 92
		n=65	n=65	n=61	n=34	n=19
	200 mg/kg/day	29 ± 7.5	160 ± 24.4	295 ± 64.5	403 ± 78.2	434 ± 92.9*
		n=65	n=65	n=63	n=38	n=27
	750 mg/kg/day	27 ± 7.9	155 ± 25.6	301 ± 74	398 ± 124	404 ± 134.7
		n=65	n=65	n=64	n=33	n=21
	2500 mg/kg/day	26 ± 7.5	143 ± 25.7	255 ± 64.4*	335 ± 86.2	343 ± 78.1
		n=65	n=65	n=60	n=33	n=26

[§] Weeks 0-103 for males and Weeks 0-99 for females. * p<0.05 vs Control; ** p<0.01 vs control.

Changes in hematology were limited to minor increases in red blood cell parameters, (Table 95), which were within the physiological variation and may be related to increased iron uptake. Plasma chemistry showed higher levels of iron in both sexes at weeks 52 and/or 103/100 at doses 750 and/or 2500 mg/kg/day. There were also higher plasma levels of urea, phosphorus in males and/or females at weeks 52 and/or 103/100, at doses 750 and/or 2500 mg/kg/day. Difference in plasma calcium levels were minor and within the physiological variation (Table 96). At doses 750 and/or 2500 mg/kg/day, both males and/or females showed lower urine phosphorus and higher urine calcium at weeks 52 and/or 103/100 (Table 97), lower serum 25-hydroxyvitamin D and higher serum 1,25-dihydroxyvitamin D at week 103/100, higher serum osteocalcin and deoxypyridinoline at weeks 52 and/or 103/100 (Table 98). These changes in serum chemistry and slightly elevated bone turnover markers, subsequent to reduced GI phosphate uptake, are likely to be the mechanism for normal animals to maintain serum phosphorus levels while on diet containing phosphate binder. At week 103/100, iron levels in liver, kidney, and spleen were higher in males and/females at doses 750 and/or 2500 mg/kg/day (Table 100). Other changes were minimal and within the physiological variation and are toxicologically insignificant.

Table 95. Hematological findings (Mean \pm SD)

Sex	Group/PA21 dose	Hct (L/L)	Hb (g/dL)	RBC ($\times 10^{12}$ /L)	Retic (%)	MCH (pg)	MCHC (g/dL)	MCV (fL)
Week 52 (n=53-63)								
Male	Control	0.461 \pm 0.021	14.7 \pm 0.8	8.75 \pm 0.45	2.51 \pm 0.6	16.9 \pm 0.89	32 \pm 0.66	52.7 \pm 2.12
	200 mg/kg/day	0.462 \pm 0.022	14.9 \pm 0.8	8.73 \pm 0.52	2.44 \pm 0.56	17.1 \pm 0.9	32.3 \pm 0.67*	53 \pm 2.01
	750 mg/kg/day	0.455 \pm 0.016	14.9 \pm 0.6	8.45 \pm 0.34**	2.37 \pm 0.46	17.6 \pm 0.72**	32.7 \pm 0.53**	53.8 \pm 1.81**
	2500 mg/kg/day	0.476 \pm 0.019**	15.7 \pm 0.6**	8.49 \pm 0.37**	2.37 \pm 0.34	18.5 \pm 0.7**	33 \pm 0.57**	56.1 \pm 2.01**
Female	Control	0.417 \pm 0.019	14.2 \pm 0.6	7.56 \pm 0.35	2.20 \pm 0.47	18.7 \pm 0.67	34 \pm 0.6	55.1 \pm 1.54
	200 mg/kg/day	0.419 \pm 0.025	14.1 \pm 0.9	7.57 \pm 0.52	2.28 \pm 0.98	18.6 \pm 0.81	33.6 \pm 0.48**	55.4 \pm 2.4
	750 mg/kg/day	0.417 \pm 0.019	14.0 \pm 0.7	7.45 \pm 0.38	2.17 \pm 0.43	18.8 \pm 0.69	33.6 \pm 0.86	56 \pm 1.58*
	2500 mg/kg/day	0.421 \pm 0.022	14.2 \pm 0.9	7.41 \pm 0.48	2.42 \pm 0.72	19.2 \pm 0.99**	33.8 \pm 1.43	56.9 \pm 1.87**
Week 103/100 [§] (n=18-30)								
Male	Control	0.409 \pm 0.054	13.9 \pm 1.9	7.81 \pm 1.26	3.91 \pm 4.42	18 \pm 2.09	34 \pm 0.97	52.9 \pm 5.72
	200 mg/kg/day	0.421 \pm 0.035	14.4 \pm 1.3	8.02 \pm 0.78	2.91 \pm 1.71	18 \pm 0.78	34.2 \pm 0.85	52.6 \pm 1.88
	750 mg/kg/day	0.427 \pm 0.018	14.8 \pm 0.7	8.09 \pm 0.50	2.27 \pm 0.64	18.4 \pm 0.99*	34.8 \pm 0.60**	52.8 \pm 2.34
	2500 mg/kg/day	0.441 \pm 0.030**	15.3 \pm 1.1**	8.29 \pm 0.61	1.99 \pm 0.62**	18.5 \pm 0.68**	34.7 \pm 0.50**	53.2 \pm 1.91*
Female	Control	0.422 \pm 0.028	14.4 \pm 0.9	7.79 \pm 0.48	1.93 \pm 0.56	18.5 \pm 0.85	34.1 \pm 0.92	54.2 \pm 1.75
	200 mg/kg/day	0.411 \pm 0.033	14.1 \pm 1.3	7.39 \pm 0.80	2.95 \pm 2.75*	19.2 \pm 1.88	34.4 \pm 0.92	56 \pm 5.28
	750 mg/kg/day	0.385 \pm 0.028**	13.6 \pm 1.0	6.95 \pm 0.62	2.46 \pm 0.98*	19.6 \pm 1.03*	35.2 \pm 0.60**	55.5 \pm 2.6
	2500 mg/kg/day	0.416 \pm 0.031	14.5 \pm 1.4	7.51 \pm 0.62	2.48 \pm 0.60*	19.3 \pm 1.19*	34.8 \pm 1.67**	55.5 \pm 2.22

[§] Week103 for males and Week 100 for females. * p<0.05 vs Control; ** p<0.01 vs control.

Table 96. Plasma chemistry findings (Mean ± SD)

Sex	Group/PA21 dose	Urea (mmol/L)	Ca (mmol/L)	Phos (mmol/L)	Fe (µmol/L)
Week 52 (n=20)					
Male	Control	3.94 ± 0.87	2.67 ± 0.06	1.73 ± 0.15	35 ± 0.89
	200 mg/kg/day	3.92 ± 0.77	2.67 ± 0.06	1.73 ± 0.09	40 ± 8*
	750 mg/kg/day	3.97 ± 0.71	2.73 ± 0.09**	2.01 ± 0.31**	42 ± 6.9**
	2500 mg/kg/day	5.27 ± 1.12**	2.84 ± 0.08**	2.07 ± 0.33**	67 ± 17.7**
Female	Control	4.29 ± 0.89	2.78 ± 0.06	1.48 ± 0.17	81 ± 16.9
	200 mg/kg/day	4.13 ± 1.19	2.77 ± 0.09	1.38 ± 0.23	76 ± 16.3
	750 mg/kg/day	3.93 ± 1.45	2.76 ± 0.07	1.66 ± 0.24*	90 ± 17.3
	2500 mg/kg/day	4.85 ± 1.37	2.73 ± 0.09*	1.97 ± 0.38**	90 ± 13.5
Week 103/100 [§] (n=19-20)					
Male	Control	3.73 ± 1.26	2.69 ± 0.08	1.47 ± 0.15	40 ± 14.4
	200 mg/kg/day	3.68 ± 1.62	2.69 ± 0.07	1.46 ± 0.16	40 ± 14
	750 mg/kg/day	3.49 ± 0.91	2.68 ± 0.06	1.48 ± 0.28	39 ± 11.4
	2500 mg/kg/day	4.46 ± 1.12	2.73 ± 0.09	1.44 ± 0.26	61 ± 18.8*
Female	Control	4.84 ± 0.75	2.68 ± 0.12	1.33 ± 0.15	54 ± 11.5
	200 mg/kg/day	4.92 ± 1.05	2.74 ± 0.10	1.37 ± 0.16	55 ± 16.9
	750 mg/kg/day	4.95 ± 0.95	2.66 ± 0.11	1.41 ± 0.20	61 ± 19.7
	2500 mg/kg/day	6.49 ± 1.40**	2.74 ± 0.09	1.97 ± 0.34**	72 ± 22.3**

[§] Week103 for males and Week 100 for females. * p<0.05 vs Control; ** p<0.01 vs control.

Table 97. Urinalysis findings (Mean ± SD)

Sex	Group/PA21 dose	Vol (ml)	pH	SG (g/L)	U-ca (mmol/L)	U-IP (µmol/L)
Week 52 (n=19-20)						
Male	Control	12.4 ± 3.8	7.6 ± 0.43	1028 ± 3.3	2.88 ± 2.07	28 ± 9
	200 mg/kg/day	12.3 ± 3.3	7.7 ± 0.39	1028 ± 5.5	2.20 ± 1.72	30 ± 12
	750 mg/kg/day	10.4 ± 3.4	7.9 ± 0.52**	1029 ± 6.5	4.05 ± 3.23	13 ± 14**
	2500 mg/kg/day	6.2 ± 2.7**	8.4 ± 0.29**	1039 ± 7.3**	22.99 ± 8.66**	0.3 ± 0.2**
Female	Control	7.8 ± 2.6	6.6 ± 0.58	1026 ± 4.3	8.59 ± 3.00	49 ± 13
	200 mg/kg/day	9.3 ± 3.0	6.6 ± 0.57	1026 ± 5.9	9.10 ± 4.24	48 ± 16
	750 mg/kg/day	9.0 ± 3.1	6.8 ± 0.55	1024 ± 3.5	7.45 ± 3.15	44 ± 12
	2500 mg/kg/day	6.8 ± 2.8	8.1 ± 0.63**	1026 ± 5.1	11.46 ± 7.11	10 ± 16**
Week 103/100 [§] (n=18-20)						
Male	Control	10.9 ± 5.0	8.2 ± 0.59	1026 ± 6.0	3.43 ± 4.86	28 ± 13
	200 mg/kg/day	14.7 ± 4.8*	8.1 ± 0.75	1024 ± 5.8	3.95 ± 3.39	21 ± 10*
	750 mg/kg/day	14.5 ± 4.1*	7.9 ± 0.56	1022 ± 3.6	2.7 ± 1.50	13 ± 11**
	2500 mg/kg/day	8.2 ± 3.4	8.6 ± 0.45	1031 ± 7.5**	17.69 ± 14.68**	2.1 ± 8.4**
Female	Control	10.5 ± 4.3	6.6 ± 0.54	1021 ± 4.4	7.89 ± 3.9	32 ± 12
	200 mg/kg/day	13.9 ± 3.3	6.8 ± 0.47	1019 ± 3.8	7.59 ± 2.88	28 ± 9
	750 mg/kg/day	11.4 ± 5.3	7.2 ± 0.79**	1021 ± 6.7	8.78 ± 4.11	19 ± 16*
	2500 mg/kg/day	9.8 ± 3.9	8.1 ± 0.41**	1020 ± 3.7	13.42 ± 6.66**	2.6 ± 5.7**

[§] Week103 for males and Week 100 for females. * p<0.05 vs Control; ** p<0.01 vs control.

Table 98. Vitamin D and bone turnover makers in serum (Mean \pm SD)

Sex	Group/ PA21 dose (mg/kg/day)	25-hydroxy vitamin D (ng/ml)	1,25-dihydroxy vitamin D (ng/ml)	Osteocalcin (ng/ml)	Deoxypyridinoline/ creatinine (nM/mM)
Week 52 (n=20)					
Male	Control			7.25 \pm 0.73	44.52 \pm 3.15
	200			8.26 \pm 0.90	40.83 \pm 3.12
	750			12.63 \pm 0.61*	53.97 \pm 3.06*
	2500			12.82 \pm 1.78*	98.86 \pm 9.73*
Female	Control			8.32 \pm 0.44	12.29 \pm 0.79
	200			9.88 \pm 0.65	12.24 \pm 1.08
	750			11.35 \pm 0.59**	15.63 \pm 1.40
	2500			13.77 \pm 0.60**	43.11 \pm 4.07*
Week 103/100 [§] (n=19-20)					
Male	Control	8.98 \pm 1.09	49.81 \pm 20.53	11.94 \pm 0.77	41.73 \pm 4.52
	200	6.61 \pm 1.28	33.33 \pm 12.10	18.05 \pm 2.82*	35.15 \pm 2.64
	750	7.73 \pm 0.98	49.77 \pm 16.72	11.63 \pm 0.78	40.15 \pm 4.08
	2500	4.54 \pm 0.73**	547.09 \pm 87.51*	11.67 \pm 0.91	89.87 \pm 10.44*
Female	Control	8.62 \pm 1.14	36.22 \pm 14.87	8.87 \pm 0.50	21.19 \pm 3.83
	200	6.71 \pm 0.86	36.39 \pm 19.16	8.92 \pm 0.52	19.02 \pm 3.63
	750	5.75 \pm 0.49**	15.53 \pm 5.68	8.45 \pm 0.45	13.91 \pm 1.40
	2500	6.75 \pm 0.66	275.5 \pm 103.6	10.61 \pm 0.55*	28.49 \pm 5.18

[§] Week103 for males and Week 100 for females. * p<0.05 vs Control; ** p<0.01 vs control.

Table 99. Serum vitamin levels in week 56*

PA21 Dose (g/kg/day)	Vitamin A (μ mol/L)		25-hydroxyvitamin D (nmol/L)		25-dihydroxyvitamin D (pmol/L)	
	Males	Females	Males	Females	Males	Females
0	1.21 \pm 0.27	0.78 \pm 0.19	66 \pm 30	57 \pm 21	64 \pm 53	23 \pm 6
0.2	1.34 \pm 0.26	0.84 \pm 0.20	75 \pm 31	38 \pm 12	25 ^a \pm 18	30 \pm 5
0.75	1.21 \pm 0.19	0.69 \pm 0.10	60 \pm 16	43 \pm 17	75 \pm 79	35 ^a \pm 14
2.5	1.33 \pm 0.20	0.72 \pm 0.10	57 \pm 14	56 \pm 19	461 ^b \pm 103	211 ^b \pm 114

a - p<0.05; b - p<0.01 vs control (0)

* Study validations (Study number WLY0013) indicated that these assays with the exception of 25-hydroxyvitamin D failed to meet acceptance criteria set for precision and accuracy. Thus, data here were just for reference

Table 100. Tissue iron levels at the end of 2-year study

Sex	Group/PA21 dose (mg/kg/day)	Liver (mg/kg)	Kidney (mg/kg)	Spleen (mg/kg)
Male	Control	322 ± 117	231 ± 106	1961 ± 817
	200	348 ± 109	229 ± 180	2080 ± 1182
	750	423 ± 109*	202 ± 82	3090 ± 1505*
	2500	780 ± 472**	230 ± 66	7310 ± 3203**
Female	Control	427 ± 120	165 ± 35	4444 ± 1371
	200	495 ± 131	171 ± 38	4327 ± 1902
	750	786 ± 307**	211 ± 72*	5989 ± 2016
	2500	1315 ± 808**	315 ± 40**	9301 ± 2008**

* p<0.05 vs Control; ** p<0.01 vs control.

Necropsy revealed cystic mesenteric lymph nodes, thickened cecum and colon in both sexes at 2500 mg/kg/day, and increased incidence of prostate with mass(es) at doses ≥750 mg/kg/day (Table 101). Histological examination revealed a slight increased incidence of benign thyroid c-cell adenoma in males at the dose 2500 mg/kg/day (Table 102). Treatment-related histological non-neoplastic findings (Table 103) included higher level of pigmented macrophages (iron content) in gastro-intestinal tract, liver, kidney, spleen, pancreas, adrenal gland, mesenteric, mandibular, and axillary lymph nodes; and epithelial hyperplasia in gastro-intestinal tract, lymphoid aggregation, inflammation, and cyst in various organs/tissues.

Table 101. Necropsy findings

Sex		Male				Female			
Group/PA21 dose (mg/kg/day)		Control	200	750	2500	Control	200	750	2500
Organ	Findings	n=65	n=65	n=65	n=65	n=65	n=65	n=65	n=65
Lymph node, mesenteric	Cyst(s)	0	0	2	14**	0	0	2	11**
	Dark	4	1	0	5	0	2	6*	5*
Cecum	Thickened	2	3	5	15*	0	1	1	13**
Colon	Thickened	0	1	0	5*	0	0	0	9**
Prostate	Mass(es)	1	5	8*	8*	-	-	-	-
Tail	Scab(s)	3	4	5	20**	1	1	1	0
	Brown stained	0	4	51**	57**	0	1	2	55**

* p<0.05 vs control; **p<0.01 vs control

Table 102. Number of animals with histological neoplastic findings in the 2-year rat study with PA21(Group size: 65/sex)

Male		Group/dose (mg/kg/kg)				Female		Group/dose (mg/kg/kg)			
Organ Name	Tumor Name	0	200	750	2500	Organ Name	Tumor Name	0	200	750	2500
ABDOMEN	HISTIOCYTIC SARCOMA	0	1	0	0	ABDOMEN	MESOTHELIOMA	1	1	0	0
	MESOTHELIOMA	0	0	1	0	ADIPOSE TISSUE	SARCOMA NOS	0	1	0	0
ADRENALS	CORTICAL ADENOMA	3	1	3	0	ADRENALS	CORTICAL ADENOMA	1	1	2	2
	MALIGNANT PHAEOCHROM	5	3	1	1		CORTICAL CARCINOMA	0	0	0	1
	MIXED MEDULLARY TUMO	2	0	0	0		MALIGNANT PHAEOCHROM	1	2	0	0
	PHAEOCHROMOCYTOMA	12	12	4	7		PHAEOCHROMOCYTOMA	1	7	6	0
BONE	FIBROSARCOMA	1	0	0	0	BONE	OSTEOMA	0	0	1	0
	OSTEOSARCOMA	0	1	0	0	BRAIN	ASTROCYTOMA	0	0	2	1
BRAIN	ASTROCYTOMA	0	1	2	0		GRANULAR CELL TUMOUR	1	0	0	0
	GRANULAR CELL TUMOUR	2	1	0	1		OLIGODENDROGLIOMA	0	0	0	1
	MALIGNANT GRANULAR C	0	0	0	1	H-POIETIC TUMOU	HISTIOCYTIC SARCOMA	1	0	2	0
	MALIGNANT MENINGIOMA	1	0	0	0		MYELOID CELL LEUKAEM	0	0	0	1
H-POIETIC TUMOU	HISTIOCYTIC SARCOMA	0	1	1	1	HEAD	SQUAMOUS CELL CARCIN	1	0	0	0
	MALIGNANT LYMPHOMA	3	1	0	1	LIVER	HEPATOCELLULAR ADENO	1	2	5	3
JEJUNUM	ADENOCARCINOMA	0	1	0	0		HEPATOCELLULAR CARCI	0	0	0	1
KIDNEYS	TUBULAR ADENOMA	1	0	0	1	LN MESENTERIC	HAEMANGIOMA	0	1	3	0
LIVER	HEPATOCELLULAR ADENO	1	1	2	2		PARAGANGLIOMA (THORA	0	0	1	0
	HEPATOCELLULAR CARCI	1	1	1	0	MAMMARY	FIBROSARCOMA	0	1	0	1
LN MESENTERIC	HAEMANGIOMA	4	1	5	2		MAMMARY ADENOCARCINO	23	13	21	11
LN THYMIC	PARAGANGLIOMA (THORA	0	0	1	0		MAMMARY ADENOMA	0	0	0	1
MAMMARY	FIBROMA	1	3	2	2		MAMMARY FIBROADENOMA	26	32	24	28
	FIBROSARCOMA	0	3	0	0		MYXOLIPOMA	0	0	0	1
	MAMMARY ADENOMA	0	1	0	0	OVARIES	DYSGERMINOMA	0	0	1	0
	MAMMARY FIBROADENOMA	0	0	1	0	PANCREAS	ACINAR CELL ADENOMA	1	2	1	2
NASAL TURBINATE	SQUAMOUS CELL CARCIN	0	0	1	0		ISLET CELL ADENOMA	0	1	1	0
PANCREAS	ACINAR CELL ADENOMA	5	2	5	1		ISLET CELL CARCINOMA	1	1	0	0
	ISLET CELL ADENOMA	2	5	2	2		MIXED CELL ADENOMA	0	1	0	0
	ISLET CELL CARCINOMA	1	3	2	2	PARATHYROID	ADENOMA	3	3	0	0
PARATHYROID	ADENOMA	4	3	2	0	PITUITARY	ADENOMA, PARS DISTAL	55	48	48	44
PITUITARY	ADENOMA, PARS DISTAL	38	34	38	38		CARCINOMA, PARS DIST	0	0	2	2
	ADENOMA, PARS INTERM	0	1	0	0	SKIN	BASAL CELL CARCINOMA	0	1	0	0
	CARCINOMA, PARS DIST	0	0	0	1		FIBROMA	0	0	1	0
PREPUTIAL GLAND	SQUAMOUS CELL CARCIN	0	1	0	0		FIBROSARCOMA	0	1	0	0
PROSTATE	ADENOCARCINOMA	0	0	2	0		KERATOACANTHOMA	1	0	0	0
	ADENOMA	0	0	0	1		SARCOMA NOS	0	1	0	0
SKELETAL MUSCLE	CHONDROSARCOMA	0	0	0	1	STOMACH	SQUAMOUS CELL PAPILL	0	1	0	0
	HAEMANGIOSARCOMA	0	0	0	1	THYMUS	THYMOMA (EPITHELIAL)	0	0	0	1
	MYXOMA	0	1	0	0		THYMOMA (LYMPHOID)	0	1	0	0
	SARCOMA NOS	0	1	0	0	THYROID	C-CELL ADENOMA	9	6	7	6
SKIN	BASAL CELL CARCINOMA	0	1	0	0		C-CELL CARCINOMA	2	1	0	0
	BASAL CELL TUMOUR	3	0	2	1		FOLLICULAR CELL ADEN	2	0	0	0
	FIBROMA	3	0	3	3	UTERINE CERVIX	GRANULAR CELL TUMOUR	1	1	0	0
	FIBROSARCOMA	1	1	0	0		SCHWANNOMA	0	1	0	0
	KERATOACANTHOMA	1	4	3	4	UTERUS	ENDOMETRIAL ADENOCAR	0	1	1	1
	LIPOMA	0	1	0	0		ENDOMETRIAL POLYP	4	3	4	2
	SEBACEOUS CELL ADENO	1	0	1	1		ENDOMETRIAL STROMAL	0	0	0	1
	SQUAMOUS CELL PAPILL	0	2	2	0		SQUAMOUS CELL CARCIN	0	1	0	0
	TRICHIOEPITHELIOMA	1	0	0	0	VAGINA	MALIGNANT SCHWANNOMA	1	0	0	0
STOMACH	LEIOMYOSARCOMA	0	0	1	0		SQUAMOUS CELL CARCIN	0	1	0	0
TESTES	INTERSTITIAL (LEYDIG)	1	6	1	4						
	cell adenoma										
THYMUS	THYMIC ADENOCARCINOM	0	0	0	1						
THYROID	C-CELL ADENOMA	6	10	10	14						
	C-CELL CARCINOMA	3	1	3	2						
	FOLLICULAR CELL ADEN	2	1	2	2						
	FOLLICULAR CELL CARC	2	0	1	0						
	GANGLIONEUROMA	0	0	0	1						

Table 103. Number of rats with non-neoplastic findings (Group size: 55-65/sex for histological examination; 65/sex for Perl's stain)

Organ	Findings	Male (PA21: mg/kg/day)				Female (PA21: mg/kg/day)			
		Control	200	750	2500	Control	200	750	2500
Adrenals	Pigmented Macrophages	9	11	18*	24**	23	26	31	35*
Liver	Pigmented Macrophages	18	20	33**	57**	11	9	30**	45**
	Positive Perl's Stain	37	45	57**	61**	29	41**	45**	60**
Kidneys	Cortical Pigment	7	7	6	13	5	3	3	16**
	Positive Perl's Stain	37	40	42	49*	14	16	26*	54**
Spleen	Hemosiderosis	45	48	62**	62**	60	59	64	64
	Positive Perl's Stain	62	61	65	65	64	64	65	65
Lymph node, mesenteric	Dilated/Cystic Sinuses	13	11	13	17	3	4	0	12*
	Mastocytosis	4	3	4	4	1	5	9**	4
	Pigmented Macrophages	13	21	39**	56**	18	28	39**	54**
	Positive Perl's Stain	16	21	41**	59**	12	30**	39**	59**
Lymph node, mandibular	Pigmented Macrophages	6	1	7	20**	9	8	25**	35**
	Plasmacytosis	12	20	29**	18	21	25	23	27
	Sinus Histiocytosis	1	3	0	1	1	6	4	7*
	Positive Perl's Stain	18	36**	39**	56**	49	50	58*	62**
Lymph node, axillary (left)	Increased Cellularity - Generalized	13	17	22*	17	10	21*	9	10
	Pigmented Macrophages	5	5	7	30**	11	17	25**	42**
Duodenum	Epithelial Hyperplasia	5	2	2	5	3	6	6	10*
	Pigmented Macrophages	0	1	1	15**	0	0	0	14**
	Positive Perl's Stain	1	8*	15**	47**	1	17**	24**	55**
Ileum/Peyers	Pigmented Macrophages	0	0	0	1	0	0	0	5*
	Positive Perl's Stain	0	2	2	19**	1	6	9*	18**
Cecum	Epithelial Hyperplasia	1	0	2	9*	0	0	0	8*
	Pigmented Macrophages	0	0	0	21**	0	0	0	6*
	Positive Perl's Stain	0	1	14**	53**	0	0	10**	47**
Colon	Epithelial Hyperplasia	0	0	1	6*	0	0	0	8**
	Pigmented Macrophages	1	10**	37**	58**	3	7	51**	57**
	Submucosal Inflammation	1	0	0	4	1	0	1	7*
	Positive Perl's Stain	2	26**	55**	63**	2	33**	59**	63**
Jejunum	Pigmented Macrophages	0	0	1	7**	0	1	0	3
	Positive Perl's Stain	1	4	6	29**	3	9	17**	33**
Rectum	Pigmented Macrophages	0	0	0	16**	0	0	0	7**
	Positive Perl's Stain	0	1	22*	57**	0	0	20**	55**
Thyroid	C-cell Hyperplasia	4	8	10	11	9	14	16	11
Prostate	Abscessation	12	16	24*	23*	-	-	-	-

* p<0.05 vs control; ** p<0.01 vs control

Summary of FDA statistical analysis on survival rate and tumor findings

FDA statistical analysis concluded that the studies showed no statistically significant dose response relationship in mortality across treatment groups or increased mortality in the treated groups in either sex (Table 104).

Table 104. Intercurrent mortality comparison (from FDA statistical review)

	Test	Statistic	P_valies
Male	Dose-response	Likelihood ratio	0.7125
	Homogeneity	Log-Rank	0.7185
Female	Dose-response	Likelihood ratio	0.5847
	Homogeneity	Log-Rank	0.3907

For tumor data analysis, multiple testing adjustment was applied. Briefly, the FDA guidance for the carcinogenicity study design and data analysis suggests the use of test levels $\alpha=0.005$ for common tumors and $\alpha=0.025$ for rare tumors for a submission with two species, and a significance level $\alpha=0.01$ for common tumors and $\alpha=0.05$ for rare tumors for a submission with one species study in order to keep the false-positive rate at the nominal level of approximately 10%. For multiple pairwise comparisons of treated group with control the FDA guidance suggested the use of test levels $\alpha=0.01$ for common tumors and $\alpha=0.05$ for rare tumors, in order to keep the false-positive rate at the nominal level of approximately 10% for both submissions with two or one species.

For tumor findings, FDA statistical analysis concluded that the studies did not show statistically significant positive dose response relationship in any of the tested tumor types. The pairwise comparisons also did not show statistically significant increased incidence of any tumor type in any of the treated groups compared to the control in either sex (Table 105).

Table 105. Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons in Rats (from FDA statistical review)

Sex	Organ	Tumor	Group : PA21, mg/kg/day: N=	Cont	Low	Mid	High	P_value			
				0 65	200 65	750 65	2500 65	Dose resp	C vs L	C vs M	C vs H
Male	Testes	Interstitial (Leydig) cell adenoma	1	6	1	4	0.2866	0.0464	0.7474	0.1617	
	Thyroids	C-cell adenoma	6	10	10	14	0.0420	0.1798	0.2331	0.0384	
Female	Adrenals	Pheochromocytoma	1	7	6	0	0.9677	0.0417	0.0662	1.0000	

Reviewer's evaluation

The rat study with PA21 in diet over 99 weeks for females and 103 weeks for males is adequate and appropriate for testing the carcinogenic potential of PA21 in animals. A slightly increased incidence of benign thyroid c-cell adenoma in males at the dose 2500 mg/kg/day was neither statistically significant nor toxicologically significant. There was no evidence for increased incidence of any tumor type in any of the treated groups. However, there were treatment-related inflammation and epithelial hyperplasia in gastro-intestinal tract of both sexes at 2500 mg/kg/day.

8.2 PA21: Carcinogenicity study by dietary administration to CD-1 mice for 104 weeks

Study no.: VFR0115
Study report location: (b) (4)
Conducting laboratory and location: (b) (4)
Date of study initiation: March 17, 2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: PA21, 010307J1/95.8%; 020307J1/96%
CAC concurrence: Yes
Deviation from study protocol: No impact to the results

Key Study Findings

Treatment-related colon and/or cecum adenocarcinomas were seen in all male treatment groups and in females at high dose group with a single incidence in a female at low dose group. There were roughened forestomach, cystic area, masses, thickened or raised areas in cecum and/or colon at doses 5000 mg/kg/day, associated with dose-related increases in incidences and severity of colon and/or cecum epithelial hyperplasia and mucosal diverticulum/cysts/hyperplasia, and epithelial hyperplasia and hyperkeratosis in the non-glandular of forestomach at the high dose. Increased incidence of urinary bladder calculus(i) was seen in males at 5000 mg/kg/day, associated with transitional epithelial hyperplasia in male urinary bladder and dilated medulla tubules in the kidney of both sexes. Higher level of iron content in gastro-intestinal tract, liver, kidney, mesenteric lymph nodes, and mesenteric lymphoid aggregation, inflammation, and cyst were seen in males and/or females at doses 1250, 2500, and/or 5000 mg/kg/day.

Methods

CrI:CD1 (ICR) mice, initial age of ~6 weeks, were on diet containing PA21 at 0, 1250, 2500 and 5000 mg/kg/day (n = 60/sex/group). The doses selected for this study were based on a 13-week toxicity studies in mice (VFR0105) conducted at 0, 2.5, 5, and 10 g/kg/day. Notable findings in study VFR0105 included elevated ALP, decreased urinary volumes, and urinary calculi after 13 weeks of treatment at 10 g/kg/day, with marked reductions in urinary phosphorus at 5 and 10 g/kg/day. At 5 and/or 10 g/kg/day, there were an increase in mitotic activity in male liver, and increased incidences of stomach hyperplasia and hyperkeratosis. Colon mucosal proliferative changes were also seen in some animals at 2.5 g/kg/day. The dose 10 g/kg/day was too high for the 104-week carcinogenic study because of the urinary calculi and decreased urinary volume in the 13-week study. Therefore, 5000 mg/kg/day was selected to be the high dose. The 1250 mg/kg/day represented a low multiple of the maximum likely human dosage. The study was designed to continue for 104 weeks. However, the study with male rats was terminated at week 101 because of poor survival. Samples of the drug-diet formulations

were analyzed at 1, 13, 26, 39, 52, 65, 78, 91, 103 of treatment to confirm the concentrations of PA21 in the treated diets and the absence in the control diet

During the study, rats were observed at least twice daily for clinical signs and morbidity/death. Physical examination, including palpation, was weekly performed on each animal. Body weight was recorded at study initiation, weekly for first 16 weeks, every 2 weeks from Week 16 to 28, every four weeks thereafter, and at the end of the final week of treatment. Mean weekly food consumption per animal was assessed at week -1, each week during weeks 1-16, once every two week during weeks 17-28, once every 4 weeks during weeks 29-104. Ophthalmoscopy was performed prior to treatment on all animals, at week 52 and before study termination on 20 animals/sex of control and high dose groups. Blood samples were collected from all surviving animals at the end of treatment period, and from animals killed prematurely when possible, for determining hematology (n=13-30/sex/group) and plasma chemistry (n=15-20/sex/group, Table 91). Overnight urine samples were collected from 10 mice/sex/group during week 59 and from all surviving mice at week 99/100 for urinalysis (Table 45) and for determining deoxypyridinoline (bone turnover marker) and creatinine (for normalization of deoxypyridinoline values).

A detailed necropsy was performed on each animal killed either prematurely or at the end of scheduled treatment period. Tissue samples from liver (residue of left lobe), kidney (poles from left kidney), and spleen (poles) of 10 males and 10 females/group were weighed, and snap-frozen in liquid nitrogen for determination of iron content. Lumbar vertebra 6 and right tibia of 10 males and 10 females/group were collected for histomorphometry analysis. Organs/tissues listed in Table 92 were sampled from all necropsied animals, and histologically processed. A complete histopathological examination was performed on all animals from all groups found dead, killed moribund, or sacrificed during or at the end of the experiment. Samples of any macroscopic abnormal tissues were also retained and processed for histopathological examination.

Results

The achieved drug doses (in diet) at during weeks 1-101/104 were 1254, 2533, and 5149 mg/kg/day for males and 1268, 2526, and 5025 mg/kg/day for females, which were within the acceptance criteria of 15% nominal doses.

There was no treatment-related effect on mortality (Table 106). Feces in the treated group were darker than in control group due to the brown color of PA21. There were no treatment-related effects on body weight gain, food consumption, and ophthalmoscopy.

Table 106. Intercurrent mortality rate in the 2-year mouse study with PA21

Group Week	Control		1250mg/kg/day		2500mg/kg/day		5000mg/kg/day	
	No. of Death	Cum. %						
Males								
0 - 52	8	13.33	1	1.67	6	10.00	4	6.67
53 - 78	11	31.67	8	15.00	13	31.67	22	43.33
79 - 91	10	48.33	15	40.00	18	61.67	10	60.00
92 - 101	10	65.00	11	58.33	5	70.00	8	73.33
Ter. Sac.	21	35.00	25	41.67	18	30.00	16	26.67
Total	N=60		N=60		N=60		N=60	
Females								
0 - 52	3	5.00	4	6.67	3	5.00	5	8.33
53 - 78	12	25.00	9	21.67	16	31.67	9	23.33
79 - 91	12	45.00	8	35.00	10	48.33	7	35.00
92 - 104	10	61.67	10	51.67	11	66.67	8	48.33
Ter. Sac.	23	38.33	29	48.33	20	33.33	31	51.67
Total	N=60		N=60		N=60		N=60	

Changes in hematology were limited to minor changes in red blood cell parameters mostly in males at all dose levels (Table 107), which were probably due to minor chronic bleeding in GI tract. Terminal plasma chemistry showed tendencies of higher iron and lower phosphorus concentrations at the dose 5000 mg/kg/day (Table 108). Urinary analysis showed minimally higher calcium and lower phosphorus contents in males and/or females at doses 1250, 2500, or 5000 mg/kg/day during weeks 59 and/or 99/100 (Table 108). There were no treatment-related effects on urinary deoxypyridinoline (Table 108). At the end of treatment period, iron levels in liver trended to be higher in males at 5000 mg/kg/day (Table 109). All these and other changes were minimal and within the range of physiological variation, and/or pharmacological effects of PA21, and were toxicologically insignificant.

Table 107. Hematological findings at the end of dose period[§] (Mean ± SD)

Sex	Group/PA21 mg/kg/day	Hct (L/L)	Hb (g/dL)	RBC (x10 ¹² /L)	Retic (%)	MCH (pg)	MCHC (g/dL)	MCV (fL)
Male	Control	0.434 ± 0.071	13.7 ± 2.1	9.15 ± 1.34	3.04 ± 0.99	15.0 ± 0.86	31.7 ± 1.29	47.4 ± 2.26
	1250	0.393 ± 0.064*	12.3 ± 2.4*	8.19 ± 1.50*	4.94 ± 3.17**	15.0 ± 0.91	31.1 ± 1.60	48.3 ± 3.49
	2500	0.393 ± 0.031*	12.3 ± 1.2*	7.99 ± 0.77*	4.53 ± 1.79**	15.4 ± 0.81	31.3 ± 1.47	49.2 ± 2.32
	5000	0.393 ± 0.043*	12.1 ± 1.5	7.98 ± 1.29*	7.48 ± 10.60**	15.3 ± 1.49	30.7 ± 1.43	49.9 ± 5.04*
Female	Control	0.398 ± 0.027	12.4 ± 0.9	8.38 ± 0.71	2.93 ± 0.77	14.9 ± 1.16	31.2 ± 1.75	47.7 ± 2.89
	1250	0.374 ± 0.055	12.0 ± 1.7	7.59 ± 1.25*	4.73 ± 4.28	15.9 ± 1.43	32.2 ± 1.86	49.6 ± 4.73
	2500	0.398 ± 0.026	12.7 ± 0.9	8.39 ± 0.65	3.10 ± 0.74	15.2 ± 0.88	31.9 ± 1.45	47.6 ± 2.29
	5000	0.389 ± 0.037	12.5 ± 1.6	7.89 ± 0.99	4.44 ± 2.43*	15.9 ± 0.69**	32.1 ± 1.58	49.6 ± 2.36*

[§] Week101 for males and Week 104 for females. * p<0.05 vs Control; ** p<0.01 vs control.

Table 108. Plasma chemistry, urinalysis, and bone turnover marker (Mean± SD)

Sex	Group/PA21 mg/kg/day	Plasma chemistry		Urinalysis		Deoxypyridinoline /Creatinine (in urine, nM/mM)
		Phos (mmol/L)	Fe (µmol/L)	U-CA (mmol/L)	U-IP (mmol/L)	
Week 59						
Male	Control			1.32 ± 0.64	21.0 ± 8.9	11.00 ± 3.46
	1250			2.07 ± 0.54*	18.6 ± 10.3	10.89 ± 5.71
	2500			1.97 ± 0.60**	16.5 ± 4.4	9.57 ± 2.64
	5000			4.94 ± 2.15**	17.6 ± 12.9	10.43 ± 6.82
Female	Control			0.84 ± 0.15	24.8 ± 5.1	14.86 ± 4.77
	1250			1.33 ± 0.59	10.8 ± 5.4**	14.13 ± 5.08
	2500			1.61 ± 0.64*	9.4 ± 4.1**	17.51 ± 5.12
	5000			2.87 ± 2.35**	7.3 ± 5.2**	20.73 ± 10.72
Prior to termination						
Male	Control	1.90 ± 0.35	47 ± 14.4	1.25 ± 0.51	17.8 ± 9.5	7.36 ± 7.09
	1250	2.10 ± 0.51	49 ± 20.2	1.18 ± 0.30	17.8 ± 7.1	6.36 ± 1.99
	2500	1.85 ± 0.58	56 ± 21.0	1.98 ± 1.79	14.4 ± 12.3	5.61 ± 2.39
	5000	1.46 ± 0.31**	55 ± 18.0	2.94 ± 2.12*	13.2 ± 7.9	5.19 ± 2.06
Female	Control	2.14 ± 0.38	39 ± 12.1	0.85 ± 0.72	16.7 ± 9.7	13.47 ± 5.71
	1250	2.25 ± 0.51	44 ± 10.1	0.62 ± 0.26	20.1 ± 8.0	10.41 ± 3.52
	2500	2.30 ± 0.54	45 ± 11.7	0.77 ± 0.49	15.8 ± 9.5	15.18 ± 13.22
	5000	1.81 ± 0.60	54 ± 17.3**	1.67 ± 1.69	8.8 ± 4.9**	12.63 ± 3.44

* p<0.05 vs Control; ** p<0.01 vs control.

Table 109. Tissue iron levels (Mean± SD)

Sex	Group/PA21 dose	Liver (mg/kg)	Kidney (mg/kg)	Spleen (mg/kg)
Male	Control	560 ± 296	132 ± 34	1216 ± 433
	1250 mg/kg/day	477 ± 347	137 ± 31	986 ± 725
	2500 mg/kg/day	409 ± 256	130 ± 18	835 ± 451
	5000 mg/kg/day	826 ± 537	162 ± 37*	1051 ± 656
Female	Control	617 ± 258	243 ± 88	1316 ± 973
	1250 mg/kg/day	728 ± 463	331 ± 250	1448 ± 743
	2500 mg/kg/day	870 ± 418	329 ± 162	1935 ± 1170
	5000 mg/kg/day	1066 ± 534*	256 ± 101	1710 ± 766

* p<0.05 vs Control.

Necropsy revealed increased incidences of urinary bladder calculus in males at dose 5000 mg/kg/day, cystic and/or enlarged mesenteric lymph nodes in both sexes at doses ≥ 1250 mg/kg/day, roughened forestomach, cystic area, masses, thickened or raised areas in cecum and/or colon at doses 5000 mg/kg/day (Table 110). There were increased incidences and severity of colon and or cecum epithelial adenocarcinoma, viewed under histological examination, in males and/or females at doses 1250, 2500, and/or 5000 mg/kg/day (Table 111). Treatment-related histological non-neoplastic findings (Table 112) included higher level of iron content in gastro-intestinal tract, liver, mesenteric lymph nodes; GI-tract epithelial hyperplasia and hyperkeratosis; urinary bladder transitional epithelial hyperplasia and dilated kidney medulla tubules; and dilated and cystic sinuses in mesenteric lymph nodes in males and/or females at doses 1250, 2500, and/or 5000 mg/kg/day.

Table 110. Number of animals with positive necropsy findings

Sex		Male				Female			
Group/PA21 dose: mg/kg/day		Control	1250	2500	5000	Control	1250	2500	5000
Organ	Findings	n=60	n=60	n=60	n=60	n=60	n=60	n=60	n=60
Urinary bladder	Contained calculus(i)	0	0	1	5*	0	0	0	0
Lymph node, mandibular	Enlarged	8	11	8	9	11	7	17	20*
Lymph node, mesenteric	Congested	3	8	10*	9	7	8	11	12
	Cystic enlargement	0	4	2	9**	0	1	0	1
	Enlarged	7	18*	14	21**	10	10	14	23
GI tract	Dark contents	2	27**	37**	43**	3	21**	36**	49**
Stomach	Forestomach roughened	4	4	8	14**	2	2	5	12**
Cecum	Cystic area(s)	0	4	4	6*	0	0	0	2
	Mass(es)	0	1	1	6*	0	1	0	0
	Raised area(s)	0	4	1	3	0	0	0	5*
Colon	Cystic area(s)	1	4	5	10**	0	0	2	4

* p<0.05 vs control; **p<0.01 vs control

Table 111. Number of animals with histological neoplastic findings in the 2-year mouse study with PA21 (Group size: 50-60 mice/sex)

Male		Group/dose (mg/kg/day)				Female		Group/dose (mg/kg/day)				
Organ Name	Tumor Name	0	1250	2500	5000	Organ Name	Tumor Name	0	1250	2500	5000	
ADIPOSE TISSUE	HIBERNOMA	7	2	5	5	ADIPOSE TISSUE	SARCOMA (NOS)	0	0	0	1	
	LIPOMA	0	2	1	0		ADRENALS	CORTICAL ADENOMA	0	0	1	0
ADRENALS	SUBCAPSULAR CELL ADE	3	3	3	2			SUBCAPSULAR CELL ADE	3	0	0	0
BONE	OSTEOMA	1	0	0	0	BONE	OSTEOMA	0	0	0	1	
BRAIN	MIXED GLIOMA	0	0	1	0	CAECUM	ADENOCARCINOMA	0	1	0	0	
CAECUM	ADENOCARCINOMA	0	1	2	1	CLITORAL GLANDS	LEIOMYOMA	0	0	1	0	
	ADENOMA	0	0	0	1		COLON	ADENOCARCINOMA	0	0	0	3
COLON	ADENOCARCINOMA	1	3	5	9		HAEMANGIOSARCOMA	0	0	1	0	
EPIDIDYMIDES	SCHWANNOMA	0	0	1	0	FEMUR INC. JOIN	OSTEOMA	0	0	1	0	
H-POIETIC TUMOU	HISTIOCYTIC SARCOMA	2	0	1	2	H-POIETIC TUMOU	HISTIOCYTIC SARCOMA	5	5	6	3	
	MALIGNANT LYMPHOMA	8	7	6	7			MALIGNANT LYMPHOMA	12	13	11	11
	MYELOID CELL LEUKAEM	1	0	1	3			MYELOID CELL LEUKAEM	1	0	0	1
HARDERIAN GLAND	ADENOMA	9	5	4	6	HARDERIAN GLAND	ADENOCARCINOMA	1	0	0	0	
KIDNEYS	TUBULAR ADENOMA	0	1	0	0		ADENOMA	3	4	3	1	
						LIVER	HAEMANGIOMA	1	1	0	0	
LIVER	CHOLANGIOCARCINOMA	0	0	1	0		HEPATOCELLULAR ADENO	1	0	0	0	
	HAEMANGIOMA	1	8	2	2	LN MESENTERIC	HAEMANGIOMA	0	0	0	1	
	HAEMANGIOSARCOMA	0	1	0	0	LUNGS + BRONCHI	BRONCHIOLOALVEOLAR A	2	2	1	2	
	HEPATOCELLULAR ADENO	7	3	7	8				7	10	8	7
	HEPATOCELLULAR CARCI	1	1	0	0	MAMMARY	ADENOACANTHOMA	1	0	2	2	
LUNGS + BRONCHI	BRONCHIOLOALVEOLAR A	15	19	10	14			MAMMARY ADENOCARCINO	2	3	4	2
								MAMMARY ADENOMA	0	2	1	2
		8	7	3	3	OVARIES	CYSTADENOMA	2	1	2	1	
PANCREAS	ISLET CELL ADENOMA	1	0	0	1		GRANULOSA CELL TUMOU	0	1	0	1	
PITUITARY	CARCINOMA, PARS DIST	0	0	1	0		LUTEOMA	2	3	2	3	
SALIVARY GLANDS	ANAPLASTIC CARCINOMA	0	0	0	1		MESOVARIAN LEIOMYOMA	1	1	0	0	
SEMINAL VESICLE	CARCINOSARCOMA	0	0	1	0	PANCREAS	ISLET CELL ADENOMA	0	0	1	0	
SKELETAL MUSCLE	HAEMANGIOSARCOMA	0	2	0	0	PITUITARY	ADENOMA, PARS DISTA	2	0	1	0	
	OSTEOSARCOMA	1	0	0	0	SKELETAL MUSCLE	OSTEOSARCOMA	1	0	0	0	
SKIN	ANAPLASTIC CARCINOMA	0	0	1	0		RHABDOMYOSARCOMA	0	1	0	0	
	FIBROSARCOMA	0	4	2	4		SARCOMA NOS	0	1	0	0	
	HISTIOCYTOMA, FIBROU	1	0	0	0	SKIN	HISTIOCYTOMA, FIBROU	0	0	1	0	
	SARCOMA NOS	0	0	1	0		LIPOSARCOMA	0	0	0	1	
	SQUAMOUS CELL CARCIN	0	1	0	0		SQUAMOUS CELL PAPILL	1	1	0	0	
SPLEEN	HAEMANGIOMA	2	1	0	0	STOMACH	SQUAMOUS CELL CARCIN	1	0	0	0	
STOMACH	SQUAMOUS CELL CARCIN	0	0	0	1		SQUAMOUS CELL PAPILL	0	1	0	0	
	SQUAMOUS CELL PAPILL	0	0	0	1	URINARY BLADDER	LEIOMYOMA	0	0	0	1	
TESTES	INTERSTITIAL (LEYDIG	1	1	1	0	UTERINE CERVIX	ENDOMETRIAL STROMAL	0	0	3	0	
THYMUS	THYMOMA (LYMPHOID)	0	0	0	1	UTERUS	ENDOMETRIAL ADENOCAR	0	1	0	0	
THYROIDS	FOLLICULAR CELL ADEN	1	0	0	0			ENDOMETRIAL ADENOMA	1	0	0	0
								ENDOMETRIAL POLYP	6	4	6	9
								ENDOMETRIAL STROMAL	1	7	3	3
								HAEMANGIOMA	0	1	1	0
URINARY BLADDER	MESENCHYMAL TUMOUR	1	0	0	0		LEIOMYOMA	1	2	1	2	

Table 112. Histological non-neoplastic findings (Group size: 50-60 mice/sex)

Organ	Findings	Male (PA21: mg/kg/day)				Female (PA21: mg/kg/day)			
		Control	1250	2500	5000	Control	1250	2500	5000
Liver	Pigment in Kupffer Cells	0	1	2	6*	7	12	4	12
	Positive Perl's Stain	23	35*	35*	45**	32	36	41	55**
Kidneys	Medulla- Dilated Tubules	2	0	0	2	0	1	1	5*
Urinary bladder	Transitional Epithelium-Hyperplasia	0	0	0	5*	0	0	0	0
Spleen	Extramedullary Hemopoiesis	11	18	18	25**	20	20	23	22
	Pigmented Macrophages	6	7	12	14*	10	11	20*	17
Lymph node, mesenteric	Dilated/Cystic Sinuses	0	8**	5*	11**	1	1	1	1
	Positive Perl's Stain	31	40	47**	53**	43	47	50	53*
Stomach, Nonglandular region	Epithelium Hyperplasia	3	1	3	9	0	0	2	3
	Hyperkeratosis	3	1	5	12*	1	4	4	5
Duodenum	Positive Perl's Stain	32	40	37	39	29	41**	46**	48**
Pey. Patch	Positive Perl's Stain	4	3	9	10	1	5	5	12**
Cecum	Epithelial Hyperplasia	2	3	3	15**	0	0	0	8**
	Mucosal Diverticulum/Cysts/Epithelail Hyperplasia	0	3	4	12**	0	0	0	3
	Positive Perl's Stain	1	0	3	10**	0	1	1	9**
Colon	Epithelial Hyperplasia	5	16*	22**	25**	3	5	6	21**
	Positive Perl's Stain	2	10*	4	18**	3	5	16**	23**
Oesophagus	Inflammatory Cells	0	0	2	0	0	0	3	5*
Jejunum	Positive Perl's Stain	1	1	0	0	0	1	2	5*
Rectum	Positive Perl's Stain	0	1	5*	9**	1	8*	11**	35**
Ovaries	Cystic Ovarian Bursa	-	-	-	-	10	22**	17	24**

* p<0.05 vs control; ** p<0.01 vs control

Summary of FDA statistical analysis on survival rate and tumor findings

FDA statistical analysis concluded that the studies did not show statistically significant dose response relationship in mortality across treatment groups in either sex (Table 113).

Table 113. Intercurrent mortality comparison (from FDA statistical review)

	Test	Statistic	P_Value
Male	Dose-Response	Likelihood Ratio	0.0774
	Homogeneity	Log-Rank	0.1090
Female	Dose-Response	Likelihood Ratio	0.3307
	Homogeneity	Log-Rank	0.1424

For tumor data analysis, multiple testing adjustment was applied. FDA statistical analysis concluded that the studies showed statistically significant dose response relationship in the incidence of adenocarcinoma in colon in both sexes. The pairwise comparison of high dose group with control in male mice was considered to be statistically significant for the increased incidence of adenocarcinoma in colon (Table 114).

Table 114. Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons (from FDA statistical review)

Sex	Organ	Tumor	PA21, mg/kg/day: N=	Group :				P_value			
				Cont 0 60	Low 1250 60	Mid 2500 60	High 5000 60	Dose resp	C vs L	C vs M	C vs H
Male	Colon	Adenocarcinoma		1	3	5	9	0.0012*	0.3715	0.0889	0.0056*
	Liver	Hemangioma		1	8	2	2	0.6656	0.0274	0.4901	0.4696
Female	Colon	Adenocarcinoma		0	0	0	3	0.0164*			0.1239
	Uterus	Endometrial stromal		1	7	3	3	0.5020	0.0363	0.2809	0.3256

Reviewer's evaluation

The mouse study with PA21 in diet over 101 weeks for males and 104 weeks for females appears adequate and appropriate for testing the carcinogenic potential of PA21 in animals. Treatment with PA21 resulted in increased incidences of adenocarcinomas in colon and cecum in males at all dose levels and in females at 5000 mg/kg/day with a single incidence in a low dose female. There were also dose-related epithelial hyperplasia and mucosal diverticuli/cysts/hyperplasia in colon and cecum, adenoma and evidence of local irritation in non-glandular forestomach with increased epithelial hyperplasia and hyperkeratosis at 5000 mg/kg/day, dilated/cystic sinuses in enlarged mesenteric lymph nodes of males at all doses, and inflammatory cells in esophagus of females at the mid and high doses. The correlation between PA21-induced hyperplasia and the presence of adenocarcinomas in both colon and cecum, and the presence of adenoma and local irritation in non-glandular forestomach suggested that the neoplastic changes were part of a continuum that originated from chronic irritation, and subsequent proliferative response of the GI-tract to oral administered PA21.

9 Reproductive and Developmental Toxicology

9.1 PA21 - Fertility and early embryonic development study in CD rats by oral gavage administration

Study no.:	VFR0098/064305
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Sept 22, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PA21, 429000, and 95.4%
Deviation from study protocol:	No impact to the results

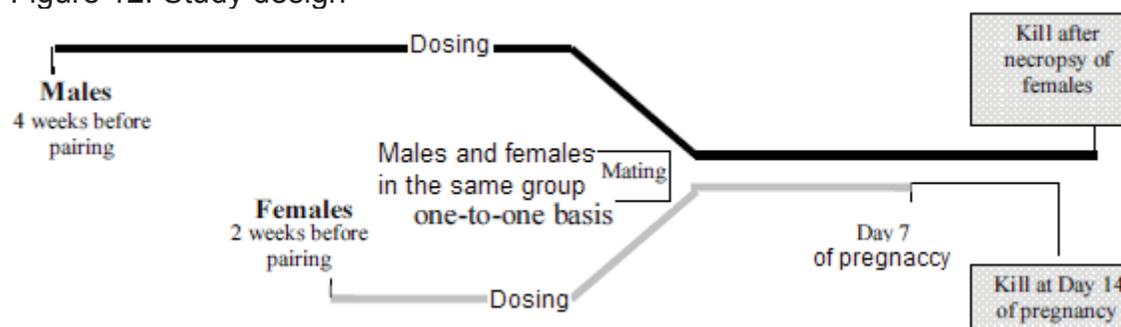
Key Findings

There were dark feces and dark contents in GI tract of PA21 treated animals. At PA21 4000 mg/kg/day, there were slightly less body weight gain and slightly higher plasma phosphorus in males, and slightly higher spleen weight in both sexes. Post implantation loss was higher at 4000 mg/kg/day. NOAELs were 4000 mg/kg/day for fertility and 1400 mg/kg/day for early embryonic development in CD rats.

Methods

Four groups of 22 male and 22 female Crl:CD (SD) IGS BR rats (Male: body weight 296-344 g, ~62 days old. Female: body weight 197-254 g, ~ 66 days old) were orally gavaged with PA21 at doses of 500, 1400, or 4000 mg/kg/day, or vehicle 1% w/v methylcellulose. Males were treated daily for four weeks before pairing, throughout pairing for total ~7 weeks. Females were treated daily for two weeks before pairing, throughout pairing, and until Day 7 of gestation. Doses selected for this study were based on previous oral repeat dose toxicity studies in rats (VFR0090, VFR0075, and VFR 0087). In a 2-week rat study using oral gavage administration (VFR0090), the highest dose of 4000 mg/kg/day resulted in slight transient weight loss, lower food consumption and loose feces at the start of treatment. Doses up to 4000 mg/kg/day in a 4-week dietary study (VFR 075) and doses up to 3000 mg/kg/day in a 13-week dietary study (VFR0087) revealed no overt changes in the reproductive organs and no treatment-related histological findings. The lowest dose of 500 mg/kg/day was ~2-fold the highest expected clinical dose. Animal pairing/mating and termination are illustrated in Figure 12.

Figure 12. Study design



During the study, rats were observed at least twice daily for clinical signs and morbidity/death. Physical examination was weekly performed on each animal. Body weight was recorded on Day 1, Day 2 and then twice-weekly, and before necropsy for males; on Day 1, Day 2, and then twice-weekly until mating was detected, and on Days 0, 4, 8, 11 and 14 of gestation. Food consumption was assessed twice weekly and mean daily food consumption was calculated. Starting from 10 days before pairing, daily vaginal smears were taken and examined for the estrous cycle of all females until evidence of mating was observed. Before pairing the animals, blood samples were obtained from 11/sex/groups after overnight fast for determination of hematology and plasma calcium (Ca) and inorganic phosphorus (Phos). Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and vaginal

smears were examined for the presence of spermatozoa. The day on which evidence of mating was found was designated Day 0 of gestation. The pre-coital interval was calculated for each female as the time elapsing between initial pairing and detection of mating. At the study termination, all animals were macroscopically examined; organ weights, tissue sampling and processing were performed (Table 115). Female uterine contents were examined. Reproductive assessments for all females included the number of corpora lutea in each ovary, the number of implantation sites, the number and distribution of resorption sites (classified as early or late), and live and dead embryos in each uterine horn.

Table 115. List of organs for weight, sampling and processing

	Organ weight	Histologically processed	Tissue iron content
Epididymides	x	x	
Kidney	x	x	x
Liver	x	x	x
Mesenteric lymph nodes		x	
Seminal vesicles	x	x	
Spleen	x	x	x
Testes	x	x	
Prostate	x	x	

Tissue samples for iron content were stored for any possible future assay requirement.

Results

There were no premature deaths in any group.

Dark feces were observed during the treatment period and 1 week post-dose at all doses. Body weight gain was lower in males at PA21 4000 mg/kg/day (Table 116), without changes in food consumption. Prior to pairing, plasma phosphate levels were slightly but statistically significantly higher (x1.14 of control) only in males at 4000 mg/kg/day. Plasma phosphate concentrations in females and plasma calcium concentrations in both sexes were not affected by PA21 treatment.

Table 116. Body weight and body weight gain (g)

Group mg/kg/day	Males									Females						
	Treatment day									Treatment day				Gestation day		
	1	1-2	1-8	1-15	1-29	1-36	1-50	1-53	1	1-2	1-8	1-15	0	0-8	0-14	
Control	Mean	321	7	45	85	139	156	199	203	225	3	14	22	254	44	78
	SD	9.9	3.3	6.7	11.1	18.4	19.9	23.1	24.5	10.6	6.4	10.2	12.6	12.3	9.6	10.4
500	Mean	320	6	40	83	136	153	193	200	226	2	17	24	259	44	79
	SD	10.1	2.8	6.6	8.5	16.1	19.4	23.7	24.7	14.2	6.0	6.6	10.5	19.7	7.4	8.5
1400	Mean	321	5	44	87	142	159	202	207	228	1	16	24	259	44	80
	SD	12.9	3.2	8.7	15.8	23.6	26.6	32.2	34.1	12.1	6.0	5.7	10.3	13.8	6.0	7.4
4000	Mean	326	0**	37**	78	128	145	180*	182*	230	0	14	21	257	46	80
	SD	10.5	4.0	7.4	10.6	17.7	22.4	29.4	29.9	12.7	7.6	7.6	9.0	20.7	6.3	9.3

* p<0.05 vs control; ** p<0.01 vs control

There were no PA21-related effects on the regularity of estrous cycles, pre-coital interval, percentage mating, conception rate or fertility index; fertility index was maximal in all groups (Table 117). Ten out of 88 animals had 1-3 more implantations than corpora lutea (1/22, 3/22, 4/22, and 2/22 in the control, low, mid, and high dose groups, respectively). At 4000 mg/kg/day, post-implantation loss was slightly, but significantly higher than concurrent control ($p < 0.05$) and concurrent background (Table 118). The sponsor excluded Litter #175 with 11 early resorptions based on the dam's small right kidney with a few punctate depressions, and concluded that the post-implantation loss was within normal background ranges. Since the average kidney weight of dam 175 was similar to other dams, the sponsor's exclusion was not acceptable. There were no PA21-related effects on the other litter data (Table 118).

Table 117. Estrous cycle, mating performance, and fertility

Group mg/kg/day Sex	Number of animals	Oestrous cycles			Pre-coital interval (days) 1-4	Group mg/kg/day Sex	Mating performance and fertility					
		Regular 4 or 5 day cycles	Irregular cycle λ				Number paired mating pregnancy	% mating	Conception rate (%)	Fertility index (%)		
Females PA21	Control	n	22	0	22	Control	22	22	22	100	100	100
		(%)	(100)		(100)	- PA21 - 500 Males	22	22	22	100	100	100
	500	n	22	0	22	- PA21 - 1400 Males	22	22	22	100	100	100
		(%)	(100)		(100)	- PA21 - 4000 Males	22	22	22	100	100	100
	1400	n	21	1	22	Control	22	22	22	100	100	100
		(%)	(95)	(5)	(100)	- PA21 - 500 Females	22	22	22	100	100	100
	4000	n	22	0	22	- PA21 - 1400 Females	22	22	22	100	100	100
		(%)	(100)		(100)	- PA21 - 4000 Females	22	22	22	100	100	100

Table 118. Litter data on Day 14 of gestation

Group mg/kg/day		Corpora Lutea		Implantations			Resorptions			Live Embryos	Implantation Loss (%)	
				Early	Late	Total	Pre-	Post-				
Control	Mean	16.0	15.4	0.6	0.0	0.6	14.8	4.0	3.9			
	SD	2.24	1.89	0.96	0	0.96	2.09	8.30	6.44			
	N	22	22	22	22	22	22	22	22			
PA21 500	Mean	16.5	15.8	1.0	0.0	1.0	14.8	4.8	6.0			
	SD	2.32	2.37	0.98	0	0.98	2.07	8.49	5.77			
	N	22	22	22	22	22	22	22	22			
PA21 1400	Mean	16.2	15.9	0.6	0.0	0.6	15.3	3.2	3.7			
	SD	2.24	2.10	0.80	0	0.80	2.14	4.76	5.12			
	N	22	22	22	22	22	22	22	22			
PA21 4000	Mean	15.8	15.6	1.5	0.0	1.5	14.1	2.3	9.5*			
	SD	1.27	1.26	2.52	0	2.52	2.92	3.68	16.39			
	N	22	22	22	22	22	22	22	22			
Background#	Average					0.96			6.32			
	Low-high range					0.8-1.3			5.1-8.6			

* $p < 0.05$ vs control

Control data from five fertility studies between 2005-2006 in the same lab

The only necropsy findings considered to be related to PA21 treatment were dark contents of the gastro-intestinal tract; this generally affected the cecum, and also the rectum, colon, ileum and jejunum. Spleen weights were slightly higher in both sexes at 4000 mg/kg/day ($p < 0.05$) (Table 119), but there were no treatment-related effects on weights of other organs.

Table 119. Body weight and organ weight (g) at necropsy

Group (mg/kg/day)		Males				Females			
		Terminal bodyweight	Kidneys	Liver	Spleen	Terminal bodyweight	Kidneys	Liver	Spleen
Unadjusted									
Control	Mean	533.8	3.84	20.01	0.846	331.9	2.46	15.03	0.726
	SD	30.64	0.304	1.433	0.1025	15.63	0.192	1.245	0.1131
PA21 500	Mean	525.8	3.85	21.77	0.880	339.2	2.49	15.64	0.791
	SD	26.27	0.286	1.940	0.0983	23.60	0.220	1.465	0.1101
PA21 1400	Mean	534.4	3.85	20.94	0.857	339.6	2.49	15.77	0.781
	SD	41.15	0.355	2.099	0.1358	16.88	0.270	1.129	0.0961
PA21 4000	Mean	511.9*	3.87	19.75	0.885	337.4	2.44	15.52	0.807
	SD	35.86	0.374	2.691	0.1247	22.76	0.220	1.672	0.1215
Adjusted Means									
Control			3.81	19.77	0.836		2.49	15.29	0.736
PA21 500			3.85	21.79**	0.881		2.47	15.53	0.787
PA21 1400			3.82	20.67	0.846		2.48	15.64	0.776
PA21 4000			3.94	20.23	0.906*		2.44	15.50	0.806*

* < 0.05 vs control; ** $p < 0.01$ vs control

The tissue iron content was not included in the report. The mean concentrations of iron in formulations prepared for dosing during the first and last weeks of treatment were generally between 103 and 109 % of intended for formulations at 50, 140 or 400 mg/ml respectively and were considered satisfactory.

In conclusion, oral administration of PA21 to male and female CD rats was well tolerated at dosages up to 4000 mg/kg/day. PA21 4000 mg/kg/day resulted in less body weight gain in males and slight higher spleen weight in both sexes. Post-implantation loss was higher at PA21 4000 mg/kg/day. NOAEL was 1400 mg/kg/day for maternal toxicity (lower in body weight gain at 4000 mg/kg/day). NOAELs were 4000 mg/kg/day for fertility and 1400 mg/kg/day for early embryonic development in this rat study.

9.2 Embryonic Fetal Development

9.2.1 PA21 – Embryo-fetal toxicity study in CD rats

Study no.:	VFR0095/063605	
Study report location:		(b) (4)
Conducting laboratory and location:		(b) (4)
Date of study initiation:	July 6, 2006	
GLP compliance:	Yes	
QA statement:	Yes	
Drug, lot #, and % purity:	PA21, 429000, 94%	
Deviation from study protocol:	No impact to the results	

Key Findings

Majority of the animals in the 4000 mg/kg/day group slightly lost weight at the start of treatment (Day 6 to 7), which was fully recovered thereafter and overall weight gain during gestation was similar to control values. After 12 days of treatment, plasma phosphorus concentration was lower in females at 1400 or 4000 mg/kg/day. At gestation Day 20, dam liver iron contents were slightly higher at doses 1400 and 4000 mg/kg/day. Post implantation loss was higher in the 4000 mg/kg/day group than in control group. There were no PA21 treatment-related incidences of major or minor fetal abnormalities or skeletal variants. NOAELs were 1400 mg/kg/day for maternal toxicity and for embryo-fetal development.

Methods

Four groups of 22 pregnant female CrI:CD® (SD) rats (~12 weeks old, body weight 235-295 g) received PA21 by gavage at doses of 500, 1400 or 4000 mg/kg/day, or vehicle 1% w/v methylcellulose 10 ml/kg from gestation Days 6 to 17. The dosages used in this study were based on data from previous work (VFR0090 and VFR0091) with this compound performed in the same laboratories. In Study VFR0090, PA21 treatment, 1000, 2000, or 4000 mg/kg/day for 2 weeks resulted PA21-related dark feces and dark content in GI tract at all dose levels, and slightly lower body weight and food consumption at high dose. In the preliminary embryo-fetal toxicity study in rats (VFR0091, n=6/group), there were no clear maternal or fetal effects up to the highest dose of 4000 mg/kg/day. Therefore the 4000 mg/kg/day was chosen as the high dose, and the lowest dose of 500 mg/kg/day was approximately 2-fold the maximum expected clinical dose.

During the study, clinical signs were observed twice daily, and detailed physical examination was performed on each animal on gestation Days 0, 5, 12, 18 and 20. Bodyweight was recorded on Days 0, 3, 6-18 and 20 of gestation, and food consumption were monitored and recorded for gestation Days 0-2, 3-5, 6-9, 10-13, 14-17 and 18-19. For 11 females from each group, blood chemistry parameters were assessed on Day 17 of gestation (the last day of treatment). Animals were killed on gestation Day 20 for necropsy, and fetuses were examined macroscopically, and subsequently by detailed internal visceral examination or skeletal examination. Liver, kidney, and spleen were sampled from dams and fetuses at necropsy on Day 20 of gestation for assessing iron content.

The homogeneity and stability of PA21 in 1% methylcellulose formulations were assessed for each formulation at preparation and after refrigerated storage for 24 and 48 hours and 3 days.

Results

A female at 4000 mg/kg/day was killed for welfare reasons on Day 16 of gestation due to traumatic injury to the tail. This isolated event was not related to PA21 treatment.

A treatment-related increase in incidence of dark feces was observed in the cage trays of PA21-treated animals. At 4000 mg/kg/day, the majority of animals had a transient slight weight loss in the first day at the start of treatment (Day 6 to 7). There was full recovery thereafter and overall weight gain during gestation was similar to control values (Table 120). There was no effect of PA21 treatment on the food consumption during gestation.

Table 120. Body weight and body weight gain during the experiment

Group	Body weight (g)	Body weight gain (g)																	
		PA21 treatment																	
		Days 0-3	Days 0-6	Days 0-6	Days 6-7	Days 6-8	Days 6-9	Days 6-10	Days 6-11	Days 6-12	Days 6-13	Days 6-14	Days 6-15	Days 6-16	Days 6-17	Days 6-18	Days 6-20	Days 18-20	
PA21 mg/kg/day	Day 0																		
Control	Mean	266	20	28	8	3	6	11	16	21	27	31	37	43	53	66	79	110	31
	SD	14.2	7.7	8.1	5.2	4.2	4.5	4.6	4.5	3.9	4.9	5.1	3.9	4.5	5.5	6.1	8.0	7.9	4.0
500	Mean	265	20	30	11	4	8	12	18	23	30	34	39	45	55	69	83	114	31
	SD	16.5	7.5	9.0	4.8	3.9	3.6	4.5	4.6	4.8	4.5	6.3	7.5	7.8	8.8	10.5	11.1	16.0	9.0
1400	Mean	266	22	33	11	2	5	14	19	24	31	36	41	47	57	70	84	116	33
	SD	14.6	6.7	8.6	5.9	5.5	8.9	4.8	5.3	4.8	6.2	5.2	5.3	5.9	6.1	8.3	10.2	12.8	6.1
4000	Mean	264	23	32	9	-1**	3	9	14	21	28	32	37	42	53	64	77	108	31
	SD	13.1	6.9	8.2	4.3	4.4	4.5	6.0	6.2	5.2	5.2	6.6	7.5	6.9	6.6	8.3	9.1	12.6	5.6

** p<0.01 vs control

At Day 17 of gestation (after 12 days of treatment), plasma phosphorus concentration was slightly lower at PA21 doses 1400 or 4000 mg/kg/day attributed to the pharmacological effect of PA21 in reduced GI uptake of phosphorus, but plasma calcium levels were unaffected by PA21 treatment (Table 121). There were no PA21 treatment-related effects on maternal macropathology findings. The Dams at 1400 and 4000 mg/kg/day showed slightly higher iron content in the livers on Day 20 of gestation (Table 121). No other disturbances were evident.

Table 121. Plasma calcium and phosphorus concentrations on gestation Day 17, and maternal and fetal tissue iron content on gestation Day 20 (n=11)

Group PA21 mg/kg/day	Plasma		Dam tissue iron content			Fetal tissue content (mg/kg)					
	Ca mmol/L	Phos mmol/L	Liver mg/kg	Kidney mg/kg	Spleen mg/kg	Liver		Kidney	Spleen†		
						Male	Female		Male	Female	
Control	Mean	2.64	1.84	91	77	1058	223	234	94	240	220
	SD	0.063	0.180	27.0	20.9	159.7	51.5	32.0	53.9		
500	Mean	2.66	1.70	101	78	1196	239	245	47 *	170	140
	SD	0.094	0.364	20.1	25.7	358.2	26.6	28.4	16.0		
1400	Mean	2.66	1.52 **	137 †	81	1077	259 *	259	33 **	170	160
	SD	0.057	0.248	42.1	14.1	210.6	21.7	28.8	4.8		
4000	Mean	2.64	1.35 **	193 **	75	1193	265 *	254	35 **	130	140
	SD	0.052	0.244	114.2	10.7	277.3	31.1	25.8	8.4		

† Samples pooled from all litters in group * p<0.05 vs control ** p<0.01 vs control

On Day 20 of gestation, post-implantation loss was higher in the PA21 4000 mg/kg/day group than concurrent control (p<0.05, Table 123). The sponsor excluded Litter #84 with 4 early resorptions and 2 late resorptions considered as “atypical of the group as a whole”, and concluded the post-implantation loss being within normal background ranges. The sponsor’s exclusion was not acceptable. Although the post-implantation loss in the PA21 4000 mg/kg/day group was within the background control range, it cannot be considered as normal since it was also seen in the fertility and early embryonic development study in CD rats (VFR0098, Table 118). Five out of 87 animals had 1-2 more implantation than corpora lutea (3/22 and 2/22 in the low and mid dose groups, respectively). No other litter parameters were affected by treatment with PA21 (Table 122, Table 123).

Table 122. Placental, litter and fetal weights on gestation Day 20 (from the application)

Group mg/kg/day		Placental Weight	Litter Weight	Litter Size	Male Fetal Weight	Female Fetal Weight	Overall Fetal Weight
Control	Mean	0.55	54.69	14.91	3.78	3.56	3.67
	SD	0.058	6.505	1.630	0.193	0.188	0.176
	N	22	22	22	22	22	22
PA21 500	Mean	0.56	53.60	14.45	3.84	3.57	3.71
	SD	0.050	6.325	1.765	0.211	0.183	0.184
	N	22	22	22	22	22	22
PA21 1400	Mean	0.57	54.64	14.68	3.81	3.63	3.73
	SD	0.064	6.526	1.783	0.211	0.215	0.201
	N	22	22	22	22	22	22
PA21 4000	Mean	0.54	51.99	14.10	3.82	3.56	3.68
	SD	0.055	8.819	2.211	0.284	0.218	0.247
	N	21	21	21	21	21	21

Table 123. Cesarean section litter data

Group, PA21 mg/kg/day	Corpora Lutea	Implantations	Resorptions			Live Young			Sex ratio (%M)	Implantation Loss (%)			
			Early	Late	Total	Male	Female	Total		Pre-	Post-		
Control	Mean SD	15.9 2.22	15.5 1.92	0.5 0.74	0.0 0	0.5 0.74	7.3 1.91	7.6 1.94	14.9 1.63	48.8 11.42	2.6 4.36	3.3 4.33	
500	Mean SD	15.9 1.85	15.2 1.74	0.7 0.89	0.0 0.21	0.7 0.88	7.7 2.21	6.8 1.51	14.5 1.77	52.7 11.6	5.4 7.24	4.7 5.60	
1400	Mean SD	16.7 2.25	15.5 2.02	0.7 0.77	0.2 0.53	1.0 0.90	7.8 2.49	6.9 2.47	14.7 1.78	53.0 16.62	6.9 11.22	5.3 5.54	
4000	Mean SD	16.0 1.96	15.3 1.45	1.1 1.22	0.1 0.44	1.2 1.50	7.0 2.07	7.1 1.84	14.1 2.21	49.4 11.14	3.8 4.41	8.0* 10.85	
Background^a	Average						0.72					5.05	
	Low- high Ranges						0.50-1.40					3.00-9.30	

* p<0.05 vs control

^a Control data from 10 embryo-fetal studies killed between March 2006 and January 2007

On Day 20 of gestation, the iron content of the male fetal livers was slightly higher in the groups receiving 1400 or 4000 mg/kg/day (x1.16 or x1.19 respectively, p<0.05 vs control), which was minimal, not seen in female fetuses, and not toxicologically significant (Table 121). The iron contents of the fetal kidneys and spleens were generally lower in treated groups than control group (Table 121). The lower iron contents in fetal kidney and spleen were not dose-dependent, not mechanistically plausible, possibly attributed to the higher values in control with big a variation, and not toxicologically conclusive.

A number of fetal findings were observed in all groups, but there were no PA21 treatment –related incidences of major and minor abnormalities or skeletal variants (Table 124, Table 125, Table 126).

Table 124. Major fetal abnormalities - group incidences (from the application)

Group, PA21 mg/kg/day	Fetuses				Litters			
	0	500	1400	4000	0	500	1400	4000
Number examined	328	318	323	296	22	22	22	21
Number affected	1	4	1	0	1	3	1	0
Folded retina	1	1	-	-	1	1	-	-
Dorsoventral distortion of sternum: interrupted vertebral column thoracic and lumbar regions: absent sacral vertebrae: anus imperforate: brachyury: tail rudimentary and swollen	-	1	-	-	-	1	-	-
Bent scapula	-	2 ^a	-	-	-	1	-	-
Short/thickened/bent humerus	-	1 ^a	1 ^b	-	-	1	1	-
Medially thickened/kinked ribs, marked	-	-	1 ^b	-	-	-	1	-

Superscript denotes fetuses with more than one abnormality

Table 125. Minor fetal visceral abnormalities - group incidences (from the application)

Group, PA21, mg/kg/day	Fetuses				Litters			
	0	500	1400	4000	0	500	1400	4000
Number examined	165	159	158	148	22	22	22	21
Number affected	30	31	31	29	17	18	15	13
Visceral abnormalities								
Eye(s)	variation in lens size	-	1	2	-	1	2	-
Thyroid	rudimentary	1	-	-	-	1	-	-
Thymus	partially undescended	1	-	2	1	1	-	1
Innominate artery	variation in origin	1	-	-	1	1	-	1
Diaphragm	thin with liver protrusion(s)/adhered to liver	4	2	3	3	4	2	3
Liver	additional lobe	-	1	-	-	-	1	-
	misshapen/bilobed posterior caudate lobe	3	-	-	2	3	-	-
Kidney(s)	rudimentary/absent papilla	1	1	2	2	1	1	1
Ureter(s)	dilated	1	1	1	2	1	1	1
Testis(es)	displaced	4	7	6	2	4	6	5
Umbilical artery	left	-	3	-	1	-	3	-
Haemorrhage(s)	brain/spinal cord	3	4	3	-	3	3	3
	eye/surrounding tissue	-	-	2	1	-	-	1
	thymus gland	-	1	-	-	-	1	-
	lungs	-	-	-	1	-	-	1
	abdominal cavity	3	2	6	3	3	2	4
	liver lobe(s)	9	6	8	15	8	4	5
	subcutaneous	1	2	2	2	1	2	2

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

Table 126. Minor fetal skeletal abnormalities/variants - group incidences (from the application)

Group, PA21, mg/kg/day Number examined	Fetuses				Litters					
	0 162	500 155	1400 164	4000 148	0 22	500 22	1400 22	4000 21		
Skeletal abnormalities										
Cranial	sutural bone		1	2	1	1	1	2	1	1
	fissure		-	-	1	-	-	1	-	-
Vertebral element abnormality										
	thoracic		-	-	-	1	-	-	-	1
Ribs	medially thickened/kinked		5	3	4	3	2	3	3	3
	irregularly ossified		-	2	1	-	-	2	1	-
Sternebrae	offset alignment		1	-	-	1	1	-	-	1
	additional centre		1	-	-	-	1	-	-	-
	bipartite ossified/misshapen		1	-	-	3	1	-	-	2
	ventral cleft		-	-	-	2	-	-	-	1
Costal cartilage	partially fused		-	-	-	3	-	-	-	1
	offset alignment		1	-	-	-	1	-	-	-
	7 th not connected to sternum		4	-	-	2	2	-	-	1
Appendicular	misshapen cranial margin scapula		-	1	-	-	-	1	-	-
Total affected by one or more of the above			11	6	7	8	6	6	4	6
Rib and vertebral configuration										
Cervical rib/with costal cartilage			4	-	-	3	4	-	-	1
Short/rudimentary 13 th rib/with/absent costal cartilage			2	1	2	1	1	1	2	1
Number with 13/14 or 14/14 ribs			8	15	12	16	7	12	7	9
Complete 14 th rib(s)			-	-	1	-	-	-	1	-
18 thoracolumbar vertebrae			2	-	-	-	1	-	-	-
Offset alignment pelvic girdle			1	2	-	-	1	2	-	-
Incomplete ossification / unossified										
Cranial centres/marked			7	12	10	8	7	9	5	7
Hyoid			2	14	8	4	2	7	5	3
Vertebrae	cervical		2	3	4	3	2	2	3	2
	thoracic		7	8	6	12	6	7	5	9
	lumbar		1	2	1	-	1	2	1	-
	sacrocaudal		6	6	5	6	5	4	5	4
Sternebrae	5 th and/or 6 th		87	88	96	80	21	20	22	20
	other		4	5	6	8	3	5	4	7
	total		88	88	96	80	21	20	22	20
Rib			1	-	-	-	1	-	-	-
Pelvic bones			2	-	3	-	2	-	3	-
Metacarpals/metatarsals			-	1	1	-	-	1	1	-
Precocious ossification										
Cervical vertebral centra (>4 ossified)			4	4	3	3	4	3	3	2
Additional observations at necropsy										
Left umbilical artery			1	-	1	3	1	-	1	3

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded

All formulations were homogenous in the vehicle for up to 48 hours following refrigerated storage (approximately 4°C), were stable in the vehicle at preparation and following resuspension after refrigerated storage (approximately 2-8°C) for 24 hours or 8 days. The mean concentrations of PA21 prepared for administration on the first and last days of treatment were between 103 and 107 % of intended concentrations and were therefore considered satisfactory.

9.2.2 PA21 – Embryo-fetal toxicity study in the rabbit by oral gavage administration

Study no.:	VFR0092/064068	
Study report location:		(b) (4)
Conducting laboratory and location:		(b) (4)
Date of study initiation:	Sept 25, 2006	
GLP compliance:	Yes	
QA statement:	Yes	
Drug, lot #, and % purity:	PA21, 426000, 95.4%	
Deviation from study protocol:	No impact to the results	

Key Findings

There was one death at PA21 1000 mg/kg/day, possibly related to the PA21 treatment. At PA21 1000 mg/kg/day, dam body weight, food consumption, and plasma phosphorus were lower, and liver iron content was higher, when compared with values in control group. There was an increased incidence of fetuses with incomplete/unossified epiphyses and metacarpals/phalanges at 1000 mg/kg/day. NOAELs were 500 mg/kg/day for both maternal toxicity and embryo-fetal development.

Methods

Four groups of 22 pregnant female New Zealand White rabbits (22-26 weeks old, body weight 3.49-4.51 kg) received PA21 by gavage at doses of 250, 500 or 1000 mg/kg/day, or vehicle 1% w/v methylcellulose 5 ml/kg from gestation Days 6 to 19. The dosages used in this study were based on data from previous studies (VFR0093 and VFR0089) performed in the same laboratories. In Study VFR0089, PA21 at 1000, 1500, or 2000 mg/kg/day for 5 days to 2 weeks resulted lower body weight and food consumption at mid and high doses. In the preliminary embryo-fetal toxicity study in rabbits (VFR0093, oral PA21 300, 600 or 900 mg/kg/day during gestation Day 6-19, n=6/group), there were no clear maternal or fetal effects up to the highest dose of 900 mg/kg/day. Therefore the increased high dose of 1000 mg/kg/day was selected for this study with lower low and intermediate doses of 250 and 500 mg/kg/day to give a two fold increase between each level.

During the study, clinical signs were observed twice daily, and detailed physical examination was performed on each animal on gestation Days 0, 6, 12, 18, 23 and 29. Bodyweight was recorded on Days 0, 3-20, 23, 26, and 29 of gestation, and food consumption was estimated daily. On gestation Day 19, blood samples were obtained from 11 animals per group for determining plasma calcium (Ca) and inorganic phosphorus (Phos). Animals were killed on gestation Day 29 for necropsy, and fetuses were examined macroscopically and subsequently by detailed internal visceral examination or skeletal examination. Meanwhile, samples of liver, kidney, and spleen were collected from up to 11 dams per group (the same animals bled for blood chemistry) and their fetuses for determining the tissue iron content.

The homogeneity and stability of PA21 in 1% methylcellulose formulations were assessed for each formulation at preparation and after refrigerated storage for various time periods.

Results

Animals # 3 and #14 from control group and # 70 from high dose group were prematurely killed for welfare reasons (Table 127). Animal #3 was sacrificed on gestation Day 28 due to suspected back trauma (limited use of hindlimbs). The exact location of damage to the back was not found at necropsy. Observations at necropsy included thickened adipose tissue, minimal contents of the gastro-intestinal tract and an enlarged gall bladder. Uterine examination revealed 13 fetuses (8 males and 5 females), that appeared viable, but none were observed to breathe and internal examination revealed that all had unexpanded lungs. Animal #14 was sacrificed on gestation Day 23 due to suspected abortion (a large quantity of blood on the cage tray). Examination of the uterus at necropsy revealed that none of the 11 fetuses had been expelled, but all were dead. A quantity of red fluid was noted within each uterine horn and the uterine tissue was dark. There was bright green gelatinous material within the gastro-intestinal tract and the spleen was enlarged.

The female #70 at 1000 mg/kg/day was killed on Day 21 of gestation. This animal had shown poor food consumption for 9 days prior to sacrifice and had lost 300 g since the start of treatment. Clinical signs observed included brown urine, dark/few/small feces, little hay eaten and little water drunk (visual assessment). This female was pregnant and necropsy confirmed the in-life signs of gastric disturbance. The reviewer agrees with the sponsor's interpretation that this death was likely attributable to PA21 treatment based on the similarity of this death to those observed on previous rabbit studies with PA21.

Table 127. Summary of female performance

Category	PA21 Dose Group, mg/kg/day	Number of females in Group			
		0	250	500	1000
Allocated to the embryo-fetal phase		22	22	22	22
Not pregnant		0	2	0	1
Animals killed for welfare reasons before Day 29		2*	0	0	1
Total of pregnant animals with litters at Day 29		20	20	22	20

* Includes one animal with total litter death

Dark feces and brown urine were observed in all treated groups during treatment due to the test material. Little hay eaten and a higher incidence of thin build were observed in several animals dosed at 1000 mg/kg/day. The bodyweight gain of animals dosed at 1000 mg/kg/day trended lower (<3%) during and post dosing when compared with control values (Figure 13). There were also other fluctuations in body weight changes during and post PA21 dosing. The lower or higher values in body weight gain during and post dosing were minimal (<3%) and not dose-dependent, and not of toxicological significance (Figure 13). Food consumption was also lower in the PA21 1000 mg/kg/day group (Table 128) during gestation Days 14-20, which was not statistically significant due to a high degree of inter-animal variability.

Figure 13. Mean body weight changes during and post-dose

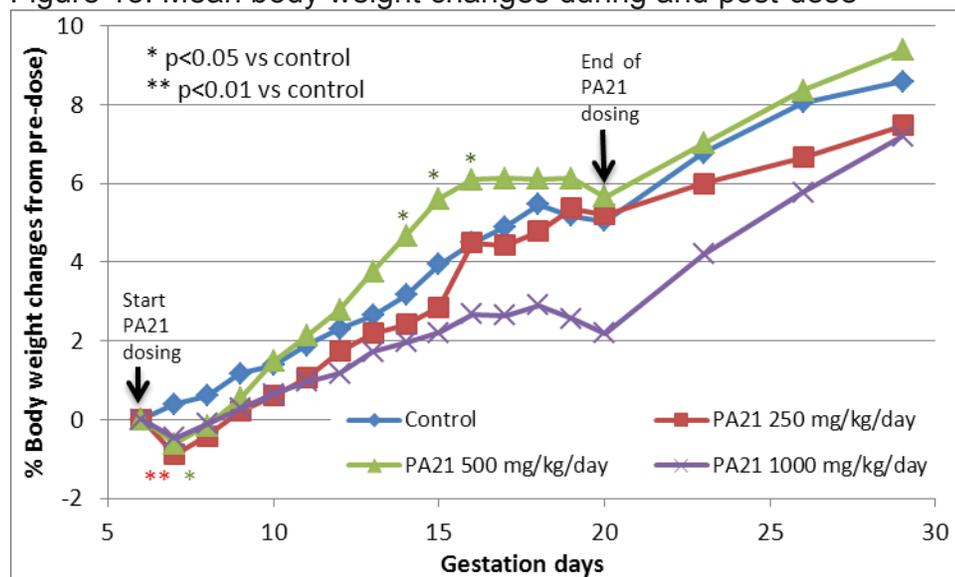


Table 128. Findings in food consumption (modified from the application)

Group		Day													
mg/kg/day		12	13	14	15	16	17	18	19	20	21	22	23	24	25
Control	Mean	154	159	155	164	173	176	174	165	165	152	151	142	139	126
	SD	39.5	44.2	41.9	35.5	27.0	31.8	30.7	47.7	45.2	52.4	47.8	54.4	55.2	54.2
PA21 250	Mean	155	140	149	163	165	156	161	158	159	143	137	121	121	99
	SD	29.0	48.9	42.3	33.8	43.3	49.4	37.3	40.4	35.1	45.3	50.9	44.0	41.5	43.6
PA21 500	Mean	170	166	164	160	162	163	158	146	154	152	138	133	126	119
	SD	30.8	28.7	35.6	40.7	52.0	58.6	58.2	57.2	42.2	42.8	45.7	47.3	38.2	38.7
PA21 1000	Mean	148	138	133	131	133	134	139	120	133	140	136	129	123	121
	SD	57.8	63.1	58.5	75.4	79.3	80.5	79.8	86.0	69.9	62.6	62.0	60.0	47.0	45.1

At Day 19 of gestation, plasma phosphorus concentration was lower in the PA21 1000 mg/kg/day group than in control group (1.48 ± 0.22 vs 1.26 ± 0.21 mmol/L in control, $p < 0.05$). Plasma calcium levels were unaffected by PA21 treatment.

There were no PA21-related findings at necropsy. The liver iron content was higher in dams at the dose 1000 mg/kg/day. The tissue iron content in dam kidney and spleen, and fetal liver, kidney and spleen was unaffected by treatment with PA21 (Table 129). Of the animals reaching the terminal kill on Day 29 of gestation, two animals in the 250 mg/kg/day treatment group and one in the 1000 mg/kg/day treatment group were found not to be pregnant. At least 20 litters were available for evaluation in each group (Table 127).

Table 129. Tissue iron content (mg/kg/day, from the application)

Group	mg/kg/day	Dam tissue samples			Fetal tissue samples					
		Liver	Kidney	Spleen	Male			Female		
					Liver	Kidney	Spleen	Liver	Kidney	Spleen
Control	Mean	138.5	58.3	359.5	1184.0	43.2	152.5	1231.7	43.7	130.0
	SD	43.7	17.9	84.3	350.6	7.0	43.5	331.8	11.1	20.0
	N	10	10	10	10	10	4	9	9	5
PA21 250	Mean	206.4	62.8	372.3	1094.0	42.4	126.0	964.4	36.2	140.0
	SD	50.3	15.8	143.9	255.9	5.0	11.4	209.9	4.1	14.1
	N	11	11	11	10	10	5	9	9	5
PA21 500	Mean	154.5	62.6	320.5	1163.6	40.5	140.0	1161.4	42.3	137.6
	SD	46.0	12.2	142.7	341.3	3.8	27.6	334.1	7.9	37.3
	N	11	11	11	11	11	6	11	11	7
PA21 1000	Mean	260.6**	65.3	575.9	1237.0	43.8	137.5	1232.0	43.4	154.0
	SD	135.9	16.6	717.4	260.9	7.1	12.6	310.4	7.9	18.2
	N	11	11	11	10	10	4	10	10	5

** $p < 0.01$ vs control

There were no adverse effects of PA21 treatment on corpora lutea, resorptions, live young, sex ratio and implantation loss (Table 130). Animals dosed at 1000 mg/kg/day showed slightly lower fetal weights and slightly larger litter size, which were not of toxicological significance (Table 131).

Table 130. Cesarean section litter data (from the application)

Group mg/kg/day	Corpora Lutea	Implantations	Resorptions			Live Young			Sex ratio (%M)	Implantation Loss (%)		
			Early	Late	Total	Male	Female	Total		Pre-	Post-	
Control	Mean	11.0	8.9	0.7	0.3	1.0	4.0	3.9	7.9	51.9	18.6	12.1
	SD	2.37	2.98				1.79	2.25	2.85			
	N	20	20	20	20	20	20	20	20	20	20	20
PA21 250	Mean	11.2	10.1	0.6	0.3	0.9	5.0	4.3	9.3	55.3	10.7	9.3
	SD	1.64	2.47				2.25	2.27	2.67			
	N	20	20	20	20	20	20	20	20	20	20	20
PA21 500	Mean	11.0	10.0	0.5	0.4	0.8	4.1	5.0	9.2	46.1	8.3*	7.5
	SD	2.61	2.49				1.28	1.94	2.24			
	N	22	22	22	22	22	22	22	22	22	22	22
PA21 1000	Mean	12.1	10.8*	1.0	0.3	1.3	4.7	4.9	9.6	47.1	10.5	10.9
	SD	2.27	2.24				2.45	1.73	2.76			
	N	20	20	20	20	20	20	20	20	20	20	20

* p<0.05 vs control

Table 131. Placental, litter and fetal weights on gestation Day 29 (from the application)

Group mg/kg/day		Placental Weight (g)	Litter Weight (g)	Litter Size	Fetal Weight (g)		Overall Fetal Weight (g)
					Male	Female	
Control	Mean	5.8	301.0	7.9	40.6	40.2	40.8
	SD	1.24	83.09	2.85	7.83	8.99	8.78
	N	20	20	20	19	19	20
PA21 250	Mean	5.6	356.1	9.3	40.1	38.6	39.6
	SD	0.86	78.90	2.67	5.38	5.30	5.25
	N	20	20	20	20	19	20
PA21 500	Mean	5.6	359.8	9.2	39.4	39.7	39.6
	SD	0.90	74.50	2.24	3.66	4.34	3.57
	N	22	22	22	22	22	22
PA21 1000	Mean	5.2	345.9	9.6	36.9	36.2	36.3*
	SD	0.93	98.38	2.76	3.74	4.29	3.79
	N	20	20	20	20	20	20

* p<0.05 vs control

There were no differences in the incidences of major and minor skeletal and visceral abnormalities among control and PA21 treatment groups (Table 132, Table 133, Table 134). There was an increased incidence of fetuses with incomplete/unossified epiphyses and metacarpals/phalanges at 1000 mg/kg/day (Table 133). These abnormalities possibly being consistent with lower mean fetal weight at this dose, and may be associated with the slightly higher litter size.

Table 132. Major fetal abnormalities - group incidences (from the application)

Group, mg/kg/day	Fetuses				Litters			
	0	250	500	1000	0	250	500	1000
Number examined	157	185	202	191	20	20	22	20
Number affected	11	3	7	6	7	3	4	2
Hydrocephaly, domed cranium	1	-	-	-	1	-	-	-
Anophthalmia with small/misshapen orbital socket: flattened cranium, partially fused nasals, small/disorganised frontals/parietals with hole	1 ^b	-	-	-	1	-	-	-
Absent nasals, small/misshapen frontals, cyclopia, medially displaced orbital sockets, proboscis, single nare and incisor socket, fused/ misshapen premaxillae, misshapen palate/zygomatic arches: fused/shortened mandibles	-	-	1	-	-	-	1	-
Partially fused frontals with bump/frontal ridge	1	-	-	1 ^c	1	-	-	1
Open/partially open eyelid(s)	5	-	3	5	2	-	1	1
Misshapen palate	-	-	-	1 ^c	-	-	-	1
Dilated aorta/aortic arch, transposition of ascending aorta and pulmonary trunk	1	-	-	-	1	-	-	-
Dilated aorta/aortic arch and or narrow pulmonary trunk	1 ^b	1	2	-	1	1	2	-
Absent kidney/ureter	1 ^a	1	-	-	1	1	-	-
Absent uterus/ovaries	-	-	1	-	-	-	1	-
Umbilical hernia	1 ^a	1	-	1 ^c	1	1	-	1
Forepaw flexure	2 ^{ab}	-	-	1 ^c	2	-	-	1
Malrotated hindpaw	1	-	-	-	1	-	-	-

Superscript denotes fetuses with more than one abnormality

Table 133. Findings in minor fetal skeletal abnormalities/variants - group incidences

Group, mg/kg/day	Fetuses				Litters			
	0	250	500	1000	0	250	500	1000
Number examined	146	182	195	185	20	20	22	20
Number intact	92	115	123	120	20	20	22	20
Skeletal abnormalities								
Cranial fissures/extra sutures	3	1	6	8	3	1	4	7
small/fissure/bipartite interparietal	1	-	1	2	1	-	1	2
hyoid cornua bent	1	-	2	3	1	-	2	3
Ribs medially thickened/kinked	-	1	-	3	-	1	-	2
interrupted 13 th	1	1	-	2	1	1	-	2
Number with 12/13 or 13/13 ribs	73	107	121	93	19	17	22	17
20 thoracolumbar vertebrae	44	66	78	63	13	14	18	14
Offset alignment pelvic girdle	1	2	6	2	1	2	5	2
Incomplete ossification/unossified								
Vertebral element cervical	-	4	-	4	-	4	-	3
Epiphyses	7	3	3	9	4	2	3	6
Metacarpals/phalanges	10	8	15	31	4	7	8	9

Fetuses with major abnormalities excluded.

Table 134. Group incidences of minor fetal visceral abnormalities in PA21 treatment groups

Group, mg/kg/day	Fetuses				Litters			
	0	250	500	1000	0	250	500	1000
Number examined at necropsy	146	182	195	185	20	20	22	20
Number heads examined at detailed visceral	54	67	72	65	20	20	22	19
Head subdural haemorrhage	-	-	-	1	-	-	-	1
Additional observations at necropsy								
Lower incisors haemorrhagic	-	-	1	-	-	-	1	-
Liver misshapen	-	-	1	-	-	-	1	-
Forelimb/paw flexure	-	-	1	-	-	-	1	-

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

The mean concentrations of PA21 on Day 6 and 19 of gestation (Day 1 and 14 of treatment) were between 93 and 101 % of intended and were therefore considered satisfactory.

In conclusion, NOAELs for maternal toxicity and embryo-fetal development were 500 mg/kg/day.

9.3 PA21: Pre- and post-natal development study in the CD rat by oral administration

Study no.:	VFR0106
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Aug 6, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot # / % purity:	PA21, 429000 / 95.3%

Key Findings

There was a slightly increased incidence of females with hair loss on the forelimbs/forepaws at 4000 mg/kg/day, affecting 11/22 females compared with 5/22 controls. Body weight was lower in F0 females at the dose 4000 mg/kg/day during lactation. Body weight gain in F1 males was lower at the dose 4000 mg/kg/day during age 5-13 weeks. At the dose 4000 mg/kg/day, neuromuscular function assessed at day 26/27 of age with the Accelerating Rotarod was lower in F1 males (maximum time achieved during three trials 196±41 vs 219±41 seconds in control, p<0.05), which became similar to the control at day 51-54 of age. NOAELs for maternal and for offspring post-natal development were both 1400 mg/kg/day, and NOAEL for offspring pre-natal development was 4000 mg/kg/day.

Methods

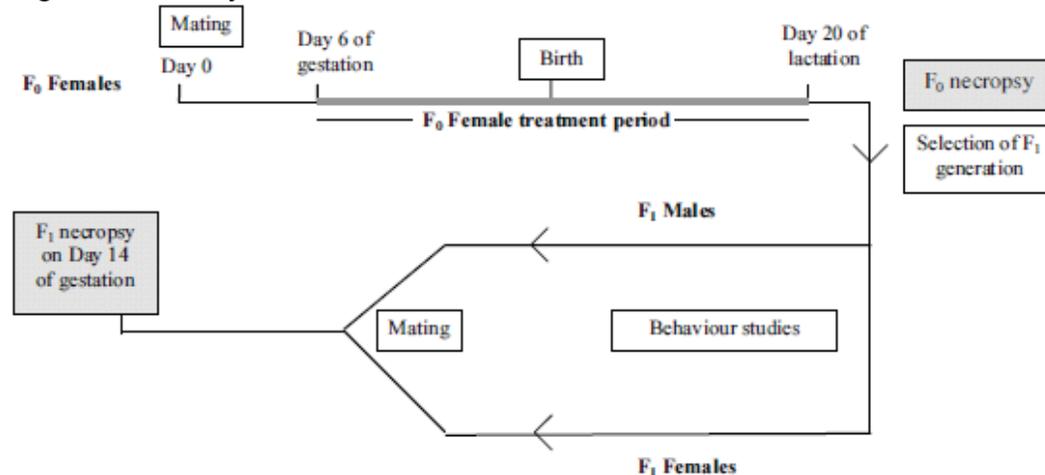
As shown in Figure 14, F₀ female Crl:CD® (SD) rats were gavaged with PA21 at 500, 1400, 4000 mg/kg/day, or vehicle 1% methylcellulose solution at 10 ml/kg/day during gestation Day 6 – lactation Day 20 (n=22/group). PA21 doses used here were based on a preliminary pre- and post-natal rat study with PA21 performed in the same laboratories (study # VFR0107). In study VFR0107, PA21 dosed during gestation Day 6 – lactation Day 10 at 2000 or 4000 mg/kg/day resulted in a slight and transient body weight loss at the start of dosing. Dosages of 2000 or 4000 mg/kg/day were well tolerated and did not cause any treatment-related problems during parturition. Females successfully reared their offsprings to day 11 of age, and bodyweights of the offsprings were slightly lower in the treated groups without clear dose-response, possibly due to slight higher litter sizes in the treated groups. Therefore, 4000 mg/kg/day was chosen to be the high dose in the current study. The low dose 500 mg/kg/day was about 2 times the maximal expected human dose.

F₀ females were allowed to litter and rear their offspring to weaning. These females were observed/examined for clinical signs, bodyweight, food consumption, gestation length, and parturition between mating day 0 and lactation day 21, and examined for plasma phosphate and calcium levels on lactation day 14. All F₁ litters were examined at ~ 24 hours after birth and then daily thereafter; clinical conditions, litter size, survival, sex ratio, and body weight were assessed. On day 4 of age, litters containing more than 10 offspring were reduced to ten (5 males and 5 females) by culling. Pre-weaning reflex developmental tests (surface righting, air righting, auditory function, and visual function) were performed on each F₁ offspring at certain days during lactation. On lactation day 21, all F₀ generation dams were sacrificed and macroscopically examined for gross lesions. Liver, kidney, and spleen samples from 11 F₀ females were collected for determining tissue iron content. At Day 28 of age, unselected F₁ pups were necropsied. Liver, kidney, and spleen samples from one male and one female of each F₁ litter were collected for determining tissue iron content.

20/sex/group F₁ generation rats were chosen for continued evaluation. Viabilities, clinical observations, body weights, food consumption were recorded. These rats were also evaluated for sexual maturation and motor activity, neuromuscular function, and learning and memory (Morris water maze).

At 9 weeks of age, the F₁ generation rats from the same treatment groups were paired on a one-to-one basis for a period of up to 3 weeks. Once mating occurred, the males and females were separated. The day on which evidence of mating was found was designated gestation Day 0. F₁ adult females were killed on day 14 of mating. C-section and gross necropsy were then performed. The reproductive assessment included the number of corpora lutea in each ovary and the number of implantation sites, the number and distribution of resorption sites, and live and dead fetuses of each uterine horn. Each fetus was weighed and examined for any gross external alterations. Females that failed to mate were killed on day 14 after the last day of pairing. Selected F₁ males were killed soon after the necropsy of the majority of the females and a gross necropsy was performed. Testes and epididymides weights were recorded.

Figure 14. Study scheme

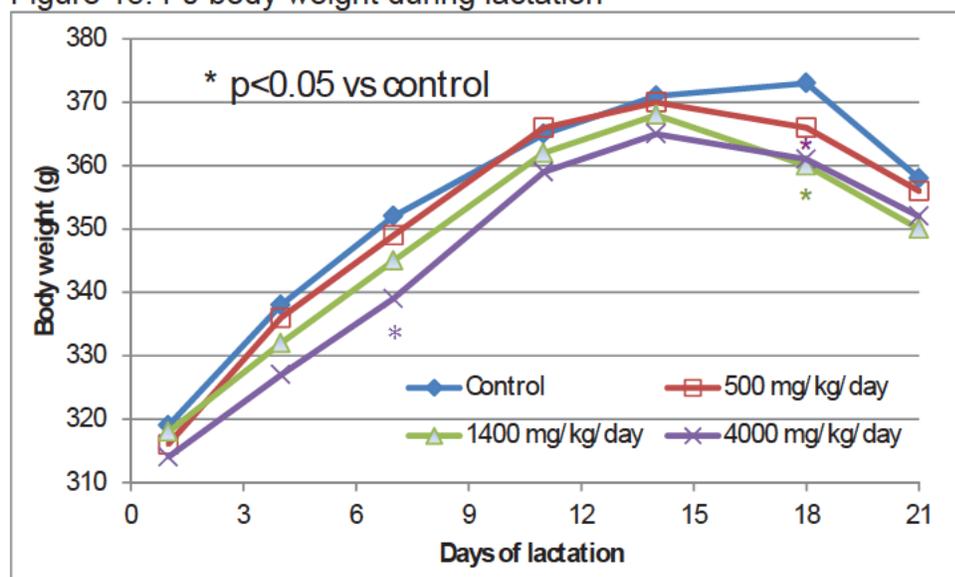


Results

There were no premature deaths during the course of the study. There were no PA21-related effects on food consumption, gestation length, parturition, gestation index, gross pathology in F₀ females. There were no PA21-related effects on clinical signs, litter size and survival, sex ratio, reflex development, motor activity, learning and memory, sexual maturation, mating performance, fertility, reproductive capacity, or macroscopic pathology of F₁ and F₂ generation offspring.

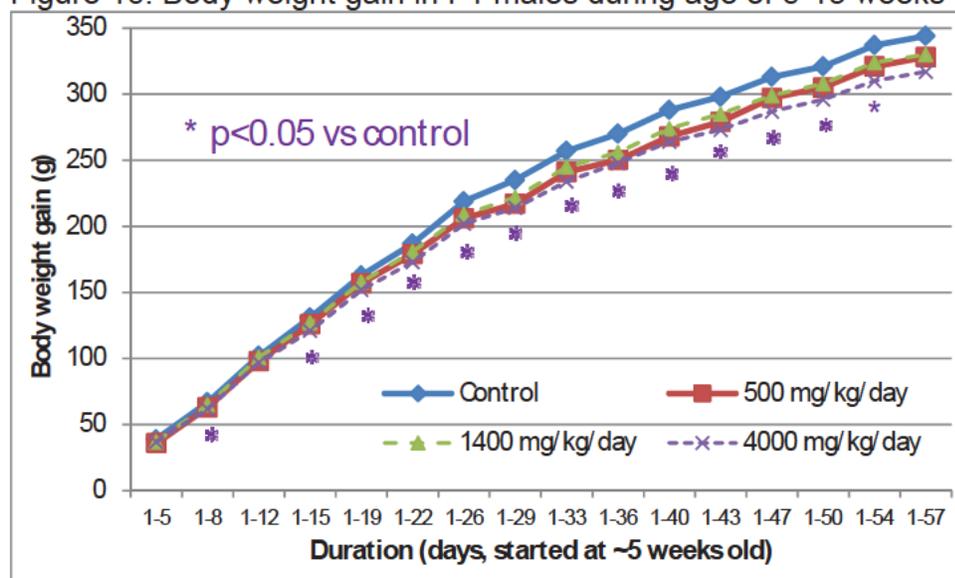
There was a treatment-related increase in the incidence of F₀ animals displaying dark feces at all dose groups during gestation and lactation, which was due to the color of the test material and was of no toxicological significance. There was a slightly increased incidence of females with hair loss on the forelimbs/forepaws at 4000 mg/kg/day, affecting 11/22 females compared with 5/22 controls. Body weight of F₀ females was similar among groups during gestation, and was lower in the 4000 mg/kg/day group during lactation (reached statistical significance at lactation days 7 and 18), and was also lower in the 1400 mg/kg/day group at lactation day 18 (Figure 15). Plasma phosphate concentration trended lower in 4000 mg/kg/day group, but did not reach statistical significance (0.85±0.33, 0.80±0.41, 0.89±0.37, and 0.64±0.21 mmol/L, respectively in control, 500, 1400, and 4000 mg/kg/day groups).

Figure 15. F0 body weight during lactation



In the F1 generation offspring, neuromuscular function assessed at day 26/27 of age with the Accelerating Rotarod was lower in males at the dose 4000 mg/kg/day (maximum time achieved during three trials 196 ± 41 vs 219 ± 41 seconds in control, $p < 0.05$), which became similar to the control at day 51-54 of age. Started at ~6 weeks of age, body weight gain was slightly but statistically lower in males at the dose 4000 mg/kg/day, which was persistent during the rest of study (Figure 16). There were no differences in body weight gain in F1 males during other period of study and in F1 females.

Figure 16. Body weight gain in F1 males during age of 5-13 weeks



Thus, NOAELs for maternal and for offspring pre-natal development were both 1400 mg/kg/day, and NOAEL for offspring post-natal development was 4000 mg/kg/day.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

PA21, a mixture of polynuclear iron (III)-oxyhydroxide, starch and sucrose, is a practically insoluble iron (b) (4)-based phosphate binder. PA21 is being developed for the control of serum phosphorus levels in patients with end stage renal disease (ESRD). The recommended regimen starts with chewing one tablet (500 mg iron) 3 times daily with meals and is adjusted by 1 tablet per day as needed until an acceptable serum phosphorus level (≤ 5.5 mg/dL) is reached, with a maximal daily dose of 6 tablets (3000 mg iron).

The intended mechanism of action for PA21 to control serum phosphorus levels in patients with ESRD is to bind dietary phosphate in the gastrointestinal (GI) tract, resulting in phosphate excretion with the feces, and thereby preventing phosphate absorption. The PA21 active component iron oxide hydroxide binds phosphate through a direct ionic interaction between the negatively charged oxygen ions on the phosphate and the ferric ions in ferric oxide. In vitro studies demonstrated efficient phosphate binding by PA21 under simulated GI tract conditions (pH range of 1.2 to 8.5), with a phosphate binding capacity at least equivalent to currently available phosphate binders. In rat models of chronic renal failure (CRF), PA21 was as effective as calcium carbonate, sevelamer carbonate and lanthanum carbonate in correcting the hyperphosphatemia and associated secondary hyperparathyroidism, was more effective than calcium carbonate and, to some extent, lanthanum carbonate in preventing vascular calcification in the thoracic aorta, and did not induce defective bone mineralization. The elevated bone turnover observed in CRF rats was corrected by PA21, with no evidence of iron deposition in the bone.

No adverse effects of PA21 on cardiovascular, central nervous, or respiratory systems were observed in safety pharmacology studies. There was no biologically significant effect on GI motility in a charcoal propulsion study conducted in mice.

A short-term study in mice showed no influence of dietary components or drugs on absorption of iron from PA21. Under simulating physiological conditions in vitro, strong/complete adsorption or interaction of furosemide, losartan, atorvastatin, doxycycline, alendronate, levothyroxine and paricalcitol to/with PA21 were observed. The observed adsorption of levothyroxine, paricalcitol and atorvastatin was less pronounced in the presence of phosphate. No significant adsorption to or interaction with PA21 was found in the case of ciprofloxacin, digoxin, enalapril, metoprolol, nifedipine, warfarin, hydrochlorothiazide, metformin, quinidine, bile acids, water soluble B vitamins, amino acids, fluoride, or oxalate. Adsorption of pioglitazone to PA21 was

moderate, and PA21 affected the solubility of omeprazole in vitro. Adsorptions of cinacalcet, glipizide, candesartan cilexetil, or enalaprilate to PA21 were inconclusive due to inconsistent solubility at different pH levels or poor tablet dissolution. Furthermore, no influence of the macronutrient oxalate on phosphate binding capacity of PA21 was observed in in vitro interaction studies simulating GI tract conditions.

Pharmacokinetic studies were conducted in mice, rats and dogs with oral ^{59}Fe -PA21 at clinically relevant dosages. After a single oral administration of ^{59}Fe -labeled PA21 in SD rats, < 3% of the administered radioactivity was recovered from sampled tissues of iron storage or utilization (blood cells, liver, spleen, and bone marrow) with small amounts (< 0.5%) in the walls of small and large intestines at 24 hours post-dose. A major portion of the administered dose was recovered from feces during the first 48 hours postdose. No radioactivity was recovered from urine, and there was neither biliary excretion nor entero-hepatic circulation of ^{59}Fe . Studies in pigmented (Lister Hooded) rats showed findings similar to those in SD rats, indicating a lack of binding or interactions of PA21 with melanin. In dogs, a low level of absorption was observed in only 1 of 3 animals studied. Excretion studies showed that virtually the total iron dose was excreted in the feces of all species within 48 hours of oral administration of PA21.

In vitro studies conducted under simulated GI tract conditions showed highest iron release from PA21 (~6%) under low pH conditions as present in the lumen of the stomach in the fasting state, and minimal release of iron (0-0.04%) under pH conditions in the stomach in the fed state as well as in the duodenum, jejunum and colon. PA21 was degraded to simple endogenous and/or innocuous molecules in vitro similar to mixtures of its components and the carbohydrates that stabilized the iron (III)-oxyhydroxide core.

A study in mice showed that iron uptake from PA21 was not influenced by a number of common foodstuffs or pharmaceutical products. Oral daily PA21 treatment for 14 days in rats did not affect the amounts or activities of cytochrome P450 (CYP) isoenzymes in liver and small intestines, indicating low potential for drug-drug interactions at the metabolic level.

A set of preclinical toxicological studies was performed with PA21 and the pivotal GLP studies are listed in Table 135.

Table 135. The pivotal GLP toxicity studies (from the application)

Study Type and Duration	Route of Administration	Species	Compound Administered
<u>Single-dose toxicity</u>	PO	Rat	PA21
<u>Repeat-dose toxicity</u>			
1 month	PO	Rat and dog	PA21
1 month comparative	PO	Rat	PA21/PA21-2
1 month	PO (fasted)	Dog	PA21
3 months	PO	Rat and dog	PA21
6 months	PO	Rat and dog	PA21
9 months	PO	Dog	PA21
<u>Genotoxicity</u>			
Bacterial mutation	In vitro	<i>S. typhimurium/E. coli</i>	PA21/PA21-2
Chromosome aberration	In vitro	CHL cells	PA21/PA21-2
Comet assay (GI tract)	PO	Rat	PA21
In vivo micronucleus ⁽¹⁾	PO	Rat	PA21
<u>Carcinogenicity</u>			
101-104 weeks	PO	Mouse	PA21
99-103 weeks	PO	Rat	PA21
<u>Reproductive toxicity</u>			
Fertility and early embryonic development	PO	Rat	PA21
Embryo-foetal toxicity	PO	Rat and rabbit	PA21
Pre- and postnatal toxicity	PO	Rat	PA21

¹ Conducted as part of 26-week toxicity study in rats.

Notes: CHL = Chinese Hamster Lung; GI = Gastrointestinal; PO = Per os (oral).

In the single dose toxicity study with PA21 5000 mg/kg, there were no PA21-related findings in rats. Thus, the lowest lethal oral dose of PA21 in rats was greater than 5,000 mg/kg.

Repeated-dose oral toxicity studies were conducted in rats and dogs up to durations of 26 weeks (rats) and 39 weeks (dogs). In addition, comparative toxicity of PA21 and PA21-2 was examined in a 4-week rat study, and toxicity under fasting conditions was also studied in a 4-week dog study. Toxicokinetic data were not collected because of the lack of significant absorption of PA21 or its iron. However, potential for iron accumulation in liver, spleen and kidney after repeat administration was investigated in these studies. Serum vitamin levels were determined in several of the repeated-dose toxicity studies, the results of which were inconclusive since retrospective validation of the assay methods applied to dog serum samples for vitamins A, E, K1 and 1,25-dihydroxyvitamin D failed to meet acceptance criteria and showed problems of sample stability.

In repeated-dose toxicity studies in rats, the PA21 NOAEL dosage tended to decrease with increasing study duration, from 1,000 mg/kg/day in the 4-week studies to 200 mg/kg/day in the 26-week study. In the 26-week repeat dose toxicity study in CD rats (followed by a 6-week recovery period), dietary administration of PA21 (dose levels 200, 750, and 2500 mg/kg/day) resulted in -

- Lower body weight gain in males during treatment at dose 2500 mg/kg/day and transiently and slightly lower body weight in males at dose 750 mg/kg/day.
- Thickened and darkened cecum and/or colon in both sexes at doses 750 and/or 2500 mg/kg/day at the end of treatment; Mucosal hyperplasia with or without submucosal inflammation/edema in the cecum, colon, and/or rectum of one male given 750 mg/kg/day and of 3 males given 2500 mg/kg/day; Mucosal hyperplasia in the colon of one male given 2500 mg/kg/day after 6 weeks of recovery.
- Higher plasma levels of ALP, urea, and phosphorus; lower urine volume and phosphorus, and higher urine pH in males and/or females at doses 750 and/or 2500 mg/kg/day at weeks 13 and/or 26; Thickened urinary bladder containing calculi and ureter distension in two males, kidney pelvic dilatation in 3 males, bladder transitional cell hyperplasia in 3 males, and ureter mucosal hyperplasia in 2 males at 2500 mg/kg/day at the end of treatment. Findings in the urinary duct were probably secondary to the marked changes in urine composition that occurred as a result of the intended pharmacological action in normophosphatemic healthy rats.
- A range of other findings were attributed to the reduction phosphate uptake as a result of the action of PA21, the coloration of the test material (due to its high iron content), the uptake of iron from GI, or to metabolic changes related to the phosphate-depleting effects of PA21. These findings included higher serum and tissue levels of iron, altered bone turnover and levels of vitamin D metabolites, and increased degree of hemosiderosis and the presence of Perls' positive material in the epithelium and lamina propria/submucosal macrophages of the GI tract, as well as in the lymph nodes and iron storage tissues (liver, spleen and kidney). These changes were minimal to moderate (2-fold or less), never associated with toxic injury, and not considered to be toxicologically significant.
- NOAEL was 200 mg/kg/day.

In a 4-week comparative or bridging toxicity study in CD rats, dietary administration of PA21 (0, 1000 or 4000 mg/kg/day) or PA21-2 (1000 or 4000 mg/kg/day) resulted in -

- Dark feces, brown staining of the tail and other body parts, dark contents in the GI tract, and darkened rectum in all treated groups with higher incidences in the groups of 4000 mg/kg/day PA21 or PA21-2. These findings were considered to be due the color of the test materials, and were not of toxicological significance.
- Significantly less body weight gains in males receiving either PA21 or PA21-2 at 4000 mg/kg/day, and in females receiving PA21-2 at 4000 mg/kg/day, associated with slightly reduction (3-7%) of food consumption.
- Slightly higher levels of plasma ALP, urea, and calcium, slightly lower plasma potassium; darker urine color, higher urine ketones, lower urinary volumes associated with higher specific gravity, higher urinary pH, and slightly higher levels of urine calcium, sodium and potassium, and negligible levels of urine phosphorus in males and females receiving 4000 mg/kg/day of either test material; Decreased

urinary phosphorus excretion in the low dose groups of PA21 and PA21-2. These findings indicated a slightly elevated bone turnover and renal function impairment.

- Higher plasma iron levels, higher iron contents in liver and spleen (trend in kidney), and higher incidences of positive Perls' staining (iron pigment) in the liver, mesenteric lymph node, and in the small and large intestines at 4000 mg/kg/day of PA21 and PA21-2; similar changes at lower incidence or low extent at the dose 1000 mg/kg/day. These findings were attributed to GI iron uptake from the test materials.
- Epithelial hyperplasia of the large intestine mostly in male groups (one incidence in PA21-treated females) at PA21 and PA21-2 4000 mg/kg/day.
- There was no apparent difference between PA21 and PA21-2. Findings at 1000 mg/kg/day were minimal and not of toxicological significance. NOAELs were 1000 mg/kg/day for both PA21 and PA21-2 in this study.

In repeat dose toxicity studies in dogs, dietary administration of PA21 at 0, 0.1, 0.3, or 1.0 g/kg, b.i.d was well tolerated for duration up to 39 weeks, with higher incidence of liquid feces in all treated male groups and in females receiving 0.1 or 1.0 g/kg b.i.d., when compared to control animals. This increased incidence of liquid feces, rather than inflammation or epithelial hyperplasia, was likely a kind of GI tract irritation that may have limited drug residence time, but was not of toxicological significance. Some minimal – moderate changes related to the pharmacological action of PA21 (altered urine and, to some extent, plasma calcium and phosphorus, and plasma 1,25-dihydroxyvitamin D levels) and its iron content (dark feces, Perls' staining of epithelium and macrophages in the GI tract or macrophages in liver/spleen) were observed, but were not considered to be toxicological significant. In contrast to the rat and mouse studies, no hyperplastic changes were observed in the GI tract or urinary bladder at histopathological examination of tissues. NOAEL was 1 g/kg b.i.d. in the 39-week repeat dose toxicity study in dogs.

A 4-week bridging toxicity study with oral PA21 capsule (1 g/kg, b.i.d) administration to fasted beagle dogs showed increases in incidences of emesis/irritation and iron uptake into the mucosa of the GI tract, and a decrease in urinary phosphate. The only difference compared to previous studies in which PA21 was administered immediately prior to feeding related to locally-mediated effects, i.e. emesis/irritation. There was no difference in apparent systemic toxicity of PA21 between fed and fasted animals at the maximum feasible dose of 2 g/kg/day.

PA21 (and PA21-2) showed no evidence of genotoxic activity in a range of in vitro and in vivo assays, including "site of contact" assays for DNA damage in the GI tract (Comet assays).

In a rat carcinogenicity study with dietary administration of PA21 at 0, 200, 750, and 2500 mg/kg/day, tumor findings were considered to be negative. All findings included –

- A slightly increased incidence of benign thyroid c-cell adenoma in males at the dose 2500 mg/kg/day, which was not dose-dependent or statistically significant.
- A slightly increased incidence of adrenal pheochromocytoma in females at the doses 200 and 750 mg/kg/day, which was not dose-dependent or statistically significant.

- Slightly lower body weight gain, markedly higher plasma and tissue iron level, slightly depressed kidney function (higher plasma urea and phosphorus, lower urine volume and urine phosphorus), and slightly elevated bone turnover due to reduced GI phosphate uptake (slightly higher levels of plasma calcium, urine calcium, serum 1,25-dihydroxyvitamin D, osteocalcin and deoxy pyridinoline) at doses 750 and/or 2500 mg/kg/day.
- Higher level of pigmented macrophages (iron content) in GI tract, liver, kidney, spleen, pancreas, adrenal gland, mesenteric, mandibular, and axillary lymph nodes at doses 750 and 2500 mg/kg/day; and epithelial hyperplasia in GI tract, lymphoid aggregation, inflammation, and cyst in various organs/tissues at 2500 mg/kg/day.

In a mouse carcinogenicity study, dietary administration of PA21 at 0, 1250, 2500, and 5000 mg/kg/day resulted in –

- Treatment-related colon and/or cecum adenocarcinomas in all male treatment groups and in females at 5000 mg/kg/day with a single incidence in a female at 1250 mg/kg/day.
- Roughened forestomach, cystic area, masses, thickened or raised areas in cecum and/or colon at doses 5000 mg/kg/day, associated with dose-related increases in incidences and severity of colon and/or cecum epithelial hyperplasia and mucosal diverticuli/cysts/hyperplasia, and epithelial hyperplasia and hyperkeratosis in the non-glandular of forestomach at 5000 mg/kg/day.
- Increased incidence of urinary bladder calculus(i) in males at 5000 mg/kg/day, associated with transitional epithelial hyperplasia in male urinary bladder and dilated medulla tubules in the kidney of both sexes.
- Higher level of iron content in GI tract, liver, kidney, mesenteric lymph nodes, and mesenteric lymphoid aggregation, inflammation, and cyst in males and/or females at doses 1250, 2500, and/or 5000 mg/kg/day.

Four definitive studies in rats and rabbits were performed to assess reproductive and developmental toxicity of PA21. The fertility and early embryonic development study was done in CD rats with oral gavage of PA21 at 0, 500, 1400, and 4000 mg/kg/day. At PA21 dose 4000 mg/kg/day, there were slightly less body weight gain and slightly higher plasma phosphorus in males, and slightly higher spleen weight in both sexes. Post implantation loss was higher at 4000 mg/kg/day. NOAELs were 4000 mg/kg/day for fertility and 1400 mg/kg/day for early embryonic development in CD rats.

The embryo-fetal toxicity studies were done with oral gavage of PA21 at 0, 500, 1400, and 4000 mg/kg/day in CD rats, and with oral gavage of PA21 at 0, 250, 500, and 1000 mg/kg/day in rabbits.

- In rats, PA21-related effects on dams included slightly lower body weights in the 4000 mg/kg/day group at the start of treatment (Day 6 to 7); statistically significantly lower plasma phosphorus concentration at 1400 or 4000 mg/kg/day after 12 days of treatment, and slightly higher liver iron contents at doses 1400 and 4000 mg/kg/day at gestation Day 20. Post implantation loss was higher in the 4000 mg/kg/day group than in control group. There were no PA21 treatment-related incidences of major or

minor fetal abnormalities or skeletal variants. NOAELs were 1400 mg/kg/day for maternal toxicity and for embryo-fetal development.

- In rabbits, there was one death at PA21 1000 mg/kg/day, possibly related to the PA21 treatment. At PA21 1000 mg/kg/day, dam body weight, food consumption, and plasma phosphorus were lower, and liver iron content was higher, when compared with values in control group. There was an increased incidence of fetuses with incomplete/unossified epiphyses and metacarpals/phalanges at 1000 mg/kg/day. NOAELs were 500 mg/kg/day for both maternal toxicity and embryo-fetal development.

In the pre- and post-natal development study in the CD rat, PA21 was orally gavaged at doses 0, 500, 1400, and 4000 mg/kg/day. PA21 treatment-related findings included –

- A slightly increased incidence of F0 females with hair loss on the forelimbs/forepaws at 4000 mg/kg/day, affecting 11/22 females compared with 5/22 controls. Lower body weight in F0 females at the dose 4000 mg/kg/day during lactation.
- Lower body weight gain in F1 males at the dose 4000 mg/kg/day during age 5-13 weeks. At the dose 4000 mg/kg/day, neuromuscular function assessed at day 26/27 of age with the Accelerating Rotarod was lower in F1 males (maximum time achieved during three trials 196 ± 41 vs 219 ± 41 seconds in control, $p < 0.05$), which became similar to the control at day 51-54 of age.
- NOAELs for maternal and for offspring post-natal development were both 1400 mg/kg/day, and NOAEL for offspring pre-natal development was 4000 mg/kg/day.

Thus, oral PA21 was not teratogenic in rats (up to 4000 mg/kg/day) and rabbits (up to 1000 mg/kg/day), and did not affect rat fertility up to 4000 mg/kg/day. Maternal NOAELs were 1400 mg/kg/day for rats and 500 mg/kg/day for rabbits based on reduced body weight gain in dams and one death at high doses. NOAELs for embryo-fetal toxicity were 1400 mg/kg/day for rats (based higher post implantation loss at high dose) and 500 mg/kg/day for rabbits (based on more fetuses with incomplete/unossified epiphyses and metacarpals/phalanges at high dose). NOAEL for offspring pre-natal development was 4000 mg/kg/day, and NOAEL for offspring post-natal development was 1400 mg/kg/day

The preclinical studies were generally well designed and conducted. Findings and NOAEL from toxicological studies were summarized in Table 136. Issues identified included (1) binding/interaction of drugs to/with PA21 in vitro; (2) the uptake of iron from GI and metabolic changes related to the phosphate-depleting effects of PA21; (3) colon and/or cecum adenocarcinomas, GI epithelial hyperplasia and mucosal diverticulum/cysts/hyperplasia; and (4) increased embryo resorptions and delayed post-natal development.

In vitro studies simulating physiological conditions showed strong/complete adsorption or interaction of furosemide, losartan, atorvastatin, doxycycline, alendronate, levothyroxine and paricalcitol to/with PA21. Since patients with chronic kidney disease are most likely treated with other drugs, the binding/interaction of the drugs to/with PA21 will affect the pharmacological effects of other drugs. According to the sponsor, PA21

did not alter the systemic exposure of a single dose of losartan, furosemide, digoxin, warfarin, or omeprazole in health volunteers. Thus, drugs may bind/interact to/with PA21 in GI include alendronate, doxycycline, levothyroxine, atorvastatin, and paricalcitol.

Repeat dose studies in mice, rats, rabbits, and dogs demonstrated uptake of iron from degraded PA21 active component polynuclear iron (III)-oxyhydroxide in GI, evidenced by higher serum and/or tissue levels of iron and/or positive Perls' staining (for iron) of epithelium and macrophages in the GI tract or macrophages in liver/spleen. However, the iron accumulation was minimal (<2 times), and not associated with toxicological injury. The iron release from polynuclear iron (III)-oxyhydroxide in vitro under pH conditions in the stomach in the fed state was minimal (0-0.04%). Iron absorption in PA21 ADME studies was <3% in rats and negligible in dogs. The daily iron uptake from PA21 at maximal dose of 3000 mg iron/day may not be an issue to cause any concern in patients with ESRD, providing patients with ESRD usually need more daily iron intake.

Changes related to pharmacological action of PA21 (altered urine and, to some extent, plasma calcium and phosphorus, and altered bone turnover and levels of vitamin D metabolites) were observed in repeat dose studies in mice, rats, rabbits, and dogs. Chronic treatment with high dose PA21 resulted in a higher incidence of urinary bladder calculus(i), associated with transitional epithelial hyperplasia in urinary bladder and dilated medulla tubules in the kidney of mice and rats, slightly impaired renal function and a slightly increased incidence of benign thyroid c-cell adenoma in rats. These findings were attributed to the exaggerated pharmacological response or physiological response to phosphate depletion induced by PA21 treatment in normophosphatemic animals. In chronic kidney disease (CKD) patients with hyperphosphatemia, PA21 treatment will correct the higher plasma phosphate but not lead to phosphate depletion. Therefore, the exaggerated pharmacological response or physiological response to phosphate depletion induced by PA21 treatment is not applicable in CKD patients.

The mouse carcinogenic study with PA21 in diet over 101 weeks for males and 104 weeks for females showed increased incidences of adenocarcinomas in colon and cecum in males at all dose levels and in females at 5000 mg/kg/day with a single incidence in a low dose female. There were also dose-related epithelial hyperplasia and mucosal diverticuli/cysts/hyperplasia in colon and cecum, adenoma and evidence of local irritation in non-glandular forestomach with increased epithelial hyperplasia and hyperkeratosis at 5000 mg/kg/day, dilated/cystic sinuses in enlarged mesenteric lymph nodes of males at all doses, and inflammatory cells in esophagus of females at the mid and high doses. The correlation between PA21-induced hyperplasia and the presence of adenocarcinomas in both colon and cecum, and the presence of adenoma and local irritation in non-glandular forestomach suggested that the neoplastic changes were part of a continuum that originated from chronic irritation, and subsequent proliferative response of the GI-tract to oral administered PA21.

In the 2-year carcinogenic study in rats (VFR0104), there were PA21-related inflammation and epithelial hyperplasia in gastro-intestinal tract of both sexes at 2500 mg/kg/day. In the 26-week toxicity study in rats (VFR0096), mucosal hyperplasia with or without submucosal inflammation/edema was also seen in the cecum, colon, and/or rectum of 3/15 males at 2500 mg/kg/day. Therefore, the PA21-associated epithelial hyperplasia in response to PA21-associated chronic irritation/inflammation in gastro-intestinal tract is not a mouse species-specific finding. It has been known for long time that chronic irritation/inflammation to epithelium in humans is risk factor for cancer (1). Oral administration of PA21 in human may also be a risk factor for cancer in GI due to possible PA21-associated chronic irritation/inflammation. In a dog repeat dose toxicity study with oral capsule PA21 for 26 and 39 week following by 6 weeks recovery (VFR0097), high incidence of liquid feces occurred in all treated male groups and in females of low and high dose groups, when compared to control animals. The increased incidence of liquid feces, rather than inflammation or epithelial hyperplasia, is a kind of GI tract irritation. But, this kind of irritation, expressed as liquid feces, may be at a functional response level (i.e., accelerated transit time), thus preventing prolonged exposure to the intestinal epithelium and preventing further changes in GI.

In Study REP000122TC-EN03v.1, iron release of PA21 reached 6.3% at the lowest pH value of 1.2 (which represents the fasting state of the stomach). In all the carcinogenic studies in rats (VFR0104) and mice (VFR 0115) and repeat dose toxicity studies in rats (VFR0096) and dogs (VFR0097), PA21 in diet resulted in higher incidences of pigmented macrophages and/or positive Perl's stain (for iron) in GI tract and mesenteric and mandibular lymph nodes, and elevation of iron contents in serum, liver, kidney, and/or spleen. Iron overload induces oxidative stress and DNA damage (2), which can enhance carcinogenic risk (3). Oral PA21-released iron when given at relatively high doses may contribute to colon cancer via production of reactive oxygen species (ROS). This mechanism of action for direct carcinogenesis may exist for excessive oral consumption of iron in humans.

The reproductive and developmental studies demonstrated that oral PA21 increased incidence of post implantation loss in rats at 4000 mg/kg/day, led to more fetuses with incomplete/unossified epiphyses and metacarpals/phalanges in rabbits at 1000 mg/kg/day, and resulted in lower body weight gain in F1 male rats during age 5-13 weeks and delayed neuromuscular function F1 male rats at the dose 4000 mg/kg/day. These findings may be attributed to maternal toxicity of PA21, e.g., reduced body weight gain in dams and one death. Considering oral PA21 was not teratogenic in rats and rabbits, and did not affect rat fertility, the risk of PA21 treatment-related reproductive toxicity in humans may be negligible if maternal health is not affected.

The recommended maximum PA21 dose in humans is 3000 mg iron/day, or 46.15 mg iron/kg/day for a 65-kg human. The safety margin or multiples with respect to carcinogenic and reproductive toxicity are summarized in Table 136.

Table 136. Safety margins

Study type	Species	Dosing route	PA21 dose*, mg/ kg/ day	Findings	Multiples**	
					LOAEL	NOAEL
Carcinogenicity	Mouse	PO	1250, 2500, 5000	Colon and/ or cecum adenocarcinomas at all doses	5	N/A
Carcinogenicity	Rat	PO	200, 750, 2500	Epithelial hyperplasia in GI tract at 2500 mg/ kg/ day	10	3
Fertility and early embryonic development study	Rat	PO	500, 1400, 4000	No effects on fertility	N/A	16
				Lower dam body weight, higher post implantation loss at 4000 mg/ kg/ day	16	5.6
Embryo-foetal toxicity study	Rat	PO	500, 1400, 4000	Lower dam body weights at 4000 mg/ kg/ day	16	5.6
				Higher post-implantation loss at 4000 mg/ kg/ day	16	5.6
Embryo-foetal toxicity study	Rabbit	PO	250, 500, 1000	One death, low dam body weight & food consumption at 1000 mg/ kg/ day	4	2
				More fetuses with incomplete/ unossified epiphyses and metacarpals/ phalanges at 1000 mg/ kg/ day	4	2
Pre- and postnatal development toxicity study	Rat	PO	500, 1400, 4000	Lower body weight in F0 females at 4000 mg/ kg/ day	16	5.6
				Lower body weight gain and delayed neuromuscular function in F1 males at the dose 4000 mg/ kg/ day	16	5.6

* PA21 contains 20% iron. ** Multiples at LOAEL or NOAEL over maximal human PA21 dose 3000 mg iron/ day in a 60-kg human on a mg/ kg base because of unabsorbable PA21 in GI

12 References

1. Dyas FG. Chronic irritation as a cause of cancer. JAMA 1928; 90: 457 (doi:10.1001/jama.1928.92690330003008c).

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3. Huang X. Iron overload and its association with cancer risk in humans: evidence for iron as a carcinogenic metal. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis.* 2003; 533: 153–171.

13 Appendix/Attachments

None

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

/s/

BAICHUN YANG
04/25/2013

THOMAS PAPOIAN
04/25/2013
Concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 205109

**Applicant: Vifor Fresenius
Medical Care Renal Pharma
France**

Stamp Date: 2/1/2013

Drug Name: PA21

NDA/BLA Type: 505(b)(1)

On **initial** overview of the NDA/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?	Yes		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	Yes		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	Yes		
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)?	Yes		No juvenile study was requested or studied.
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).			N/A
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?	Yes		
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?	Yes		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement
010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			N/A
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?		No	8.1 Pregnancy, multiples in mg/kg/day (OK) but did not state so. 8.2 Labor and Delivery, 8.3 Nursing Mothers, no multiples. 13.1 and 13.2 Nonclinical Toxicology, multiples in mg/kg/day (OK) but did not state so.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			N/A
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

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.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Baichun Yang 3/8/13
 Reviewing Pharmacologist Date

Thomas Papoian 3/8/13
 Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement
010908

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/s/

BAICHUN YANG
03/08/2013

THOMAS PAPOIAN
03/11/2013
Concur.