

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**203971Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## MEMORANDUM

Xofigo (radium Ra 223 dichloride)

**Date:** April 8, 2013

**To:** File for NDA 203971

**From:** John K. Leighton, PhD, DABT

Acting Director, Division of Hematology Oncology Toxicology  
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review for Xofigo conducted by Dr. Chen and secondary memorandum and labeling provided by Dr. Palmby. Of note, Dr. Palmby summarized the rationale that dedicated studies for genotoxicity and reproduction toxicology based on mechanism of action and the proposed indication for radium Ra-223 dichloride were not warranted. I concur with Dr. Palmby's conclusion that Xofigo may be approved and that no additional nonclinical studies are needed to support the proposed indication.

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/s/  
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JOHN K LEIGHTON  
04/08/2013

## MEMORANDUM

**Date:** April 5, 2013  
**From:** Todd R. Palmby, Ph.D.  
Pharmacology/Toxicology Supervisor  
Division of Hematology Oncology Toxicology (DHOT)  
Office of Hematology and Oncology Products (OHOP)  
**To:** File for NDA 203971 Xofigo (radium Ra 223 dichloride)  
**Re:** Approvability for Pharmacology and Toxicology  
**Indication:** Treatment of patients with castration-resistant prostate cancer with bone metastases and no evidence of visceral metastatic disease

Non-clinical pharmacology and toxicology studies to support Xofigo (radium Ra 223 dichloride) NDA 203415 for the treatment of patients with castration-resistant prostate cancer with bone metastases were reviewed by Wei Chen, Ph.D. Studies conducted with intravenously administered radium Ra dichloride included pharmacology, toxicokinetics and ADME, safety pharmacology and general toxicology.

Radium Ra 223 dichloride is an alpha particle-emitting pharmaceutical containing the radioactive isotope radium-223 as the active moiety. Radium-223 is a calcium mimetic and forms complexes with hydroxyapatite in areas of increased bone turnover, such as metastases. The energy transfer from alpha emitters leads to a high frequency of double-strand DNA breaks in nearby cells. Pharmacology studies showed that radium Ra 223 dichloride induced cytotoxicity and cell cycle arrest in tumor cell lines and inhibited osteoclast differentiation and osteoblast activity *in vitro*, and resulted in prolonged survival, reduced osteolytic area and increased bone formation in a mouse metastasis model. Thus, the appropriate Established Pharmacologic Class for radium Ra 223 dichloride was determined to be "radioactive therapeutic agent," which has been used for other FDA approved products. There are currently no alpha particle-emitting pharmaceuticals approved by FDA.

Repeat-dose toxicology studies were conducted in rats and dogs with intravenous radium Ra 223 dichloride administration once every 4 weeks for up to 1 year and 6 months, respectively. Overall, toxicities were consistent with the exposure primarily limited to bone. Toxicities were also generally predictive of clinical adverse events, which included decreased white blood cell, platelet and red blood cell counts, decreased myeloid to erythroid cells in the bone marrow and compensatory effects of increased reticulocytes and extramedullary hematopoiesis in the spleen. Acute retinal detachment and hypertrophy of the retinal pigmented epithelium was observed following a single dose of 450 kBq/kg radium Ra 223 dichloride, but was not observed in rats or in repeat-dose studies in dogs at lower doses. Radium-223 accumulates in the *tapetum lucidum*, a pigmented structure, in the canine eye, which is a structure that is absent in the human eye. This suggests retinal detachment is likely a species specific

phenomenon, so this finding was not included in the package insert. Osteosarcomas were observed after 6 months of the initial administration of radium Ra 223 dichloride in rats, which often included metastases. Mammary carcinoma was observed in one female rat and lymphoma in multiple organs was observed in one male rat following repeated administration. There were effects on bone and teeth observed in rats primarily affecting areas of active growth.

Genetic toxicology studies were not conducted with radium Ra 223 dichloride. The radioactive properties and mechanism of action of radium Ra 223 dichloride are sufficient to characterize it as genotoxic.

No nonclinical reproductive or developmental toxicity studies were conducted with radium Ra 223 dichloride. In general, an embryo-fetal developmental toxicity study would be expected for a product to be used in patients with advanced cancer, as described in the Guidance for Industry: ICH S9 Nonclinical Evaluation of Anticancer Pharmaceuticals. However, this study was deemed unwarranted to support approval of Xofigo given the patient population and the properties of this product. Radium Ra 223 dichloride is genotoxic and caused toxicity to hematopoietic cells in animals. In addition, due to the calcium mimetic properties of radium-223 and its incorporation into sites of active bone turnover, embryo-fetal exposure to Xofigo would be predicted to cause teratogenicity. Since no clinical trials including the use of Xofigo in female patients were reviewed with this NDA submission and no clinical benefit was established in female patients, Xofigo was assigned a Pregnancy Category X in the package insert.

**Recommendation:** I concur with Dr. Chen's conclusion that pharmacology and toxicology data support the approval of NDA 203971 for Xofigo. There are no outstanding nonclinical issues that would preclude the approval of Xofigo for the proposed indication.

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/s/  
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TODD R PALMBY  
04/05/2013

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

**PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION**

Application number: 203,971  
Supporting document/s: 1  
Applicant's letter date: December 14, 2012  
CDER stamp date: December 14, 2012  
Product: Xofigo (radium Ra 223 dichloride)  
Indication: castration-resistant prostate cancer patients with bone metastases  
Applicant: Bayer Healthcare Pharmaceuticals  
Review Division: Division of Hematology Oncology Toxicology  
(for Division of Oncology Products 1)  
Reviewer: Wei Chen, Ph.D.  
Supervisor/Team Leader: Todd Palmby, Ph.D.  
Division Director: John Leighton, Ph.D., D.A.B.T. (acting)  
(Robert Justice, M.D., M.S.)  
Project Manager: Elleni Alebachew

**Disclaimer**

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 203,971 are owned by Bayer Healthcare Pharmaceuticals or are data for Bayer Healthcare Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of NDA 203,971 that Bayer Healthcare Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 203,971.

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# 1 Executive Summary

## 1.1 Introduction

Radium-223 dichloride solution for injection (BAY 88-8223) is an alpha particle-emitting pharmaceutical with the divalent cation  $^{223}\text{Ra}^{2+}$  as the active moiety. The United States Adopted Names (USAN) established name for radium-223 dichloride is radium Ra 223 dichloride. However, this product will be referred to as radium-223 dichloride throughout this review. An important characteristic of radium-223 is its targeting of areas of increased bone turnover, as in bone metastases. The isotope radium-223 mimics calcium and targets bone, specifically areas of bone metastases, by forming complexes with the bone mineral hydroxyapatite. The high linear energy transfer of alpha emitters (80 keV/micrometer) leads to a high frequency of double-strand DNA breaks in adjacent cells, resulting in a localized anti-tumor effect. Radium-223 dichloride showed cell killing effects *in vitro* in various tumor cell lines including multidrug-resistant cell lines and noncycling cells. In addition to this direct cytotoxic effect, radium-223 has been shown to inhibit osteoclast differentiation and osteoblast activity *in vitro*. The efficacy of radium-223 has been shown *in vivo* with an osteolytic experimental skeletal metastases model in nude mice intraventricularly inoculated with human breast cancer cells. As such, radium-223 has been developed for the treatment of castration-resistant prostate cancer (CRPC) patients with bone metastases. With this NDA, the Applicant submitted nonclinical pharmacology, pharmacokinetic, and toxicology studies to support the approval of radium-223 for the treatment of CRPC with bone metastases.

## 1.2 Brief Discussion of Nonclinical Findings

The active moiety in radium-223 dichloride is the alpha particle-emitting nuclide radium-223 with a radioactive half-life of 11.4 days. The nonclinical repeat-dose general toxicity studies with radium-223 were conducted in rats and dogs, consistent with the clinical route of administration. Nonclinical studies also included safety pharmacology studies to evaluate the effects of radium-223 dichloride on vital organ systems. No nonclinical studies of radium-223 dichloride evaluating reproductive and developmental toxicity in male and female animals were conducted considering the proposed indication, which does not include female patients, and the radioactive nature of the proposed product. The repeat-dose toxicology studies were conducted in compliance with Good Laboratory Practice regulations. Nonclinical pharmacokinetic and toxicokinetic studies of radium-223 dichloride were also evaluated in rats and dogs.

### *Pharmacology*

Radium-223 dichloride is an alpha particle-emitting radiopharmaceutical; it delivers alpha radiation to areas of increased bone turnover, as in bone metastases. Radium-223 dichloride induced DNA double strand breaks, and showed cell killing effects *in vitro* in various tumor cell lines including multi drug resistant cell lines and non-cycling cells. Beside this direct cytotoxic effect, radium-223 dichloride was also shown to induce cell cycle arrest and to have inhibitory effects on osteoclast differentiation and osteoblast activity *in vitro*. Radium-223 dichloride treatment resulted in prolonged survival in the

mouse metastasis model, with reduction of the total osteolytic area, increase of bone formation and improvement of related symptoms.

#### *Safety pharmacology*

Safety pharmacology studies revealed no adverse effects of radium-223 dichloride on the functions of the central nervous, respiratory or cardiovascular systems after a single intravenous bolus injection at doses of up to 1,000 kBq/kg in rats (for function of the central nervous system or respiratory system), and at doses up to 450 KBq/kg in conscious telemetered dogs (for cardiovascular function and ECG).

#### *General toxicology*

Radium-223 dichloride was tested in single dose toxicity studies in mice, rats and dogs, and in repeat-dose toxicity studies in rats and dogs. The toxicology studies in rats and dogs have shown that administration of radium-223 dichloride resulted in lower body weights, lower food consumption, alterations in hematology parameters including a decrease in white blood cell, platelet and red blood cell counts, accompanied by a compensatory increase in reticulocyte count, and microscopic observations of extramedullary hematopoiesis in the white pulp of the spleen and a decrease in the myeloid to erythroid cell ratio in bone marrow. No adverse effect of radium-223 dichloride on the central nervous, respiratory or cardiovascular system was noted at the doses tested in the repeat-dose toxicology studies in rats and dogs. Clinical and anatomical pathology findings associated with the treatment of radium-223 dichloride were generally reversible after a recovery period. The reduction in white blood cell count was not reversed until approximately 40 weeks after dosing in rats with repeated administration of radium-223 dichloride. Osteosarcomas, often with metastasis, were seen after 6 months following a single or repeated administration of radium-223 dichloride in rats. Other neoplastic changes were observed in rats after repeat-dosing of radium-223 dichloride, including mammary carcinoma in one female and lymphoma in one male. Non-neoplastic lesions observed in rats were bone depletion/fibrosis, osteocyte depletion associated with disorganized growth lines and changes in the bone socket of the teeth. Toxicities observed are mostly direct effects based on the pharmacology of radium-223 dichloride.

#### *Genetic toxicology*

No studies of the genotoxic effects of radium-223 dichloride *in vitro* and/or *in vivo* were conducted due to the radioactive nature of this product and its mechanism of action to induce double-strand DNA breaks.

#### *Reproductive toxicology*

No stand-alone non-clinical studies of radium-223 dichloride evaluating reproductive and developmental toxicity in animals were conducted. However, in the general toxicology studies, minimal numbers of abnormal spermatocytes and tubular atrophy in the testes were observed in rats.

*Carcinogenicity studies*

No studies were conducted as the indicated population have advanced cancer.

**1.3 Recommendations****1.3.1 Approvability**

Recommending approval. The nonclinical studies adequately support the safety of radium-223 dichloride by intravenous administration in castration-resistant prostate cancer patients with bone metastases.

**1.3.2 Additional Non Clinical Recommendations**

Additional nonclinical studies are not needed at this time.

**1.3.3 Labeling**

Information needed for nonclinical sections of the label are provided in this review. Therefore, a separate labeling review is not deemed necessary.

**2 Drug Information****2.1 Drug**

CAS Registry Number: 444811-40-9

Trade name: Xofigo

Note: Alpharadin is the former, interim name

Generic Name: radium Ra 223 dichloride, radium-223 dichloride, radium-223 chloride

Code Name: BAY 88-8223

Chemical Name: radium-223 dichloride

Molecular Formula/Molecular Weight:  $^{223}\text{RaCl}_2$  / For  $^{223}\text{Ra}^{2+}$  = 223.0 g/mole

Structure or Biochemical Description:  $^{223}\text{RaCl}_2$

Pharmacologic Class: radioactive therapeutic agent

Mechanism of action: alpha particle-emitting pharmaceutical with anti-tumor effect on bone metastases

**2.2 Relevant INDs, NDAs, BLAs and DMFs: IND 67,521**

**2.3 Drug Formulation: 1000 kBq/mL, a ready-to-use solution for injection**

Composition of Radium-223 chloride solution for injection  
(copied from Applicant's submission)

Each 10 ML vial contains

Composition	Reference to Standard	Function	Amount per vial	Amount per mL
<b>Drug substance</b>				
Radium-223 chloride	In-house	Drug substance	6000 kBq (3.2 ng) <sup>a</sup> at reference date	1000 kBq (0.53 ng) <sup>a</sup> at reference date
<b>Excipients<sup>b</sup></b>				
Sodium chloride	Current Ph. Eur. / USP	Tonicity agent	37.8 mg	6.3 mg
Sodium citrate <sup>c</sup>	Current Ph. Eur. / USP	pH adjuster	43.2 mg	7.2 mg
Hydrochloric acid	Current Ph. Eur. / USP	pH adjuster	1.2 mg <sup>d</sup>	0.2 mg <sup>d</sup>
Water for injection	Current Ph. Eur. / USP			(b) (4)

a Calculated as radium-223

b [Redacted] (b) (4)

c

d

**2.4 Comments on Novel Excipients: N/A**

**2.5 Comments on Impurities/Degradants of Concern:**

The CMC review team requested input from the pharmacology/toxicology team regarding the proposed [Redacted] (b) (4)

[Redacted]

[Redacted] administered to patients in clinical trials and animals in nonclinical toxicology studies from previous batches of radium-223 dichloride were similar to the amounts that would be administered to patients if levels were at the proposed specifications. Therefore, the Applicant's proposed limits for [Redacted] (b) (4)

[Redacted] are acceptable from a pharmacology/toxicology perspective.

## **2.6 Proposed Clinical Population and Dosing Regimen**

Proposed clinical population: castration-resistant prostate cancer (CRPC) patients with bone metastases

Proposed dose and dose regimen:

50 kilobecquerel (kBq) (= 1.35  $\mu$ Ci) per kilogram body weight (50 kBq/kg),  
once every 4 weeks (q4w) for a total of 6 injections

Route of administration: slow intravenous (iv) injection (generally up to 1 minute)

### 3 Studies Submitted

#### Studies Reviewed

##### Safety Pharmacology

	Title	Study no.	Folder/file name
1	Safety pharmacology study of alpharadin (radium-223) on central nervous system function on rats	Report No. R-8659	M4.2.1.1
2	Safety pharmacology study of alpharadin (radium-223) on respiratory function on rats	Report No. R-8657	M4.2.1.1
3	Safety pharmacology study of alpharadin (radium-223) on cardiovascular system function in telemetered beagle dogs	Report No. R-8658	M4.2.1.1

##### ADME

	Title	Study no.	Folder/file
1	Pharmacokinetics and biodistribution of radium-223 (Alpharadin <sup>TM</sup> ) in mice	148-001; BC-1Tr001-2007	M4.2.2.3
2	Pharmacokinetics and biodistribution of radium-223 (Alpharadin <sup>TM</sup> ) in mice	148-003; BC-1Tr001-2008	M4.2.2.3
3	Pharmacokinetics and biodistribution of radium-223 (Alpharadin <sup>TM</sup> ) in mice. Drug Substance Production Process II	148-011; BC-1Tr002-2008	M4.2.2.3
4	Urinary and fecal clearance of radium-223 (Alpharadin <sup>TM</sup> ) in mice	148-002; BC-1Tr002-2007	M4.2.2.4
5	Urinary and fecal clearance of radium-223 (Alpharadin <sup>TM</sup> ) in mice. Drug Substance Production Process II	148-012; BC-1Tr003-2008	M4.2.2.5
6	Pharmacokinetics and biodistribution of radium-223 (Alpharadin <sup>TM</sup> ) during treatment with zoledronic acid (Zometa) in mice	148-013; BC-1Tr004-2008	M4.2.2.5

Toxicology studies

## Repeat dose

	Title	Study no.	Folder/file name
1	Alpharadin (radium-223): single and repeated dose toxicity study in rats with an extended recovery period	62985	M4.2.3.2
2	Alpharadin (radium-223): 12-month repeat dose toxicity study in rats	67071	M4.2.3.2
3	Long term radiotoxicity study of repeat dose intravenous radium-223 in normal dogs (an original report and an amendment)	MS RA 2	M4.2.3.2

## Local tolerance study

	Title	Study no.	Folder/file name
1	Alpharadin: Local Irritation Study in Rabbits	69865	M4.2.3.6

**Studies summarized**Pharmacology

	Title	Study no.	Folder/file name
1	Cellular effects of alpha particle radiation from radium-223, Alphasarin: III. DNA damages, double strand breaks observed by gammaH2A.X flowcytometry.	Report No. R-8689	M4.2.1.1
2	Cellular effects of alpha particle radiation from radium-223, Alphasarin: I. Survival and dose rate.	Report No. R-8687	M4.2.1.1
3	Cellular effects of alpha particle radiation from radium-223, Alphasarin: II. Survival of multidrug resistant and non-cycling cells.	Report No. R-8688	M4.2.1.1
4	Cellular effects of alpha particle radiation from radium-223, Alphasarin: V. Age-response	Report No. R-8691	M4.2.1.1
5	Cellular effects of alpha particle radiation from radium-223, Alphasarin: IV. Cell cycle effects.	Report No. R-8690	M4.2.1.1
6	The effects of 6 doses of Alphasarin on human osteoclast differentiation and activity in vitro	Report No. R-8693	M4.2.1.1
7	The effects of 6 doses of Alphasarin on mouse osteoblast differentiation and activity in vitro	Report No. R-8694	M4.2.1.1
8	Dose finding study for Alphasarin in a breast cancer bone metastasis model	Report No. R-8695	M4.2.1.1
9	Method of action of Alphasarin in a breast cancer bone metastasis model	Report No. R-8696	M4.2.1.1
10	Survival study with Alphasarin in a breast cancer bone metastasis model	Report No. R-8697	M4.2.1.1

Toxicology studiesSingle dose

	Title	Study no.	Folder/file name
1	Alphasarin (radium-223): single dose toxicity study in mice	52158	M4.2.3.1
2	Alphasarin (radium-223): single dose toxicity study in rats	52159	M4.2.3.1
3	Biodistribution and acute radiotoxicity study of single dose intravenous radium-223 in normal dogs	MS RA1	M4.2.3.1

**Studies submitted, but not reviewed**Pharmacology

	Title	Study no.	Folder/file name
1	Cellular dosimetry for radium-223	Report No. R-8692	M4.2.1.1

Secondary Pharmacodynamics

	Title	Study no.	Folder/file name
1	Combination treatment with Alpharadin in a breast cancer bone metastasis model	Report No. R-8698	M4.2.1.2

ADME

	Title	Study no.	Folder/file
1	Retrospective validation of Wizard gamma counter	Report No. R-8659	M4.2.2.2

Toxicology studies

	Title	Study no.	Folder/file name
1	Taxotere and Alpharadin: Maximum Tolerated Dose (MTD) Study in Rats	68997	M4.2.3.7
2	Taxotere® and Alpharadin™: A 3-Month Single Dose Toxicity Study in Rats	69120	M4.2.3.7
3	Taxotere® and Alpharadin: A 22-Weeks Repeat Dose Toxicity Study in Rats	69202	M4.2.3.7

## 4 Pharmacology

### 4.1 Primary Pharmacology

#### Brief summary

Radium-223 delivers alpha radiation to areas of increased bone turnover, as in bone metastases. The pharmacodynamic effects of radium-223 dichloride administration on tumor cells, osteoclasts and osteoblasts were tested *in vitro*, and the anti-tumor efficacy was assessed *in vivo*. Radium-223 dichloride treatment resulted in DNA double-strand breaks and showed cytotoxic effects *in vitro* in various tumor cell lines including multidrug-resistant cell lines and non-cycling cells. *In vitro* studies also showed radium-223 dichloride induced cell cycle arrest and an inhibitory effect of radium-223 dichloride on osteoclast differentiation and osteoblast activity. Anti-tumor effects of radium were demonstrated in *in vivo* studies. Radium-223 dichloride administration improved survival time with a lower incidence of cachexia, reduction of the whole tumor burden, reduction of the total osteolytic area and an increase of bone formation in the nude mice model using a human breast cancer cell line.

Safety pharmacology assessments of radium-223 dichloride included respiratory and central nervous system studies in rats and a cardiovascular study in conscious telemetered dogs. No significant effects on the function of the central nervous system or respiratory system in rats were observed after a single intravenous administration of radium-223 dichloride at doses of up to 1,000 kBq/kg (27  $\mu$ Ci/kg), and no treatment-related cardiovascular effects including effects on ECG measurements (PR, QRS, RR, and QT durations), heart rate, arterial blood pressure, or left ventricular pressure were observed after single intravenous administration of up to 450 KBq/kg (12  $\mu$ Ci/kg) in dogs.

#### *Primary pharmacodynamics*

##### Mechanism of action:

Radium-223 dichloride is an alpha particle-emitting radiopharmaceutical, and is a natural bone seeking isotope, with a physical half-life of 11.4 days and radioactive daughter isotopes with short half-life including radon-219 ( $t_{1/2} = 4$  sec). Because the intrinsic bone targeting property of radium-223 is similar to that of other alkaline earth elements like calcium, radium-223 targets areas of increased bone turnover in bone metastases and concentrates by forming a complex with hydroxyapatite which constitutes 50% of the bone structure. The high linear energy transfer (LET) alpha particle radiation (80 keV/ $\mu$ m) induces a high frequency of double-strand DNA breaks, resulting in a localized antitumor effect in the target areas containing metastatic cancer cells. The short path length (less than 100 micrometers) of the alpha particles limits the effect on adjacent healthy tissue such as the bone marrow. Radium-223 dichloride showed cell killing effects *in vitro* on tumor cells, osteoclasts and osteoblasts by inducing DNA double-strand breaks. The anti-tumor efficacy was demonstrated *in vivo* in skeletal metastases models in nude mice. These results suggest that radium-223 dichloride delivers alpha radiation to areas of increased bone turnover, as in bone metastases, and has anti-tumor effects on bone metastases.

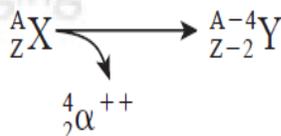
Drug activity related to proposed indication:1- alpha emitters leads to double-strand DNA breaksThe Applicant cited publications

**Radiobiologic principles in radionuclide therapy**, Amin I. Kassis, PhD; and S. Jame Adelstein, The journal of nuclear medicine (2005) 46, No.1 (Suppl): 4s-12s

**Advanced drug delivery reviews**, George Sgouros, Advanced Drug Delivery Reviews 60 (2008) 1402–1406

These reference articles are review papers; the following summary is excerpted from the above review articles.

*Summary:* Alpha particle-emitting radionuclides are positively charged with a mass and charge equal to the helium nucleus, and their emission leads to a daughter nucleus with 2 fewer protons and 2 fewer neutrons:



The alpha particles have energies ranging from 5–9 MeV with corresponding tissue ranges of 5-10 cell diameters, travel in straight lines, and deposit 80–100 keV/μm along most of their track (rate of energy deposition increases to ~300 keV/μm toward the end of the track). Alpha-particle emitting radionuclides are of interest in targeted therapy because of the short range and high linear energy transfer (LET) of these emissions. The former provides the specificity to target a chosen cell population with minimal effect on non-targeted cells; the latter leads to a high frequency of double-stranded DNA damage, much of which is irreparable. DNA lesions may be produced by direct ionization of DNA (direct effect) or by the interaction of free radicals (mostly hydroxyl radicals produced in water molecules that diffuse a few nanometers) with DNA, an interaction that may be modified by radical scavengers.

Applicant-conducted study

**Title:** Cellular effects of alpha particle radiation from radium-223, Alpharadin:

III. DNA damages, double strand breaks observed by gammaH2A.X flowcytometry

Test facility: Algeta ASA  
P.O. Box 54 Kjelsas  
N-0411 Oslam Norway

Report date: December 12, 2008

*Summary:*

This study showed that alpha particle radiation from radium-223 induced double-strand breaks in the DNA molecule.

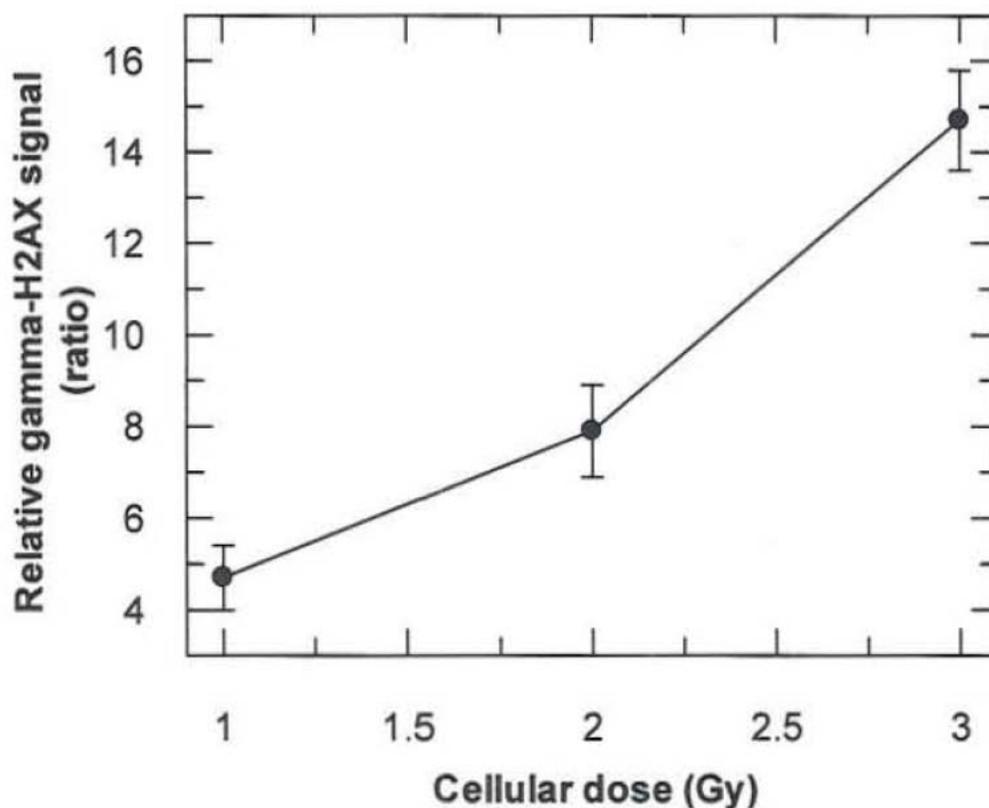
**Methods:** The NHIK 3025 cells were irradiated in flasks (T75) by growth medium containing different activity concentrations of radium-223. After treatment the cells were washed three times with PBS before they were given fresh medium and incubated for 30 min. The cells were then washed; double strand breaks (DSB) in the DNA molecule were detected by using fluorescent  $\gamma$ H2AX-specific antibodies and flowcytometry.

**Results:**

Study results showed an increase of  $\gamma$ H2AX signal, or DSBs, with increasing doses of radium-223.

The following figure is copied from the Applicant's submission.

Relative  $\gamma$ H2AX signal as a function of cellular dose



2- in vitro cytotoxicity

**Cellular effect of radium-223 dichloride *in vitro***

**Title:** Cellular effects of alpha particle radiation from radium-223, Alpharadin:  
I. Survival and dose rate

Test facility: Algeta ASA  
P.O. Box 54 Kjelsas  
N-0411 Oslam Norway

Report date: December 8, 2008

*Summary:*

- The cell survival curve is almost straight in a semilogarithmic plot, indicating that alpha particle radiation from  $^{223}\text{Ra}$  is an effective cell killer;
- The dose rate effect is minimal.

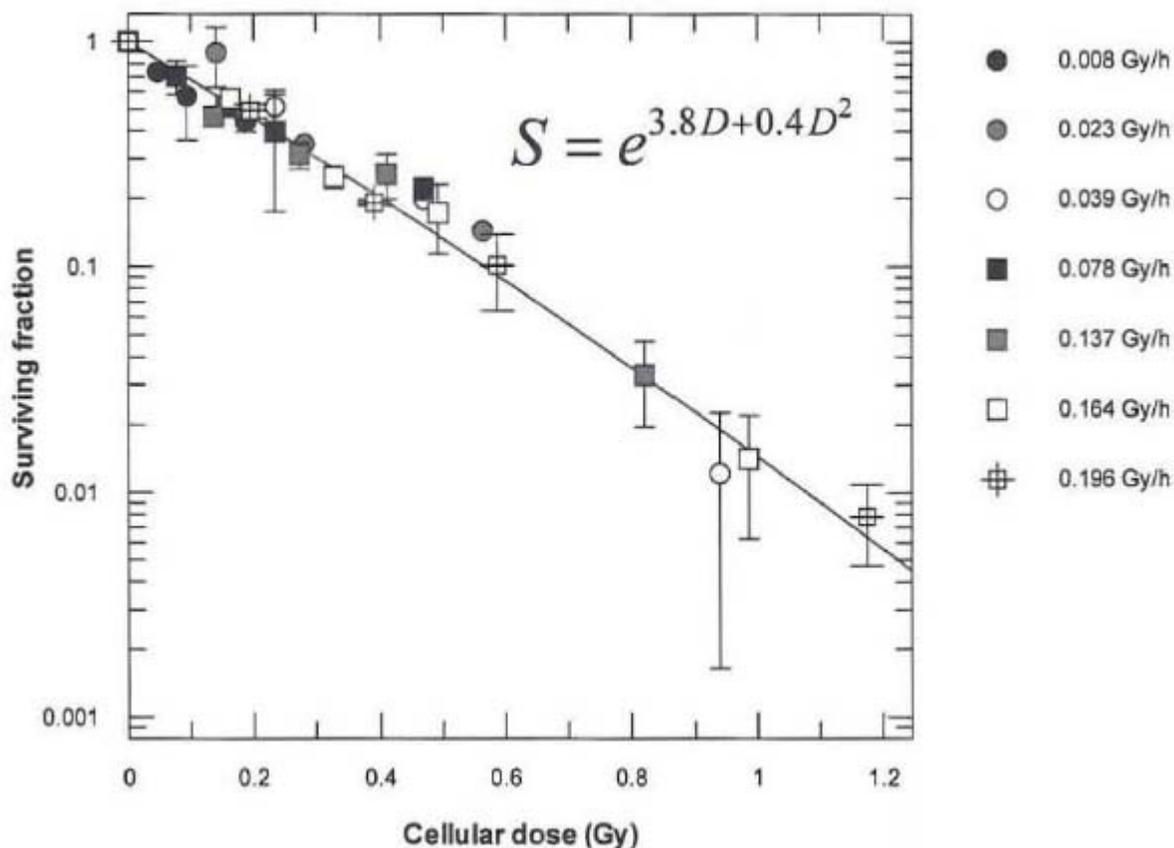
*Methods:* Human cell line, NHIK 3025, was given a radioactive dose from radium-223. This radiopharmaceutical is in liquid form and consists of radium-223 in secular equilibrium with its daughters. Different concentrations of radioactivity from radium-223 were mixed with the growth medium. NHIK 3025 cells were exposed to different doses of radium-223 (1-25 kBq/mL) for 1-24 h. This resulted in cellular dose rates between 0.008-0.196 Gy/h, and a cellular dose range from 0.05 to 1.2 Gy, calculated using a calculation system that combines the computational ease of the Medical Internal Radiation Dose (MIRD) schema with additional information provided by microdosimetry for use with alpha particle emitters. Cell survival was measured by colony survival. The cells were seeded out and treated with radioactive medium for a predefined time in six well trays. After treatment was ended (washing three times with Hanks' buffer) the cells got fresh medium and were incubated. The six well trays were incubated for 10-14 days before the colonies were fixed and stained. The colonies containing more than 40 cells were scored as survivors. The surviving fraction was expressed as the number of colonies after treatment relative to the number of colonies in an untreated control.

*Results:*

The cell surviving fractions (2-3 experiments) were plotted against the different dose levels in the program "GraFit 5" (Version 5.0. II, Erithaus Software, Ltd., UK). The dose response curve is shown in the following figure (copied from the Applicant's submission). This fit corresponds to the  $\alpha\beta$ -model with an  $\alpha$  term of  $3.8 \pm 0.2$  and a  $\beta$ -term of  $0.04 \pm 0.2$ .

Note: The  $\alpha$ -term represents double strand break (DSB) caused by a single radiation particle, while the  $\beta$ -term represents DSB caused by two different radiation particles. The value of the  $\alpha$ -term tells something about the radiation sensitivity for this specific type of radiation and cell type. The small  $\beta$ -term indicated that there is only a small fraction of DSB induced by two different radiation particles.

Fraction surviving NHIK 3025 cells plotted against cellular dose



$$SF = e^{-\alpha D - \beta D^2}$$

$SF$  is the surviving fraction of cells treated with a radiation dose  $D$ , where  $\alpha$  and  $\beta$  are constants.

### Effects on drug-resistant cell lines

**Title:** Cellular effects of alpha particle radiation from radium-223, Alphasar:

II. Survival of multidrug resistant and non-cycling cells

Test facility: Algeta ASA

P.O. Box 54 Kjelsås

N-0411 Oslo Norway

Report date: December 9, 2008

### Summary:

- The effect of radium-223 on cell survival was not cell type specific;
- Alpha particle radiation from radium-223 killed multidrug resistant cells as effectively as non-resistant cells;
- Alpha particle radiation from radium-223 killed non-cycling cells as effectively as exponentially growing cells.

**Methods:** the cell lines NHIK 3025, NHIK 3025 (dox), A549 and A549 (dox) were used in this experiment. The cells in exponential growth plateau phase were given a radioactive dose by mixing different activity concentrations of radium-223 with the growth medium. The cellular doses ranged from 0.19-0.94 Gy. Cell survival was measured by colony survival.

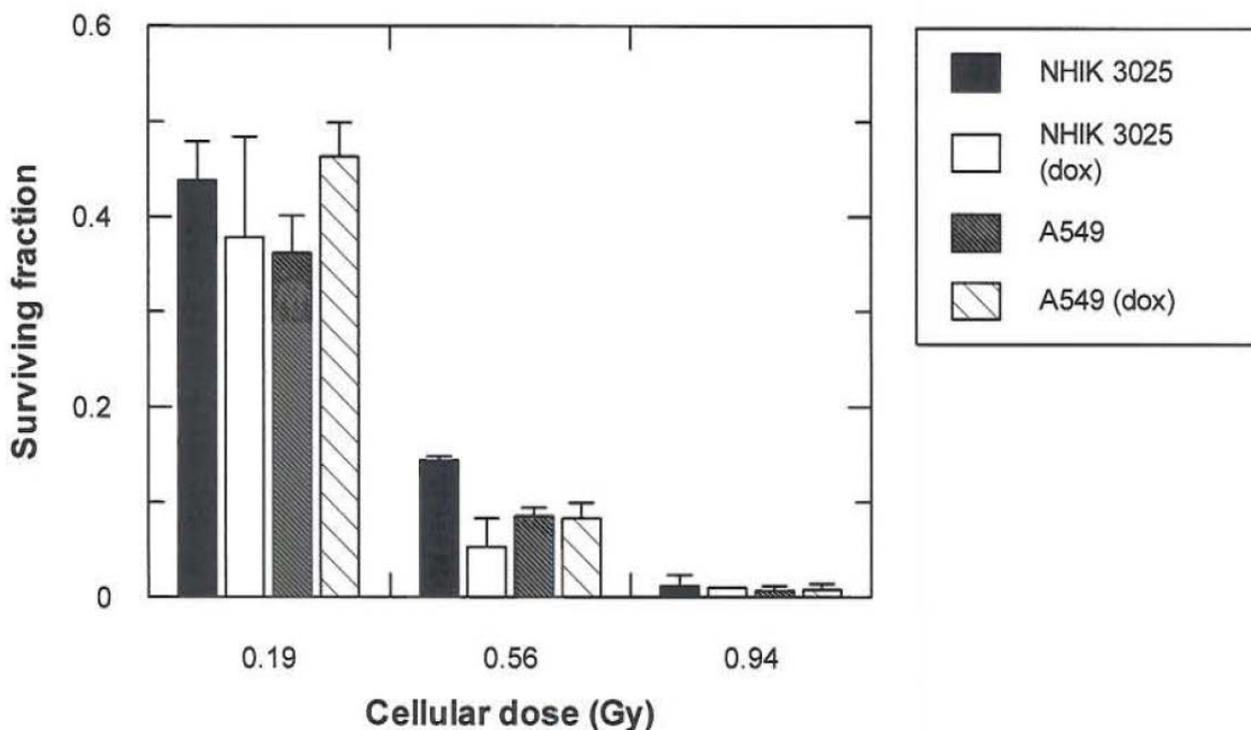
Note: NHIK 3025 (dox) and A549 (dox) are sister cell lines to NHIK 3025 and A549, which developed multidrug resistance (MDR) by treatment with doxorubicin.

**Results:**

The surviving fractions were similar for the different cell types, when given the same cellular dose. NHIK 3025 and A549 cells are killed with the same efficiency independent of multidrug resistance.

The following figure is copied from the Applicant's submission.

Comparison of surviving fractions for cells treated with  $^{223}\text{Ra}$  for 24 hours in exponential growth plateau phase - NHIK 3025 and NHIK 3025 (dox), A549 and A549 (dox)



**Radium-induced cell deaths at different phases of cell cycle**

**Title:** Cellular effects of alpha particle radiation from radium-223, Alphasar:  
V. Age-response

Test facility: Algeta ASA  
P.O. Box 54 Kjelsas  
N-0411 Oslam Norway

Report date: December 18, 2008

*Summary:*

The effect on cell survival after treatment with alpha particle radiation is independent of cell cycle phase.

*Methods:* "Mitotic harvest" method was used to synchronize the cells. Synchronized NHIK 3025 cells at different cell cycle phases were treated with radium-223 at a dose rate of 0.43 Gy/h for 1 hour, and then incubated for 10-14 days before the colonies were fixed and stained. The colonies containing more than 40 cells were scored as survivors. The surviving fraction was expressed as the number of colonies after treatment relative to the number of colonies in an untreated control.

*Results:*

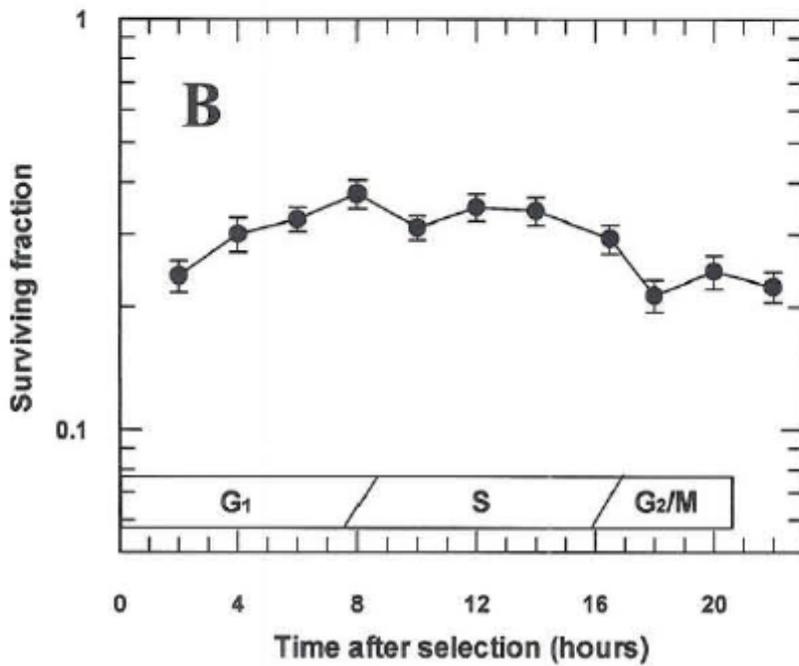
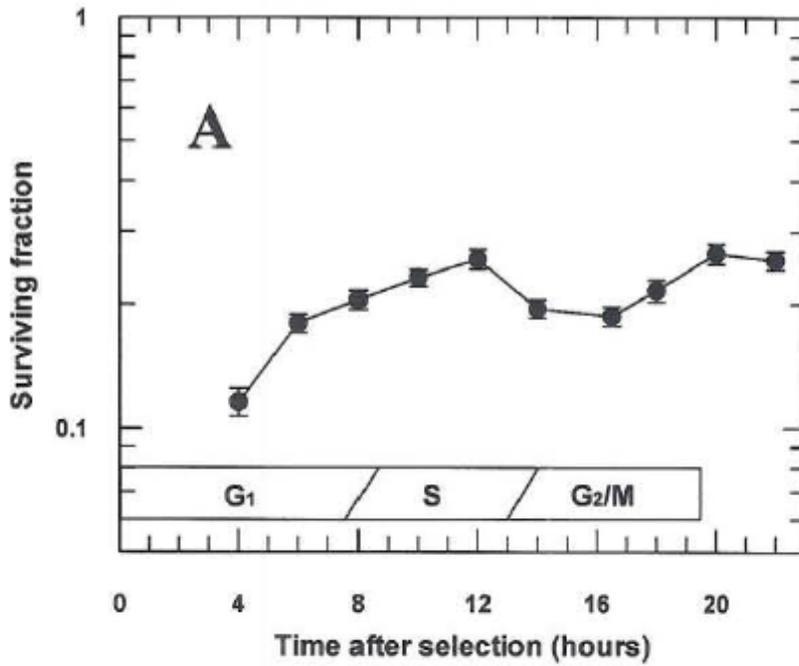
Cell survival fractions after treatment with alpha particle radiation from radium-223 were similar in cells at different cell cycle phases.

The following table and figures are copied from the Applicant's submission.

Median time length (hour) to synchronize cells into different cell cycle phases  
in the two age-response experiments

	<b>G<sub>1</sub>-phase (h)</b>	<b>S-phase (h)</b>	<b>G<sub>2</sub>/M-phase (h)</b>	<b>Median time of cell cycle (h)</b>
<b>Exp 1</b>	8,5	5,5	5,5	19,5
<b>Exp 2</b>	8,5	8,5	3,5	20,5

Age-response curve for synchronized NHIK 3025 cells treated with radium-223 in different parts of the cell cycle



Note: Figure A shows the results from experiment 1, and Figure B shows the results from experiment 2. The surviving fraction was expressed as the number of colonies after treatment relative to the number of colonies in an untreated control.

**3- Radium-223 induces cell cycle arrest**

**Title:** Cellular effects of alpha particle radiation from radium-223, Alpharadin™:  
IV. Cell cycle effects.

Test facility: Algeta ASA  
P.O. Box 54 Kjelsas  
N-0411 Oslam Norway

Report date: December 17, 2008

**Summary:**

The study suggested that alpha particle radiation from radium-223 induces G2 arrest.

**Methods:** NHIK 3025 (-/- p53, -/- pRb) cells were given a radioactive cellular doses from 0.05 -1.2 Gy by mixing different activity concentrations of radium-223 with the growth medium. Cells with dose rates 0.008-0.039 Gy/h were irradiated for 6, 12 and 24 hours, and cells with dose rates 0.08-0.2 Gy/h were irradiated for 1, 2, 3 and 6 hours. Cell cycle analysis was measured by using fluorescing dye, propidium iodide and flowcytometre method.

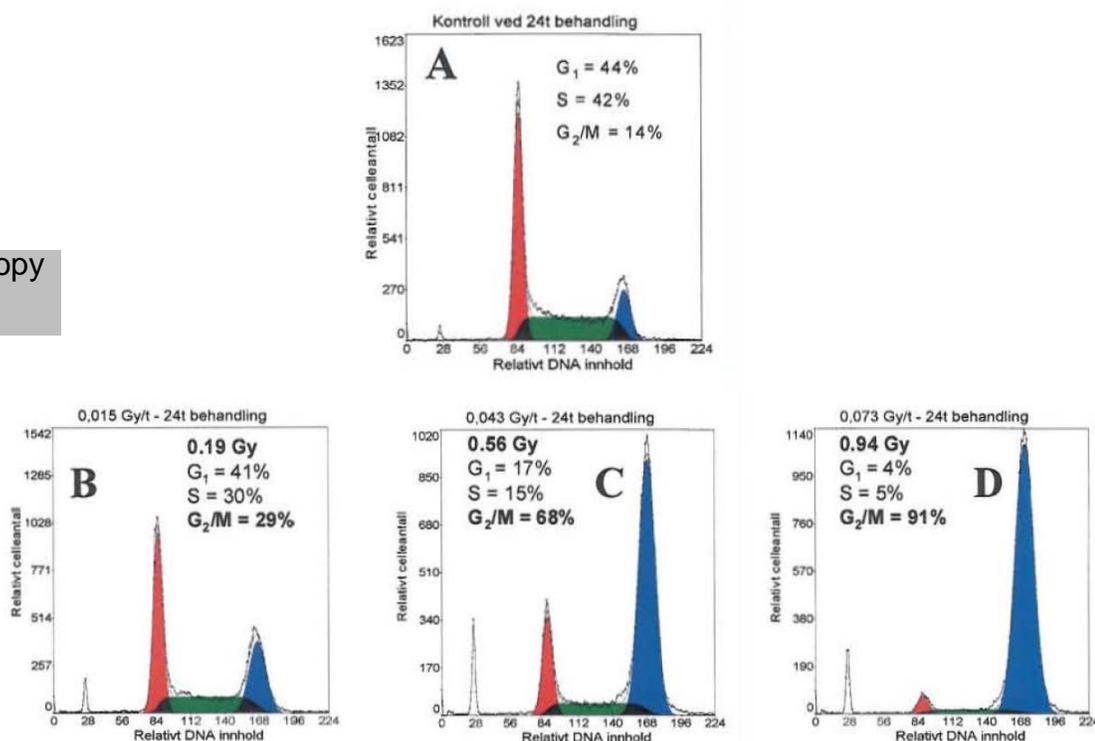
**Results:**

A fraction of cells in G2-phase increased with increasing dose of radium-223. The fraction of cells in G2 phase was as large as 91% after the treatment of radium-223 at a cellular dose of 0.94 Gy.

The following figures are copied from the Applicant's submission.

DNA histograms for cells treated with different cellular doses of radium-223

Best Available Copy



**4- Effect of radium-223 on osteoclasts**

**Title:** The effects of 6 doses of Alphasradin on human osteoclast differentiation and activity *in vitro*

Test facility: [REDACTED] (b) (4)

Report date: September 27, 2010

**Summary:**

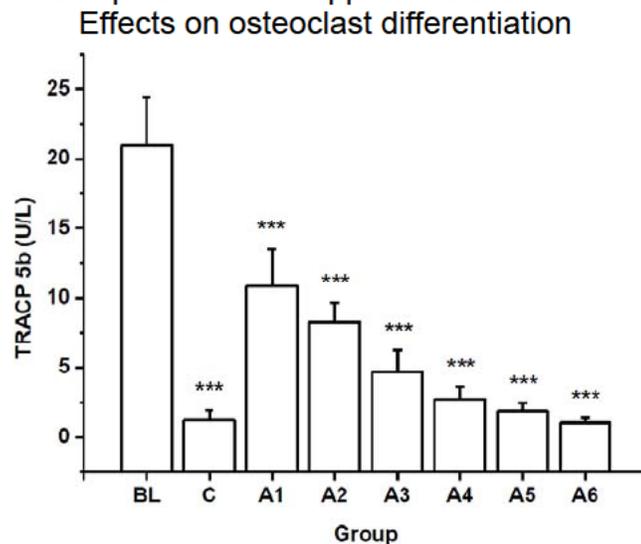
- Radium-223 showed a dose-dependent inhibition of osteoclast differentiation;
- No significant effects were observed in the osteoclast activity assay.

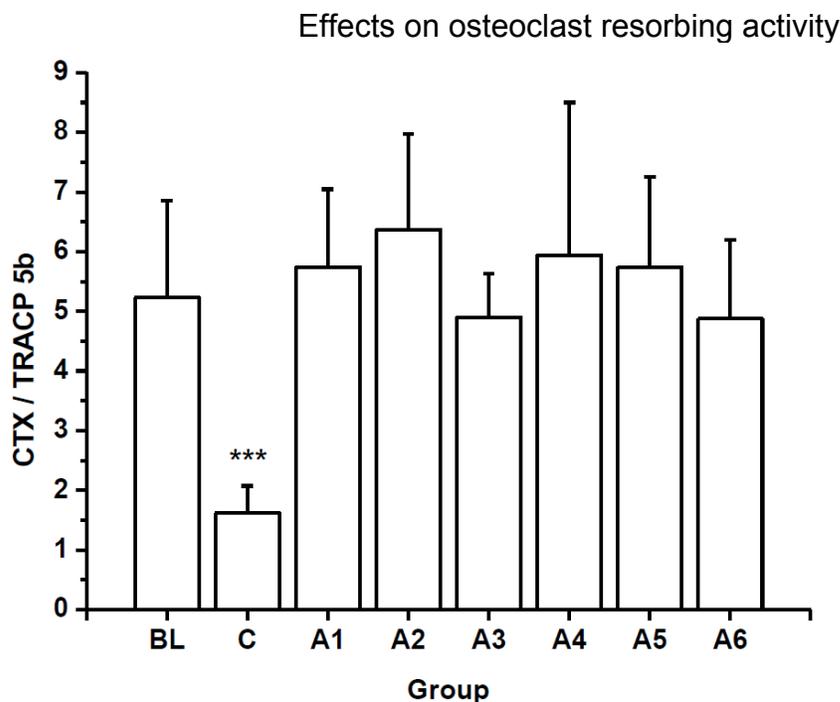
**Methods:** Human bone marrow-derived CD34+ osteoclast precursor cells were cultured on bovine bone slices. The effects of radium-223 on differentiation and activity of human osteoclasts were investigated *in vitro* at 6 concentrations of radium-223 (50, 100, 200, 400, 800 and 1600 Bq/mL). Osteoclasts were quantitated by measuring tartrate-resistant acid phosphatase 5b activity (TRACP 5b) in the culture medium after precursor cells were cultured on bovine bone slices for 7 days. Osteoprotegerin (OPG) was included as a reference inhibitor of osteoclast differentiation. In the second part of the experiment, the culture medium was replaced by new medium at day 7, and the formed mature osteoclasts were cultured for an additional 3 days, allowing them to resorb bone. Radium-223 was added into the culture medium at day 7. C-terminal crosslinked telopeptides of type I collagen (CTX) were measured in the culture medium collected at day 10 to quantitate bone resorption during days 7-10.

**Results:**

Radium-223 inhibited osteoclast differentiation at all tested concentrations in a dose-dependent manner. Radium-223 treatment did not affect osteoclast activity in mature osteoclasts.

The following figures are copied from the Applicant's submission.





Note: The results are shown as TRACP 5b activity (U/L) secreted into the culture medium or as CTX/TRACP 5b activity. The groups are: BL =Baseline (no added compounds); C = Control (5.0 nM OPG); A1 = 50 Bq/mL Alparadin<sup>TM</sup>; A2= 100 Bq/mL Alparadin<sup>TM</sup>; A3 = 200 Bq/mL Alparadin<sup>TM</sup>; A4 = 400 Bq/mL Alparadin<sup>TM</sup>; A5 = 800 Bq/mL Alparadin<sup>TM</sup>; A6 = 1600 Bq/mL Alparadin<sup>TM</sup>. Figure 1 was prepared from the original data shown in Table 1. The results of all other groups were compared separately with the results of the baseline group using one-way ANOVA ( $p < 0.001$  between all groups). Three asterisks (\*\*\*) indicate a statistically significant inhibitory effect with a  $p$ -value  $< 0.001$ .

#### Osteoclast differentiation assay

50 Bq/ml	100 Bq/ml	200 Bq/ml	400 Bq/ml	800 Bq/ml	1600 Bq/ml
52% (***)	39% (***)	22% (***)	13% (***)	9% (***)	5% (***)

#### Osteoclast activity assay

50 Bq/ml	100 Bq/ml	200 Bq/ml	400 Bq/ml	800 Bq/ml	1600 Bq/ml
110% (NS)	122% (NS)	94% (NS)	114% (NS)	110% (NS)	93% (NS)

The results are shown above as % of activity compared with the results of the baseline group, the mean value of the baseline group being 100%. Three asterisks (\*\*\*) indicate a statistically significant inhibitory effect with a  $p$ -value  $< 0.001$ . NS = Not significantly different from the baseline group.

**5-Effects of radium on osteoblasts**

**Title:** The effects of 6 doses of Alphanadin on mouse osteoblast differentiation and activity *in vitro*

Test facility: (b) (4)

Report date: September 27, 2010

**Summary:**

Radium-223 has mild beneficial effects on mouse osteoblasts with 100 and 200 Bq/mL concentrations, and non-beneficial effects with 400, 800 and 1600 Bq/mL concentrations.

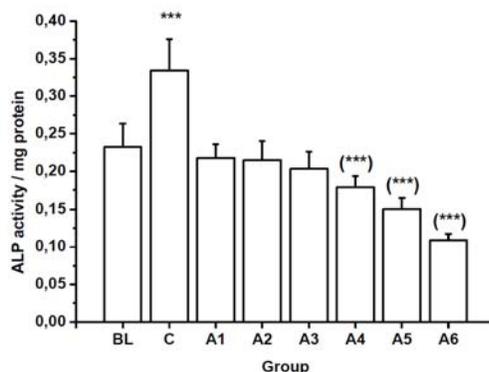
**Methods:** KS483 mouse osteoprogenitor cells were used to investigate the effects of 6 doses (50, 100, 200, 400, 800 and 1600 Bq/mL) of radium-223 on differentiation and activity.  $17\beta$ -estradiol was included in the study as a reference compound that stimulates osteoblasts. This study was performed in two parts. In the first part, the cells were cultured for 8 days, after which the formed mature osteoblasts were quantitated by measuring the amount of intracellular alkaline phosphatase (ALP) activity. In the second part of the study, the cells were cultured for 13 days, during which N-terminal propeptide of type I procollagen (PINP) secreted into the culture medium was determined at day 11 to demonstrate effects on organic bone matrix formation, and the amount of calcium deposited into the formed bone matrix was determined at day 13 to demonstrate effects on inorganic bone matrix formation.

**Results:**

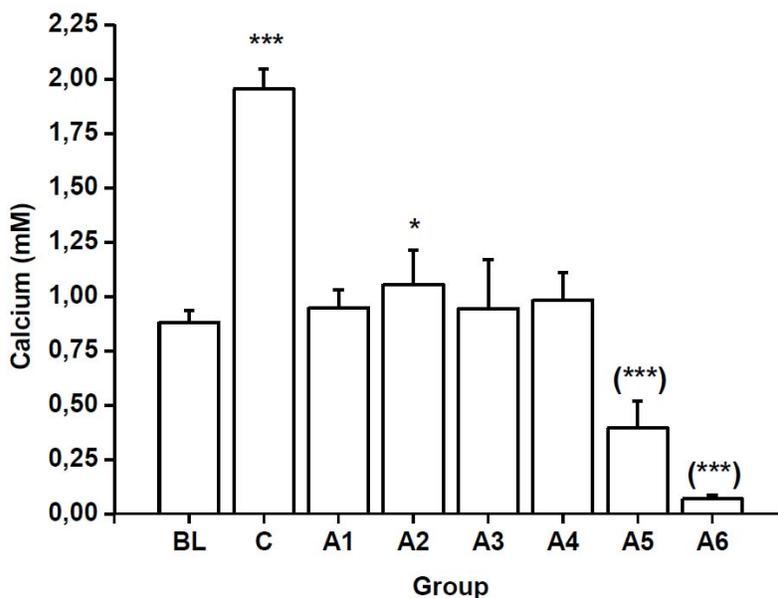
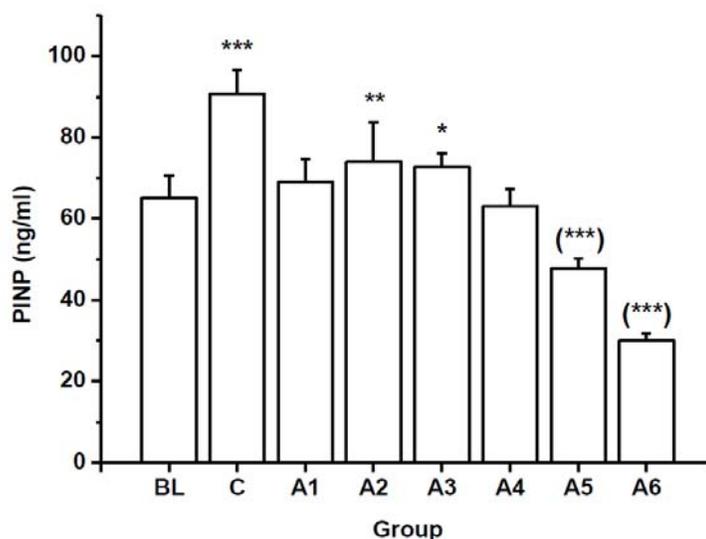
Radium-223 dichloride, at a concentration of 100 Bq/mL, increased slightly both PINP and calcium values, and at 200 Bq/mL increased slightly PINP values in the osteoblast activity assay. The concentrations of 800 and 1600 Bq/mL decreased PINP and calcium values in the osteoblast activity assay. The concentrations 400, 800 and 1600 Bq/mL decreased ALP values in the osteoblast differentiation assay. These results demonstrate that radium-223 has mild beneficial effects on osteoblasts at 100 and 200 Bq/mL concentrations, and non-beneficial effects with 400, 800 and 1600 Bq/mL concentrations. The most beneficial concentration was 100 Bq/mL.

The following table and figures are copied from the Applicant's submission.

Effects on osteoblast differentiation



Effects on osteoblast activity



Note: The groups are: BL = Baseline (no added compounds); C = Control (10 nM 17β-estradiol); A1 = 50 Bq/mL <sup>223</sup>Ra; A2 = 100 Bq/mL <sup>23</sup>Ra; A3 = 200 Bq/mL <sup>23</sup>Ra; A4 = 400 Bq/mL <sup>23</sup>Ra; A5 = 800 Bq/mL <sup>23</sup>Ra; A6 = 1600 Bq/mL <sup>23</sup>Ra.

Osteoblast differentiation assay

ALP activity at day 8

50 Bq/ml	100 Bq/ml	200 Bq/ml	400 Bq/ml	800 Bq/ml	1600 Bq/ml
94% (NS)	93% (NS)	88% (NS)	77% ([***])	65% ([***])	47% ([***])

Osteoblast activity assay

PINP secretion at day 11

50 Bq/ml	100 Bq/ml	200 Bq/ml	400 Bq/ml	800 Bq/ml	1600 Bq/ml
106% (NS)	114% (**)	112% (*)	97% (NS)	73% ([***])	46% ([***])

## Calcium deposition at day 13

50 Bq/ml	100 Bq/ml	200 Bq/ml	400 Bq/ml	800 Bq/ml	1600 Bq/ml
108% (NS)	120% (*)	107% (NS)	112% (NS)	45% ([***])	8% ([***])

The results above are shown as % of activity compared with the results of the baseline group, the mean value of the baseline group being 100%. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, NS = Not significantly different compared with the baseline group.

**6-In vivo activity**

**Title:** Dose finding study for Alpharadin in a breast cancer bone metastasis model

**Test facility:** (b) (4)

**Report date:** September 17, 2010

**Summary:**

The radium-223 dichloride dose of 300 kBq/kg was the most effective dose in maintaining body weight and decreasing tumor burden and osteolytic lesions.

**Methods:** The effect of radium-223 dichloride on breast cancer bone metastases was studied in female athymic nude mice. On day 0, the animals (4-5 weeks old, 7 mice/group) were given intracardiac inoculation of human breast cancer cells. Body weights were determined twice a week. The development of osteolytic lesions was followed by x-ray and fluorescence imaging at day 14 and at sacrifice. Based on the presence of osteolytic lesions and body weight at day 14 the animals were randomized to four groups. The test compound and vehicle were administered intravenously at day 15. The animals were sacrificed at day 25, or earlier if they are moribund. Tissue samples were collected for histology and embedded in paraffin from left and right tibia and femur for possible analysis in a separate study later. Gross necropsy was performed on all animals at the end of the study, and all macroscopic signs were recorded.

The following are the 4 experimental groups in the study:

1. Control group receiving vehicle 5 mL/kg i.v. (single dose)
2. Test group receiving radium-223 dichloride 300 kBq/kg i.v. (single dose)
3. Test group receiving radium-223 dichloride 600 kBq/kg i.v. (single dose)
4. Test group receiving radium-223 dichloride 1200 kBq/kg i.v. (single dose)

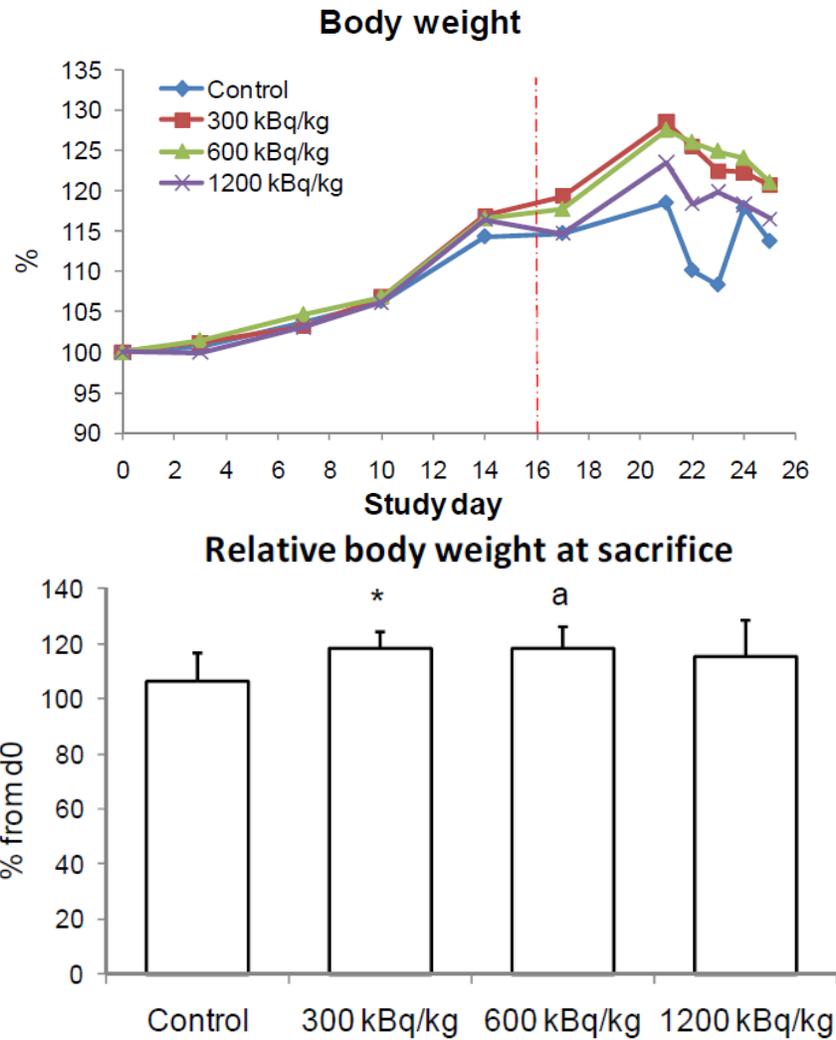
**Results:**

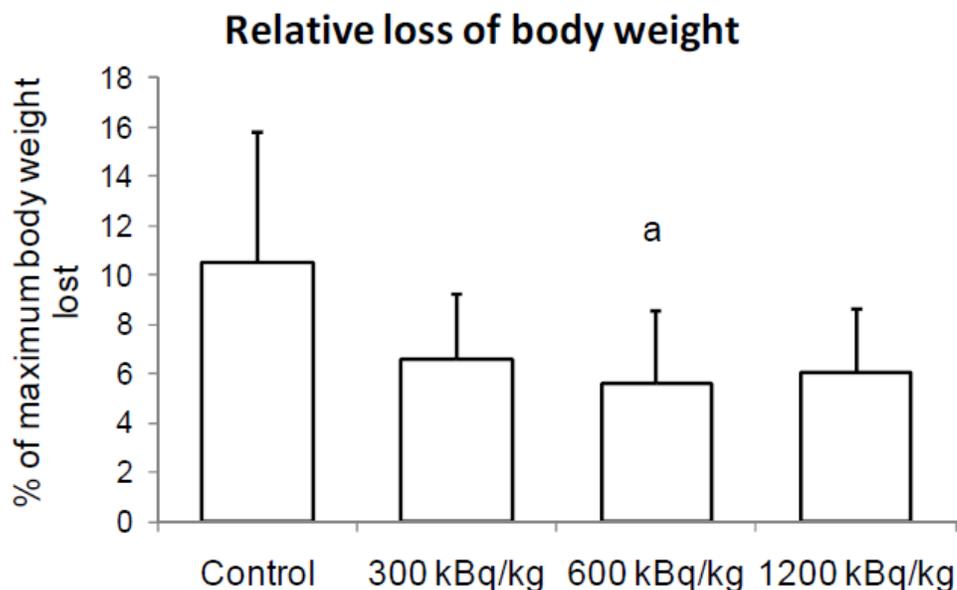
- Body weights were maintained better in animals receiving 300 kBq/kg and 600 kBq/kg of radium-223 dichloride;
- Paraplegia and cachexia were prevented by doses of 600 and 1200 kBq/kg of radium-223 dichloride;
- Whole body tumor burden as analyzed by GFP positive area was decreased by radium-223 dichloride at doses  $\geq$  300 kBq/kg, with the maximal inhibitory effect at 300 kBq/kg;

- The development of osteolytic lesions was inhibited by radium-223 dichloride at a dose of 300 kBq/kg, as shown by the reduced number of osteolytic lesions and the trend of decreased total osteolytic area.

The following figures are copied from the Applicant’s submission.

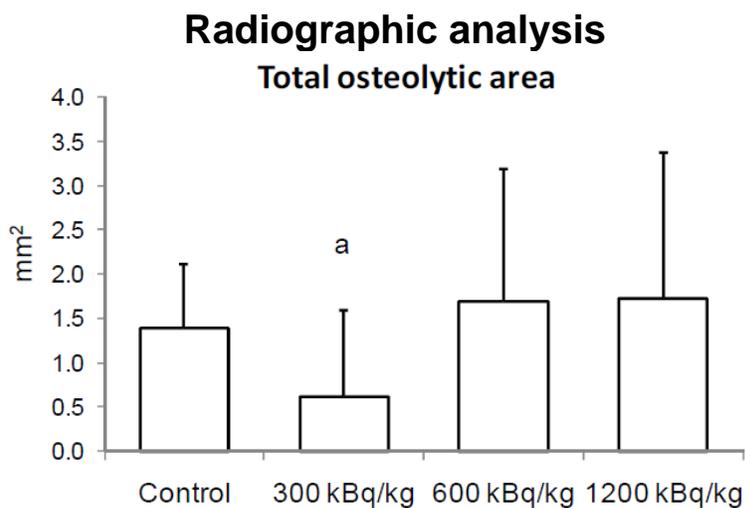
Note: In the following figures, One asterisk (\*) indicates a statistically significant difference with a p-value < 0.05 and letter “a” indicates a trend with p-value < 0.1.



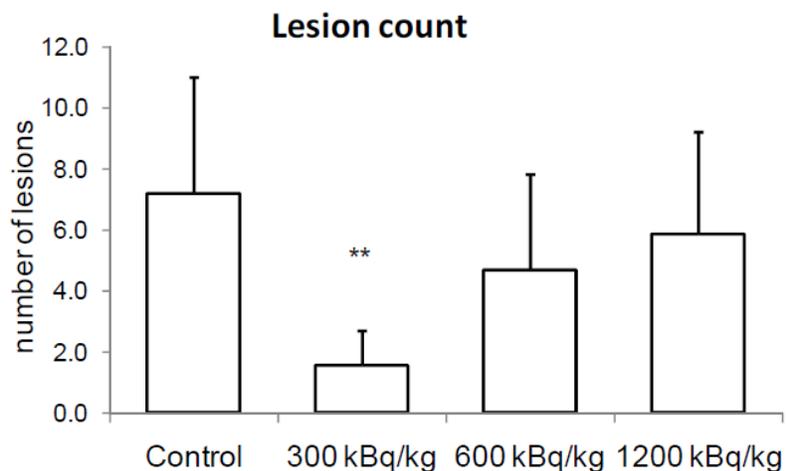


### Proportion of paraplegic and cachectic animals

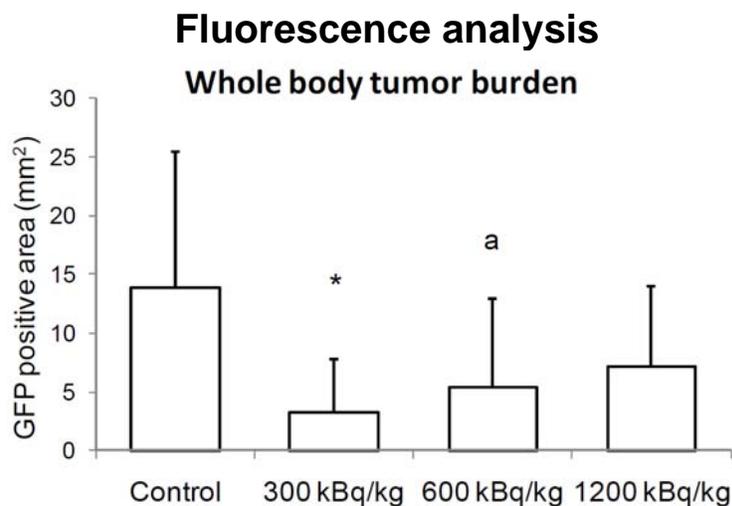
	Control	300 kBq/kg	600 kBq/kg	1200 kBq/kg
<b>Cachectic</b>	20% (1/5)	0 (0/7)	0 (0/7)	0 (0/7)
<b>p-value</b>		0.417	0.417	0.417
<b>Significance</b>		NS	NS	NS
<b>Paraplegic</b>	60% (3/5)	14.3% (1/7)	0 (0/7)	0 (0/7)
<b>p-value</b>		0.222	0.045	0.045
<b>Significance</b>		NS	*	*



-Total osteolytic lesion area (mm<sup>2</sup>) at sacrifice (mean+SD).



-Number of osteolytic lesions at sacrifice (mean+SD).



**Title:** Method of action of Alpharadin™ in a breast cancer bone metastasis model

**Test facility:** (b) (4)

**Report date:** December 28, 2010

**Summary:**

- Body weights were maintained better in animals receiving radium-223 dichloride;
- Cachexia and paraplegia were inhibited by radium-223 dichloride;
- The development of osteolytic lesions was inhibited by radium-223 dichloride as shown by reduced total and means osteolytic area in radiographic analysis and increased bone area in histomorphometric analysis.
- The number of osteoclasts at the tumor-bone interface was decreased by radium-223 dichloride.

**Method:** mode of action (MOA) of radium-223 dichloride on breast cancer bone metastases in female athymic nude mice was studied in a 25-day study with the following 2 experimental groups:

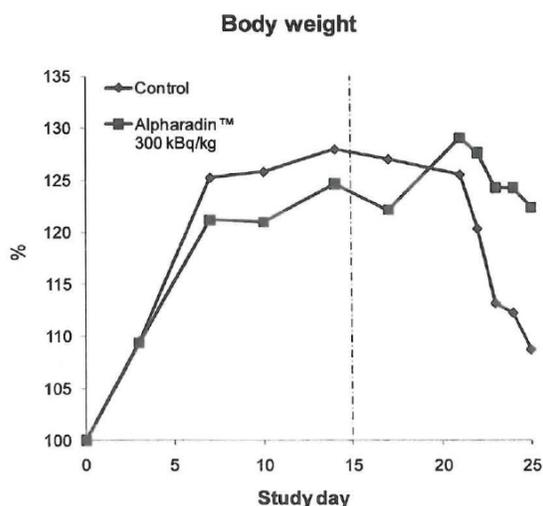
1. Control group receiving vehicle 5 mL/kg i.v. (single dose)
2. Test group receiving Alpharadin 300 kBq/kg i.v. (single dose)

On day 0, the animals (4 weeks old) were given intracardiac inoculation of human breast cancer cells. Body weights were determined twice a week. The development of osteolytic lesions was followed by x-ray and fluorescence imaging at day 14 and at sacrifice. Based on the presence of osteolytic lesions and body weight at day 14 the animals were randomized to two groups (15 animals/group). The test compound and vehicle was administered intravenously at day 15. Blood samples for analyzing TRACP 5b and PINP were collected before the inoculation of cancer cells and at days 14 and 24. The animals were sacrificed at day 25, or earlier if they became moribund.

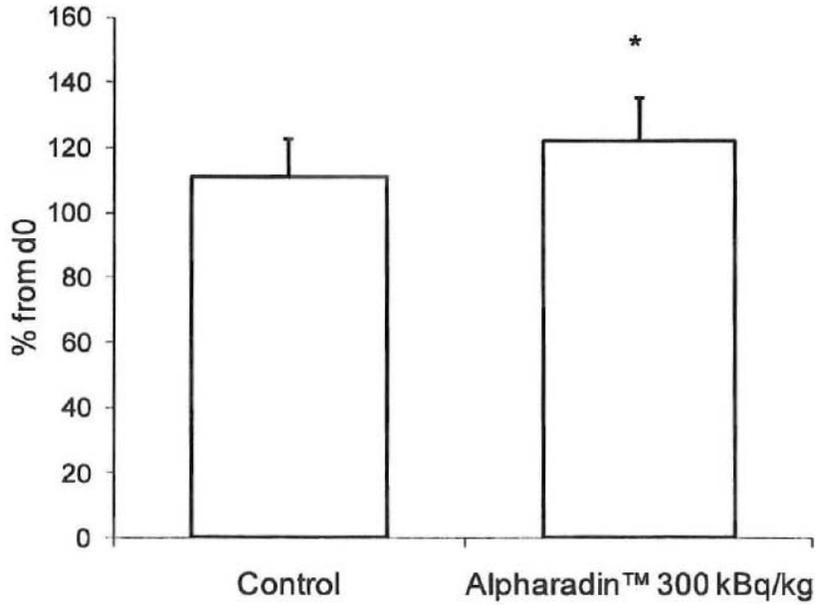
Tissue samples were collected for histology and embedded in paraffin from left and right tibia and femur for histomorphometric analysis. Gross necropsy was performed to all animals at the end.

**Results:** The development of osteolytic lesions was inhibited by radium-223 dichloride as shown by reduced total and mean osteolytic area in radiographic analyses and increased bone area in histomorphometric analyses. Increase in relative PINP values after start of the treatment suggests increased bone formation in animals receiving radium-223 dichloride.

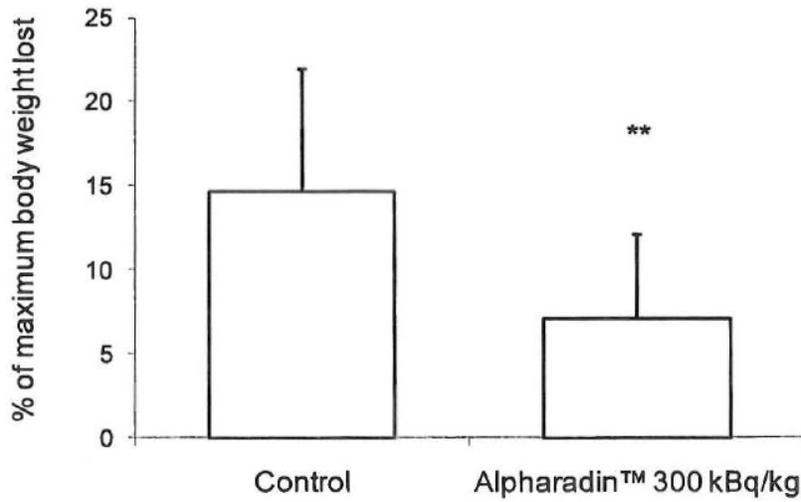
The following table and figures are copied from the Applicant's submission.



**Relative body weight at sacrifice**



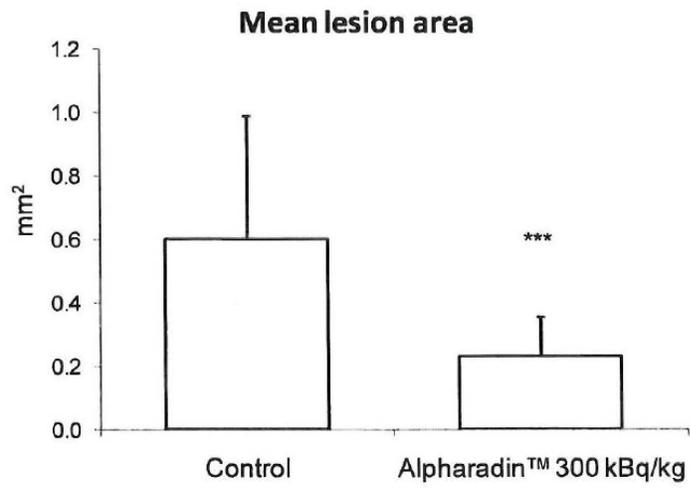
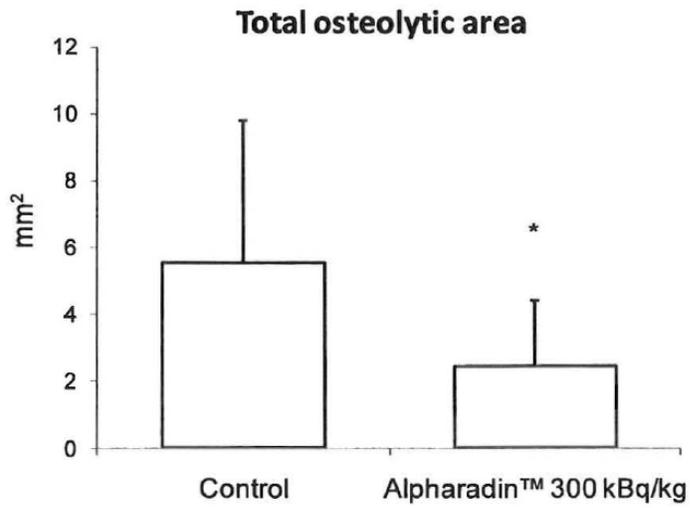
**Relative loss of body weight**



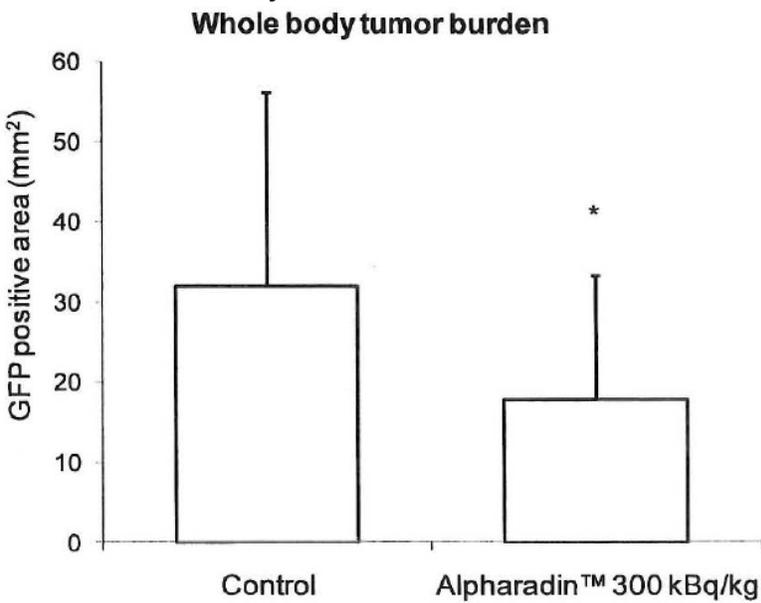
*Paraplegia and cachexia*

	Control	Alpharadin™ 300 kBq	p-value	Significance
<b>Cachectic</b>	7/14	0/14	0.006	<b>**</b>
<b>Paraplegic</b>	3/14	1/14	0.596	<b>NS</b>

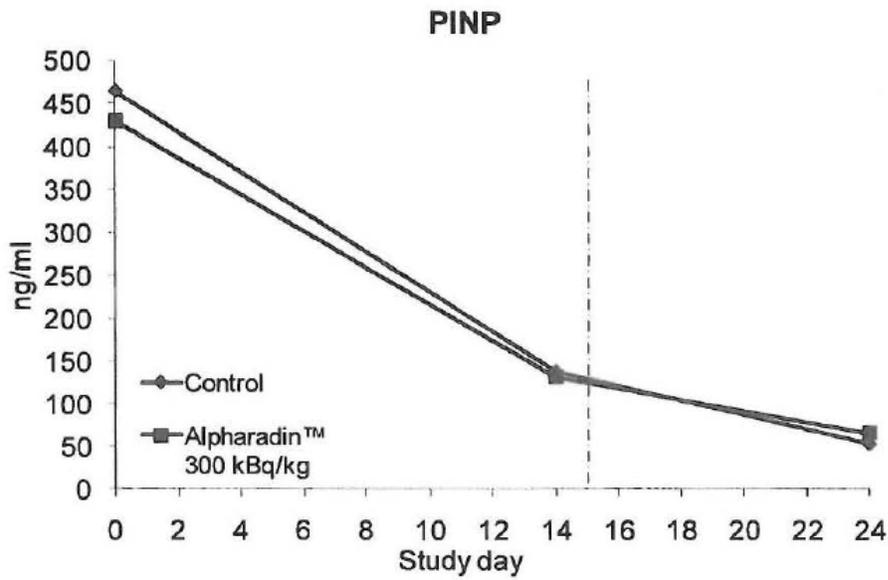
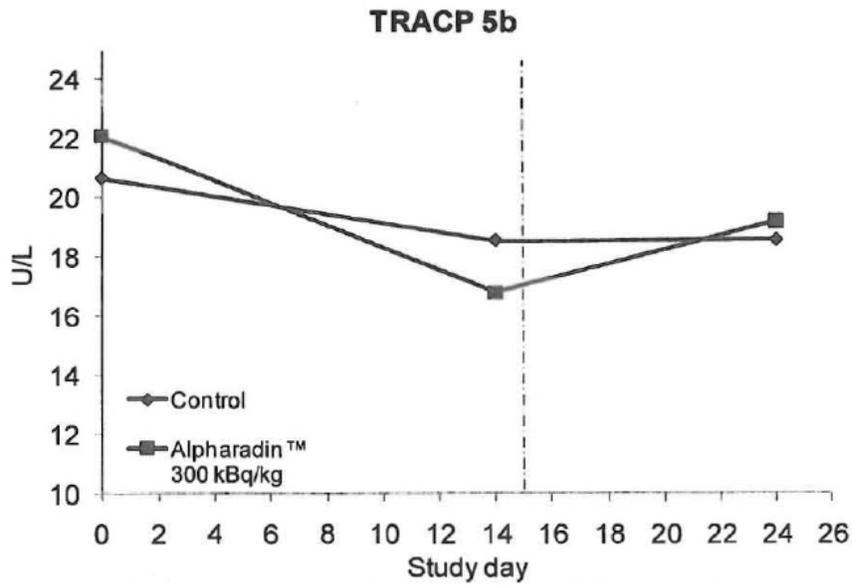
*Radiographic analysis*



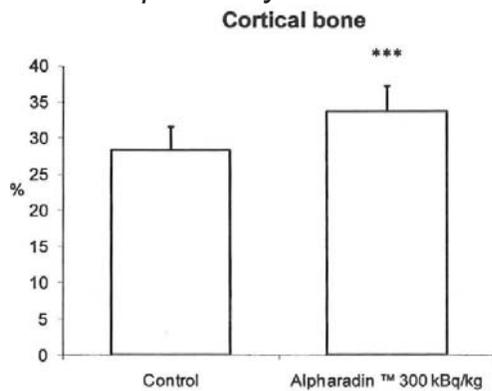
*Fluorescence analysis*

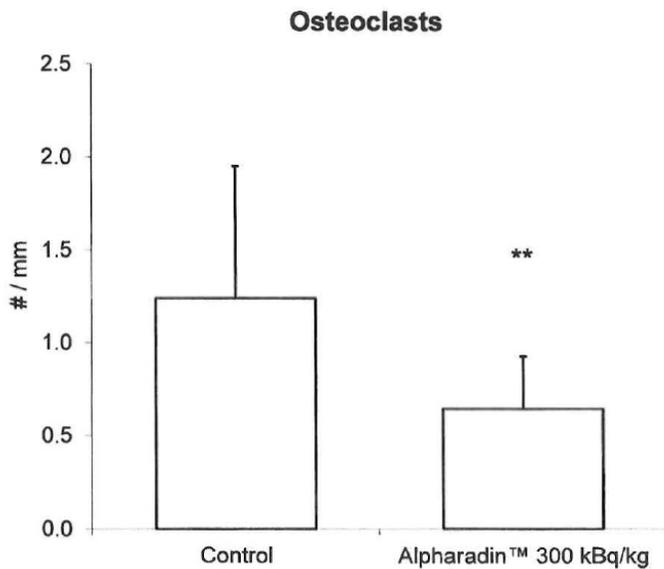
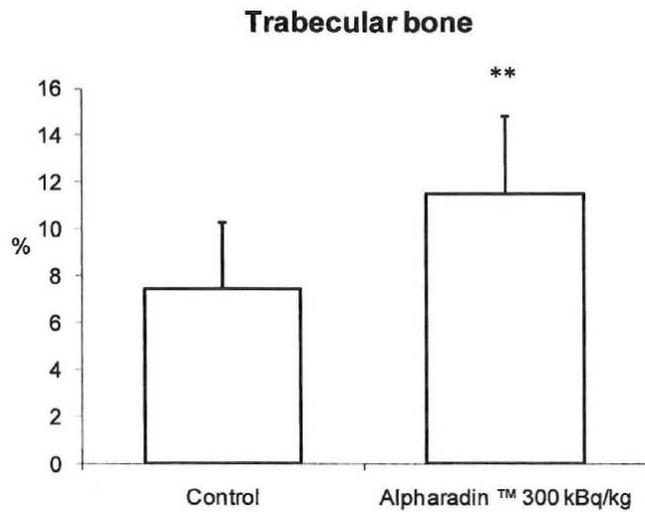


Bone marker measurements



Histomorphometry





**Title:** Survival study with Alpharadin in a breast cancer bone metastasis model

**Test facility:** (b) (4)

**Report date:** December 28, 2012

**Summary:** Body weights were maintained better in animals receiving radium-223 dichloride and the time to sacrifice was markedly increased by radium-223 dichloride.

**Method:** The effects of radium-223 dichloride on survival of female athymic nude mice carrying breast cancer bone metastases were studied in a 52-day study with the following 2 experimental groups:

1. Control group receiving vehicle 5 mL/kg iv (single dose)

## 2. Test group receiving 300 kBq/kg radium-223 dichloride, iv. (single dose)

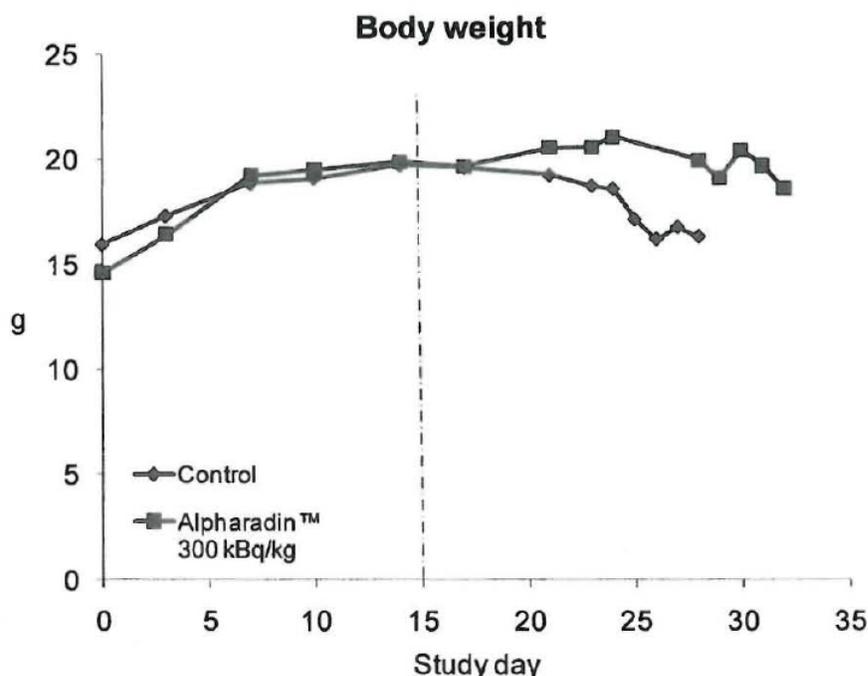
On day 0, the animals (4 weeks old) were given intracardiac inoculation of human breast cancer cells. Body weights were determined twice a week. The development of osteolytic lesions was followed by x-ray and fluorescence imaging at days 14, 24 and at sacrifice. Based on the presence of osteolytic lesions and body weight at day 14 the animals were randomized to two groups (10 animals/group). The test compound and vehicle were administered intravenously at day 15. Blood samples for analyzing TRACP 5b and PINP were collected before the inoculation of cancer cells and at days 14, 24 and at sacrifice. The animals were sacrificed at day 52, or when they became moribund. Tissue samples were collected for histology and embedded in paraffin from left and right tibia and femur for possible histomorphometric analysis. Gross necropsy was performed on all animals at the end of the study, and all macroscopic signs were recorded.

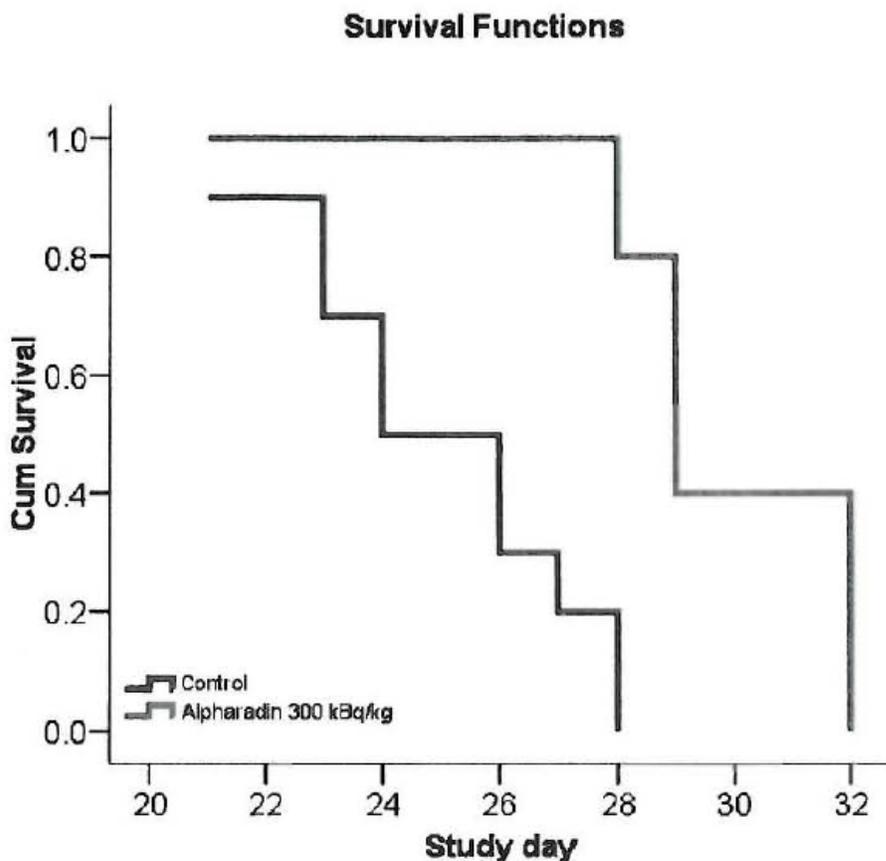
### Results:

Only body weight changes and survive curves are shown here since the results from this study were similar to the findings from the studies described above.

- Radium-223 dichloride treatment improved body weight loss.
- Animals receiving radium-223 dichloride survived longer;

The following table and figures are copied from the Applicant's submission.





#### 4.2 Secondary Pharmacology

No study conducted.

#### 4.3 Safety Pharmacology

##### Safety pharmacology summary:

No clinically relevant adverse effects of radium-223 dichloride on neurological, cardiovascular or pulmonary functional parameters were observed in conducted safety pharmacology studies.

##### Neurological effects:

##### Safety pharmacology study of alpharadin (radium-223) on central nervous system function on rats

Study no: (b) (4) Project No. 2148-001 Study No: 3

Volume #, and page #: electronic submission, Module 4

M4\42-study-rep\421-pharmac\4213-safety-pharmacol\R-8659.pdf 1-229

Conducting laboratory and location: (b) (4)

Date of study initiation: January 23, 2007

GLP compliance: yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** radium-223

Batch No.: A 702001 or A 701004,

Purity: >99% for both batches-radiochemical purity

**Formulation/vehicle:** 55/1000 kBq/mL, no other details were provided.

The control vehicle was 0.028M sodium citrate in sodium chloride.

**Methods:** The effect of radium-223 dichloride on the central nervous system was studied in male Sprague-Dawley rats following a single intravenous (i.v.) administration of radium-223 dichloride at dose levels of 50, 250 and 1000 kBq/kg or control article. The functional observational battery (FOB) and locomotor activity were evaluated before dosing, and at approximately 1 and 24 hours after dosing. The following parameters were monitored per standard operating procedures for FOB: home cage observation, hand-held observation, hearing/startle response, hearing/click response, body temperature, open field (mobility/gait), tail pinch, pupil response, eye blink response, vision, hindlimb extension, catalepsy, grip strength (forelimb and hindlimb), righting reflex (acrial and ground), foot splay and body weights (24 hour post-dose body weights only performed for Groups 1, 2 and 3). No FOB body weights were taken for all groups for the pre-dose and 1-hour post-dose measurements and for the Group 4 24-hour post-dose measurement. Locomotor activity (number and duration of ambulatory movement, inactivity duration and rearing) was captured using an automated system (Photobeam Activity System, San Diego Instruments, Inc., version 2.0.5.100, San Diego, CA).

**Dosing:**

Species/strain: male Sprague-Dawley [CrI:CD@(SD)IGS BR] rats

#/sex/group or time point: 8/group

Age: approximately 5 weeks

Weight: 84 -106 g

Doses in administered units: 50, 250, 1000 kBq/kg

Route, form, volume, and infusion rate: IV, the dosing volume is calculated as follows.

dosing volume (mL) = dose (kBq/kg) x body weight (kg) x decay factor / test article conc (kBq/mL).

**Results:**

Functional Observational Battery: There were some statistically significant decreases in foot splay, hindlimb and forelimb grip in all treated group animals, however the effects were not consistent and not dose dependent.

Locomotor Activity: unremarkable

In addition to the above results, the Applicant also provided the following conclusion for clinical observation part of the study, the data were not reviewed.

“No death or clinical signs were observed following administration of control article or Alparadin up to 1000 kBq/kg in any of the rats at any dose groups throughout the duration of the study, No differences were noted in body weights and total body weight gains on Day 7 when compared to the control article group, No effects on total body weight gains were observed in either the control group or test article groups.”

Respiratory effects:**Safety pharmacology study of alpharadin (radium-223) on respiratory function on rats****Study no:** (b) (4) Project No. 2148-001 Study No: 1**Volume #, and page #:** electronic submission, Module 4  
M4\42-study-rep\421-pharmacol\4213-safety-pharmacol\R-8657.pdf 1-64**Conducting laboratory and location:** (b) (4)**Date of study initiation:** January 23, 2007**GLP compliance:** yes**QA report:** yes ( x ) no ( )**Drug, lot #, radiolabel, and % purity:** Alpharadin (radium-223)  
Batch No.: A 702001 or A 701004,  
Purity: >99% for both batches-radiochemical purity**Formulation/vehicle:** 55/1000 kBq/mL, no other details were provided.  
The control vehicle was 0.028M sodium citrate in sodium chloride.**Methods:** The effect of radium-223 dichloride on the respiratory system was studied in male Sprague-Dawley rats following a single intravenous (iv.) administration of radium-223 dichloride at dose levels of 50, 250 and 1000 kBq/kg or control article. The respiratory rate and tidal volume were monitored, and minute volume calculated for 20 to 30 minutes prior to dosing, and up to four hours post-dosing. The measurements were taken while the animals were in the restrained tubes. Measurements consisted of tidal volume, frequency of breathing (Rate) and minute volume.

In addition to pulmonary physiological function measurements, clinical observations, body weights and body weight gains were evaluated. Each group of rats was sacrificed without necropsy and tissue collection on their respective Day 8.

**Dosing:**

Species/strain: male Sprague-Dawley [CrI:CD@(SD)IGS BR] rats

#/sex/group or time point: 8/group

Age: approximately 5 weeks

Weight: 83 -109 g

Doses in administered units: 50, 250, 1000 kBq/kg mg/kg

Route, form, volume, and infusion rate: IV, the dosing volume is calculated as follows.

$$\text{dosing volume (mL)} = \text{dose (kBq/kg)} \times \text{body weight (kg)} \times \text{decay factor} / \text{test article conc (kBq/mL)}.$$
**Results:**

There were no statistically or biologically relevant differences between any of the treatment groups with respect to respiratory rate, tidal volume or minute volume. No mortality, morbidity, or clinical signs of toxicity were observed in animals.

Cardiovascular effects:**Safety pharmacology study of alfaradin (radium-223) on cardiovascular system function in telemetered beagle dogs****Study no:** (b) (4) Project No. 2148-001 Study No: 2**Volume #, and page #:** electronic submission, Module 4

M4\42-study-rep\421-pharmaco\4213-safety-pharmaco\IR-8658, Page 1-191

**Conducting laboratory and location:** (b) (4)**Date of study initiation:** January 24, 2007**GLP compliance:** yes**QA report:** yes ( x ) no ( )**Drug, lot #, radiolabel, and % purity:** Alfaradin (radium-223)

Batch No.: A702009, A703007, A 704004, and A 705003

Purity: &gt; 99% (radiochemical purity)

**Formulation/vehicle:** 1000 kBq/mL, no other details provided.

The control article is sodium citrate 0.028M in sodium chloride.

**Methods:** Telemetered male beagle dogs were used in the study. Each dog was intravenously (iv) administered 4 dose levels of radium-223 dichloride at: Control (0 kBq/kg), Low (50 kBq/kg), Mid (150 kBq/kg) and High (450 kBq/kg) on Study Days 1, 29, 57 and 85 (once every four weeks with 4-week washout period between doses). The following table shows the study design.

Animal (ID)	Study Day			
	1	29	57	85
Dog 1 (404)	50 kBq/kg	450 kBq/kg	150 kBq/kg	0 kBq/kg
Dog 2 (308)	150 kBq/kg	50 kBq/kg	0 kBq/kg	450 kBq/kg
Dog 3 (402)	0 kBq/kg	150 kBq/kg	450 kBq/kg	50 kBq/kg
Dog 4 (403)	450 kBq/kg	0 kBq/kg	50 kBq/kg	150 kBq/kg

Each dog was continuously recorded the following for at least two hours prior to and through at least 24 hours following each dose: cardiovascular function measurements as well as body temperature and locomotor activity. Animals were weighed prior to each dose for dosage calculations and were observed within one hour after each dose and weekly during the study. Clinical chemistry and hematology evaluations were performed three days prior to the second, third and fourth doses and

necropsy. After the final washout period (Study Day 113), the animals were necropsied and tissues collected for histopathologic evaluation.

A telemetry system (Data Sciences International, St. Paul, MN) consisted of an implantable transmitter unit (TL II M3-D70-PCTP) for the measurement of systemic blood pressure, left ventricular pressure, heart rate, electrocardiogram (ECG), body temperature and locomotor activity, signal receiver (RMC-I), ambient pressure monitor (APR-I), a Data Exchange Matrix (OEM) and a data acquisition system using DSI DataquestART™ software.

### Dosing:

Species/strain: male Beagle dogs

#/sex/group or time point: 4/group

Age: 6-7 months

Weight: 9-12 kg

Doses administered in units: 50 kBq/kg, 150 kBq/kg, 450 kBq/kg

Route, form, volume, and infusion rate: IV, the dosing volume is calculated as follows.

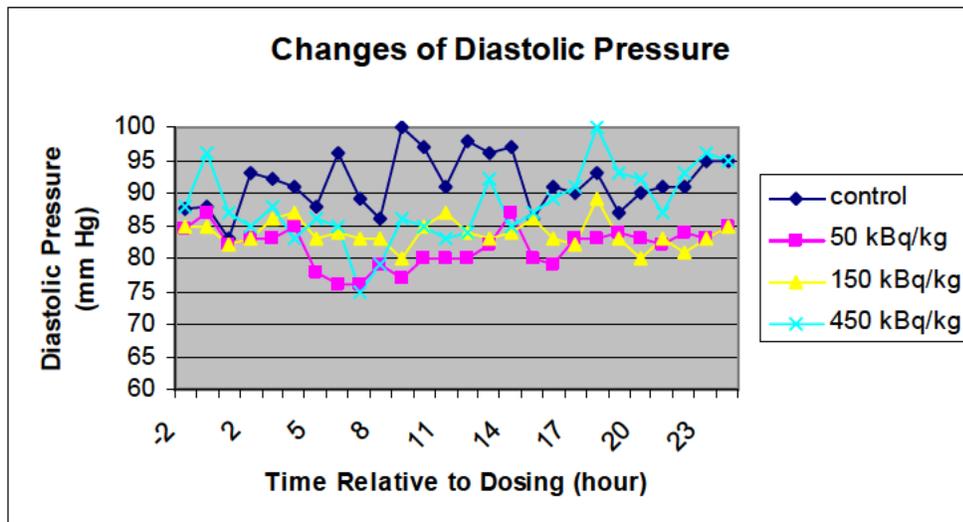
$$\text{dosing volume (mL)} = \text{dose (kBq/kg)} \times \text{body weight (kg)} \times \text{decay factor} / \text{test article conc (kBq/mL)}$$

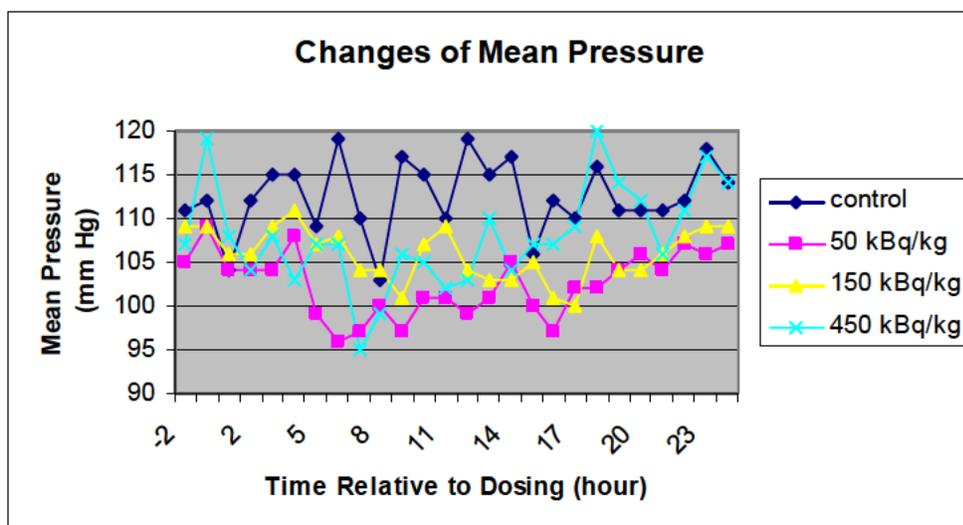
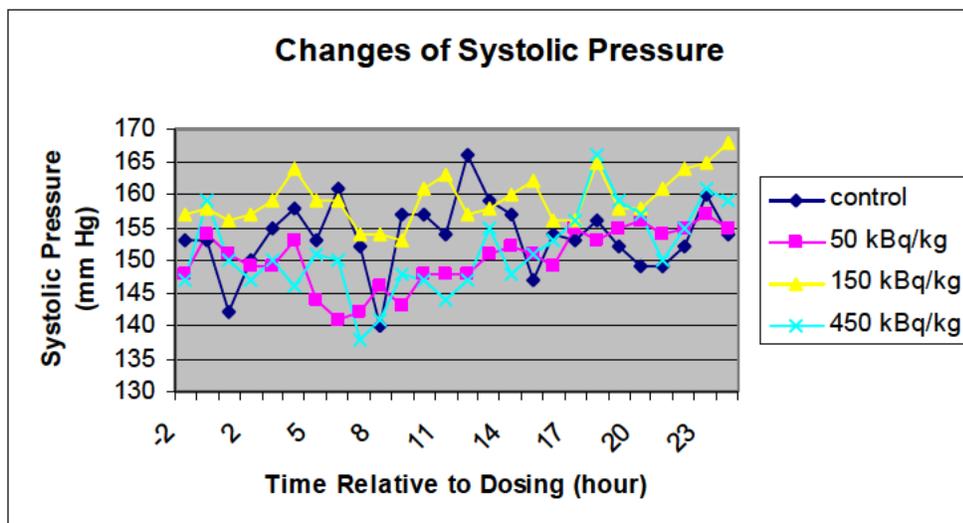
### Results:

#### Cardiovascular Parameter Measurements:

1. Left Ventricular Pressure: unremarkable

2. Arterial Blood Pressure:





Summary: Blood pressure values (diastolic, systolic and mean) were generally lower after administration of radium-223 dichloride when compared to the values from control groups. There were recovery trends 24hours after dosing.

Reviewer comment: The differences in the test article-treated groups compared to the control group were not statistically significant, not dose-dependent, and the changes did not correlate with heartbeat or left ventricular pressure. Therefore, the changes in blood pressure related to administration of radium-223 dichloride were not considered to be clinically meaningful.

3. ECG and Heart Rate Measurements: heart rate, QRS complex, PR duration, RR duration and QTc duration were calculated using ECG analysis, and there were no statistically significant differences between the test article-treated groups and the control group.

Renal effects: no study conducted

Gastrointestinal effects: no study conducted

Abuse liability: no study conducted

## PHARMACOLOGY TABULATED SUMMARY

Overview of primary pharmacodynamics studies *in vitro* and *in vivo*

Cell type/animal model	Measured parameter	Tested dose range
NHIK3025	Cell survival Double strand breaks (DSB) Cell cycle analysis	Cellular dose: 1-3 Gy (1-25 kBq/mL)
NHIK3025 (dox)	Cell survival	0.19-0.94 Gy
A549	Cell survival	0.19-0.94 Gy
A549 (dox)	Cell survival	0.19-0.94 Gy
Human bone marrow-derived CD34+ osteoclast precursor cells	Differentiation Activity	50-1600 Bq/mL
KS483 mouse osteoprogenitor cells (osteoblasts)	Differentiation Activity	50-1600 Bq/mL
A breast cancer bone metastasis model, mouse	Body weight Tumor burden Osteolytic lesion Cachexia Bone formation Osteoclast differentiation The number of osteoclasts at the tumor-bone interface	300-1200 kBq/kg (single dose)

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

#### Brief summary

Pharmacokinetic studies were performed in mice after intravenous single dose administration of radium-223 dichloride. Radium-223 is primarily distributed to bone tissue, with about 70% of the administered dose bound to bone tissue (<1% in soft tissue) at 12 hours after administration. Radium-223 remained within the bone at least up to approximately 5 half-lives. Excretion of radium-223 occurred by gastrointestinal and renal route. A relatively high concentration of radioactivity was found in the kidney and large intestine in mice within the first hour after administration, which is consistent with the urinary and fecal excretion of radium-223.

The pharmacokinetics profile of radium-223 dichloride was also characterized in dog single- and repeat-dose studies. The pharmacokinetics profile of radium-223 dichloride in dogs was similar to that observed in rats. The biodistribution and excretion of radium-223 was not affected by the repeat dosing of radium-223 dichloride.

The pharmacokinetics or biodistribution of radium-223 was not affected by administration of a bisphosphonate (zoledronic acid) 2 hours earlier.

#### Methods of Analysis:

The analytical method for detection and quantification of radium-223 in biological samples is based on measuring of the radioactivity of gamma component of the radioactivity emitted by radium-223 and the daughter nuclides. Biological samples were analyzed for total radioactivity of radium-223 using a Sodium Iodide (NaI) well type detector.

#### Absorption

No study conducted since radium-223 dichloride is administered intravenously.

#### Distribution

#### Pharmacokinetics and biodistribution of radium-223 (Alpharadin™) in mice

#### Key Study Findings:

- Radium-223 is rapidly cleared from the blood following an intravenous injection;
- Radium-223 quickly binds to bone tissue where it remains for at least up to 336 hours, the longest time point in this study;
- There was little accumulation of radium-223 in soft tissue with the exception of the spleen.

**Study no:** 148-001; BC-1Tr001-2007

**Volume #, and page #:** electronic submission, page 1-49

**Conducting laboratory and location:** Algeta ASA  
P .O. Box 54 Kjelsas  
N-0411 Oslo, Norway

**Date of study initiation:** November 28, 2006

**GLP compliance:** yes

**QA report:** yes (x ) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 88-8223  
batch No.: A611008  
Purity: 96.8% (radiochemical purity)

**Formulation/vehicle:** a sterile, isotonic, ready to administer solution,  
no other details provided.

**Methods:** BALB/CA female mice were intravenously given a single dose of radium-223 dichloride on day 0. Blood samples were withdrawn at all time points specified below for radioactivity measurements.

Group	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Time (h)	0	0,25	0,5	0,75	1	2	3	6	12	18	24	36	48	72	120	336
Blood	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Tissue	x		x		x			x	x		x		x	x	x	x

Tissue samples (both the femur, the sternum, the skull-defined as the skeleton of the head including the mandible, the brain, the liver, the heart, both kidneys, the spleen, the lung, the large intestine, the small intestine and the both femur muscle) were taken at time points marked above for radioactivity measurements of the tissue samples.

**Dosing:**

Species/strain: female BALB/CA mice

#/sex/time point: 3/sex

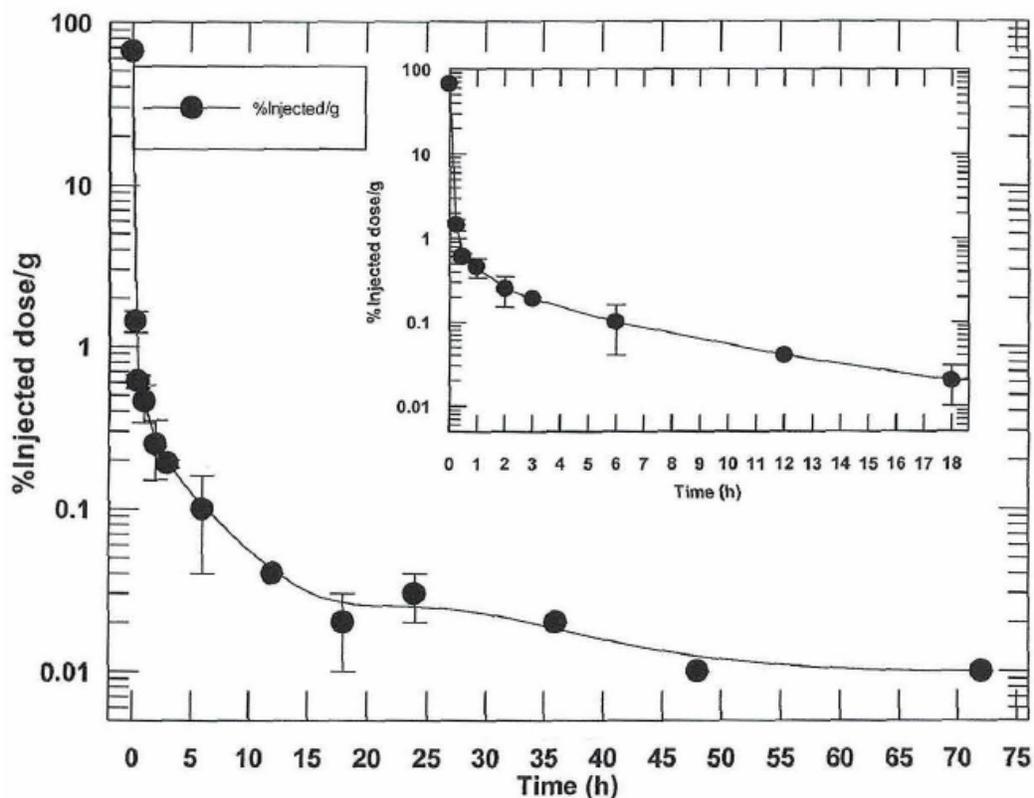
Weight: 19.5-25.9 g

Doses in administered units: 625 kBq/kg

Route, form, volume, and infusion rate: IV at dose volume of ~ 25  $\mu$ L ( 20 g body weight with solution activity at 500 kBq/mL ). The exact injection volume was calculated based upon the calibration date and conversion table supplied with the test article.

**Results:** The following figures and tables are copied from Applicant's submission.

Pharmacokinetics of radium-223 in mice (average of 3 animals)



% injected dose/gram in blood (average of 3 animals)

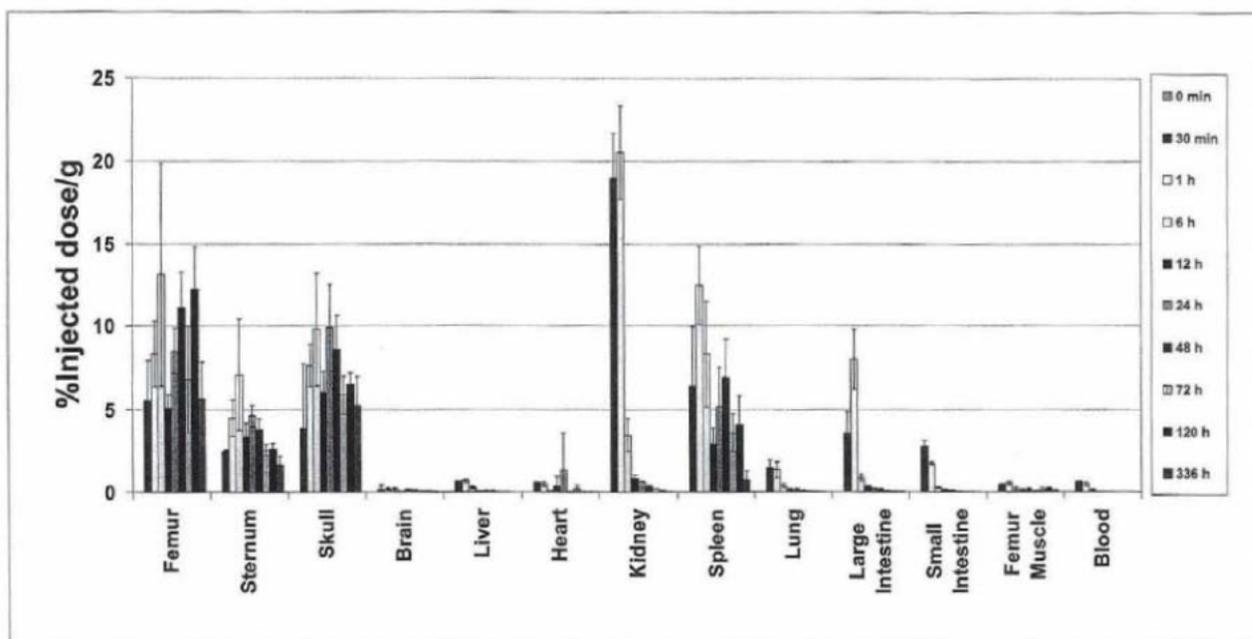
<b>Blood</b>		
<b>Hours</b>	<b>%Inj/g</b>	<b>SD</b>
0.00	0.00	0.00
0.25	1.44	0.22
0.50	0.61	0.05
0.75	0.61	0.06
1	0.46	0.12
2	0.25	0.10
3	0.19	0.01
6	0.10	0.06
12	0.04	0.00
18	0.02	0.01
24	0.03	0.01
36	0.02	0.00
48	0.01	0.00
72	0.01	0.00
120	0.00	0.00
336	0.00	0.00

Biodistribution of radium-223 in mice (average of 3 animals)

	<b>Femur</b>		<b>Sternum</b>		<b>Skull</b>		<b>Brain</b>		<b>Liver</b>		<b>Heart</b>		<b>Kidney</b>	
	<b>%Inj/g</b>	<b>SD</b>	<b>%Inj/g</b>	<b>SD</b>	<b>%Inj/g</b>	<b>SD</b>	<b>%Inj/g</b>	<b>SD</b>	<b>%Inj/g</b>	<b>SD</b>	<b>%Inj/g</b>	<b>SD</b>	<b>%Inj/g</b>	<b>SD</b>
30 min	5.56	2.39	2.47	0.08	3.87	3.86	0.19	0.21	0.63	0.03	0.57	0.02	18.99	2.71
1 h	8.33	1.95	4.49	1.11	7.63	1.27	0.17	0.08	0.67	0.13	0.46	0.11	20.53	2.84
6 h	13.13	6.72	7.07	3.34	9.82	3.38	0.16	0.08	0.27	0.07	0.09	0.02	3.45	1.00
12 h	5.09	0.80	3.34	0.83	6.03	1.26	0.07	0.01	0.06	0.02	0.38	0.56	0.84	0.17
24 h	8.50	1.34	4.62	0.68	9.91	2.61	0.13	0.05	0.08	0.03	1.33	2.24	0.61	0.03
48 h	11.12	2.18	3.77	0.70	8.59	2.03	0.12	0.03	0.07	0.02	0.02	0.01	0.34	0.06
72 h	6.80	3.19	2.53	0.36	5.88	1.13	0.08	0.02	0.03	0.00	0.18	0.25	0.18	0.02
120 h	12.24	2.56	2.59	0.37	6.51	0.71	0.09	0.01	0.03	0.01	0.01	0.01	0.11	0.01
336 h	5.64	2.17	1.63	0.53	5.25	1.74	0.04	0.01	0.01	0.01	0.00	0.01	0.03	0.01

	<b>Spleen</b>		<b>Lung</b>		<b>Large Intestine</b>		<b>Small Intestine</b>		<b>Femur Muscle</b>	
	<b>%Inj/g</b>	<b>SD</b>	<b>%Inj/g</b>	<b>SD</b>	<b>%Inj/g</b>	<b>SD</b>	<b>%Inj/g</b>	<b>SD</b>	<b>%Inj/g</b>	<b>SD</b>
30 min	6.40	3.53	1.43	0.50	3.59	1.30	2.77	0.36	0.45	0.01
1 h	12.46	2.43	1.35	0.49	7.99	1.79	1.74	0.08	0.53	0.10
6 h	8.31	3.16	0.39	0.11	0.86	0.21	0.27	0.03	0.19	0.12
12 h	2.90	1.00	0.13	0.06	0.30	0.06	0.14	0.03	0.11	0.07
24 h	5.19	2.34	0.12	0.08	0.19	0.02	0.11	0.02	0.13	0.08
48 h	6.90	2.30	0.07	0.02	0.15	0.03	0.06	0.01	0.05	0.06
72 h	3.58	1.18	0.05	0.00	0.08	0.02	0.03	0.00	0.14	0.12
120 h	4.08	1.73	0.05	0.03	0.05	0.01	0.02	0.01	0.20	0.09
336 h	0.71	0.55	0.03	0.02	0.03	0.01	0.01	0.00	0.07	0.06

Biodistribution of radium-223: radioactivity in tissues and organs (average of 3 animals)



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#### Summary:

- 1) Clearance from blood appeared to be biphasic. A calculation of the biological half-life of radium-223 in blood established the initial half-life ( $t_{1/2\alpha}$ ) to be about 0.1 hours (about 5-10 minutes), and a terminal half-life ( $t_{1/2\beta}$ ) of about 12 hours;
- 2) Radioactivity accumulated mainly in the bone;
- 3) Initially there was a high amount of radioactivity found in the kidney, but the level was rapidly reduced;  
(Reviewer note: possibly due to the radioactivity being present in the urine.)
- 4) Radioactivity from radium-223 was found in the large intestine;  
(Reviewer note: possibly due to excretion of radium isotopes directly into the fecal contents.)
- 5) There was little radioactivity detected in soft tissue with the exception of the spleen.

#### Pharmacokinetics and biodistribution of radium-223 (Alpharadin™) in mice

##### Key Study Findings:

Once bound to bone, radium-223 remains within the bone at least up to approximately 5 radioactive half-lives.

**Study no:** 148-003; BC-1Tr001-2008

**Volume #, and page #:** electronic submission, page 1-49

**Conducting laboratory and location:** Algeta ASA  
P.O. Box 54 Kjelsas  
N-0411 Oslo, Norway

**Date of study initiation:** March 20, 2007

**GLP compliance:** yes

**QA report:** yes (x ) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 88-8223

batch No.: A611 008

Purity: 96.8% (radiochemical purity)

**Formulation/vehicle:** a sterile, isotonic, ready to administer solution 125 kBq/mL,  
no other details provided.

**Methods:** BALB/CA female mice were administered a single intravenous dose of radium-223 dichloride on day 0. Samples were withdrawn at all time points specified below for sequent radioactivity measurements. Tissue samples were femur, sternum, skull-defined as the skeleton of the head including the mandible, brain, liver, heart, kidney, spleen, lungs, large intestine, small intestine and the femur muscle.

Cage No.	A	B	C	D	E	F	G	H	I
Time points (day)	0	7	14	21	28	35	42	49	56
Number of mice	6	6	6	6	6	6	6	6	6
Blood	x	x	x	x	x	x	x	x	x
Fecal pellets	x	x	x	x	x	x	x	x	x
Urine	x	x	x	x	x	x	x	x	x
Tissue	x	x	x	x	x	x	x	x	x

**Dosing:**

Species/strain: female BALB/CA mice

#/sex/ time point: 6/sex

Weight: 19.1-23.6 g

Doses in administered units: 625 kBq/kg

Route, form, volume, and infusion rate: IV at dose volume of ~ 25  $\mu$ L ( 20 g body weight with solution activity at 500 kBq/mL ). The exact injection volume was calculated based upon the calibration date and conversion table supplied with the test article

**Results:** The following figures and tables are copied from Applicant's submission.

% injected dose/gram in blood (average of 6 animals)

Comparative data from Study 148-001 (average of 3 animals)

Blood		Ra-223 This study		Ra-223 Study 148001	
Hours	Days	% inj/g	SD	% inj/g	SD
0.00				66.7	1
	7	0.00	0.00		
336	14	0.00	0.00	0.00	0.00
	21	0.00	0.00		
	28	0.00	0.00		
	35	0.00	0.00		
	42	0.00	0.00		
	49	0.00	0.00		
	56	0.00	0.00		

Biodistribution of radium-223 in tissues and organs (as % injected dose/gram) where sampling was taken at extended times (average  $\pm$  SD of 6 animals)

	Femur Muscle		Femur		Sternum		Skull		Brain		Heart		Liver	
	%Inj/g	SD	%Inj/g	SD	%Inj/g	SD	%Inj/g	SD	%Inj/g	SD	%Inj/g	SD	%Inj/g	SD
Day 7	0.00	0.01	7.08	0.96	2.71	0.29	8.13	0.98	0.06	0.06	0.32	0.36	0.01	0.00
Day 14	0.00	0.00	7.30	0.90	2.51	0.59	7.63	0.70	0.05	0.01	0.14	0.26	0.01	0.00
Day 21	0.00	0.00	5.40	0.49	0.98	0.22	7.11	0.40	0.03	0.01	0.28	0.16	0.00	0.00
Day 28	0.24	0.05	8.57	1.04	2.56	0.36	5.95	0.68	0.03	0.01	0.20	0.28	0.00	0.00
Day 35	0.01	0.01	6.23	0.47	1.85	0.21	6.33	0.38	0.02	0.00	0.17	0.16	0.00	0.00
Day 42	0.05	0.08	6.52	0.90	1.94	0.26	5.88	0.45	0.02	0.02	0.12	0.19	0.01	0.00
Day 49	0.01	0.02	5.87	0.41	1.79	0.24	5.88	0.75	0.01	0.02	0.05	0.11	0.00	0.00
Day 56	0.02	0.07	6.04	1.23	1.62	0.41	6.16	0.55	0.03	0.03	0.18	0.21	0.00	0.02

	Lung		Spleen		Kidney		Small Intestine		Large Intestine	
	%Inj/g	SD	%Inj/g	SD	%Inj/g	SD	%Inj/g	SD	%Inj/g	SD
Day 7	0.03	0.02	1.33	0.45	0.09	0.02	0.02	0.00	0.04	0.01
Day 14	0.03	0.01	0.40	0.15	0.06	0.02	0.01	0.00	0.01	0.01
Day 21	0.02	0.03	0.38	0.03	0.03	0.01	0.01	0.00	0.03	0.02
Day 28	0.02	0.03	0.35	0.15	0.04	0.01	0.00	0.00	0.01	0.01
Day 35	0.01	0.03	0.33	0.14	0.03	0.01	0.00	0.00	0.01	0.01
Day 42	0.06	0.03	0.30	0.04	0.04	0.02	0.01	0.00	0.01	0.02
Day 49	0.03	0.05	0.26	0.06	0.02	0.02	0.01	0.01	0.03	0.03
Day 56	0.04	0.08	0.44	0.13	0.05	0.04	0.02	0.02	0.03	0.03

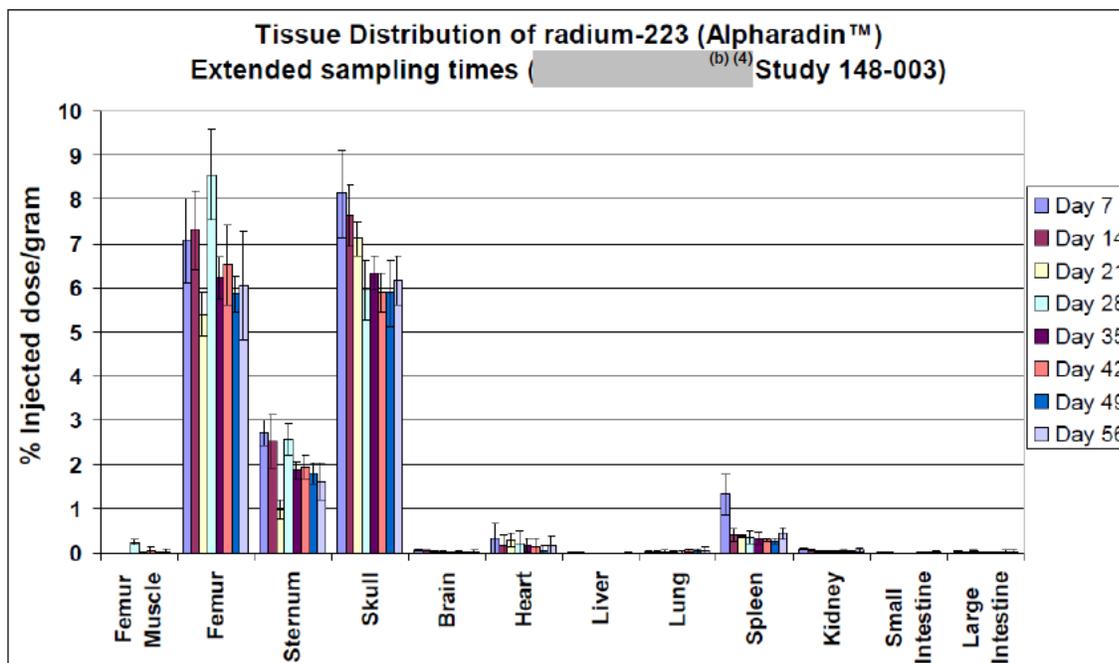
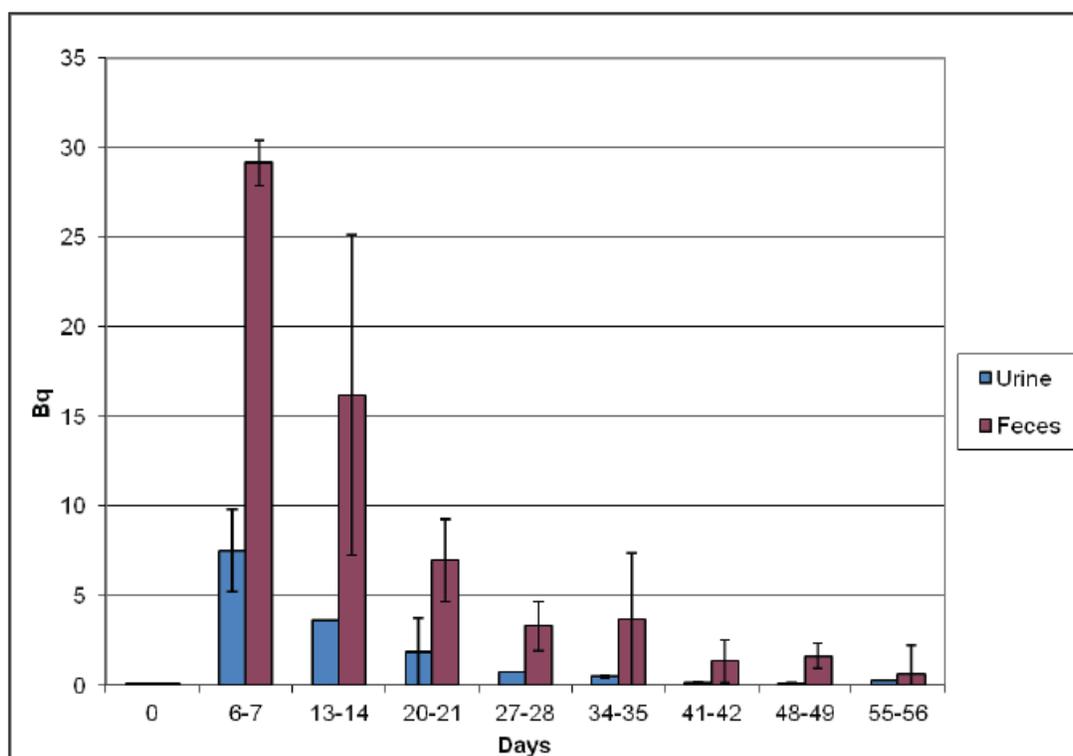


Figure 1: Biodistribution of radium-223 (Study 148-03, this study) in mice (average of 6 animals)

## Urinary and fecal excretion of radium-223 (Alpharadin™)

Days	Urine		Feces		Feces / Urine excretion ratio
	Bq*	stdev	Bq*	stdev	
0	0.06	0.14	0.15	0.05	2.27
6-7	28.59	8.83	111.03	1.25	3.88
13-14	15.66	0.00	69.61	8.97	4.45
20-21	9.10	8.99	33.82	2.28	3.72
27-28	4.36	0.00	19.15	1.40	4.40
34-35	4.23	0.82	32.91	3.70	7.77
41-42	2.12	0.66	18.34	1.20	8.66
48-49	1.65	1.15	33.60	0.71	20.33
55-56	7.69	0.35	19.54	1.60	2.54
<b>Total Bq*</b>	73.46		338.16		4.60
<b>% of Urine+Feces</b>	18		82		
<b>Injected dose [Bq/3 mice]:</b>	108822				

Urinary and fecal excretion of radium-223 (Alpharadin™), as total Bq in samples, at extended sampling times in mice (average of 6 animals  $\pm$  stdev)

**Summary:**

- 1) Radium-223 was not detected in blood samples 7 days after administration (1<sup>st</sup> time point);
- 2) Radium-223 binds to bone tissue where it remains for at least up to 56 days, the longest timepoint in this study;
- 3) There was little radioactivity detected in soft tissue with the exception of the spleen;
- 4) Small but detectable amounts of radium-223 were excreted in the urine and feces over the time course of the study.

**Pharmacokinetics and biodistribution of radium-223 (Alpharadin™) in mice.****Drug Substance Production Process II**

Note: The purpose of the study was to evaluate the bioequivalence with respect to pharmacokinetics and tissue biodistribution of radium-223 in mice where the drug substance has been manufactured according to a new production process, Production Process II. The drug substance currently used is manufactured according to Production Process III. According to the CMC reviewer, (b) (4)

**Study no:** 148-011; BC-1Tr002-2008

**Volume #, and page #:** electronic submission, page 1-37

**Conducting laboratory and location:** Algeta ASA  
P.O. Box 54 Kjelsas  
N-0411 Oslo, Norway

**Date of study initiation:** June 3, 2008

**GLP compliance:** yes

**QA report:** yes (x) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 88-8223  
batch No.: A806012  
Purity: 96.8% (radiochemical purity)  
INCB018424  
Lot No.: SS-IN2-175  
Purity: not provided

**Formulation/vehicle:** a sterile, isotonic, ready to administer solution, no other details provided

**Methods:** Mice were given a single intravenous bolus injection of 625 kBq/kg of radium-223 dichloride (Production Process II) and blood and tissues were sampled at various times after administration at time points specified below to determine plasma clearance of radium-223.

Group	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Time (h)	0	0,25	0,5	0,75	1	2	3	6	12	18	24	36	48	72	120	336
Blood	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Tissue	x		x		x			x	x		x		x	x	x	x

Tissue samples included both femurs, sternum, the skull-defined as the skeleton of the head including the mandible, brain, liver, heart, both kidneys, spleen, lung, large intestine, small intestine and the both femur muscles.

**Dosing:**

Species/strain: female BALB/CA mice

#/sex/group or time point: 3/sex

Weight: 19.0-23.6 g

Doses in administered units: 625 kBq/kg

Route, form, volume, and infusion rate: IV at dose volume of ~ 100 µL ( 20 g body weight with solution activity at 125 kBq/mL ). The exact injection volume was calculated based upon the calibration date and conversion table supplied with the test article

**Results:** The following conclusion was made by the Applicant. The data was verified by this reviewer. This reviewer agrees with Applicant's conclusion based on the data submitted.

*Summary:* Pharmacokinetics and tissue biodistribution of radium-223 where the Drug Substance was manufactured according to Production Process II appears to be identical to that found when the Drug Substance was manufactured according to Production Process 1. Radium-223 was associated with bone tissue with little soft tissue accumulation. The blood pharmacokinetic parameter of half-life was similar to that found in previous studies using radium-223 dichloride where the Drug Substance was manufactured according to Production Process I. It can be concluded that Production Process II results in a Drug Substance bioequivalent to Production Process I with respect to blood pharmacokinetics and tissue biodistribution.

**Study title: Biodistribution and acute radiotoxicity study of single dose intravenous radium-223 in normal dogs**

Study no.: MS RA1

Study report location: Electronic submission, M4.

Study report: pages 1-160

Amendment: pages 1-345

Conducting laboratory and location:

(b) (4)

Date of study initiation: August 1, 2007

GLP compliance: Yes\*

QA statement: yes ( X ) no ( )

Drug, batch #, and % purity: BAY 88-8223,  
A708003, A709002, A710005; or A710009  
>99% (radiochemical purity)

## Methods

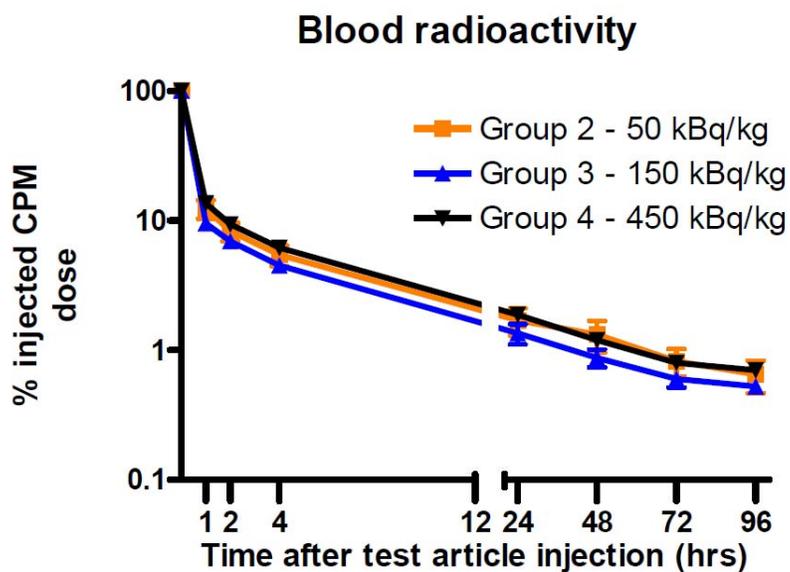
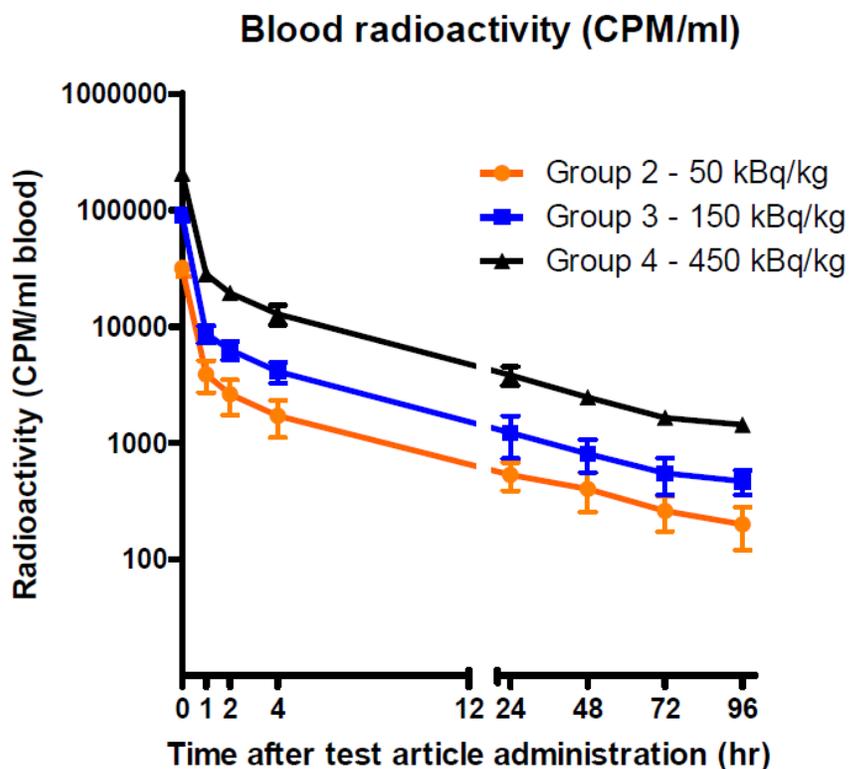
Doses:	50, 150, 450 kBq/kg*
	* the basis for dose selection was not provided
Frequency of dosing:	Single dose
Route of administration:	Iv bolus
Dose volume:	11 mL
Formulation/Vehicle:	0.9% saline
Species/Strain:	Beagle dog
Number/Sex/Group:	2/sex/group
Age:	Greater than 1 year old
Weight:	Males: 206-231 g; females: 160-187 g
Satellite groups:	none
Deviation from study protocol:	no
Radioactivity measurement	Radioactivity of blood samples were measured on a WIZARD gamma counter and recorded as counts per minute (CPM) to determine the biodistribution of Radium-223
Toxicokinetics	at 1, 2, 4, 24, 48, 72 and 96 hours after test article administration
Gamma imaging	whole body nuclear scintigraphy scans were performed at 24 and 72 hours after test article administration

**RESULTS:**Blood

Mean PK parameters  $\pm$  SD for each treatment group

	<b>Group 2 50 kBq/kg</b>	<b>Group 3 150 kBq/kg</b>	<b>Group 4 450 kBq/kg</b>
C <sub>MAX</sub> (CPM/L)	31.85 $\pm$ 4.72	90.79 $\pm$ 6.08	207.07 $\pm$ 14.36
AUC (CPM/L •HR)	72.6 $\pm$ 22.2	160.2 $\pm$ 28.1	516.5 $\pm$ 68.2
T <sub>1/2A</sub> (HR)	0.18 $\pm$ 0.03	0.10 $\pm$ 0.07	0.18 $\pm$ 0.05
T <sub>1/2B</sub> (HR)	2.23 $\pm$ 0.36	2.46 $\pm$ 1.07	3.10 $\pm$ 1.70
T <sub>1/2C</sub> (HR)	50.13 $\pm$ 11.00	56.08 $\pm$ 12.83	58.66 $\pm$ 18.41
CL (ML/HR)	330.7 $\pm$ 60.9	395.4 $\pm$ 54.2	281.0 $\pm$ 29.2
VDSS (L)	17.95 $\pm$ 5.27	23.86 $\pm$ 8.44	17.29 $\pm$ 6.70

Note: Radium-223 was rapidly eliminated from the blood with the mean half lives of all treatment groups; alpha  $\frac{1}{2}$  life = 9.27 min., beta  $\frac{1}{2}$  life = 2.60 hr., gamma  $\frac{1}{2}$  life = 54.94 hr. The mean alpha half lives of the 50, 150 and 450 kBq/kg dose groups were 10.9, 5.9 and 11.0 minutes respectively.



**Summary:**

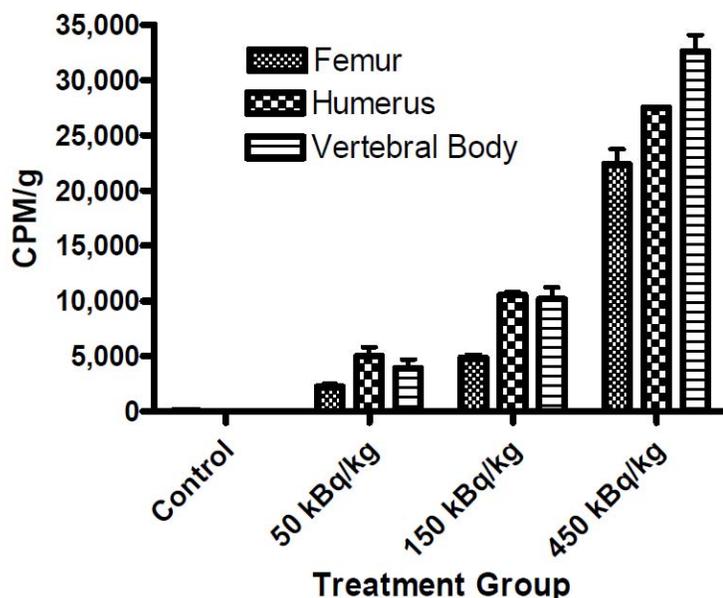
- There was a dose-dependent decrease in blood radioactivity levels during the first 24 hours after test article administration;
- Blood radioactivity was less than 1-2 % of injected dose for all treatment groups after 24 hours indicating rapid blood clearance of radium-223.
- Radium-223 was rapidly cleared from the blood volume with an alpha half life of 9.3 minutes.

Radioactivity of target organs at necropsy

Mean values of retained radium-223 radioactivity / gram of tissue (CPM/g)  
± SD in various soft tissues by treatment group

Tissue	Group 1 Saline	Group 2 50 kBq/kg	Group 3 150 kBq/kg	Group 4 450 kBq/kg
Spleen	53.73 ± 157.86	101.4 ± 87.95	168.35 ± 27.2	565.03 ± 251.48
Liver	-63.88 ± 113.39	67.45 ± 45.46	215.85 ± 33.87	501.4 ± 100.81
Kidney	25.78 ± 79.69	102.73 ± 84.96	61.65 ± 108	141.6 ± 176.73
Lung	-40.58 ± 41.97	364.65 ± 676.45	79.38 ± 73.33	85 ± 34.57
Large Intestine	32.55 ± 66.33	342.23 ± 664.32	64.4 ± 75.13	13.3 ± 114.13
Thyroid	-13.33 ± 69.2	60.78 ± 25.78	28.38 ± 14.91	164.43 ± 86.88
Pituitary	618.93 ± 1425.32	-158.3 ± 251.63	841.38 ± 941.39	723.6 ± 849.99

### Retained radioactivity in bone 30 days post treatment



**Summary:** Radium-223 activity was high in bone specimens compared to soft tissues in all treatment group dogs. There was a dose-dependent increase in the amount of radioactivity retained per gram of bone tissue with increasing dose of radium-223 dichloride. Bones with high marrow content (vertebral body and proximal humerus) had higher levels of retained radioactivity 30 days post test article administration compared to cortical bone regions. Small amounts of retained radioactivity were detected in the liver and spleen in treatment group dogs with a trend towards increased levels in the 450 kBq/kg group dogs. Levels of retained radioactivity in the other soft tissue organs (kidney, colon, lung, thyroid and pituitary) were negligible.

**Study title: Long term radiotoxicity study of repeat dose intravenous radium-223 in normal dogs**

Study no.: MS RA2

Study report location: M4.2.3.2, page 1-145

Conducting laboratory and location:  (b) (4)

Date of study initiation: March 1, 2008

GLP compliance: yes

QA statement: yes ( X ) no ( )

Drug, batch #, and % purity: Radium-223, A803004, &gt;99% (radiochemical purity)

**Key study findings:**

- Pharmacokinetic analysis showed rapid clearance of radium-223 from the blood;
- There was no significant difference in the pharmacokinetic profile between the third and sixth test article injections
- Low levels of radioactivity were detected in the femur, humerus and vertebral body 30 days after dosing.

**METHODS:**

<b>Doses:</b>	50 kBq/kg
<b>Dose Justification:</b>	The dose level for the radium-223 dichloride test article was determined based on doses used in human phase I and II trials and the results of the biodistribution and acute toxicity study in normal beagle dogs (Study MSRA1).
<b>Controls:</b>	2ml 0.9% sodium chloride
<b>Species/strain:</b>	Beagle dog
<b>Age</b>	1.4-3.5 years old
<b>Weight</b>	7-12 kg
<b>Number/sex/group</b>	2/sex (control) 4/sex (treatment group)
<b>Route, formulation, volume</b>	IV at a dose volume of 2 mL with 0.9% sodium chloride, Control vehicle: 2ml 0.9% sodium chloride
<b>Satellite groups used for toxicokinetics:</b>	none
<b>Study design:</b>	Male and female dogs were dosed once every 4 weeks for 6 treatments Note: only one dose level was used in the study

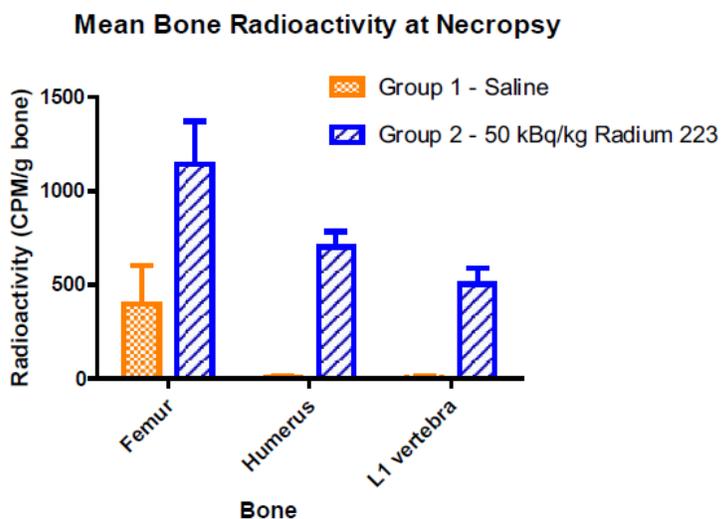
<b>Deviation from study protocol:</b>	no
<b>Radioactivity measurement</b>	Radioactivity of clinical samples were measured on a WIZARD gamma counter and recorded as counts per minute (CPM)
<b> Autoradiography</b>	using a storage phosphor screen system

**OBSERVATIONS AND TIMES:**

<u>Retained radioactivity in organs and tissues</u>	Samples (Spleen, Liver, Kidney, Small Int., Large Int., Heart, Lung, Bile, thyroid, Pituitary, prostate) were collected at the time of necropsy approximately 30 days after the final test article injection
<u>Autoradiography</u>	Conducted on sections of the L2 vertebrae, femur and humerus at the time of necropsy approximately 30 days after the final test article injection
<u>Toxicokinetics</u>	Blood: immediately before and 5, 15, 30, 45 minutes and 1, 2, 4, 6, 8 and 12 and 24, 48 and 72 hours after the third and sixth test article administrations

**RESULTS:**

Retained radioactivity in organs and tissues



*(excerpted from Applicant’s submission)*

Note: The Applicant stated that the cause of finding radioactivity in the femurs of two control group dogs is unknown

*Summary:* The soft tissues had minimal retained radioactivity with only background levels of radioactivity detected. Radioactivity was detected at low levels in the femur, humerus and vertebral body.

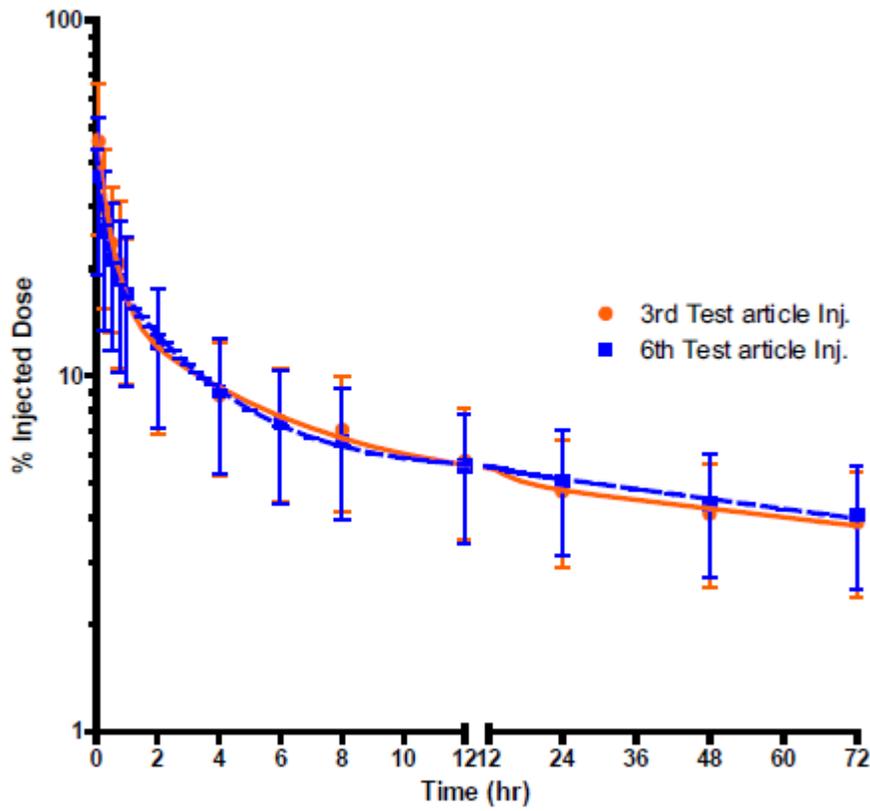
Autoradiography

The bones in the Group 2 dogs demonstrated uniform distribution of radium-223 throughout the L2 vertebral body segment and concentration in the proximal humerus and distal femur region with surface distribution cortical bone diaphyseal regions.

Toxicokinetics:

The figure and table in this section are copied from the Applicant's submission.

Graph of percent injected radioactivity in blood for the 3rd and 6th test article injections



Parameter	3 <sup>rd</sup> test article injection	6 <sup>th</sup> test article injection
Cmax	0.2818 ± 0.0751	0.3861 ± 0.3802
V1	1.6610 ± 0.6268	1.6415 ± 0.6807
K21	2.0641 ± 1.4116	2.9665 ± 1.4141
K31	0.0938 ± 0.0373	0.1038 ± 0.0199
K10	0.0531 ± 0.0233	0.0710 ± 0.0713
K12	3.0364 ± 2.5475	7.7741 ± 11.7919
K13	0.6580 ± 0.3388	1.0564 ± 1.2662
K10 ±HL	15.4386 ± 6.7564	16.4248 ± 9.7119
Alpha_HL	0.1937 ± 0.1194	0.1230 ± 0.0789
Beta_HL	2.3720 ± 1.0095	1.8456 ± 0.3865
Gamma_HL	145.823 ± 52.828	142.012 ± 40.193
AUC	5.7210 ± 1.3463	5.7147 ± 0.9086
CL	0.07905 ± 0.01953	0.07645 ± 0.02419
AUMC	1230.77 ± 708.54	1172.09 ± 516.17
MRT	200.98 ± 73.60	197.39 ± 57.91
Vss	14.858 ± 3.291	14.008 ± 1.906
V2	2.247 ± 0.573	1.996 ± 0.547
CLD2	4.276 ± 2.399	6.0823 ± 3.855
V3	10.95 ± 2.21	10.37 ± 1.81
CLD3	0.9893 ± 0.3067	1.0557 ± 0.1417

*Summary:* There was a rapid blood clearance of radium-223 with blood radioactivity less than 5 % of injected dose after 24 hours after 3<sup>rd</sup> and 6<sup>th</sup> injections. There was no difference between the 3<sup>rd</sup> and 6<sup>th</sup> test article injection for any of the PK parameters.

#### Metabolism

No studies of the metabolism of radium-223 dichloride were conducted since there are no metabolic pathways for this isotope of the element radium. The natural decay of radium-223 is known.

#### Excretion

##### Urinary and fecal clearance of radium-223 (Alpharadin™) in mice

#### Key Study Findings:

- Elimination of radium-223 occurred via the urine and feces, with 14% recovery of the administered dose 5 day after administration;
- Urinary and fecal excretion of radium-223 was found at 1 hour after injection and reaches a maximum at 6 hours after injection

- The amount of radioactivity in urine and feces decreased during 12 and 24 hours after injection; little or no radioactivity was detected 96 and 120 hours after injection;
- Excretion of radium-223 was approximately equally via the fecal and renal routes with a ratio of 1: 0.95

**Study no:** 148-002; BC-1Tr002-2007

**Volume #, and page #:** electronic submission, page 1-45

**Conducting laboratory and location:** Algeta ASA  
P.O. Box 54 Kjelsas  
N-0411 Oslo, Norway

**Date of study initiation:** November 28, 2006

**GLP compliance:** yes

**QA report:** yes (x) no ( )

**Drug, lot #, radiolabel, and % purity:** BAY 88-8223  
batch No.: not provided  
Purity: not provided

**Formulation/vehicle:** a sterile, isotonic, ready to administer solution, no other details provided

**Methods:** Female mice were administered a single intravenous dose of radium-223 dichloride on day 0. Urine and fecal pellets were collected at the time points of 0 (control), 1 h, 6 h, 12 h, 24 h, 48 h, 72 h, 96 h and 120 h. Samples were then placed in a Perkin Elmer Wizard 1480 automated Nal counter and counts (as cpm) from radium-223 (and its daughters) were measured. Corrections were applied for the weight of the samples and for the radioactive decay from the time of injection to the time of counting as well as counter efficiency and conversion from cpm to Bq.

**Dosing:**

Species/strain: female BALB/CA mice

#animals in the study: n=6\*

\*deviation from protocol- 9 mice were scheduled, however; 6 mice were dose due to insufficient test article. This deviation did not affect study conclusion.

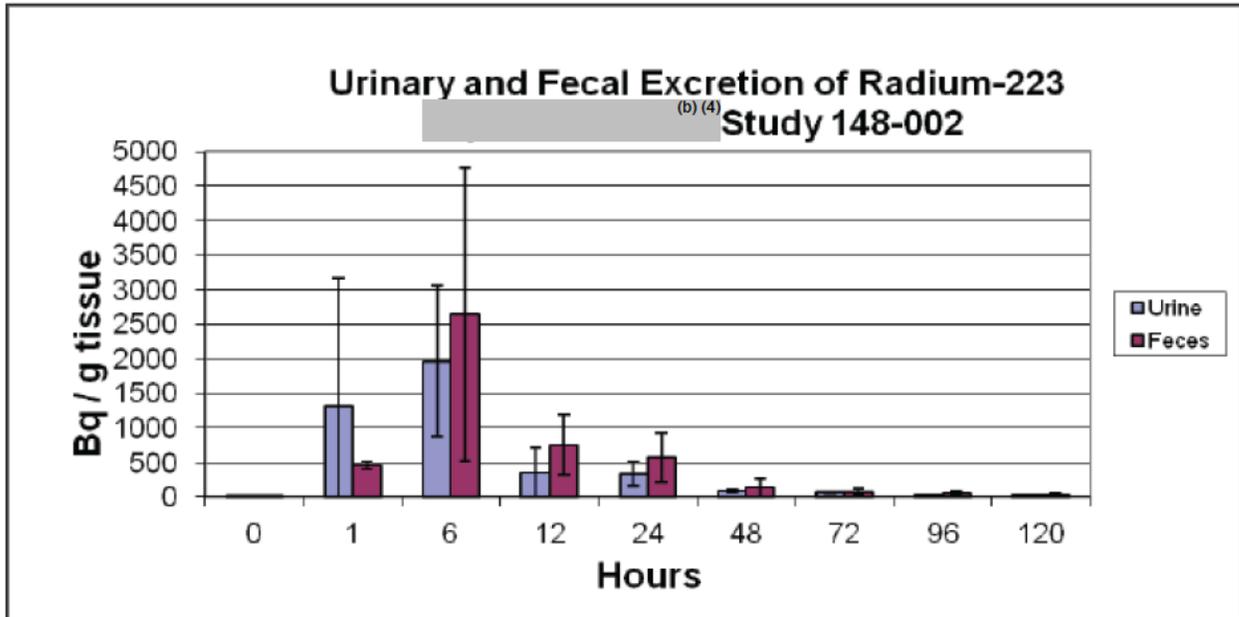
Weight: 20.7-24.0 g

Doses in administered units: 625 kBq/kg

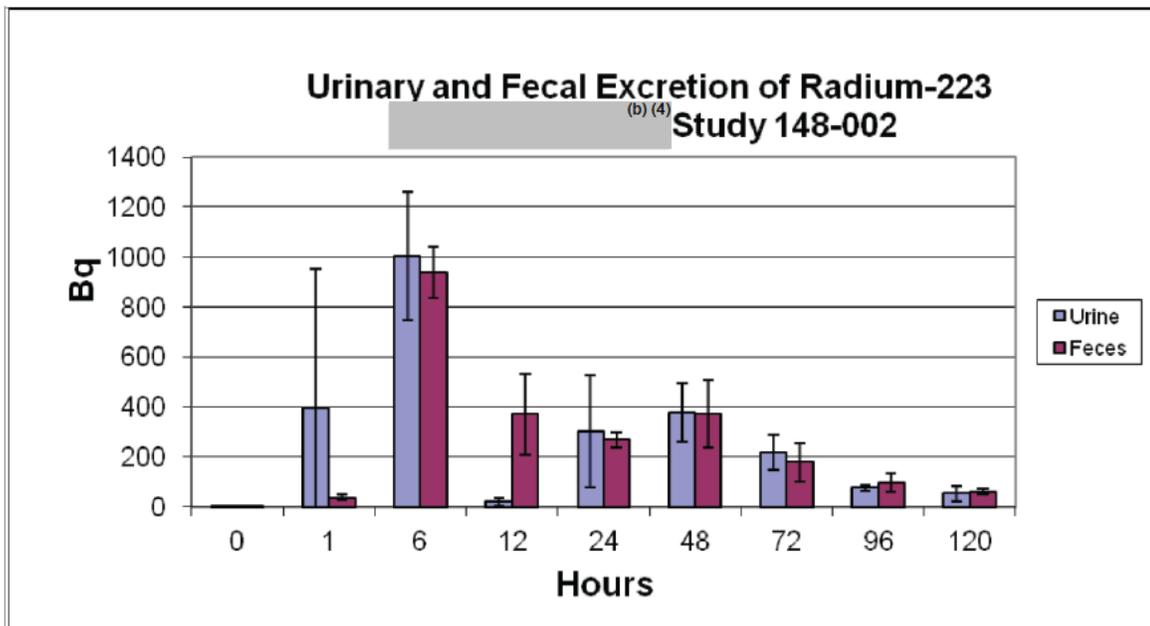
Route, form, volume, and infusion rate: IV at dose volume of ~ 25 µL ( 20 g body weight with solution activity at 500 kBq/mL ). The exact injection volume was calculated based upon the calibration date and conversion table supplied with the test article

**Results:** The following figures are excerpted from Applicant's submission.

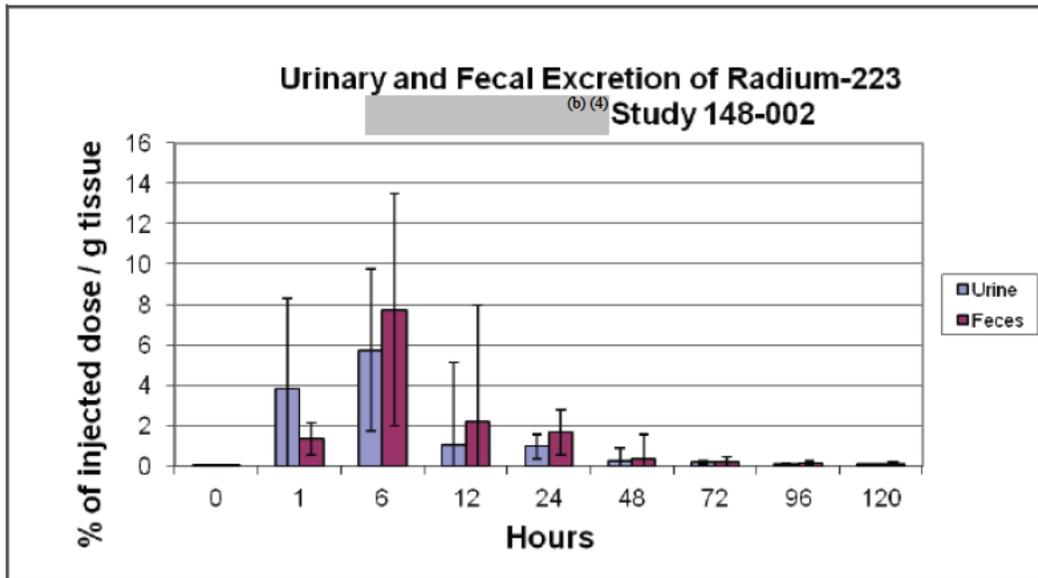
Urinary and fecal excretion of radium-223 presented as Bq per gram urine or feces (mean value of 2 cages with 3 animals each, in total 6 animals)



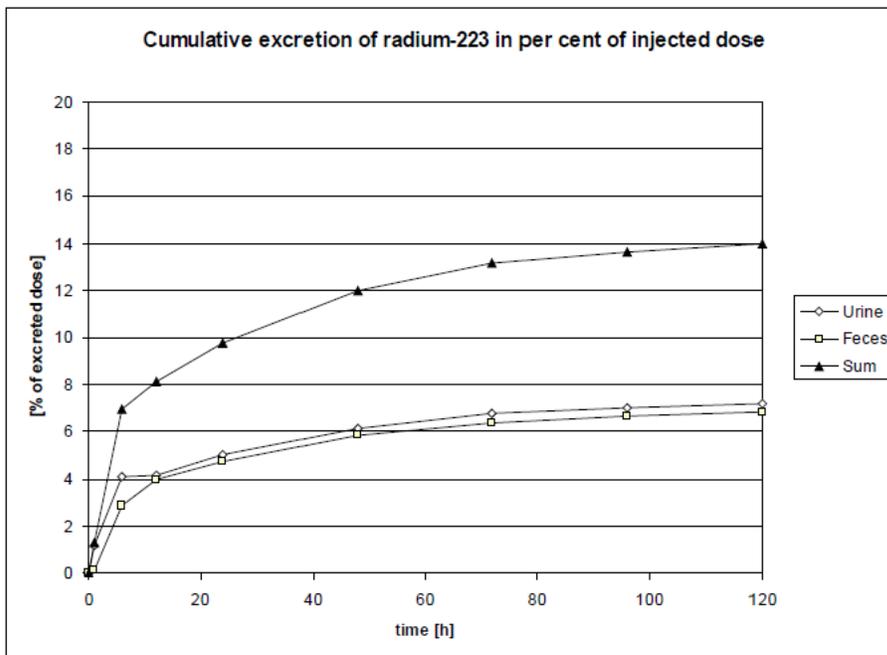
Urinary and fecal excretion of radium-223 as absolute Bq per total (average of 6 animals)



Urinary and fecal excretion of radium-223 presented as % of injected dose per g (mean values of 2 cages with 3 animals each, in total 6 animals)



Cumulative excretion of radioactivity in urine and feces (mean values of 6 animals)



**Summary:** Excretion of radium-223 is seen in the urine and feces at 1 hour after injection. Maximum urinary and fecal excretion occurs at 6 hours after injection. Little or no radioactivity was detected in the urine and feces collected 96 and 120 hours after injection. Radium-223 was about equally excreted via the biliary / fecal and renal route (7.2% urine and 6.8% feces). Five days after administration about 14% of the administered dose was recovered.

**Urinary and fecal clearance of radium-223 (Alpharadin™) in mice.  
Drug Substance Production Process II**

\* this study was performed with radium-223 dichloride which was manufactured according to a new production process, Production Process II.

**Key Study Findings:**

No relevant differences in the excretion characteristics of radium-223 dichloride in mice after iv administration were observed when the drug substance is manufactured by Process II.

**Study no:** 148-012; BC-1Tr003-2008**Volume #, and page #:** electronic submission, page 1-37**Conducting laboratory and location:** Algeta ASA  
P.O. Box 54 Kjelsas  
N-0411 Oslo, Norway**Date of study initiation:** June 3, 2008**GLP compliance:** yes**QA report:** yes (x) no ( )**Drug, lot #, radiolabel, and % purity:** BAY 88-8223  
batch No.: A806012  
Purity: not provided**Formulation/vehicle:** a sterile, isotonic, ready to administer solution, no other details provided**Methods:** Female mice were given a single dose of radium-223 dichloride on day 0 via intravenous administration. Urine and fecal pellets were collected at the time points of 0 (control), 1 h, 6 h, 12 h, 24 h, 48 h, 72 h, 96 h and 120 h. Samples were then placed in a Perkin Elmer Wizard 1480 automated NaI counter and counts (as cpm) from radium-223 (and its daughters) were measured. Corrections were applied for the weight of the samples and for the radioactive decay from the time of injection to the time of counting as well as counter efficiency and conversion from cpm to Bq.**Dosing:**

Species/strain: female BALB/CA mice

#animals in the study: n=9

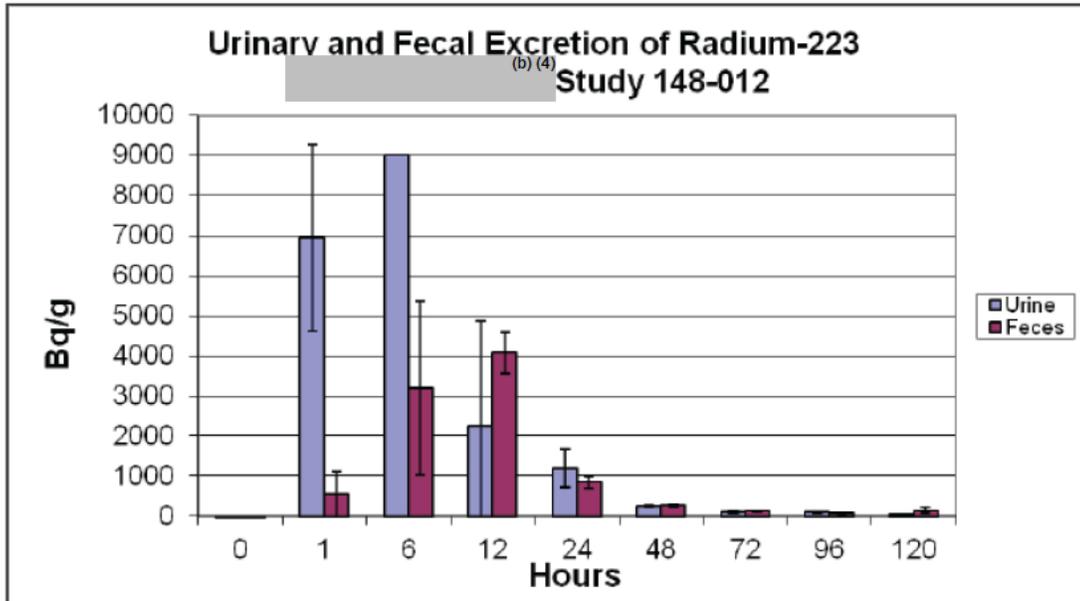
Weight: 20-22.3 g

Doses in administered units: 625 kBq/kg

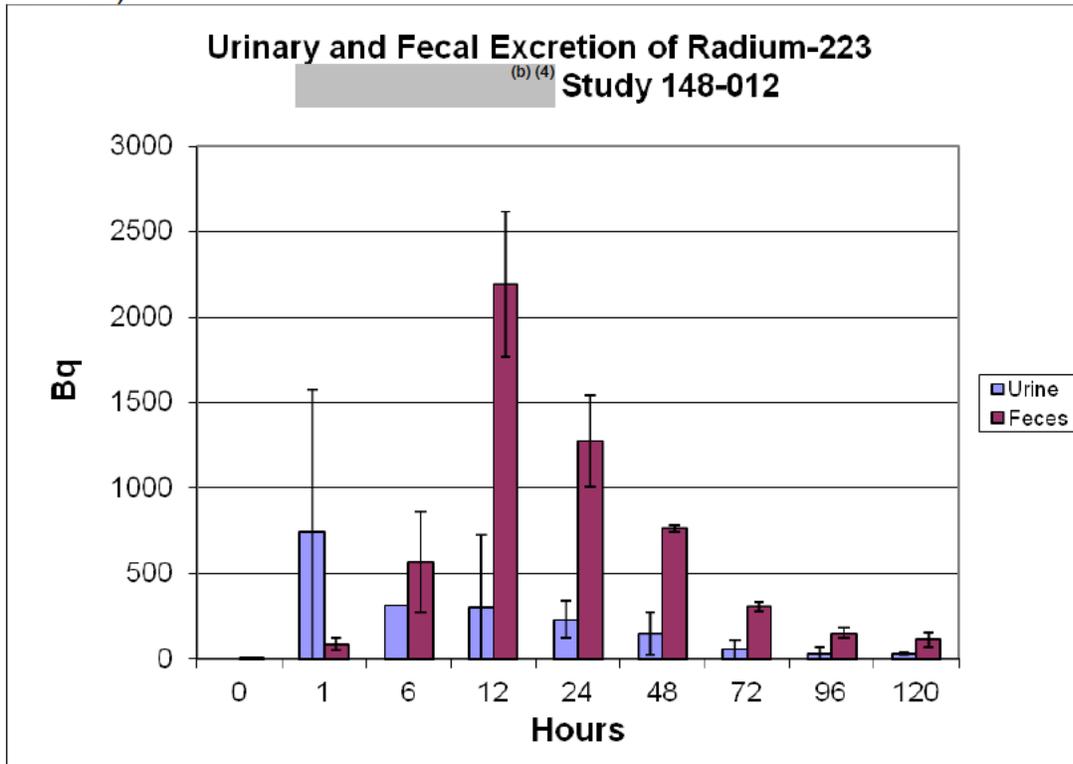
Route, form, volume, and infusion rate: IV at dose volume of 100 µL (20 g body weight with solution activity at 125 kBq/mL). The exact injection volume was calculated based upon the calibration date and conversion table supplied with the test article

**Results:** The following figures are excerpted from Applicant's submission.

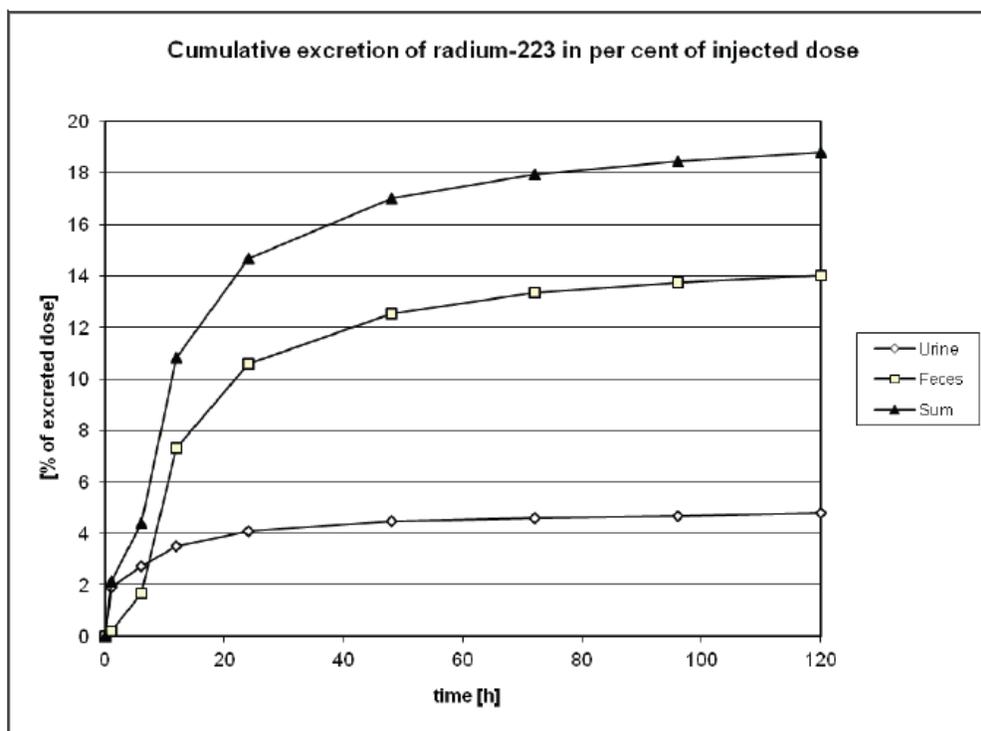
Urinary and fecal excretion of radium-223 presented as Bq per gram urine or feces (mean value (mean value of 9 animals))



Urinary and fecal excretion of radium-223 as absolute Bq per total (average of 9 animals)



Cumulative excretion of radioactivity in urine and feces (mean values of 9 animals)



**Summary:** Excretion of radium-223 is seen in the urine and feces at 1 hour after injection. Maximum urinary excretion occurred at 6 hours after injection while maximum fecal excretion occurred between 6 and 12 hours after injection. Radium-223 was excreted via the biliary / fecal and renal route (in total 4.8% in urine and 14.0% in feces). The urine to feces cumulative total ratio was 1:2.9. Five days after administration about 19% of the administered dose was recovered.

**Note:** In this study there was a greater amount of radioactivity excreted in the feces than in the urine (1:2.9) compared to the study results from Study 148-002. The urine to fecal excretion ratio from Study 148-002 (Report BC-1Tr002-2007) was about equal with a ration of 1:0.95. The recovery rate of the injected dose was 19% in this study (148-012) and 14% in study 148-002 (Production Process I). However, with large standard deviations, it cannot be concluded that excretion is different when the drug substance is manufactured either by Production Process I or Process II.

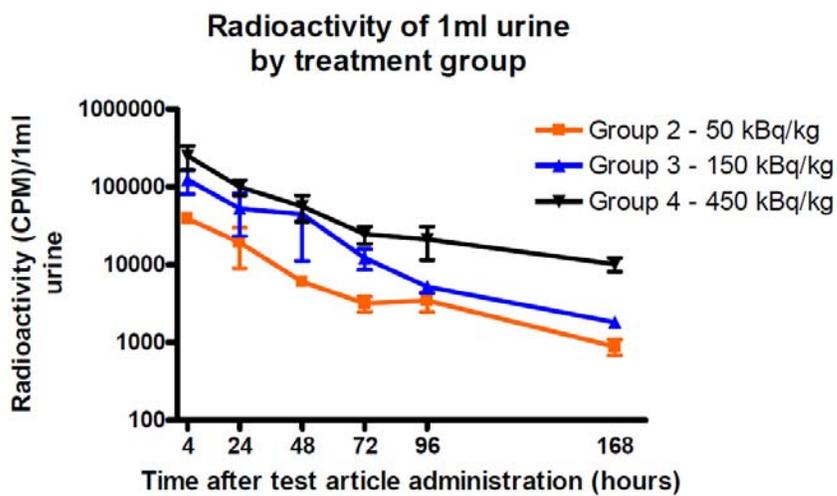
**Study title: Biodistribution and acute radiotoxicity study of single dose intravenous radium-223 in normal dogs**

Study no.: MS RA1  
 Study report location: Electronic submission, M4.  
 Study report: pages 1-160  
 Amendment: pages 1-345  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: August 1, 2007  
 GLP compliance: Yes\*  
 QA statement: yes ( X ) no ( )  
 Drug, batch #, and % purity: BAY 88-8223,  
 A708003, A709002, A710005; or A710009  
 >99% (radiochemical purity)

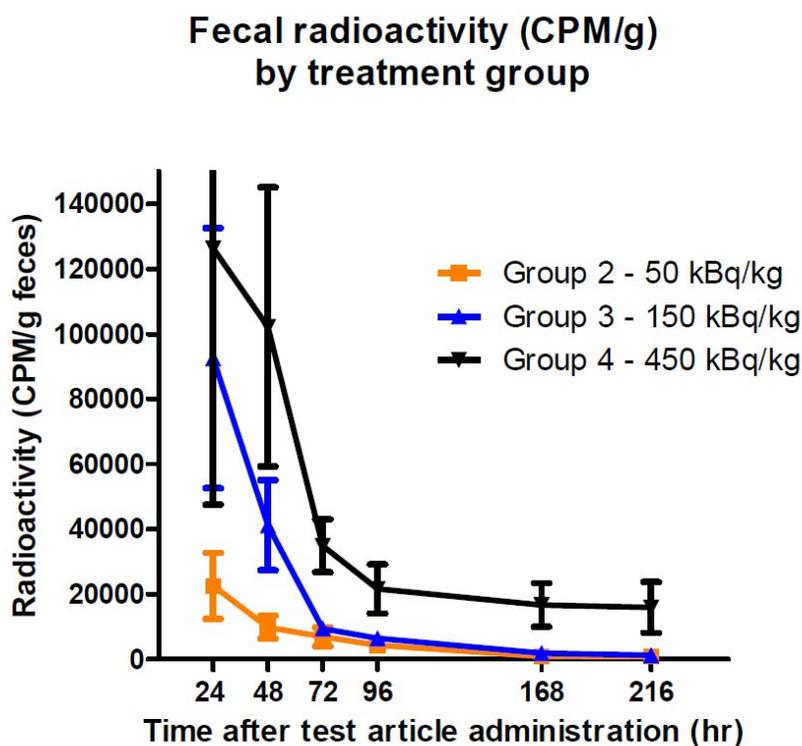
**Methods**

Doses: 50, 150, 450 kBq/kg\*  
 \* the basis for dose selection was not provided  
 Frequency of dosing: Single dose  
 Route of administration: iv bolus  
 Dose volume: 11 mL  
 Formulation/Vehicle: 0.9% saline  
 Species/Strain: Beagle dog  
 Number/Sex/Group: 2/sex/group  
 Age: Greater than 1 year old  
 Weight: Males: 206-231 g; females: 160-187 g  
 Satellite groups: none  
 Deviation from study protocol: no  
 Radioactivity measurement: Radioactivity of clinical samples were measured on a WIZARD gamma counter and recorded as counts per minute (CPM) to determine the biodistribution of Radium-223 in urine, feces and bile  
 Urine: at baseline, 4, 24, 48, 72, 96 hours and 7 days post test article injection  
 Feces: at baseline, 24, 48, 72 and 96 hours and 7 and 9 days post test article injection  
 Bile: at 4, 24 and 72 hours, and at the time of necropsy (Day 30)

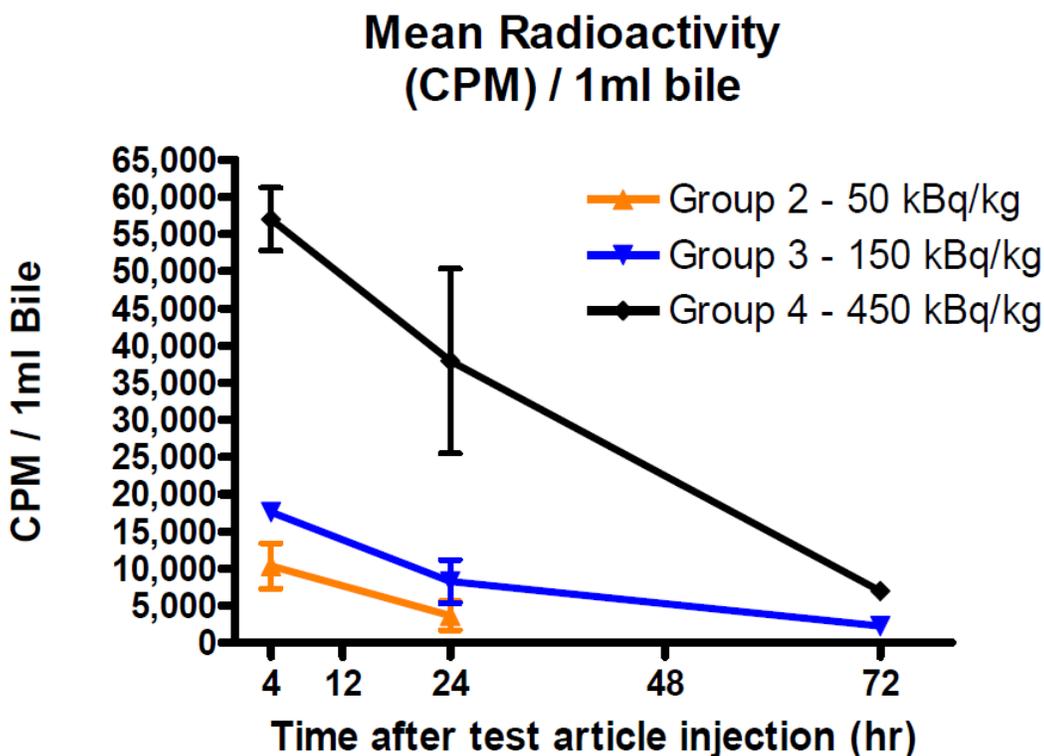
**RESULTS:** The following figures are copied from the Applicant's submission.  
Urine



Feces



Bile



*Summary:* Elimination of Radium-223 occurred in dose dependent manner by both the urinary and gastrointestinal routes.

**Study title: Long term radiotoxicity study of repeat dose intravenous radium-223 in normal dogs**

Study no.: MS RA2

Study report location: M4.2.3.2, page 1-145

Conducting laboratory and location: (b) (4)

Date of study initiation: March 1, 2008

GLP compliance: yes

QA statement: yes ( X ) no ( )

Drug, batch #, and % purity: Radium-223, A803004, >99% (radiochemical purity)

**Key study findings:**

- Excretion via the urinary and gastrointestinal systems;
- There was no significant difference in the pharmacokinetic profile between the third and sixth test article injections.

**METHODS:**

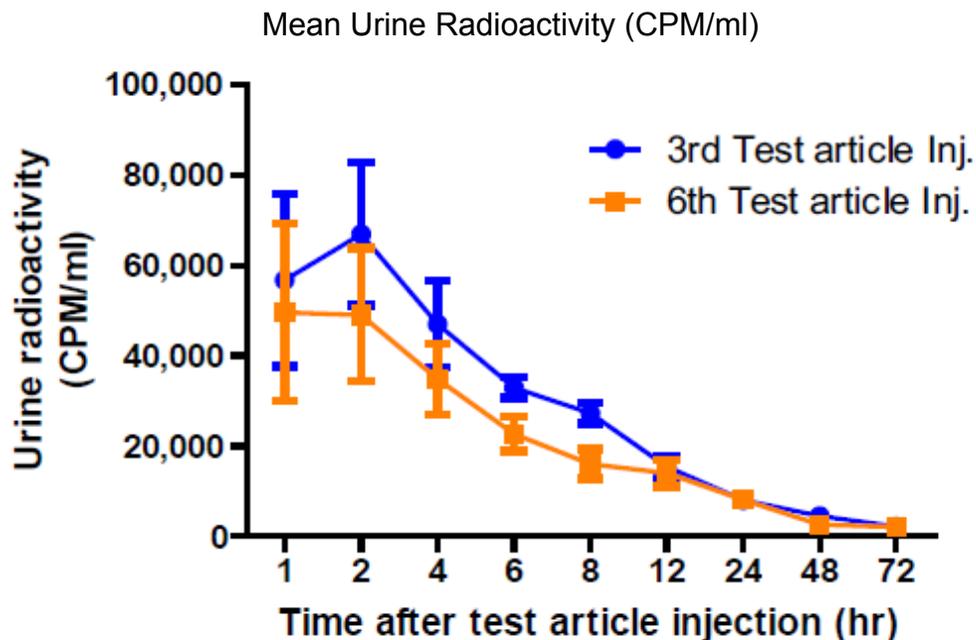
<b>Doses:</b>	50 kBq/kg
<b>Dose Justification:</b>	The dose level for the radium-223 dichloride test article was determined based on doses used in human phase I and II trials and the results of the biodistribution and acute toxicity study in normal beagle dogs (Study MSRA1).
<b>Controls:</b>	2ml 0.9% sodium chloride
<b>Species/strain:</b>	Beagle dog
<b>Age</b>	1.4-3.5 years old
<b>Weight</b>	7-12 kg
<b>Number/sex/group</b>	2/sex (control) 4/sex (treatment group)
<b>Route, formulation, volume</b>	IV at a dose volume of 2 mL with 0.9% sodium chloride, Control vehicle: 2ml 0.9% sodium chloride
<b>Satellite groups used for toxicokinetics:</b>	none
<b>Study design:</b>	Male and female dogs were dosed once every 4 weeks for 6 treatments
<b>Deviation from study protocol:</b>	no
<b>Radioactivity measurement</b>	Radioactivity of clinical samples were measured on a WIZARD gamma counter and recorded as counts per minute (CPM)

**OBSERVATIONS AND TIMES:**

<u>Toxicokinetics</u>	Urine: before and 1, 2, 4, 8, 12, 24, 48 and 72 hours after the third and sixth test article administrations Feces: 12, 24, 48, 72, 96 and 168 hours after the third and sixth test article administrations
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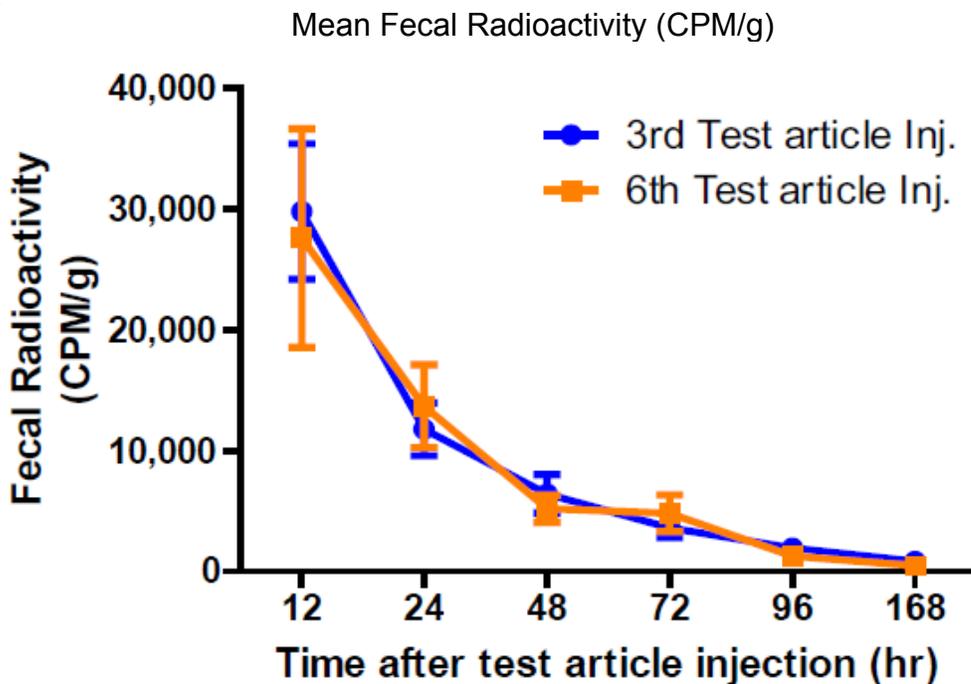
**RESULTS:**

Urine



*Summary:* Radium-223 was excreted via the urinary routes. There were no significant differences in radioactivity in urine between the two time points (3<sup>rd</sup> and 6<sup>th</sup> dose).

Feces



*(excerpted from Applicant's submission)*

**Summary:** Radium-223 was excreted via gastrointestinal routes. There were no significant differences in radioactivity in feces between the two time points (3<sup>rd</sup> and 6<sup>th</sup> dose).

Pharmacokinetic drug interactions

**Pharmacokinetics and biodistribution of radium-223 (Alpharadin™) during treatment with zoledronic acid (Zometa) in mice**

**Key Study Finding:**

Blood clearance and the tissue biodistribution of radium-223 dichloride given after zoledronic acid did not appear to differ from that of radium -223 dichloride given alone.

**Study no:** 148-013; BC-1Tr004-2008

**Volume #, and page #:** electronic submission, page 1-57

**Conducting laboratory and location:** Aigate ASA  
P.O. Box 54 Kjelsas  
N-0411 Oslo, Norway

**Date of study initiation:** May 6, 2008

**GLP compliance:** yes

**QA report:** yes (x ) no ( )

**Drug, lot #, radiolabel, and % purity:** INCB018424  
Lot SS-IN2-175  
Purity: not provided  
Zometa  
Batch#: SO 129  
Purity: not provided

**Formulation/vehicle:**

radium-223 dichloride -125 kBq/mL, a sterile, isotonic, ready to administer solution, no other details provided

Zometa-20 µ/mL in sterile salt water

**Methods:** Female mice received a single dose on day 0 via intravenous administration of salt solution or intravenous administration of Zometa. Two hours after administration of Zometa or the vehicle, radium-223 dichloride was administered. Blood samples were withdrawn at all time points specified in the following table. Subsequent blood sample analysis was used to determine blood clearance of radium-223. Tissue samples (femur bones, sternum, skull (de fined as the skeleton of the head including the mandible), brain, liver, heart, kidney, spleen, lung, large intestine, small intestine and the femur muscle) were taken at time points shown in the following table. Subsequent tissue sample analysis was used to determine the biodistribution of radium-223.

Group	0	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
Group		B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15
Hour	NA	0,25	0,5	0,75	1	2	3	6	12	18	24	36	48	72	120	336
Blood	x	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x
Tissue	x		x		x			x	x		x		x	x	x	x

**Dosing:**

Species/strain: female BALB/CA mice

#animals/time points: 3

Weight: 19.9 -24.1 g

Doses in administered units: 625 kBq/kg

Route, form, volume, and infusion rate: IV, The exact injection volume for radium-223 dichloride was calculated based upon the calibration date and conversion table supplied with the test article.

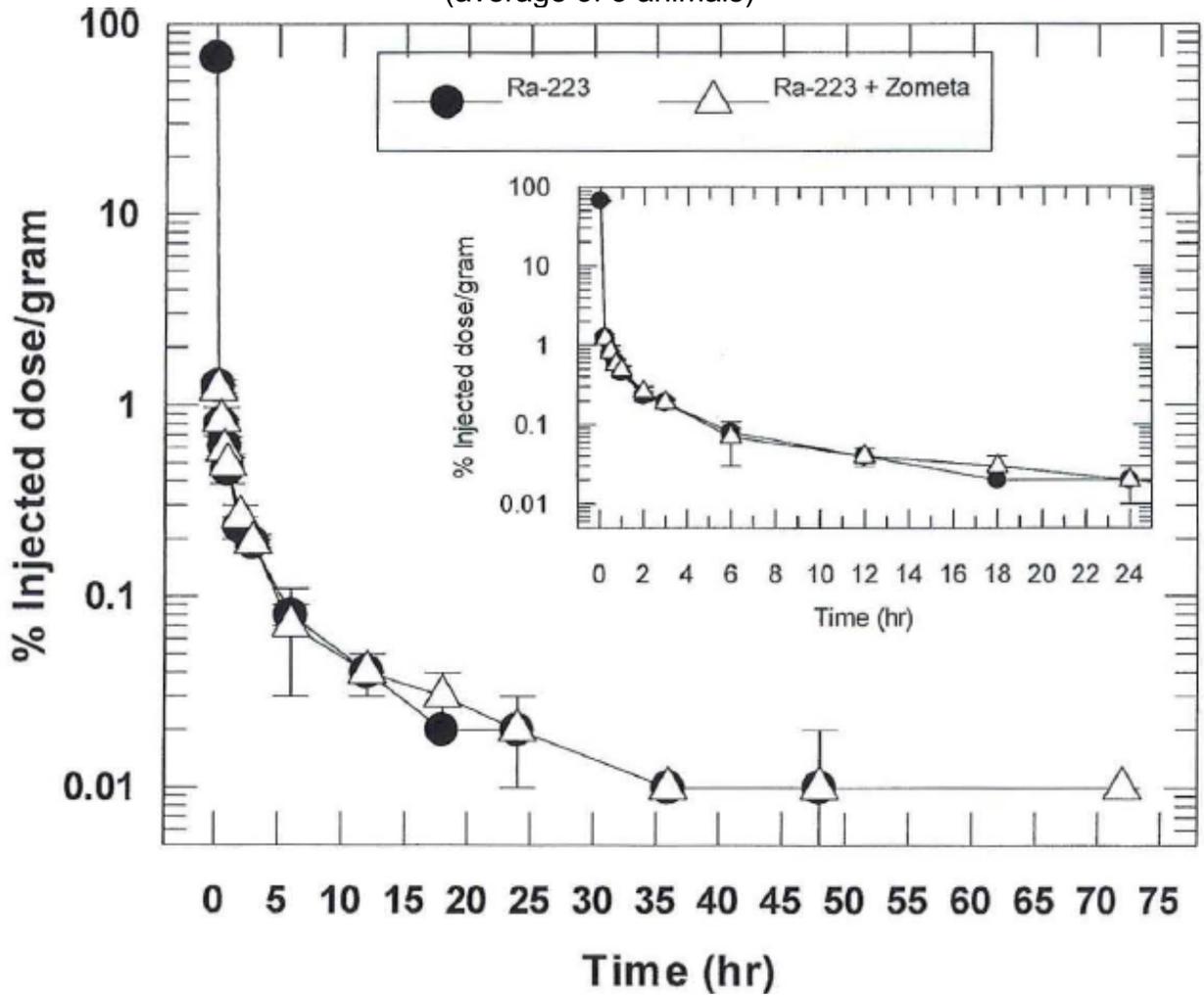
Injection volume for Zometa is 5 ml/mg.

**Results:** The following tables and figure are copied from the Applicant's submission.

% Injected dose/gram in blood (average of 3 animals)

<b>Blood</b>	<b>Ra-223</b>		<b>Ra-223 + Zometa</b>	
<b>Hours</b>	<b>% Inj/g</b>	<b>SD</b>	<b>% Inj/g</b>	<b>SD</b>
<b>0.00</b>	66.6		66.6	
<b>0.25</b>	1.26	0.10	1.21	0.05
<b>0.50</b>	0.80	0.04	0.83	0.14
<b>0.75</b>	0.61	0.07	0.58	0.06
<b>1</b>	0.46	0.07	0.49	0.06
<b>2</b>	0.23	0.03	0.26	0.04
<b>3</b>	0.19	0.01	0.19	0.02
<b>6</b>	0.08	0.01	0.07	0.04
<b>12</b>	0.04	0.00	0.04	0.01
<b>18</b>	0.02	0.00	0.03	0.01
<b>24</b>	0.02	0.01	0.02	0.01
<b>36</b>	0.01	0.00	0.01	0.00
<b>48</b>	0.01	0.01	0.01	0.00
<b>72</b>	0.00	0.01	0.01	0.00
<b>120</b>	0.00	0.00	0.00	0.01
<b>336</b>	0.00	0.01	0.00	0.00

Pharmacokinetics of radium-223 alone or 2 h after an injection of zoledronic acid in mice (average of 3 animals)



## Biodistribution of radium-223-radioactivity in tissue and organs

	Femur		Sternum		Skull		Brain		Heart		Liver	
	Ra-223	Ra-223+Z	Ra-223	Ra-223+Z	Ra-223	Ra-223+Z	Ra-223	Ra-223+Z	Ra-223	Ra-223+Z	Ra-223	Ra-223+Z
30 min	8.05±1.97	7.56±1.90	6.17±1.01	5.85±1.77	8.52±1.24	8.74±1.97	0.13±0.02	0.12±0.03	4.62±6.21	1.55±0.84	1.07±0.06	1.06±0.30
1 h	11.68±2.54	9.57±2.65	5.95±1.53	6.59±1.75	9.90±1.91	10.26±0.69	0.13±0.02	0.13±0.01	2.37±1.22	0.54±0.02	0.79±0.18	1.03±0.13
6 h	11.91±0.58	7.70±4.52	6.45±0.56	4.95±3.40	9.75±0.32	7.95±4.53	0.11±0.01	0.09±0.05	0.60±0.87	0.11±0.06	0.20±0.02	0.26±0.15
12 h	9.56±0.43	12.50±0.28	5.53±0.70	6.80±0.48	9.54±0.52	10.38±0.76	0.09±0.01	0.12±0.01	0.70±0.60	1.89±0.91	0.11±0.01	0.14±0.03
24 h	7.73±1.12	7.47±1.14	4.45±1.03	3.82±0.43	9.31±1.46	9.06±1.04	0.12±0.02	0.12±0.01	0.34±0.30	1.56±1.58	0.13±0.04	0.11±0.09
48 h	9.52±1.91	8.02±2.36	4.28±0.38	3.23±0.63	9.63±1.14	9.46±2.29	0.13±0.03	0.12±0.01	0.01±0.00	0.04±0.00	0.05±0.03	0.10±0.02
72 h	11.22±0.75	10.60±0.96	4.13±0.77	4.68±0.07	9.16±1.52	9.16±1.05	0.12±0.03	0.12±0.02	0.02±0.01	0.09±0.16	0.04±0.01	0.07±0.03
120 h	7.17±1.97	7.39±0.81	3.09±1.94	2.51±0.34	8.52±1.99	8.16±0.75	0.09±0.03	0.09±0.02	0.01±0.02	0.00±0.01	0.02±0.00	0.03±0.01
336 h	8.69±1.92	9.39±1.29	2.96±0.26	2.99±0.92	8.23±0.14	6.23±0.92	0.07±0.01	0.05±0.01	0.22±0.22	0.00±0.01	0.01±0.01	0.00±0.00

	Lung		Spleen		Kidney		Small Intestine		Large Intestine		Femur Muscle	
	Ra-223	Ra-223+Z	Ra-223	Ra-223+Z	Ra-223	Ra-223+Z	Ra-223	Ra-223+Z	Ra-223	Ra-223+Z	Ra-223	Ra-223+Z
30 min	2.17±0.53	3.17±0.83	20.39±2.38	21.43±6.41	33.52±3.42	31.10±5.96	5.63±0.34	5.42±0.64	10.33±1.22	10.96±6.04	0.88±0.07	0.82±0.17
1 h	2.25±0.25	1.77±0.55	19.55±3.40	22.24±1.13	21.07±2.71	20.32±2.24	3.31±1.23	2.74±0.73	12.35±2.88	13.54±1.67	0.68±0.20	0.54±0.07
6 h	0.39±0.22	0.59±0.31	8.26±2.44	10.78±6.56	3.08±0.47	2.66±1.39	0.51±0.26	0.31±0.15	1.40±0.76	0.93±0.47	0.06±0.02	0.11±0.09
12 h	0.15±0.04	0.21±0.07	6.03±1.65	7.40±0.86	1.70±0.19	2.01±0.16	0.14±0.02	0.19±0.03	0.35±0.03	0.51±0.04	0.07±0.09	0.16±0.12
24 h	0.17±0.02	0.14±0.02	7.87±1.34	5.11±4.41	0.85±0.10	0.82±0.18	0.09±0.02	0.10±0.02	0.34±0.02	0.25±0.01	0.03±0.02	0.02±0.01
48 h	0.09±0.08	0.14±0.06	5.56±2.62	5.64±0.68	0.15±0.15	0.45±0.01	0.05±0.02	0.07±0.02	0.12±0.04	0.18±0.06	0.06±0.07	0.07±0.09
72 h	0.06±0.03	0.04±0.03	5.64±1.95	6.46±2.25	0.21±0.02	0.23±0.05	0.03±0.00	0.03±0.01	0.10±0.06	0.07±0.04	0.03±0.06	0.05±0.08
120 h	0.04±0.01	0.03±0.05	3.05±0.96	4.25±1.96	0.10±0.02	0.12±0.01	0.02±0.00	0.03±0.01	0.06±0.01	0.04±0.01	0.05±0.07	0.03±0.05
336 h	0.04±0.03	0.01±0.01	1.16±0.19	0.75±0.16	0.06±0.02	0.03±0.01	0.02±0.01	0.01±0.00	0.04±0.01	0.05±0.02	0.00±0.01	0.01±0.02

**Summary:** There was no statistically significant difference in blood pharmacokinetics or tissue distribution between radium-223 given alone or 2 hours after an iv injection of zoledronic acid.

## Discussion and Conclusions

The pharmacokinetics of radium-223 dichloride was investigated *in vivo* in mice and dogs.

Radium-223 dichloride is administered intravenously; therefore, 100% is bioavailable.

Nonclinical pharmacokinetic studies in mice and dogs show that radium-223 is rapidly eliminated from the blood circulation. Less than 0.1% or 2% of the radioactive dose were present in blood of mice and dogs already 24 h after administration, respectively. Elimination from blood appeared to be biphasic in mice. In mice, initial half-life was about 0.1 hour while terminal half-life was about 13 hours. In dogs, following single dose injection of radium-223 dichloride the initial and intermediate half-lives were short, approximately 10 minutes and the last measured half-life was about 50 hours. The observed pharmacokinetics was comparable in dogs following single and repeated injection of radium-223 dichloride. In dogs, where three different doses were investigated, distribution and elimination from blood appeared to be independent of dose. The biodistribution studies in mice and dogs showed that radium-223 is primarily distributed to bone tissue with much higher affinity relative to soft tissues. Recalculations of organ concentration data from these studies revealed that in total about 70% of the administered dose was already bound to bone tissue (<1% in soft tissue) at 12 hours after administration. The results from mouse studies suggested that once bound to bone (femur, sternum, skull) radium-223 remained within the bone at least up to approximately 5 half-lives (entire duration of the study) and probably for the entire decay period. Little radioactivity was detected in soft tissue with the exception of the spleen in mice. This accumulation in spleen is unclear. A very limited uptake in spleen was seen in dogs (the Applicant also stated that uptake in spleen was not seen

in rats). Initially, a high level of radioactivity was found in the kidney in mice, most likely because of excretion in the urine, but the level was rapidly reduced. Also, high concentrations of radioactivity were found within the first hour after administration in the large intestine of mice, potentially due to excretion of radium isotopes directly (via bile) into the intestinal tract and contained in the fecal contents. In both the single- and repeat-dose dog biodistribution and toxicity studies, approximately 30 days after the last dose, very low radioactivity in the intestine tissue was detected, suggesting that radioactivity was mainly excreted via the fecal route without uptake in intestinal tissue.

The decay of radium-223 is known; no metabolic pathways exist for the element isotope radium-223.

Excretion studies after single dose intravenous administration of radium-223 dichloride were conducted in mice. In addition, the excretion of radium-223 was also performed in dogs with single and repeated dose administration. In both mice and dogs, excretion of radium-223 occurred by gastrointestinal and renal route. Maximum urinary and fecal excretion in mice was seen until 6 hours and in dogs at 2 and 12 hours, the first sampling times for these parameters, respectively. Small detectable amounts of radium-223 were excreted in the urine and feces in mice throughout a period of 56 days after a single dose of radium-223 dichloride administration. The cumulative urine to feces ratio in mice was 1:3 five days after administration with Production Process II. In two studies in mice, five days following administration of radium-223 dichloride an average 16% (14-18%) of the administered dose was recovered. The low recovery rate can be explained by the retention of radium-223 in bone tissue. The corresponding distribution studies in mice reveal retention of about 70% of the administered radioactivity. Thus, it can be concluded that in total about 30% of the administered dose is excreted mainly by the gastrointestinal and renal route in mice. Also, in dogs a time dependent decrease of the radioactivity concentration in urine and feces was observed. No treatment-related toxicities were observed in the kidney although radium-223 dichloride was detected in the kidney and it was a route of excretion.

Since bisphosphonates, agents that also target bone, may be used by CRPC patients with bone metastases, interaction with radium-223 dichloride was studied. The pharmacokinetics or biodistribution of radium-223 was not affected by administration of a bisphosphonate (zoledronic acid) 2 hours earlier.

Tables and figures to include comparative TK summary

The following tables and summary information are from the Applicant. Some of the studies summarized (e.g. data from rat, human) were not reviewed by this reviewer.

**Pharmacokinetics: Basic PK after a single dose, rodent**

Report no.:		R-8646	R-8649	
Study no.:		(b) (4) 148-001	(b) (4) 148-011	
Location:		Module 4.2.2.3	Module 4.2.2.3	
Species / strain:		Mouse / BALB/CA	Mouse / BALB/CA	
Admin. compound:		Radium-223 dichloride	Radium-223 dichloride	
Analyte:		Radium-223	Radium-223	
Dose [kBq/kg]		625	625	
Route		i.v.	i.v.	
Gender / No. of animals		Female / 3	Female / 3	
Feeding condition		Ad libitum	Ad libitum	
Formulation		100% isotonic solution	100% isotonic solution	
Duration [h]		336	336	
Assay		Gamma-counting	Gamma-counting	
Time [h]	% injected dose / gram in blood		% injected dose / gram in blood	
	Mean	S.D.	Mean	S.D.
0	66.7*		66.7*	
0.25	1.44	0.22	1.62	0.05
0.5	0.61	0.05	0.89	0.03
0.75	0.61	0.06	0.71	0.1
1	0.46	0.12	0.54	0.05
2	0.25	0.10	0.33	0.0
3	0.19	0.01	0.23	0.0
6	0.10	0.06	0.11	0.0
12	0.04	0.00	0.05	0.01
18	0.02	0.01	0.03	0.0
24	0.03	0.01	0.03	0.01
36	0.02	0.00	0.02	0.0
48	0.01	0.00	0.02	0.01
72	0.01	0.00	0.01	0.0
120	0.00	0.00	0.01	0.0
336	0.00	0.00	0.00	0.0

**Pharmacokinetics: Absorption / basic PK after a single dose, non-rodent**

Report no.:	R-8668 / R-8668A		R-8668 / R-8668A		R-8668 / R-8668A	
Study no.:	MS RA1		MS RA1		MS RA1	
Location:	Module 4.2.3.1		Module 4.2.3.1		Module 4.2.3.1	
Species / strain:	Dog / Beagle		Dog / Beagle		Dog / Beagle	
Admin. compound:	Radium-223 dichloride		Radium-223 dichloride		Radium-223 dichloride	
Analyte:	Radium-223		Radium-223		Radium-223	
Dose [kBq/kg]	50		150		450	
Route	i.v.		i.v.		i.v.	
Gender / No. of animals	m + f / 2 + 2		m + f / 2 + 2		m + f / 2 + 2	
Feeding condition	Ad libitum		Ad libitum		Ad libitum	
Formulation	100% isotonic solution		100% isotonic solution		100% isotonic solution	
Duration [h]	96		96		96	
Assay	Gamma-counting		Gamma-counting		Gamma-counting	
Time [h]	Arithmetic		Arithmetic		Arithmetic	
	Mean [% inj. dose]	S.D. [% inj. dose]	Mean [% inj. dose]	S.D. [% inj. dose]	Mean [% inj. dose]	S.D. [% inj. dose]
0	100	0.00	100	0.00	100	0.00
1	13.0	4.31	9.74	1.40	14.1	1.52
2	8.73	2.92	7.12	1.25	9.70	0.503
4	5.74	2.10	4.62	0.800	6.39	1.01
24	1.79	0.591	1.38	0.554	1.92	0.367
48	1.39	0.700	0.913	0.282	1.24	0.111
72	0.892	0.407	0.620	0.215	0.831	0.108
96	0.690	0.372	0.532	0.136	0.720	0.076

**Pharmacokinetics: Absorption / basic PK after a single dose, non-rodent**

Report no.:		R-8668 / R-8668A		R-8668 / R-8668A		R-8668 / R-8668A	
Study no.:		MS RA1		MS RA1		MS RA1	
Location:		Module 4.2.3.1		Module 4.2.3.1		Module 4.2.3.1	
Species / strain:		Dog / Beagle		Dog / Beagle		Dog / Beagle	
Admin. compound:		Radium-223 dichloride		Radium-223 dichloride		Radium-223 dichloride	
Analyte:		Radium-223		Radium-223		Radium-223	
Dose [kBq/kg]		50		150		450	
Route		i.v.		i.v.		i.v.	
Gender / No. of animals		m + f / 2 + 2		m + f / 2 + 2		m + f / 2 + 2	
Feeding condition		Ad libitum		Ad libitum		Ad libitum	
Formulation		100% isotonic solution		100% isotonic solution		100% isotonic solution	
Duration [h]		96		96		96	
Assay		Gamma-counting		Gamma-counting		Gamma-counting	
		Arithmetic		Arithmetic		Arithmetic	
Parameter	Unit	Mean	S.D.	Mean	S.D.	Mean	S.D.
C <sub>max</sub>	[CPM/mL]	30238	3577	88741	5282	199383	12113
V <sub>1</sub>	[mL]	799	310	720	197	727	97.869
t <sub>1/2α</sub>	[h]	0.18	0.03	0.09	0.07	0.17	0.05
t <sub>1/2β</sub>	[h]	2.20	0.33	2.52	1.34	2.40	0.75
t <sub>1/2γ</sub>	[h]	51.40	15.33	55.15	16.54	49.71	17.36
AUC	[CPM/mL·h]	73544	23250	159318	26555	492092	45895
CL	[mL/h]	326	58	398	61	294	23
AUMC	[CPM/mL·h·h]	4392537	2786308	9062093	1494835	26523094	10012619
MRT	[h]	57	20	58	15	54	19
V <sub>ss</sub>	[mL]	18367	6116	23333	7879	16047	7189

**Pharmacokinetics: Absorption / basic PK after repeated dose (after 3<sup>rd</sup> and 6<sup>th</sup> injection) , non-rodent**

Report no.:		R-8670 / R-8670A		R-8670 / R-8670A	
Study no.:		MS RA2		MS RA2	
Location:		Module 4.2.3.2		Module 4.2.3.2	
Species / strain:		Dog / Beagle		Dog / Beagle	
Admin. Compound:		Radium-223 dichloride		Radium-223 dichloride	
Analyte:		Radium-223		Radium-223	
Dose	[kBq/kg]	50 (3 <sup>rd</sup> injection)		50 (6 <sup>th</sup> injection)	
Route		i.v.		i.v.	
Gender / No. of animals		m + f / 6 + 5		m + f / 6 + 6	
Feeding condition		Ad libitum		Ad libitum	
Formulation		100% isotonic solution		100% isotonic solution	
Duration	[h]	96		96	
Assay		Gamma-counting		Gamma-counting	
		Arithmetic		Arithmetic	
Parameter	Unit	Mean	S.D.	Mean	S.D.
C <sub>max</sub>	[CPM/mL]	16503	1169	16605	634
V <sub>1</sub>	[mL]	824	142	815	117
t <sub>1/2</sub> α	[h]	0.0466	0.0137	0.0300	0.0117
t <sub>1/2</sub> β	[h]	0.752	0.222	0.956	0.448
t <sub>1/2</sub> γ	[h]	14.3	5.792	20.0	9.75
AUC	[CPM/mL·h]	36625	9480	39359	10536
CL	[mL/h]	386	86.4	382	181
AUMC	[CPM/mL·h·h]	677668	424774	1035426	565208
MRT	[h]	17.1	6.9	23.9	11.3
V <sub>ss</sub>	[mL]	6090	1306	7651	2560

**Pharmacokinetics: Biodistribution of 223Ra – radioactivity in tissues and organs, rodent (mice)**

Report no.: R-8646 Study no.: (b) (4) 148-001 Location: Module 4.2.2.3  
 Report title: Pharmacokinetics and Biodistribution of radium-223 (Alpharadin™) in mice

Species / strain:	Mouse / BALB/CA	Route:	i.v.	Assay:	Gamma-counting
Admin. compound:	Radium-223 dichloride	Gender / no.:	Female / 3		
Analyte:	Radium-223	Feeding conditions:	Ad libitum		
Dose:	625 kBq/kg	Formulation:	100% isotonic solution		

Organ / tissue	0.5 h		1 h		6 h		12 h		%injection/g		48 h		72 h		120 h		336 h	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Brain	0.19	0.21	0.17	0.08	0.16	0.08	0.07	0.01	0.13	0.05	0.12	0.03	0.08	0.02	0.09	0.01	0.04	0.01
Femur	5.56	2.39	8.33	1.95	13.1	6.72	5.09	0.80	8.50	1.34	11.1	2.18	6.80	3.19	12.24	2.56	5.64	2.17
Femur muscle	0.45	0.01	0.53	0.10	0.19	0.12	0.11	0.07	0.13	0.08	0.05	0.06	0.14	0.12	0.20	0.09	0.07	0.06
Heart	0.57	0.02	0.46	0.11	0.09	0.02	0.38	0.56	1.33	2.24	0.02	0.01	0.18	0.25	0.01	0.01	0.11	0.01
Kidney	18.99	2.71	20.53	2.84	3.45	1.00	0.84	0.17	0.61	0.03	0.34	0.06	0.18	0.02	0.00	0.01	0.03	0.01
Large intestine	3.59	1.30	7.99	1.79	0.86	0.21	0.30	0.06	0.19	0.02	0.15	0.03	0.08	0.02	0.05	0.01	0.03	0.01
Liver	0.63	0.03	0.67	0.13	0.27	0.07	0.06	0.02	0.08	0.03	0.07	0.02	0.03	0.00	0.03	0.01	0.01	0.01
Lung	1.43	0.50	1.35	0.49	0.39	0.11	0.13	0.06	0.12	0.08	0.07	0.02	0.05	0.00	0.05	0.03	0.03	0.02
Sternum	2.47	0.08	4.49	1.11	7.07	3.34	3.34	0.83	4.62	0.68	3.77	0.70	2.53	0.36	2.59	0.37	1.63	0.53
Skull	3.87	3.86	7.63	1.27	9.82	3.38	6.03	1.26	9.91	2.61	8.59	2.03	5.88	1.13	6.51	0.71	5.25	1.74
Small intestine	2.77	0.36	1.74	0.08	0.27	0.03	0.14	0.03	0.11	0.02	0.06	0.01	0.03	0.00	0.02	0.01	0.01	0.00
Spleen	6.40	3.53	12.46	2.43	8.31	3.16	2.90	1.00	5.19	2.34	6.90	2.30	3.58	1.18	4.08	1.73	0.71	0.55

Report no.: R-8648 Study no.: (b)(4) 148-003 Location: Module 4.2.2.3  
 Report title: Pharmacokinetics and Biodistribution of Radium-223 (Alpharadin™) in mice

Species / strain: Mouse / BALB/CA Route: i.v. Assay: Gamma-counting  
 Admin. compound: Radium-223 dichloride Gender / no.: Female / 6  
 Analyte: Radium-223 Feeding conditions: Ad libitum  
 Dose: 625 kBq/kg Formulation: 100% isotonic solution

Organ / tissue	%injection/g															
	Day 7		Day 14		Day 21		Day 28		Day 35		Day 42		Day 49		Day 56	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Brain	0.06	0.06	0.05	0.01	0.03	0.01	0.03	0.01	0.02	0.00	0.02	0.02	0.01	0.02	0.03	0.03
Femur	7.08	0.96	7.30	0.90	5.40	0.49	8.57	1.04	6.23	0.47	6.52	0.90	5.87	0.41	6.04	1.23
Femur muscle	0.00	0.01	0.00	0.00	0.00	0.00	0.24	0.05	0.01	0.01	0.05	0.08	0.01	0.02	0.02	0.07
Heart	0.32	0.36	0.14	0.26	0.28	0.16	0.20	0.28	0.17	0.16	0.12	0.19	0.05	0.11	0.18	0.21
Kidney	0.09	0.02	0.06	0.02	0.03	0.01	0.04	0.01	0.03	0.01	0.04	0.02	0.02	0.02	0.05	0.04
Large intestine	0.04	0.01	0.01	0.01	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.03	0.03	0.03
Liver	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02
Lung	0.03	0.02	0.03	0.01	0.02	0.03	0.02	0.03	0.01	0.03	0.06	0.03	0.03	0.05	0.04	0.08
Skull	8.13	0.98	7.63	0.70	7.11	0.40	5.95	0.68	6.33	0.38	5.88	0.45	5.88	0.75	6.16	0.55
Small intestine	0.02	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.02	0.02
Spleen	1.33	0.45	0.40	0.15	0.38	0.03	0.35	0.15	0.33	0.14	0.30	0.04	0.26	0.06	0.44	0.13
Sternum	2.71	0.29	2.51	0.59	0.98	0.22	2.56	0.36	1.85	0.21	1.94	0.26	1.79	0.24	1.62	0.41

**Pharmacokinetics: Biodistribution of 223Ra – radioactivity in tissues and organs, rodent (Rat)**

Report no.: R-8661 Study no.: (b) (4) Study 52159 Location: Module 4.2.3.1

Report title: Single Dose Toxicity Study in Rats

Species / strain:	Rat / Wistar	Route:	i.v.	Assay:	Gamma-counting
Admin. compound:	Radium-223 dichloride	Gender / no.:	Female + Male / 2 + 2		
Analyte:	Radium-223	Feeding conditions:	Ad libitum		
Dose:	1250 kBq/kg	Formulation:	100% isotonic solution		

Organ / tissue	Day 30	
	Mean [%injection/g]	S.D.
Adrenal	0.00	0.01
Aorta	0.01	0.01
Brain	0.00	0.00
Cecum	0.00	0.00
Colon	0.00	0.00
Duodenum	0.00	0.00
Epididymides	0.00	0.00
Eyes	0.00	0.00
Femur	1.17	0.15
Heart	0.00	0.00
Ileum	0.00	0.00
Injection site	0.46	0.04
Jejunum	0.00	0.00
Kidneys	0.00	0.00
Knee joint	1.97	0.27
Larynx	0.16	0.03
Liver	0.00	0.00
Lung	0.00	0.00
Lymph node mandi	0.02	0.01
Lymph node mesent	0.00	0.00
Mammary	0.00	0.00
Muscle	0.00	0.00
Oesophagous	0.00	0.00
Ovaries	0.02	0.01

**Pharmacokinetics: Biodistribution of 223Ra – radioactivity in tissues and organs, non-rodent  
Single dose (Day 30)**

Report no.:	R-8668 / R-8668A		Study no.:	MS RA1		Location:	Module 4.2.3.1		
Report title:	Biodistribution and acute Radiotoxicity Study of Single Dose intravenous Radium-223 in normal dogs								
Species / strain:	Dog / Beagle		Route:	i.v.		Assay:	Gamma-counting		
Admin. compound:	Radium-223 dichloride		Gender / no.:	Male + Female / 2 + 2					
Analyte:	Radium-223		Feeding conditions:	Ad libitum					
Dose:	0 ; 50; 150; 450 kBq/kg		Formulation:	100% isotonic solution					
<b>Day 30</b>									
<b>Dose</b>	<b>Control (0.9% saline)</b>		<b>50 kBq/kg</b>		<b>150 kBq/kg</b>		<b>450 kBq/kg</b>		
	Arithmetic		Arithmetic		Arithmetic		Arithmetic		
<b>Organ / tissue</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	
	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>	
Femur	72.7	61.0	2222	502	4861	509	22354	2365	
Humerus	2.9	4.1	5007	1146	10778	n.a. <sup>a</sup>	27522	n.a. <sup>a</sup>	
L1 / L3 vertebra	49.8	39.7	3910	1396	10166	2108	32590	2604	
Spleen	83.1	135	122	62.1	168	27.2	582	228	
Liver	22.0	32.7	83.9	28.2	231	47.8	501	101	
Prostate	77.8	n.a. <sup>a</sup>	77.8	n.a. <sup>a</sup>	n.a. <sup>a</sup>	n.a. <sup>a</sup>	0.0	n.a. <sup>a</sup>	
Kidney	60.9	48.1	117	71.1	81.3	84.3	177	144	
Lung	17.2	19.2	396	655	83.8	67.7	86.2	32.5	
Large int.	48.0	60.1	370	643	73.9	60.2	53.7	79.0	
Bile	21.7	28.0	37.6	23.7	48.5	8.0	33.6	13.4	
Thyroid	19.3	38.6	60.8	25.8	28.4	14.9	164	86.9	
Pituitary	713	1354	40.7	70.5	891	871	787	748	

**Pharmacokinetics: Biodistribution of 223Ra – radioactivity in tissues and organs, non-rodent  
Repeated dose (Day 30)**

Report no.:	R-8670 / R-8670A		Study no.:	MS RA2		Location:	Module 4.2.2.2	
Report title:	Long Term Radiotoxicity Study of Repeat Dose intravenous Radium-223 in normal dogs							
Species / strain:	Dog / Beagle		Route:	i.v.		Assay:	Gamma-counting	
Admin. compound:	Radium-223 dichloride		Gender / no.:	Male + Female / 6 + 5/6				
Analyte:	Radium-223		Feeding conditions:	Ad libitum				
Dose:	50 kBq/kg		Formulation:	100% isotonic solution				
<b>Day 30</b>								
<b>Dose</b>	<b>Control (0.9% saline)</b>				<b>50 kBq/kg</b>			
<b>Organ / tissue</b>	<b>Mean</b>	<b>S.D.</b>	<b>Mean</b>	<b>S.D.</b>	<b>Mean</b>	<b>S.D.</b>	<b>Mean</b>	<b>S.D.</b>
	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>	<b>[CPM/g]</b>
Femur	397	408	1144	649				
Humerus	12.0	13.9	704	228				
L1 vertebra	7.23	9.44	504	246				
Spleen	16.4	16.2	93.4	71.1				
Liver	11.9	14.3	17.3	10.1				
Kidney	48.1	21.7	42.0	28.0				
Small int.	13.5	n.a. <sup>a</sup>	10.3	2.97				
Large int.	22.6	23.6	12.6	15.0				
Heart	6.27	4.49	4.70	7.92				
Lung	3.45	5.89	11.5	15.1				
Bile	9.75	11.3	37.9	31.4				
Thyroid	156	272	108	141				
Pituitary	1063	1551	2763	3156				
Prostate	344	47.9	247	189				

**Human** (the following summary information is copied from applicant's submission)

At 15 minutes post injection, about 20% of the injected activity remained in the blood. At 4 hours, about 4% of the injected activity remained in the blood, decreasing to less than 1% at 24 hours after the injection. Radium-223 is incorporated primarily into bone or is excreted into the intestine. With regard to bone, the level of activity was determined to be in the range of 44% to 77% of the administered activity at 4 hours post injection. In the intestine, activity was already observed 10 minutes post injection. Most likely the secretion/excretion of radium from blood into the small intestine through the small intestine wall is through the transport mechanisms involved for other divalent cations (e.g. Ca, Mg and Ba).

**Pharmacokinetics: Excretion of 223Ra – radioactivity in tissues and organs, rodent****Single dose**

Report no.: R-8647 Study no.: (b) (4) 148-002 Location: Module 4.2.2.5  
 Report title: Urinary and Fecal Clearance of Radium-223 (Alpharadin™) in mice

Species / strain:	Mouse / BALB/CA	Route:	i.v.	Assay:	Gamma-counting
Admin. compound:	Radium-223 dichloride	Gender / no.:	Female / 6		
Analyte:	Radium-223	Feeding conditions:	Ad libitum		
Dose:	625 kBq/kg	Formulation:	100% isotonic solution		
<b>Matrix</b>	<b>Interval</b>	<b>% of administered dose</b>			
Urine	0-6 h	4.10			
	0-24 h	5.05			
	0-72 h	6.79			
	0-120 h	7.17			
Feces	0-6 h	2.87			
	0-24 h	4.74			
	0-72 h	6.36			
	0-120 h	6.82			
Recovery		14.0			

**Pharmacokinetics: Excretion of <sup>223</sup>Ra – radioactivity in tissues and organs, non-rodent  
Single dose (day 30)**

Report no.:	R-8668 / R-8668A	Study no.:	MS RA1	Location:	Module 4.2.3.1
Report title:	Biodistribution and acute Radiotoxicity Study of Single Dose intravenous Radium-223 in normal dogs				
Species / strain:	Dog / Beagle	Route:	i.v.	Assay:	Gamma-counting
Admin. compound:	Radium-223 dichloride	Gender / no.:	Male + Female / 2 + 2		
Analyte:	Radium-223	Feeding conditions:	Ad libitum		
Dose:	0, 50, 150, 450 kBq/kg	Formulation:	100% isotonic solution		

Dose	Day 30							
	Control (0.9% saline) Arithmetic		50 kBq/kg Arithmetic		150 kBq/kg Arithmetic		450 kBq/kg Arithmetic	
	Mean [CPM/mL]	SD [CPM/mL]	Mean [CPM/mL]	SD [CPM/mL]	Mean [CPM/mL]	SD [CPM/mL]	Mean [CPM/mL]	SD [CPM/mL]
Urine								
0 h	12.0	n.a. <sup>a</sup>	162.3	221	24.0	23.8	12.4	n.a. <sup>a</sup>
0-4 h	116	64.8	39146	10608	123111	72448	263067	90135
4-24 h	41.0	23.9	19278	17910	52558	58862	99890	45299
24-48	44.6	34.4	5984	1614	44603	67079	56139	41072
48-72 h	39.4	28.9	3182	1216	12194	6151	24698	12374
72-96h	18.6	7.7	3453	19841	5194	1307	21094	19286
96-168 h	13.2	9.3	884	402	1811	510	9978	3321

**Repeated dose (after 3<sup>rd</sup> and 6<sup>th</sup> injection)**

Report no.:	R-8670 / R-8670A	R-8670 / R-8670A
Study no.:	MS RA2	MS RA2
Location:	Module 4.2.3.2	Module 4.2.3.2
Species / strain:	Dog / Beagle	Dog / Beagle
Admin. Compound:	Radium-223 dichloride	Radium-223 dichloride
Analyte:	Radium-223	Radium-223
Dose [kBq/kg]	50 (3 <sup>rd</sup> injection)	50 (6 <sup>th</sup> injection)
Route	i.v.	i.v.
Gender / No. of animals	m + f / 6 + 5	m + f / 6 + 6
Feeding condition	Ad libitum	Ad libitum
Formulation	100% isotonic solution	100% isotonic solution
Duration [h]	96	96
Assay	Gamma-counting	Gamma-counting

Time period	Arithmetic		Urine		Arithmetic	
	Mean [CPM/mL]	S.D. [CPM/mL]	Mean [CPM/mL]	S.D. [CPM/mL]	Mean [CPM/mL]	S.D. [CPM/mL]
0-1 h	56755	46573	49648	47821		
1-2 h	66957	38734	49059	32901		
2-4 h	47018	23590	34839	19302		
4-6 h	29376	10560	22731	10190		
6-8 h	27261	5671	16022	7958		
8-12 h	15435	5976	14046	6631		
12-24 h	8002	3288	8154	3667		
24-48 h	4570	2392	2658	848		
48-72 h	2234	403	2086	504		
72-96 h	1552	1297	1481	88.6		

**Human** (the following summary information is copied from applicant's submission)

Fecal excretion was the major route of elimination from the body. Fecal excretion post injection at 24 hours was 2% (range < 1% to 13%) and at ~48 hours cumulative fecal excretion was 13% (range < 1% to 34%). At ~48 hours cumulative urine excretion was 2% (range <1% - 5%) of the injection radioactivity. It is estimated that approximately 5% radium-223 was excreted through urine in the first void a mean of 2.4 hours after injection. The whole body measurement at 7 days after injection indicates that a median of 76% of administered activity was excreted from the body.

## 6 General Toxicology

### Overall toxicology summary

General toxicology: Radium-223 dichloride was tested in single dose toxicity studies in mice, rats and dogs. Repeat-dose toxicology studies were conducted with administration once every 4 weeks up to 12 months in duration in rats and up to 6 months in duration in dogs. In single dose studies, treatment-related deaths were observed in mice at a dose of 2500 kBq/kg (7500 kBq/m<sup>2</sup>) in mice. Single doses of radium-223 dichloride up to 3081 kBq/kg (18486 kBq/m<sup>2</sup>) in rats and 450 kBq/kg (9000 kBq/m<sup>2</sup>) in dogs were well tolerated; these were the highest doses tested in these studies. Single and repeated dosing in rats and dogs resulted in reduced body weight gain and weight loss, bone marrow suppression characterized by decreased bone marrow hematopoietic cellularity, and hematologic changes including lower total RBC, WBC, lymphocytes, eosinophils, and platelets. The myelotoxicity observed was considered to be dose limiting. The initial acute response to treatment with radium-223 dichloride showed reversibility to some hematology parameters after a single administration of radium-223 dichloride. However, in the repeat-dose part of the study, the reduction in white blood cell count, for example, was not reversed until approximately 40 weeks after dosing. Osteosarcomas were observed in rats after 6 months from the start of treatment. The incidence of osteosarcomas was dose-dependently increased in rats, but was not time-dependent with dose increases. Other non-neoplastic lesions were observed in rats treated with radium-223 dichloride, which included bone depletion/fibrosis associated with disorganized growth lines, osteocyte depletion and changes in the bone socket of the teeth.

Genetic toxicology: not conducted

Carcinogenicity: not conducted

Reproductive toxicology: not conducted.

In conducted general toxicology studies, rats that received a single dose of radium-223 dichloride had minimal numbers of abnormal spermatocytes (giant cells) in tubules at doses  $\geq 2054$  kBq/kg, with normal content of spermatocytes in the epididymides. Marked tubular atrophy was found in one male with a single dose of radium at 2054 kBq/kg. One male with repeated dosing at 50 kBq/kg had tubular atrophy in the testes and oligospermia in the epididymides.

Radium-223 dichloride is considered to be a reproductive toxicant and a carcinogen based on its radioactive properties and mechanism of action.

Special toxicology: A local tolerance toxicity study was conducted in rabbits. Transient erythema, but no edema, hemorrhage, or histological change of the injection site was observed after a single perivenous injection of 750 kBq/animal in a GLP study in rabbits.

Radium-223 dichloride was well tolerated locally after bolus intravenous administration in the single and repeated-dose toxicity studies based on clinical observations and the histopathological evaluation of the injection site.

### **6.1 Single-Dose Toxicity**

Single dose studies in mice and rats were submitted to IND 67,521; these study reports were previously reviewed by Dr. Siham Biade.

#### **Study 52158: Alpharadin (Radium-223) single dose toxicity study in mice**

The following conclusion for study 52158 was copied from the nonclinical review by Dr. Biade on 03/31/2008 for IND 67,521:

Based on the results of the biodistribution study, Ra-223 had a pronounced affinity to calcified tissues like bone and cartilage. Alpharadin (Ra-223), administered to mice as a single i.v. treatment in dosages of 1250, 2500, and 3750 kBq/kg caused a reduced body weight gain, a dose-related minimal to moderate depletion of osteocytes and osteoblast, a minimal to marked depletion of the hematopoietic cells in the bone marrow and a minimal to slight extramedullary hematopoietic in the spleen, and in the mandibular and mesenteric lymph nodes. The NOAEL was considered to be below 1250 kBq/kg.

#### **Study 52159: Alpharadin (Radium-223) single dose toxicity study in rats**

The following conclusion was excerpted from Dr. Biade's review for IND 67,521.

Rats were well tolerated to single dose treatment of radium-223 at doses up to 3081 kBq/kg. WBC counts, neutrophil counts, lymphocyte counts were significantly lower in all treated animals. Drug related changes were observed in the bones, bone marrow, spleen, thymus, lungs, and testes.

In bone, dose related minimal to moderate depletion of osteocytes and osteoblasts was found in all treated animals. Depletion of osteoclasts was also observed. A slight to moderate proliferative fibroosseous lesion in the femur was seen in all radium-223 treated animals. Further, disruption/disorganization of the physis/growth line was observed.

Dose-related minimal to marked depletion of the hematopoietic cells was seen in bone marrow of femur and sternum in all animals at all doses. The depletion was most prominent in the metaphysis and epiphysis of the femur and around the cancellous bone in sternum. In bone marrow of sternum, minimal to slight fibrosis was seen in males at doses  $\geq$  2054 kBq/kg (MD), and in females at 3081 kBq/kg (HD).

Moderate to marked extramedullary hematopoiesis was found in the spleen of all animals. A few giant cells (most likely megakaryocytes according to the report) were observed in the liver of some animals in the high dose group and this effect was thought to be part of the increased extramedullary hematopoiesis. This effect was accompanied by an increase in spleen weight. In the lungs, minimal to slight alveolar edema was observed in the females in the mid dose group and in both sexes in the high dose group. In the testes: minimal numbers of abnormal spermatocytes (giant cells) were observed in tubules in all males at doses  $\geq$  2054 kBq/kg. Marked tubular atrophy was found in one animal at 2054 kBq/kg (1/5).

**Study title: Biodistribution and acute radiotoxicity study of single dose intravenous radium-223 in normal dogs**

Study no.: MS RA1

Study report location: Electronic submission, M4.

Study report: pages 1-160

Amendment: pages 1-345

Conducting laboratory and location:



Date of study initiation: August 1, 2007

GLP compliance: Yes\*

QA statement: yes ( X ) no ( )

Drug, batch #, and % purity: BAY 88-8223,  
A708003, A709002, A710005; or A710009  
>99% (radiochemical purity)

Note: This study was performed according to GLP. No individual animal data was provided with the original submission (R-8668). An amendment (R-8668A) to the final study report was submitted afterwards. The submitted amendment contains individual animal data as listed in the following:

- Biodistribution and excretion in blood, urine and feces
- Hematology
- Biochemistry
- Thyroid and pituitary function
- Urinalysis
- Clinical observations and medical records
- Radioactivity of target organs at necropsy
- Macroscopic findings
- Organ weights

However, the submitted individual animal data were all in hand writing, and they are illegible. Therefore, the study results described in the following section are the Applicant's conclusions; the data was not verified.

Reviewer's note: This single dose study is not a pivotal study for this NDA submission, therefore, a request to submit a better quality representation of individual animal data is not deemed necessary. The study results from this single-dose study is captured here since the repeat dose study in dogs (a pivotal study) was conducted with only one dose level and an MTD was not reached. The study results from this single-dose study may provide additional information on dose-response, DLT, and a toxicity profile at higher dose levels following radium-223 dichloride treatment.

**Key Study Findings**

- Injection of radium-223 dichloride was well tolerated to the doses up to 150 kBq/kg;
- There were dose-dependent decreases in RBC, HT, Hb, total white cell count, granulocyte count and platelet count;

- Dose limiting myelotoxicity and retinal detachment with hypertrophy of the retinal pigmented epithelium (RPE) was observed at the 450 kBq/kg dose;
- There were dose related decreases of hematopoietic cellularity and percentage bone marrow in sternal, femur, and/or vertebral bone marrow;
- There was increased splenic extramedullary hematopoiesis in radium-223 dichloride treated animals.

## Methods

Doses: 50, 150, 450 kBq/kg\*  
 \* the basis for dose selection was not provided

Frequency of dosing: Single dose  
 Route of administration: iv bolus  
 Dose volume: 11 mL  
 Formulation/Vehicle: 0.9% saline  
 Species/Strain: Beagle dog  
 Number/Sex/Group: 2/sex/group  
 Age: Greater than 1 year old  
 Weight: Males: 206-231 g; females: 160-187 g  
 Satellite groups: none

Unique study design: Biodistribution: Radioactivity of clinical samples were measured on a WIZARD gamma counter and recorded as counts per minute (CPM) (SOP# MS RA1-8) to determine the biodistribution of Radium-223 in blood, urine, feces and bile.  
 Thyroid and pituitary function: measured from blood drawn at baseline, 15 and 30 days after test article administration

Deviation from study protocol: no

## Observations and Results

### **OBSERVATIONS AND TIMES:**

<u>Mortality</u>	daily
<u>Clinical observation</u>	daily
<u>Detailed physical examinations</u>	weekly
<u>Body weights</u>	on the day of test article administration, every 7 days thereafter and immediately before euthanasia (day 30)
<u>Food consumption</u>	Not performed
<u>Clinical Pathology:</u>	Blood was collected at 24 and 72 h, 7, 15 and 30 days after injection; Urine was collected at baseline, 4, 24, 48, 72, 96 hours and 7 days post test article injection
<u>Gross pathology:</u>	All animals at death or at scheduled sacrifice, 4 weeks after the last treatment
<u>Organ weights:</u>	All animals, at death or at scheduled sacrifice

<p><u>Histopathology:</u></p>	<p>Including bone marrow evaluation, all animals, at death or at scheduled sacrifice                  Adequate Battery: yes (x), no ( ),                  Peer review: yes (x), no ( )</p>
<p><u>Toxicokinetics</u></p>	<p>Blood: at 1, 2, 4, 24, 48, 72 and 96 hours after test article administration                  Urine: at baseline, 4, 24, 48, 72, 96 hours and 7 days post test article injection                  Feces: at baseline, 24, 48, 72 and 96 hours and 7 and 9 days post test article injection</p>

**RESULTS:**

Mortality: 1 female at 450 kBq/kg on day 12 after dosing

Death cause: pneumonia with respiratory clinical signs as well as fever

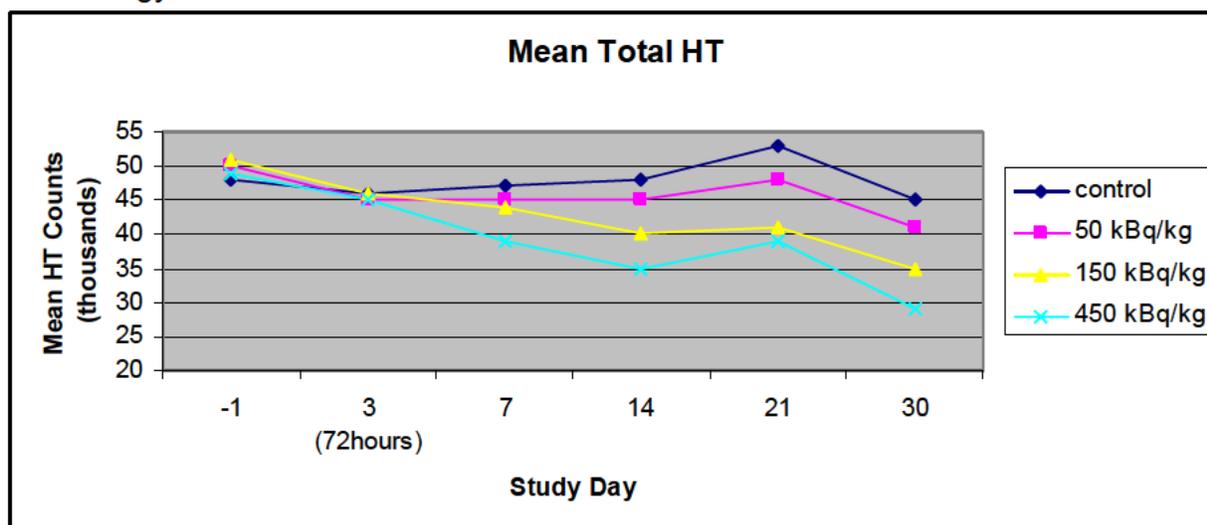
Clinical examinations

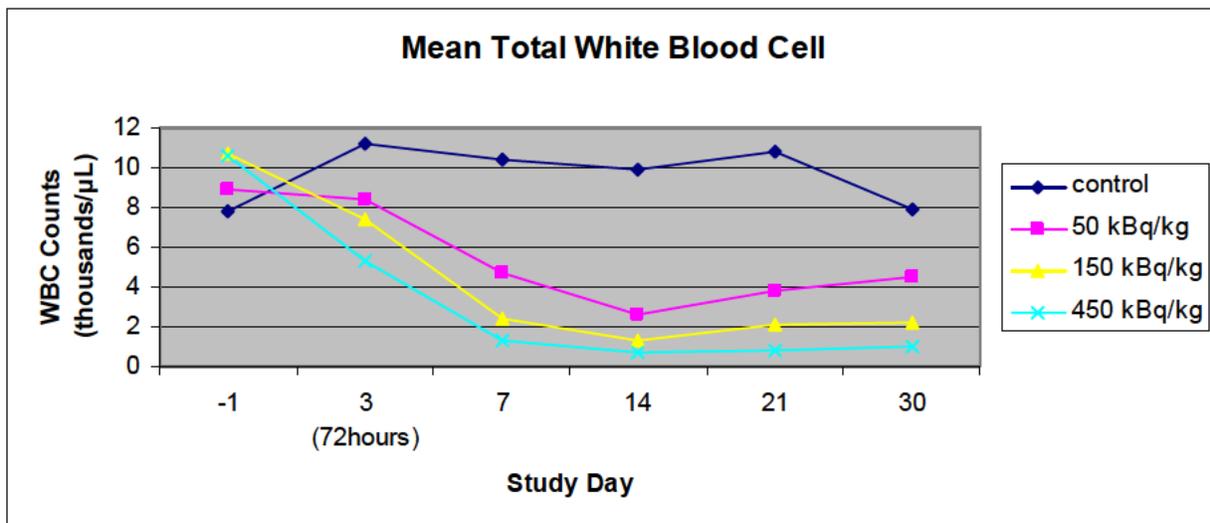
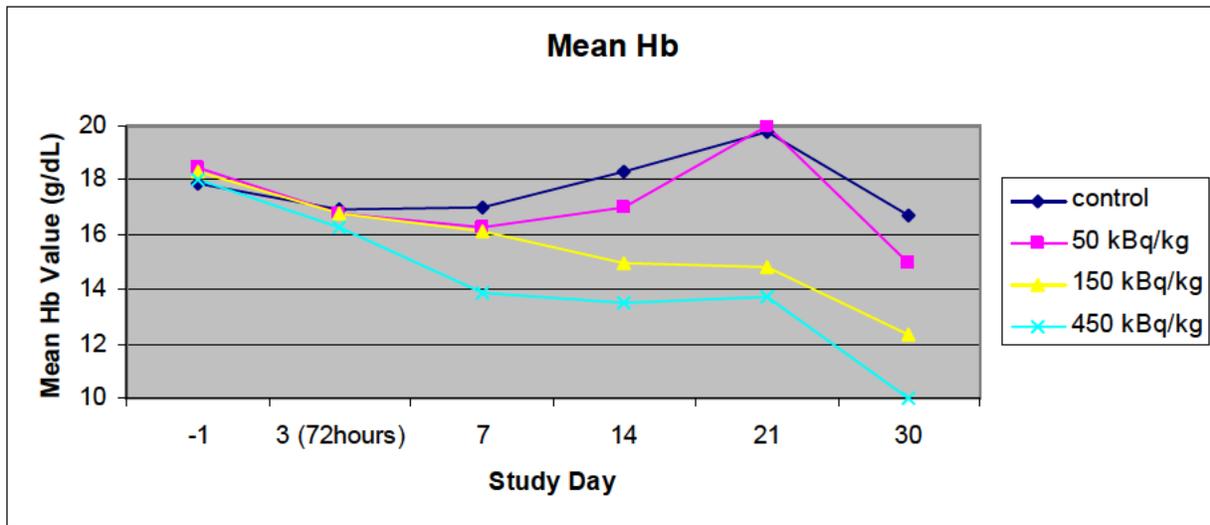
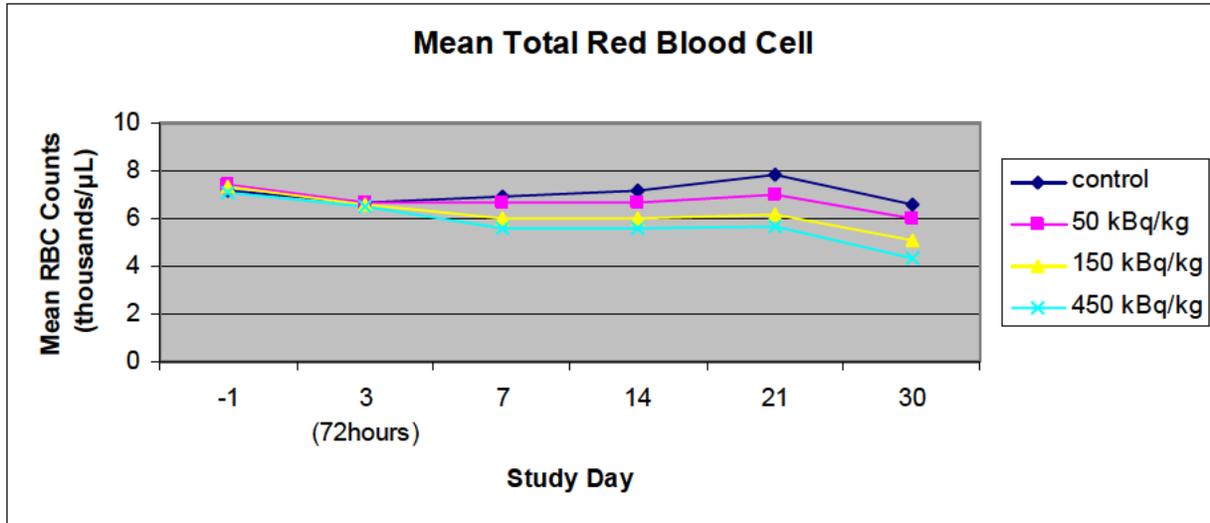
Some dogs at 450 kBq/kg developed a transient elevation in body temperature (> 103°F and < 104.5°F) that corresponded with the granulocyte nadir 7-14 days after test article administration.

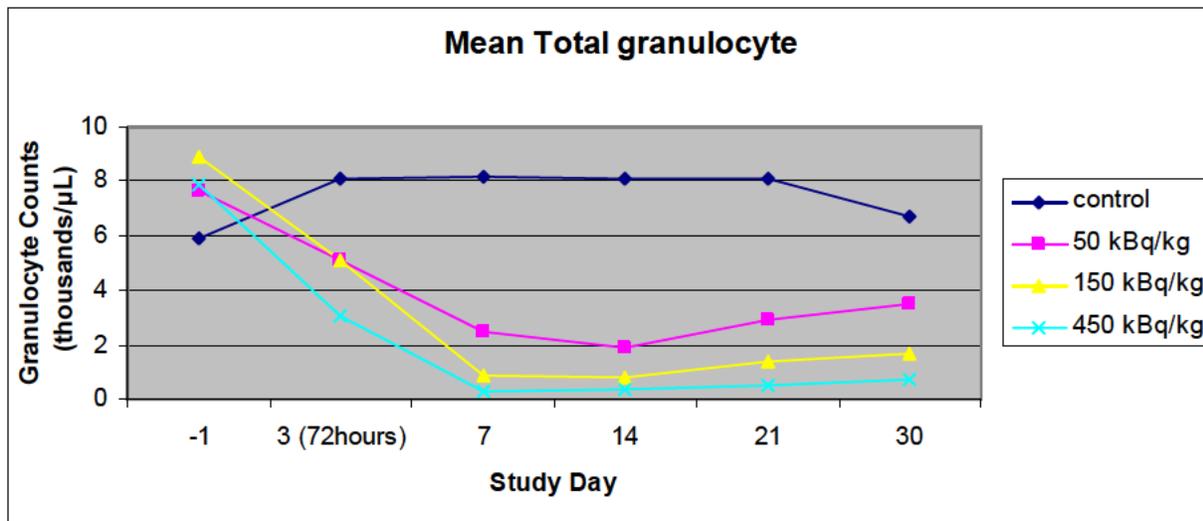
Several dogs in groups 3 and 4 had decreased fecal production 7-10 days after test article administration.

Body weight: unremarkable

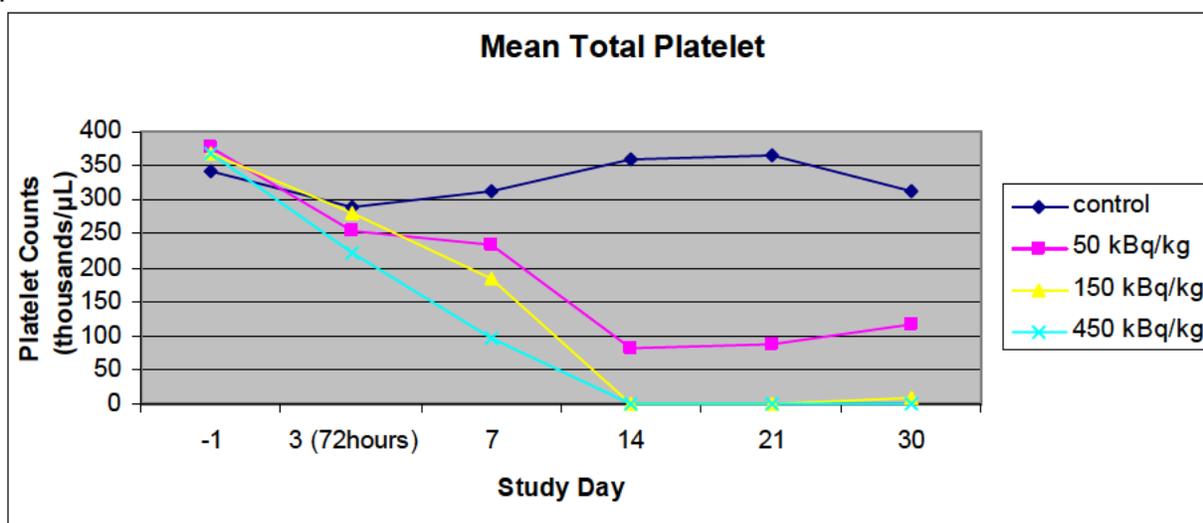
Hematology:







μ



*Summary:* Dose-dependent decreases in RBC, HT, and Hb were observed during the study with lowest levels at Day 30 after dosing. Dose-dependent decreases in WBC, granulocytes, and platelets were observed with nadir at 14 days after dosing. There were some recovery trends for the changes of WBC, granulocytes, platelet at LD group.

Biochemistry: unremarkable

Urinalysis: unremarkable

Gross pathology: unremarkable

Body and Organ Weights: unremarkable

Histopathology including bone marrow evaluation:

- A dose-dependent decline of hematopoietic cellularity in the L2 vertebral

- body segments, 7th sternbrae and distal femur sections;
- Increased splenic extramedullary hematopoiesis- a compensatory response to the marrow hypocellularity.
  - Acute bilateral retina detachment, with hypertrophy of the retinal pigmented epithelium (RPE) was seen 3 dogs at 450 kBq/kg. This change was occasionally accompanied by gliosis of the optic nerve, subretinal proteinaceous fluid, or retinal congestion/hemorrhage.

Note: Selective accumulation of radium-223 in the pigmented structure of canine eye may be a species specific phenomenon based on previous reports in the literature. The implication of these findings for human patients is unclear. Retina detachment was not seen in the repeat dog study at 50 kBq/kg.

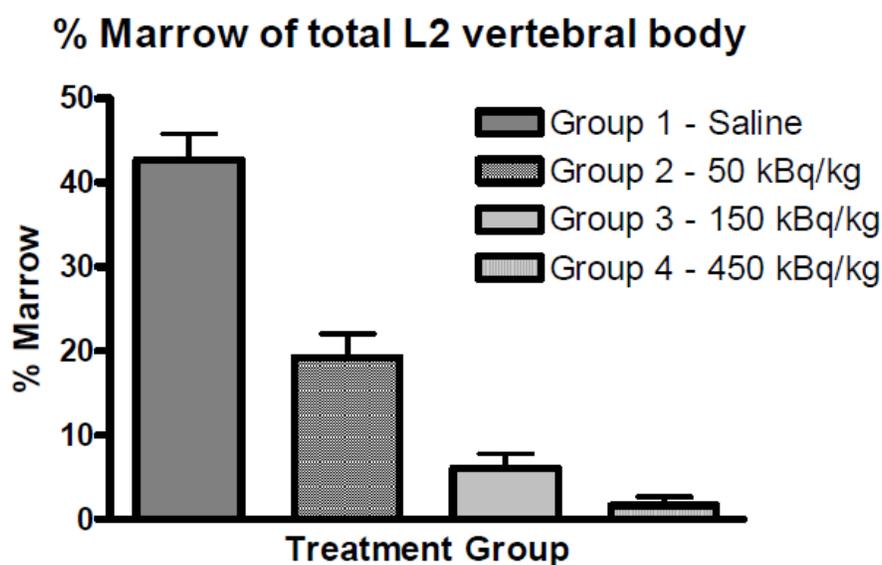
Mean bone marrow smear M:E ratios and bone marrow section sternum mean cellularity percentage estimate by treatment group

Dose (kBq/kg)	control	50	150	450
M:E Ratio	0.93	0.54	0.43	NA
Bone marrow section Sternum – Cellularity (%)	80	18	5	0

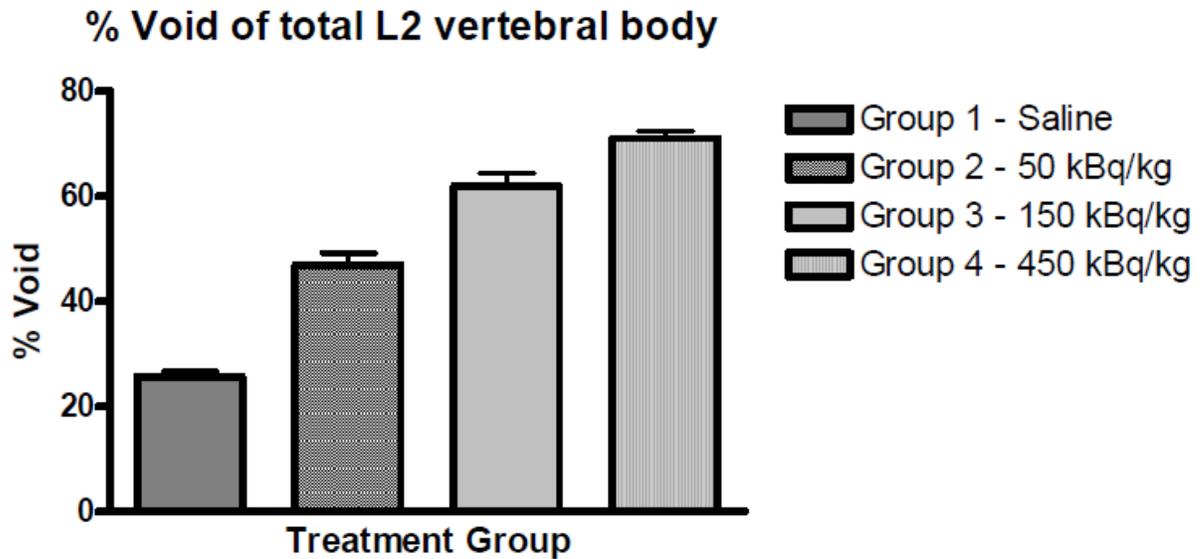
Summary results for mean percentage marrow, void and trabecular bone from L2 vertebral body histology slides

Dose (kBq/kg)	control	50	150	450
% Marrow	42.7	19.2	6.1	1.6
% Void	25.7	46.9	61.9	71.0
% Trabeculae	31.6	33.9	32.1	37.4

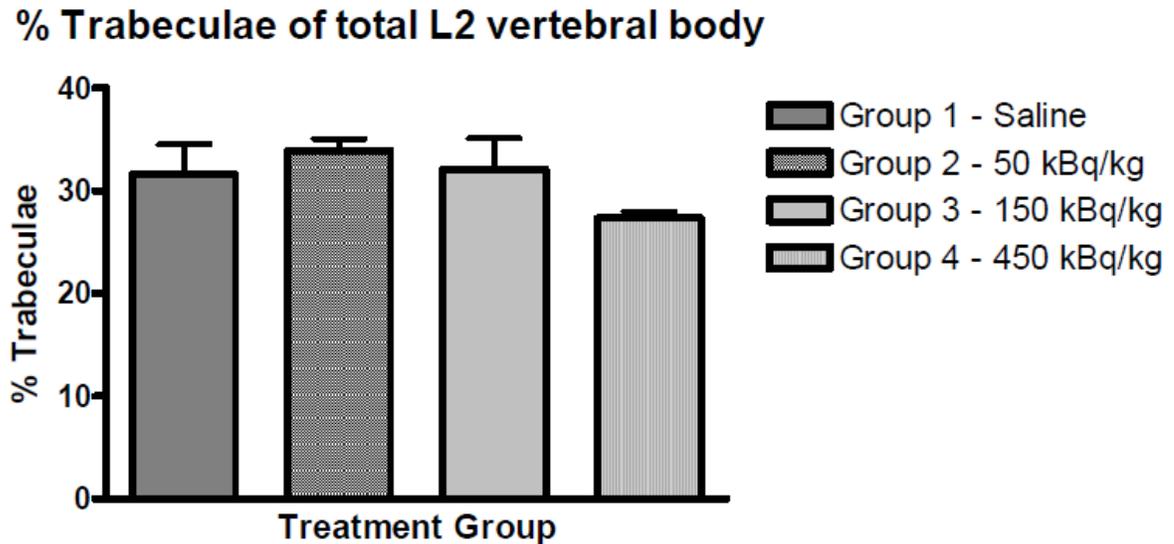
Graph of mean percentage marrow of total L2 vertebral body area by treatment group (copied from the Applicant's submission)



Graph of mean percentage void of total L2 vertebral body area by treatment group (copied from the Applicant's submission)



Graph of mean percentage trabecular bone volume in the L2 vertebral body sections by treatment group



*Summary:* Histomorphometric analysis showed that there were dose dependent decreases in marrow elements.

Toxicokinetics: refer to PK section of this review

## 6.2 Repeat-Dose Toxicity

**Study title:                           Alpharadin (radium-223)  
                          Single and Repeated Dose Toxicity Study in Rats with an Extended  
                                                  Recovery Period**

Study no.: 62985  
Study report location: Electronic submission, M4. pages 1-1389  
Conducting laboratory and location: (b) (4)  
Date of study initiation: June 7, 2006  
GLP compliance: yes  
QA statement: yes ( X ) no ( )  
Drug, lot #, and % purity: BAY 88-8223  
                                                  Batches: A606007, A606006, A607004,  
                                                  A607003, A608003, A608002, A608001,  
                                                  A608006, A6005009 and A605008  
                                                  % radiochemical purity: > 99%

Note: Late radiation toxicity was evaluated in this study since this study included 12 months post-dosing monitoring after the treatment with radium-223 dichloride.

### Key Study Findings

- Decreased body weights and reduced food consumption were observed after a single intravenous injection at doses of 20, 80, 325 or 1300 kBq/kg or 4 intravenous injections with 1 month intervals at doses of 20, 325 or 650 kBq/kg;
- There were dose-dependant decreases in the white blood cell counts, and decreased values of haemoglobin, red blood cells, haematocrit and platelets 14 days after the first dose;
- The observed hematologic changes except for white blood cell counts recovered 1 year after single or repeat dosing of radium-223 dichloride.
- The histopathological examination revealed treatment related changes in the bone socket of the teeth, in the liver, kidneys, uterus, spleen , bone;
- Osteosarcomas were observed in the single dose and repeat dose animals.

## Methods

Doses: Single dose: 0, 20, 80, 328, 1300 kBq/kg\* (group 1-5)  
 Repeat dose: 0, 20, 325, 650 kBq/kg\* (group 6-9)  
 \* the basis for dose selection was not provided

Frequency of dosing: Single dose: once followed by a 1-year recovery period  
 Repeat dose: once every 4 weeks for 4 weeks (a total of 4 injections)  
 followed by a 1-year recovery period

Route of administration: iv

Dose volume: 1 ml/kg\*  
 \* volume correction according to physical decay of radium-223

Formulation/Vehicle: a sterile, non-pyrogenic solution in a sodium citrate buffer with no preservative, traces of strontium and calcium

Species/Strain: SPF Wistar rats

Number/Sex/Group: 8/sex/group

Age: approximately 7-8 weeks old

Weight: Males: 213 - 238 g; females: 164 - 184 g

Satellite groups: none

Unique study design: none

Deviation from study protocol: Yes, but no major impact on the study results

## Observations and Results

**OBSERVATIONS AND TIMES:**

<u>Mortality</u>	daily
<u>Clinical examinations</u>	Daily including weekly examining the condition of the teeth starting from the 3 <sup>rd</sup> week of the study
<u>Detailed physical examinations</u>	Not performed
<u>Body weights</u>	weekly
<u>Food consumption</u>	weekly
<u>Condition of teeth</u>	Weekly
<u>Ophthalmoscopy</u>	Once before start of treatment, and once before termination of treatment* *no details test days are provided
<u>Clinical Pathology:</u>	pre-dosing (Day -7), on Day 15, in Week 14 (3 months after dosing in Groups 1-5 and 2 weeks after the last dose in Groups 6-9), then every third (3rd) month and at termination- 1 year after last dosing
<u>Gross pathology:</u>	All animals at death or at scheduled sacrifice, Week 52 (Day 365 -367) for

	Groups 1-5, Week 64 (Day 448 – 449) for Groups 6-9
<u>Organ weights:</u>	All animals at death or at scheduled sacrifice, Week 52 (Day 365 -367) for Groups 1-5, Week 64 (Day 448 – 449) for Groups 6-9
<u>Histopathology:</u>	All animals at death or at scheduled sacrifice, Week 52 (Day 365 -367) for Groups 1-5, Week 64 (Day 448 – 449) for Groups 6-9 Adequate Battery: yes (x), no ( ), Peer review: yes (x), no ( )

**RESULTS:**

Mortality:

Single dose (group 1-5)

Dose (kBq/kg)	Number of animals	Number of death	Sex	Animal#	Days of death
0	8	0	M	-	-
	8	0	F	-	-
20	8	1	M	23	281
	8	0	F	-	-
80	8	0	M	-	-
	8	1	F	43	101**
328	8	3	M	49	234
				52	323
				53	354
	8	4	F	57	351
			58	300	
			59	338	
			64	330	
1300	8	3	M	69	247
				71	212
				72	15**
	8	2	F	74	275
			75	288	

Repeat dose (group 6-9)

Dose (kBq/kg)	Number of animals	Number of death	Sex	Animal#	Days of death
0	8	1*	M	84	366
	8	0	F	-	-
20	8	1	M	97	343
	8	1	F	106	342

325	8	5	M	115 116 118 119 120	411 320 392 385 369
	8	5	F	122 125 126 127 128	348 421 400 446 329
650	8	6	M	129 131 132 134 135 136	190** 251 274 435 317 317
	8	6	F	137 139 140 142 143 144	391 335 323 410 366 335

\* found dead

\*\* deaths caused by blood sampling

*Summary:* Mortality was observed in animals treated with radium-223 dichloride at all dose levels. The observed early deaths were dose-dependent and all treatment-related deaths occurred 7 months after the treatment.

#### Clinical Signs

##### unscheduled deaths

##### *Single dose (group 1-5)*

Dose (kBq/kg)	Number of death	Sex	Animal#	Days of death	Clinical signs
0	0	M	-	-	-
	0	F	-	-	-
20	1	M	23	281	Animal very pale (eyes almost white).
	0	F	-	-	-
80	0	M	-	-	-
	1	F	43	101	unremarkable
328	3	M	49	234	Large weight loss, piloerection, subdued, forced respiration, vocalising. Swelling front leg, cannot use leg Swelling on hind legs
			52	323	
			53	354	

	4	F	57 58 59 64	351 300 338 330	Paralysis of hind legs Swelling on maxilla Paralysis of hind legs Abnormal very impaired gait on hind legs
1300	3	M	69	247	Paralysed left hind leg and bone mass on shoulder Abnormal stereotype behavior, head tilted to the left unremarkable
			71	212	
	2	F	72	15	Large weight loss Paralysis of hind legs
			74 75	275 288	

*Repeat dose (group 6-9)*

Dose (kBq/kg)	Number of death	Sex	Animal#	Days of death	Clinical signs
0	1	M	84	366	-
	0	F	-	-	-
20	1	M	97	343	Paralysis of hind legs
	1	F	106	342	Piloerection, animal pale, weight loss.
325	5	M	115	411	Swelling of lower jaw Swelling at the mandibular region, weight loss Swelling in mouth Swelling in mouth Swelling in the mandibular region, large weight loss
			116	320	
			118	392	
			119	385	
			120	369	
	5	F	122	348	Paralytic hind legs Swelling in abdomen Swelling in mouth Swelling in mouth Swelling on shoulder, paralysis of hind legs
			125	421	
			126	400	
			127	446	
			128	329	
650	6	M	129	190	- Paralysis of hind legs Paralysis of hind legs and convulsions Swelling lower jaw Abnormal very impaired gait on hind legs Abnormal very impaired gait on hind legs
			131	251	
			132	274	
			134	435	
			135	317	
			136	317	

	6	F	137	391	Respiratory problems
			139	335	Paralysis or hind legs
			140	323	Piloerection and limited use of hind legs
			142	410	Paralysis of legs
			143	366	Piloerection, respiration problems
			144	335	Swelling on the nose

Terminal sacrifice: unremarkable

Missing, reduced and irregularly growing teeth in all animals (scheduled deaths and terminal sacrifice)

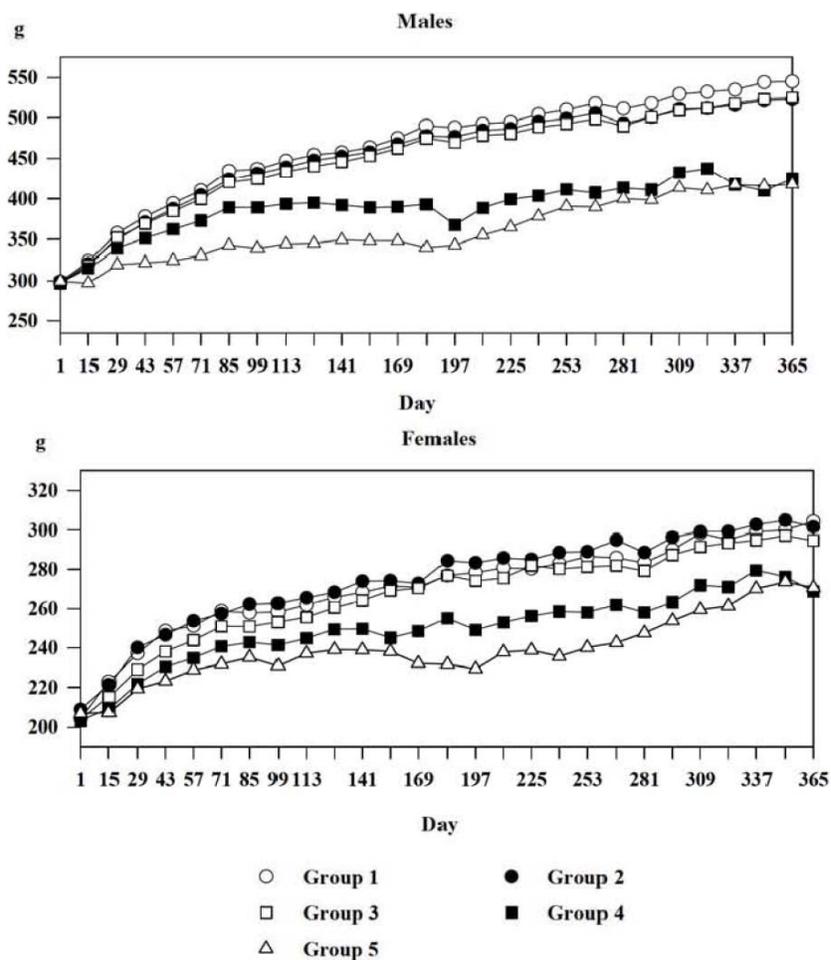
Approximately 6 month after start of treatment, several animals in Groups 5 (1300 kBq/kg), 8 (4 x 325 kBq/kg) and 9 (4 x 650 kBq/kg) had very short teeth or almost no teeth left. Most of these animals lost weight and were, therefore, offered diet softened with water. After introduction of moistened diet, these animals gained weight again. Furthermore, in Group 4, abnormal growth of the teeth in the lower jaw (very long teeth) was observed and several animals had their teeth clipped in order to eat normally. In addition, in these animals short teeth were observed in the upper jaw.

day of study	Numbers of animals affected							
	Males				Females			
	control	LD	MD	HD	control	LD	MD	HD
21-182								
189			1	3				6
196			1	3				6
203			1	4				7
210				10				8
217				11				11
224				12				12
231				12				12
238			7	12			1	12
245			8	12			1	12
252			8	12			1	12
259			8	12			2	12
266			8	12			2	11
273			8	11			2	10
280			8	11			3	10
287			8	10			5	9
294			7	10			6	7
301			7	10			2	7
308			8	10			12	6
315			6	10			12	7
322			6	10			11	7
329			5	10		1	8	7
336			5	9			-	-

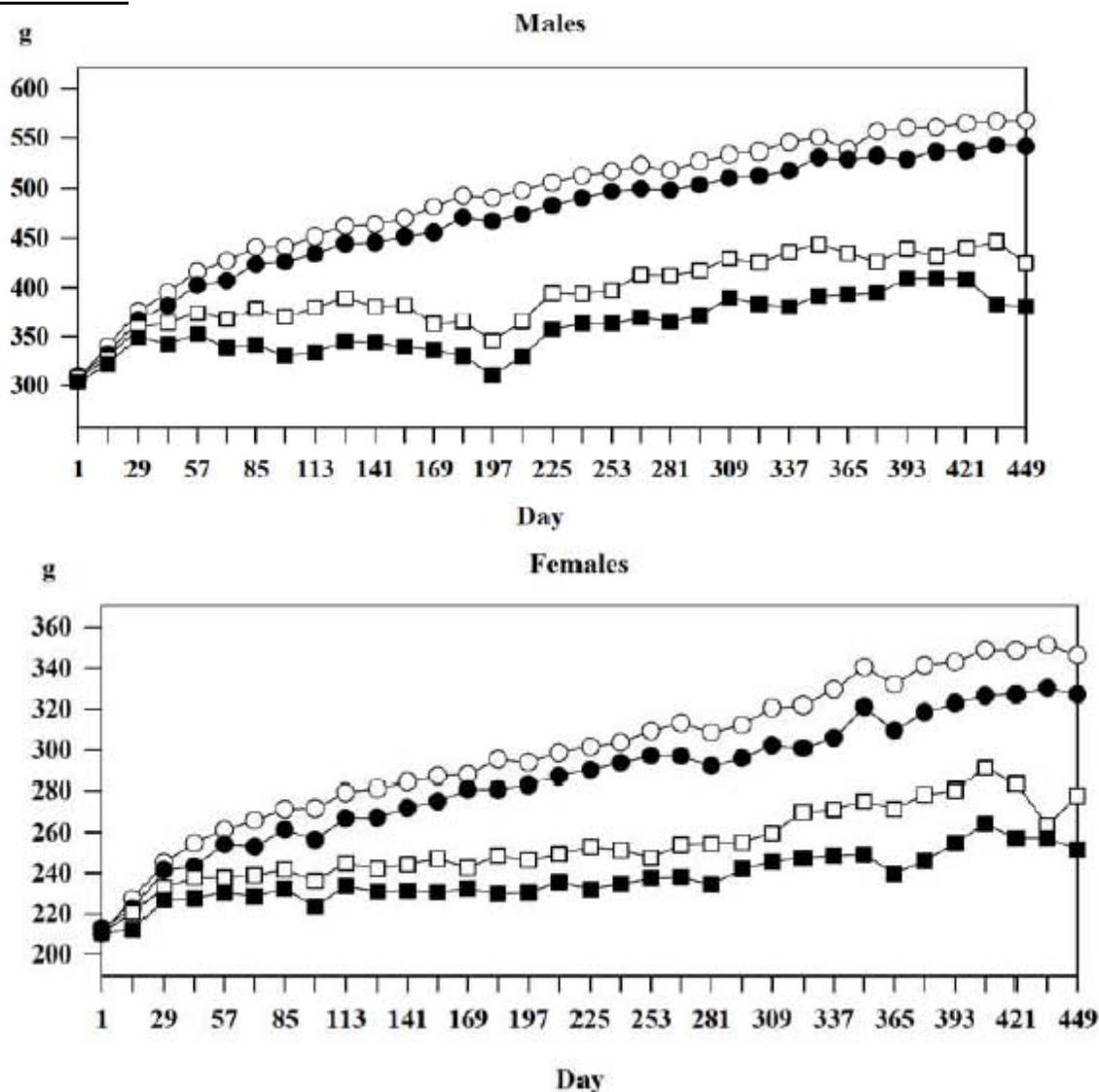
## Body Weights

Single dose

The following figures are copied from the Applicant's submission; the data are verified by the reviewer.

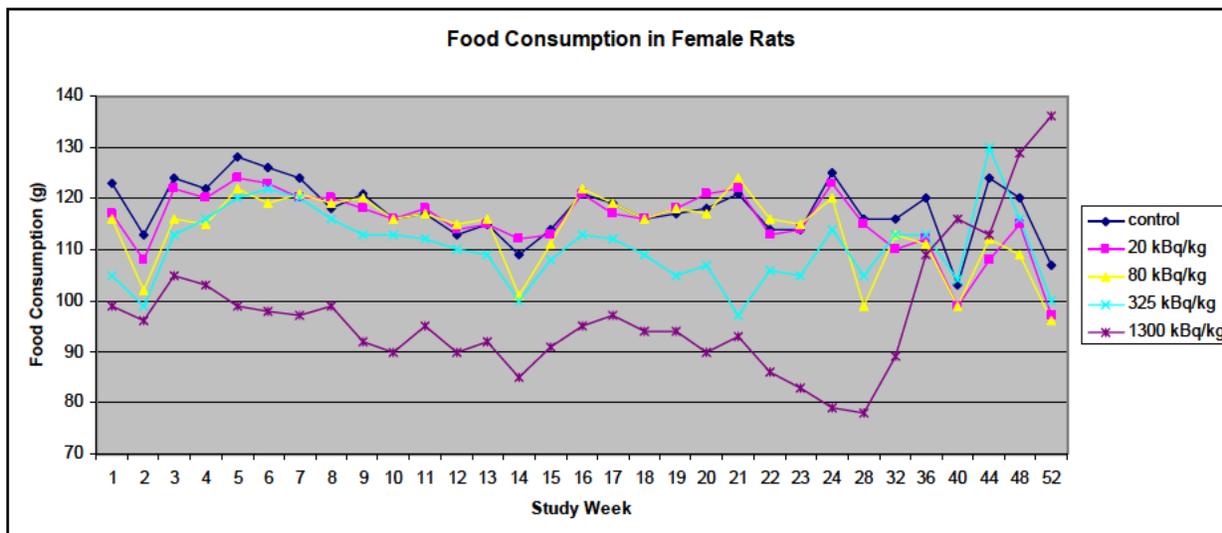
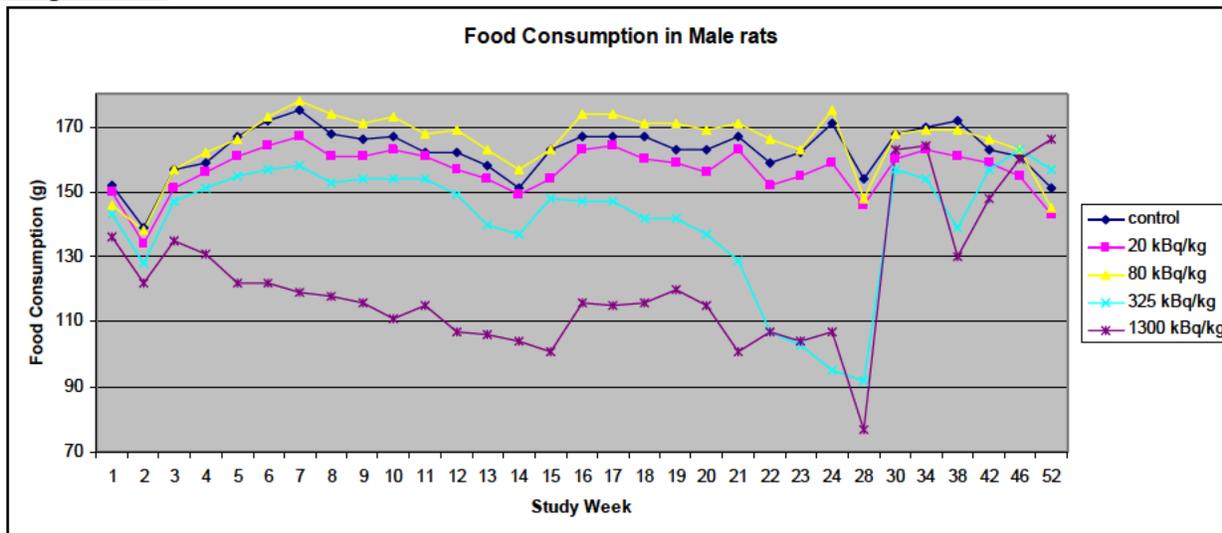


**Summary:** Treatment related, dose dependent, decreased body weights were observed in males and females during the study, and decreased body weights were statistically significant in Groups 4 (328 kBq/kg) and 5 (1300 kBq/kg). There were recovery trends; however, the observed lower body weights were not fully recovered after a year of recovery period.

Repeat dose

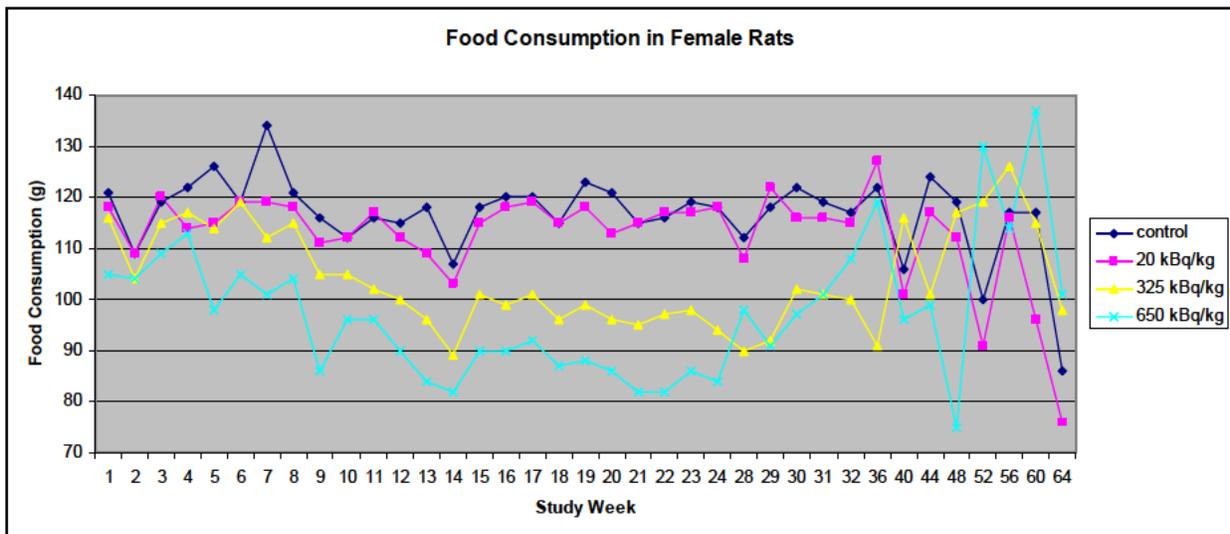
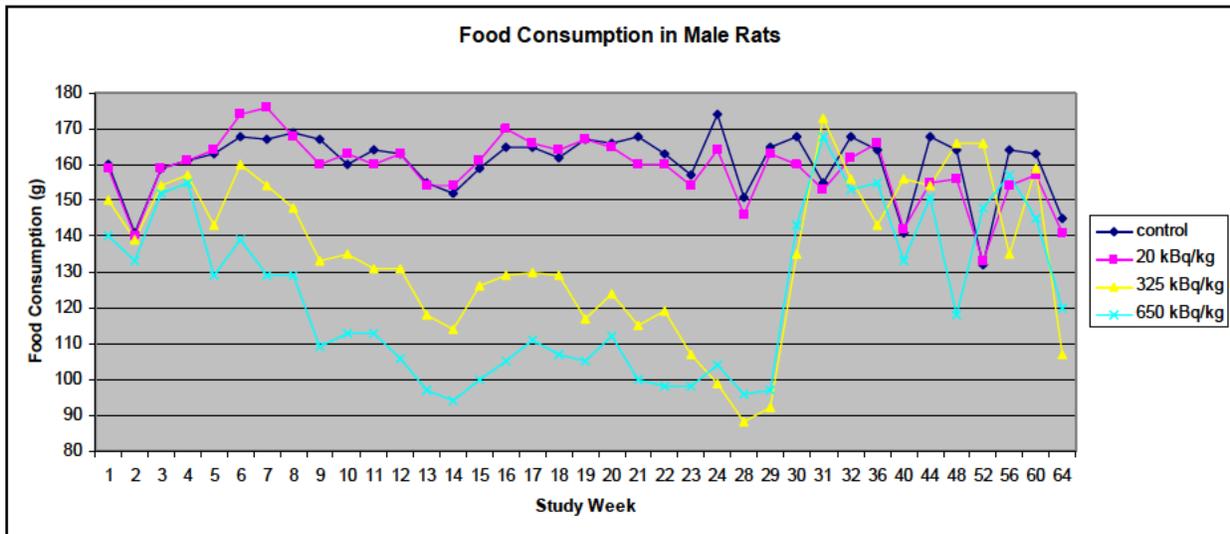
*Summary:* Body weights in all treatment groups were dose-dependently lower than the control group (↓ up to 33% in males, ↓ up to 22% in females), and the differences in body weights compared to the control group were statistically significant in MD, HD groups. There were recovery trends; however, the observed lower body weights were not fully recovered after a year of recovery period.

Food Consumption  
*Single dose*



**Summary:** Reduced food consumption (↓up to 37% in males and females) was observed in Groups 4 (150 kBq/kg) and group 5 (1300 kBq/kg) when compared to control group. Food consumption returned to normal levels after a year of recovery period.

Repeat dose



*Summary:* Reduced food consumption (↓up to 40% in males, ↓up to 29% in females) was observed in Groups 8 (325 kBq/kg) and group 9 (650 kBq/kg) when compared to control group. Food consumption returned to normal levels after a year of recovery period.

Ophthalmoscopy: unremarkable

Hematology  
Single dose

Gender	Percent Change From Vehicle Control							
	Male				Female			
	20	80	325	1300	20	80	325	1300
<b>Doses (kBq/kg)</b>								
Day 15								
Hemoglobin		-3	-9	-5		-3	-12	-13
Red Blood cells	-2	-4	-9	-7		-4	-14	-14
Hematocrit		-4	-8	-6		-3	-11	-13
White blood cells	-15	-43	-56	-71	-10	-20	-46	-64
Neutrophils -absolute	-22	-55	-76	-83	-17	-24	-72	-77
-percentage	-10	-25	-51	-49	-9	-7	-49	-37
Lymphocytes-absolute	-14	-41	-54	-70	-10	-19	-44	-63
-percentage							5	4
Eosinophils-absolute	-20	-80	-100	-100	-33	-67	-100	-100
-percentage	25	-10	-25	-75	-20	-60	-100	-70
platelet	-9	-10	-48	-74	-7	-18	-42	-72
Week 14								
Hemoglobin								-3
Red Blood cells			-4	-12		-4	-7	-15
White blood cells	-17	-17	-7	-24	-8	3	-12	-18
Neutrophils -absolute	-17	-14	-21	-73	-9	3	-12	-11
-percentage			-15	-7			6	
Lymphocytes-absolute	-17	-16	-3	-23	-8	4	-11	-18
-percentage								
Eosinophils-absolute	33	33		67			-37	-62
-percentage	50			70			-18	-18
platelet	-4	-12			5	4	11	7
APTT							17	26
Week 27								
White blood cells				-34				-25
Neutrophils -absolute				-25	-20	-10	-16	-34
-percentage					-22	-14	-14	-11
Lymphocytes-absolute	-15	-4		-35				-23
Eosinophils-absolute			-37	-12				
-percentage	60	50	-10	70				
platelet	-5	-12	-4	-20				
APTT							22	41
Pt	9		6	9				
Week 40								
Red Blood cells								-17
Neutrophils -absolute	4	-10	11	-40				
-percentage				-11				
Lymphocytes-absolute				-26				
Eosinophils-absolute	13	25	-25	75				

Gender	Percent Change From Vehicle Control							
	Male				Female			
Doses (kBq/kg)	20	80	325	1300	20	80	325	1300
-percentage	18	27		136				
platelet								-13
APTT							22	39
Week 52								
White blood cells	-21	-22	-22	-36				
Neutrophils -absolute					22	61	67	39
-percentage					39	63	63	72
Lymphocytes-absolute	-28	-24	-25	-39	-19	-15	-14	-27
Eosinophils-absolute	75	-100	-50	-100	20	-80	-40	-60
-percentage	175	-100	-50		29	-36	-29	-50
platelet		-10		-10	-5	-9	-6	-5
APTT								18

## Repeat dose

Gender	Percent Change From Vehicle Control					
	Male			Female		
Doses (kBq/kg)	20	325	650	20	325	650
Day 15						
Hemoglobin		-8	-11		-12	-13
Red Blood cells		-13	-14		-15	-14
Hemaocrit		-8	-10		-13	-13
White blood cells	-11	-58	-54	-16	-46	-43
Neutrophils -absolute	-28	-72	-77	-13	-71	-75
-percentage	-22	-36	-49		-47	-52
Lymphocytes-absolute	-10	-56	-52	-16	-44	-51
-percentage			6			
Eosinophils-absolute	-100	-87	-87	-70	-100	-100
-percentage	-62	-50	-87			
Platelet		80	46		-47	-57
Week 14						
Hemoglobin			-5		-8	
Red Blood cells		-19	-19		-19	-14
Hematocrit			-6			
White blood cells	-10	-46	-51	-17	-45	-43
Neutrophils -absolute	-31	-56	-56	-19	-52	-44
-percentage	-26	-28	-18		-22	-9
Lymphocytes-absolute	-6	-43	-49	-16	-44	-43
-percentage						
Eosinophils-absolute	300	-100	-100	-60	-100	-90
-percentage	-18	-27	-64	-20	-60	10

Platelet						-6
Week 27						
Hemoglobin			-7			-12
Red Blood cells			-11		-16	-18
Hemaocrit						-12
White blood cells	-16	-40	-47	-25	--47	-31
Neutrophils -absolute	-53	-53	-68	-24	-44	-29
-percentage	-44	-26	-44			
Lymphocytes-absolute	-5	-36	-39	-25	-48	-31
-percentage						
Eosinophils-absolute	-15	-62	-100	-50	-17	-83
-percentage	-13	-33	-73			
Platelet					-20	-13
Week 40						
Hemoglobin			-9			
Red Blood cells		-17	-18	-7	-12	-11
Hemaocrit						
White blood cells		-27	-17	-26	-24	-21
Neutrophils -absolute	-15	-26	-7			
-percentage						
Lymphocytes-absolute		-27	-21	-29	-26	-25
-percentage						
Eosinophils-absolute	-68	-79		-20	-20	-40
-percentage	-40	-40	20			
Platelet		-15	-16		-21	-29
Week 52						
Red Blood cells		-13	-19			
White blood cells		-18	-28			
Neutrophils -absolute	-22	-12	-44			
-percentage	-31		-29			
Eosinophils-absolute	-13	-50	-100			
-percentage			-70			
Platelet				-8	-15	-31
Week 64						
Hemoglobin			-9			
Red Blood cells		-11	-15			
White blood cells			-36			
Neutrophils -absolute						206
-percentage					44	118
Lymphocytes-absolute		-17	-39			
-percentage						
Eosinophils-absolute		-100	-100			
-percentage		-100	-100			



*Repeat dose (group 6-9)*

Dose (kBq/kg)	Number of death	Sex	Animal#	Days of death	Treatment related macroscopic findings
0	1	M	84	366	
	0	F	-	-	-
20	1	M	97	343	
	1	F	106	342	
325	5	M	115	411	Lower jaw: Mass Mandible: Hard swelling Lower jaw: Mass/nodule Lower jaw: Nodule Subcutis, mandible/head: Nodule
			116	320	
118			392		
119			385		
120			369		
5	F	122	348	Uterus: Polyp  Lower jaw: Mass Muscles on the shoulder: Mass	
		125	421		
		126	400		
		127	446		
		128	329		
650	6	M	129	190	Changes in urinary bladder Changes in urinary bladder
			131	251	
132			274		
134			435		
135			317		
136			317		
6	F	137	391	Uterus: Left horn thickened	
		139	335		
		140	323		
		142	410		
		143	366		
		144	335		

“-“ not applicable; blank-no significant finding

Terminal sacrifice:

*Single dose:* unremarkable

*Repeat dose*

Dose (kBq/kg)	Sex	Animal#	Treatment-related macroscopic findings
325	M	113	Tooth: Broken Lip: Thickened area
	F	123	Uterus: Left horn thickened
650	F	138	Head : Nodule

Organ Weights: unremarkable

## Histopathology

## Single dose:

*Unscheduled deaths*

Gender	Number of animal affected									
	Male					Male				
Dose (kBq)	0	20	80	325	1300	0	20	80	325	1300
No.of animals examined	0	1	0	2	3	0	0	1	4	2
<i>Kidney</i>										
Karyomegaly -minimal					1					
<i>Uterus</i>										
Polyp, endometrial stroma	-	-	-	-	-					2
<i>Bones</i>										
Osteosarcoma				1	1					
<i>Vertebrae</i>										
Osteosarcoma					1				2	
<i>Sternum</i>										
Depletion										
osteocytes/-blasts -minimal				2	1				1	1
-slight					1					1
Fibro-osseous lesion -minimal					1					
No.of animals examined	0	1	0	2	2	0	0	1	4	2
<i>Bone, femur</i>										
Depletion										
osteocytes/-blasts -minimal				2	1				4	
-slight					1					1
Hyperostosis -minimal				1					3	
-slight					2					1
Abnormal/disorganized physis				2	2				2	1

*Terminal sacrifice*

Gender	Number of animal affected									
	Male					Female				
Dose (kBq)	0	20	80	325	1300	0	20	80	325	1300
No.of animals examined	7	7	8	6	4	8	8	6	4	4
<i>Teeth</i>										
Fibril-osseous lesion (in the bone socket) -minimal					1					
-slight										1
No.of animals examined	8	7	8	6	5	8	8	7	4	6
<i>Kidney</i>										
Karyomegaly -minimal					4					
<i>Uterus</i>										
Polyp, endometrial stroma	-	-	-	-	-				1	3
<i>Bones</i>										
Osteosarcoma				1						
<i>Sternum</i>										
Depletion										
osteocytes/-blasts -minimal		5	2	2	2				1	1
-slight					3					4
-moderate										1
Fibro-osseous lesion -minimal					1					

Gender	Number of animal affected									
	Male					Female				
Dose (kBq)	0	20	80	325	1300	0	20	80	325	1300
Decreased cellularity, bone marrow	1		6	1		2	2	2	2	
-minimal		1	1	4	1	1	1			2
-slight				1	4					4
-moderate										
No.of animals examined	8	7	8	6	5	8	8	4	4	6
<i>Bone, femur</i>										
Depletion										
osteocytes/-blasts -minimal				4					4	1
-slight				2	5					4
-moderate										1
Hyperostosis -minimal				5	1	1			3	
-slight					4					6
Abnormal/disorganized physis			1	2	5				2	6

Repeat dose

*Unscheduled deaths*

Gender	Number of animal affected							
	Male				Female			
Dose (kBq)	0	20	325	650	0	20	325	650
No.of animals examined	1	1	3	3	0	0	4	5
<i>Teeth</i>								
Fibril-osseous lesion (in the bone socket) -slight					-	-	1	1
Ostcosarcoma (in the bone socket)			3		-	-		
No.of animals examined	1	0	4	6	0	0	5	5
<i>Kidney</i>								
Karyomegaly -minimal		-	4	5	-	-	3	3
-slight		-		1	-	-		2
<i>Uterus</i>								
Polyp, endometrial stroma	-	-	-	-	-	-	5	2
No.of animals examined	1	1	4	6	0	0	5	5
<i>Bones</i>								
Osteosarcoma			3		-	-		
<i>Bone, femur</i>								
Depletion								
osteocytes/-blasts -minimal			1	1	-	-	2	
-slight			3	2	-	-	3	
-moderate				3	-	-		5
Hyperostosis -minimal					-	-		
-slight			4	5	-	-	5	5
-moderate				1	-	-		
Abnormal/disorganized physis			4	6	-	-	5	5
<i>Sternum</i>								
Depletion								
osteocytes/-blasts -minimal			1		-	-	4	
-slight			3		-	-	1	
-moderate				5	-	-		5
-marked				1	-	-		
Fibro-osseous lesion -minimal				1	-	-		2
-slight			1	2	-	-		1

*Terminal sacrifice*

Gender	Number of animal affected							
	Male				Female			
Dose (kBq)	0	20	325	650	0	20	325	650
No.of animals examined	7	7	4	2	8	8	3	3
<i>Teeth</i>								
Fibril-osseous lesion (in the bone socket) -slight							1	
-moderate				2				
Osteosarcoma (in the bone socket)			1				1	1
No.of animals examined	7	7	4	2	8	8	3	3
<i>Kidney</i>								
Karyomegaly -minimal			3	1			1	1
-slight				1				2
<i>Uterus</i>								
Polyp, endometrial stroma	-	-	-	-			1	2
<i>Bones</i>								
Osteosarcoma			2					
<i>Bone, femur</i>								
Depletion osteocytes/-blasts -minimal							3	
-slight			4					
-moderate				2				3
Hyperostosis -slight			4	2			3	3
Abnormal/disorganized physis		1	4	2		2	3	3
<i>Sternum</i>								
Depletion osteocytes/-blasts -minimal	1		1	1			2	
-slight			3				1	
-moderate				1				3
Fibro-osseous lesion -minimal								2
-slight				1				

**Summary:** Treatment related changes included changes in the bone socket of the teeth, instances of karyomegaly in the kidneys, endometrial stroma polyps in the uterus, extramedullary haematopoiesis in the spleen and osteocyte depletion and fibro-osseous lesion in bone. Osteosarcomas were observed in both the single- and repeat-dose groups and occurred mainly in the 325 or 4 x 325 kBq/kg group.

**Toxicokinetics:** refer to PK section of this review

**STUDY SUMMARY:**

After single intravenous injections to rats of 20, 80, 325 or 1300 kBq/kg or 4 intravenous injections with 1 month intervals of 20, 325 or 650 kBq/kg of radium-223 dichloride, decreased body weights and reduced food consumption were observed within the first 6 months of the study. In addition, changes in clinical pathology parameters were observed starting from study day 14 after the 1<sup>st</sup> dose; which included dose-dependant decreases in the white blood cell counts and decreased values of hemoglobin, red blood cells, hematocrit and platelets. The changes were more severe

after repeat doses. The treatment-related hematologic changes were improved over time and after a 12-month recovery period, most changes (except for WHC/lymphocytes) were recovered. The histopathological examination revealed treatment related changes in the bone socket of the teeth, kidneys, uterus, spleen, and bone. The treatment related changes included malignant bone tumors (osteosarcomas). The changes in clinical pathology correlated with the histopathological findings, which are considered related to radiation toxicity.

**Study title:                   Alpharadin (radium-223)  
12-Month Repeat Dose Toxicity study in Rats**

Study no.: 67071

Study report location: Electronic submission, M4. pages 1-954

Conducting laboratory and location: (b) (4)

Date of study initiation: October 21, 2007

GLP compliance: yes

QA statement: yes ( X ) no ( )

Drug, lot #, and % purity: BAY 88-8223,

Batch No	% radiochemical purity
A710008	>99
A711009	>99
A712006	>99
A801004	>99
A802002	>99
A803002	>99
A804001	>99
A805003	>99
A806011	>99.9
A807001	>99.9
A808005	>99.9
A808012	>99.9

**Key Study Findings**

- Treatment-related death was observed at all dose groups 7 months after starting the treatment;
- Unscheduled deaths were mainly due to osteosarcomas;
- Animals at MD and HD started to loose their upper teeth from the 7th month of treatment;
- Dose-dependent lower body weights and food consumption were observed;
- Decreases in white blood cells and in red blood cells accompanied by an increase in reticulocytes were observed;
- osteosarcomas were observed in bone tissue and skeletal muscle.

## Methods

Doses: 25, 50, 100 kBq/kg\*  
 \* the basis for dose selection was not provided

Frequency of dosing: once every 4 weeks for 48 weeks (a total of 12 injections) followed by a 4-week recovery period

Route of administration: i.v.

Dose volume: approximately 2 ml/kg\*  
 \* volume correction according to physical decay of radium-223.

Formulation/Vehicle: in a sodium citrate buffer with no preservative, traces of strontium and calcium

Species/Strain: SPF Wistar rats

Number/Sex/Group: 12/sex/group

Age: approximately 7-8 weeks old

Weight: Males: 206-231 g; females: 160-187 g

Satellite groups: none

Unique study design: none

Deviation from study protocol: Yes, no major impact on the study results

## Observations and Results

**OBSERVATIONS AND TIMES:**

<u>Mortality</u>	daily
<u>Clinical examinations</u>	Two times daily
<u>Detailed physical examinations</u>	Weekly Examining the condition of the teeth: weekly starting from the 3rd week of the study.
<u>Body weights</u>	weekly
<u>Food consumption</u>	weekly
<u>Condition of teeth</u>	Weekly
<u>Clinical Pathology:</u>	before the start of treatment and every 4 weeks (23 days post-dosing or 3 days before the next dosing) thereafter
<u>Gross pathology:</u>	All animals at death or at scheduled sacrifice, 4 weeks after the last treatment
<u>Organ weights:</u>	All animals, at death or at scheduled sacrifice
<u>Histopathology:</u>	Including a bone marrow smear in all animals Adequate Battery: yes (x), no ( ), Peer review: yes (x), no ( )

**RESULTS:**

## Mortality

Dose (kBq/kg)	Number of death	Sex	Animal#	Days of death
0	0	M	-	-
	0	F	-	-
25	4	M	25	329
			28	307
30			273*	
33			294	
	1	F	40	322
50	7	M	49	294
			51	283
			52	310
			53	282
			54	322
			55	224
			59	223
	3	F	63	330
			65	322
			69	328
100	3	M	77	271*
			82	331
			83	282
	7	F	86	269*
			87	260
			88	329
			89	330
			90	281
			92	293
		95	293	

\* found dead

*Summary:* Mortality was observed in all animals treated with radium-223 dichloride. The treatment-related mortalities were dose-dependent and time-dependent, and are considered to be treatment related.

Clinical Signs  
 Unscheduled deaths

Dose (kBq/kg)	Sex	Animal #	Day of death	Clinical signs	neoplasms
25	M	25	329	Lame on fore leg, sore	Follicular cell adenoma in Thyroid gland
		28	307	Lame. Large swelling on hindleg	Osteosarcomas
		30	273	Found dead	Osteosarcomas
		33	294	Lame. Bladder enlarged, in pain when palpated in abdomen	Mammary gland: carcinoma
	F	40	322	Piloerection, swollen mandible, swelling between front teeth in mandible	Osteosarcomas
50	M	49	294	Large swelling in shoulder/neck	Osteosarcomas
		51	283	Pale mucous membranes,	Large granular lymphocyte lymphoma in multiple organs
		52	310	Lame on foreleg	Osteosarcomas
		53	282	Lame on both hind legs	Osteosarcomas
		54	322	Partly lame on both hind legs.	Osteosarcomas
		55	224	Uncoordinated movements.	Osteosarcomas
		59	223	Piloerection Passive, laboured respiration Quickly growing mass in the neck area	Osteosarcomas/ Lung adenoma
	F	63	330	Less active, piloerection, lame on foreleg	Osteosarcomas
		65	322	Swelling on foreleg and hind leg	Osteosarcomas
69		328	Lame on foreleg	Osteosarcomas	
100	M	77	271	d.267-269: some dry loose stool around anus	Osteosarcomas
		82	331	Swelling on back, lame on hind leg	Osteosarcomas
		83	282	Subdued, piloerection, circular movements	Osteosarcomas
	F	86	269	Unremarkable	
		87	260	d.258-260:Subdued, squinting.	Cerebrum Oligodendroglioma
		88	329	Squealing, Piloerection	Osteosarcomas
		89	330	Swelling and lame on hindleg	Osteosarcomas
90		281	Lame on foreleg	Osteosarcomas	
92	293	Swelling and lame on hindleg	Osteosarcomas		
95	293	Swelling and lame on hindleg	Osteosarcomas		

Terminal sacrifices: unremarkable except for observation in teeth

## Missing, reduced and irregularly growing teeth (early deaths and terminal deaths)

day of study	Numbers of animals affected							
	Males				Females			
	control	LD	MD	HD	control	LD	MD	HD
21-182								
189			1	3				6
196			1	3				6
203			1	4				7
210				10				8
217				11				11
224				12				12
231				12				12
238			7	12			1	12
245			8	12			1	12
252			8	12			1	12
259			8	12			2	12
266			8	12			2	11
273			8	11			2	10
280			8	11			3	10
287			8	10			5	9
294			7	10			6	7
301			7	10			2	7
308			8	10			12	6
315			6	10			12	7
322			6	10			11	7
329			5	10		1	8	7
336			5	9			-	-

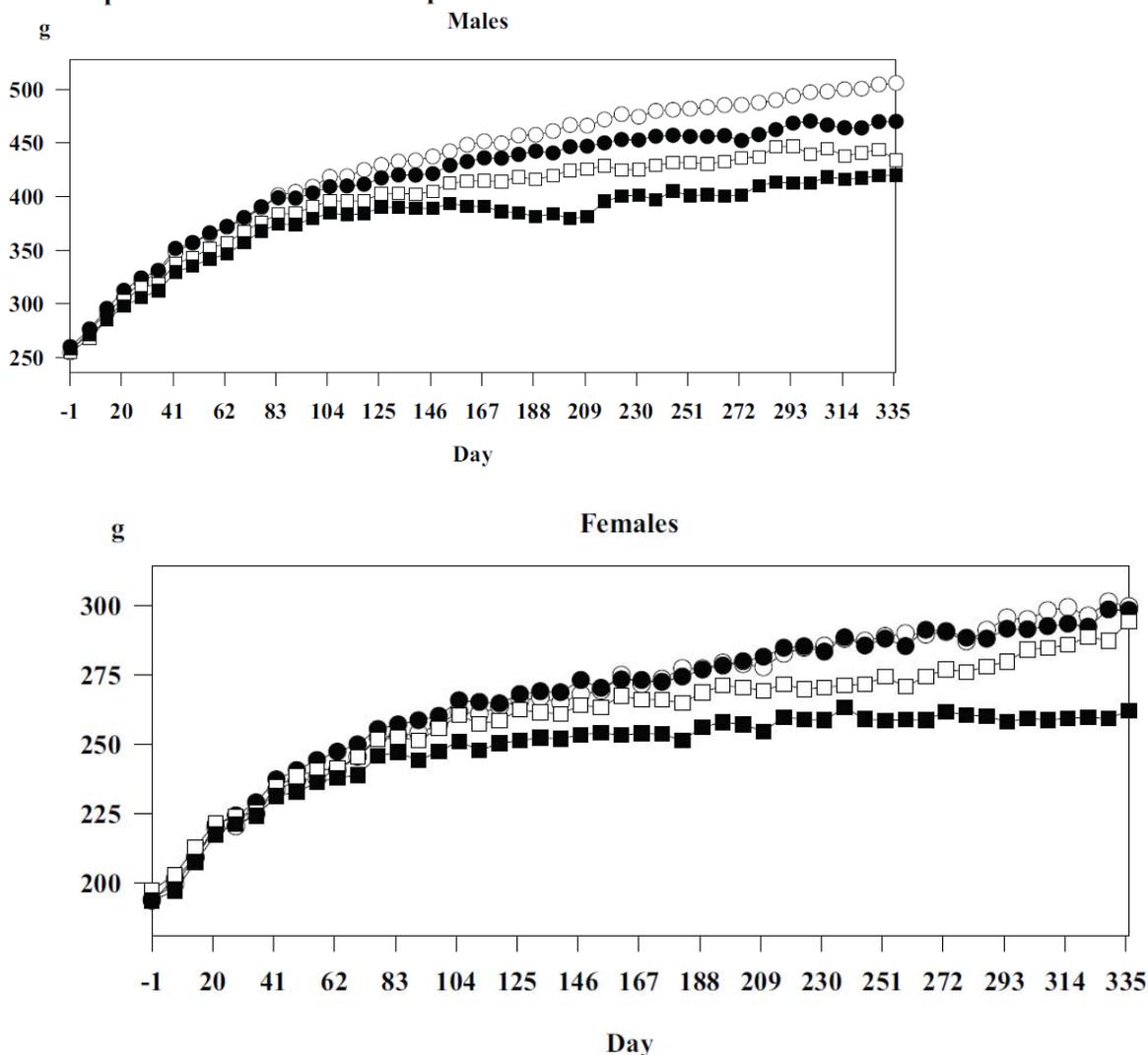
*Summary:* The observed clinical signs were generally secondary to treatment-induced osteosarcomas. Treatment related changes in teeth became clearly pronounced at the beginning of the 7<sup>th</sup> month of the study. In the highest dose group (Group 4), the loss of the teeth started to occur earlier and affected more animals than in Group 3. There was a sex difference in the occurrence of loss of teeth in Group 3: males of this group started to loose teeth at least one month earlier than females, and there were more males affected than females at all time points. No tooth loss was observed in animals in Groups 1 and 2.

#### Body Weights

The following figures are copied from the Applicant's submission; the data are verified by the reviewer.

## Summary of body weight (g)

- Group 1      ● Group 2  
 □ Group 3      ■ Group 4

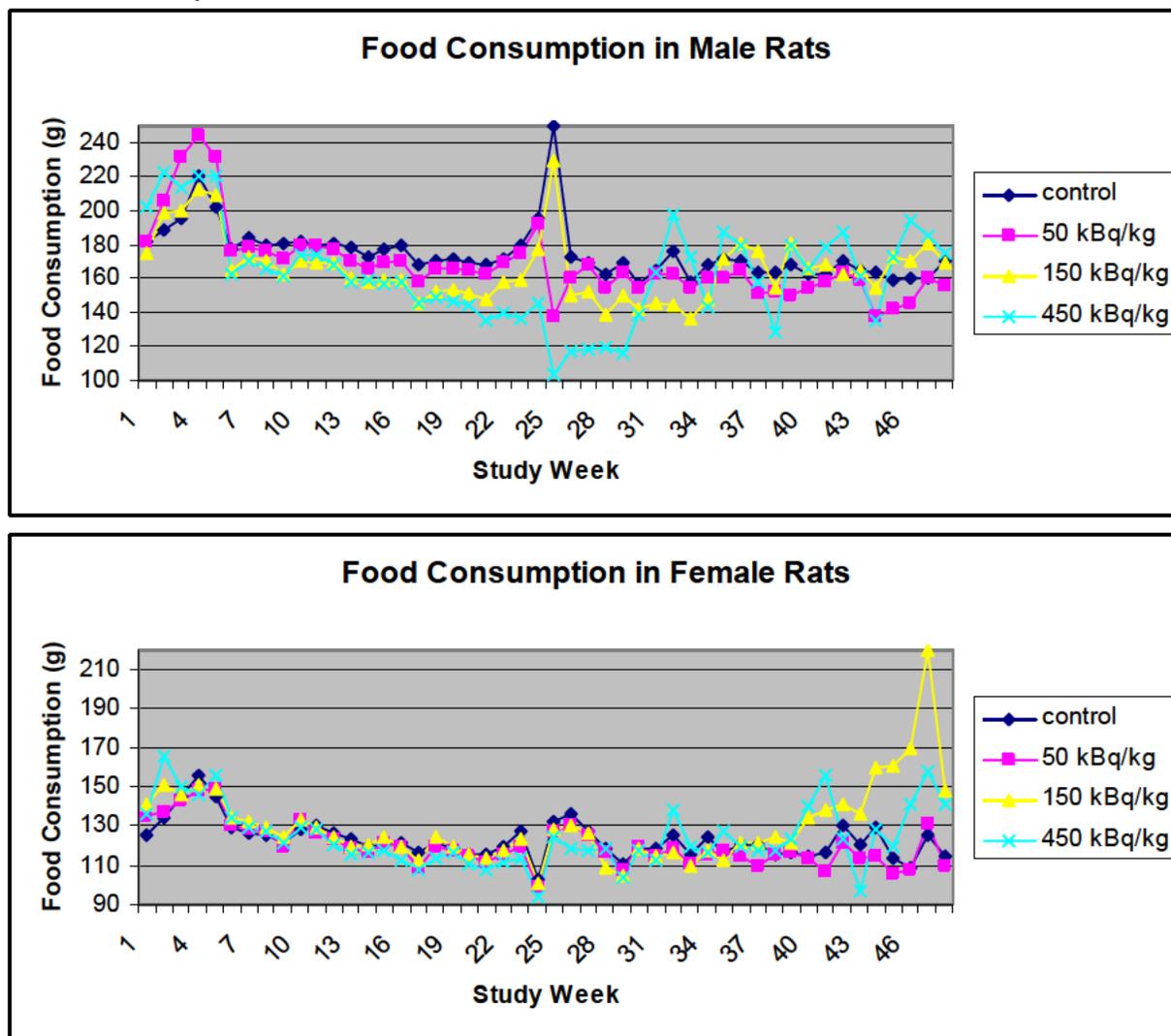
**Summary:**

Male: Body weights were dose- and time-dependently lower than the control group in all treatment groups. In HD, the difference in body weight comparing to the control group became statistically significant starting from Day 35 (↓5%) and remained significant throughout the study period. In the MD, the difference from the control group became statistically significant from Day 119 (↓7%) and thereafter throughout the study period. In the LD, lower body weight became statistically significantly from the end of the 9th month of the study (Days 273, ↓7%) until the end of the study period. Body weight gain over the whole study period was statistically significantly lower in Groups 2 - 4 compared to the control group.

Female: Body weights were dose-dependently lower than the control group in females at doses  $\geq 50$  kBq/kg. Statistically significantly lower body weight was observed in

Group 4 females from Day 161 ( $\downarrow 8\%$ ) and throughout the rest of the study period. Body weight gain over the whole study period was statistically significantly lower in HD compared to the control group.

### Food Consumption



*Summary:* Lower food consumption in treatment groups was observed in male rats after week 9. The reduction in food consumption was dose-dependent, and a prominent reduction was observed in the period between week 15-29 ( $\downarrow$  up to 59%). Changes of food consumption with the treatment were not remarkable in female rats.

## Hematology

Gender	Percent Change From Vehicle Control					
	Males			Females		
	Dose (kBq/kg)	50	150	450	50	150
WBC						
Day -7	-13	-8	-11	12	14	2
3 days before #2 injection	-17	-20	-23	7	7	-20
3 days before #3 injection	-14	-14	-20	-19	-7	-32
3 days before #4 injection	-11	-20	-33	3	9	-6
3 days before #5 injection	-16	-24	-51	-8	-3	-30
3 days before #6 injection	-24	-34	-43	-5	-16	-24
3 days before #7 injection	-29	-36	-44	-17	-24	-36
3 days before #8 injection	-28	-36	-41	-7	-21	-32
3 days before #9 injection	-26	-34	-40	-19	-28	-25
3 days before #10 injection	-40	-54	-46	-4	-15	-27
3 days before #11 injection	-34	-44	-56	-16	-23	-28
3 days before #12 injection	-25	-30	-49	-14	-21	-23
25 days after treatment	-24	-13	-46	-8	-26	-21
RBC						
Day -7		-8				-7
3 days before #2 injection		-4	-8		-7	-10
3 days before #3 injection		-4	-11		-6	-11
3 days before #4 injection	-3	-7	-15		-6	-12
3 days before #5 injection	-11	-9	-12		-10	-16
3 days before #6 injection	-7	-13	-15		-9	-16
3 days before #7 injection	-6	-13	-13		-12	-17
3 days before #8 injection	-11	-15	-19		-7	-12
3 days before #9 injection	-10	-22	-23		-10	-14
3 days before #10 injection	-11	-15	-14		-14	-20
3 days before #11 injection	-11	-15	-15		-13	-19
25 days after treatment	-14	-18	-15		-16	-15
RETIC						
Day -7	2			10		10
3 days before #2 injection	4		9			10
3 days before #3 injection	13	17	43	19	22	41
3 days before #4 injection	10	14	24	5	14	19
3 days before #5 injection	10	14	52		15	35
3 days before #6 injection	6	22	44	24	23	41
3 days before #7 injection	-5	11	11	13	50	50
3 days before #8 injection		32	26	18	41	65
3 days before #9 injection	5	35	-5	-16	-8	12
3 days before #10 injection	10	14	10	5	30	40
3 days before #11 injection		30	25	21	32	47
3 days before #12 injection	20	45	25	17	28	50
25 days after treatment		5	26	35	53	47

Blank: unremarkable changes.

*Summary:* In both in males and females, white blood cells were lower from the 1st month of treatment (note: counts for neutrophil, lymphocyte, and eosynophil were all lower); decreases in red blood cells were observed from the 1st or the 2nd month of treatment, accompanied by an increase in reticulocytes (indicating increased erythrocyte regeneration). These changes were dose- and time-dependent and did not resolve 25 days after the last dose.

#### Clinical Chemistry

Alkaline phosphatase (ALP) levels were significant higher in Group 4 after the 10<sup>th</sup> dose (↑98%) in males and after the 12th dose (↑66%) in females.

Albumin (and Albumin/Globulin ratio) was significantly lower in Groups 2-4 males after the 10<sup>th</sup> dose (↓up to 12%).

Above changes did not resolve after a 4 week recovery period.

Urinalysis: Not performed

#### Gross Pathology

##### Unscheduled deaths

Dose (kBq/kg)	Sex	Animal#	Days of death	observation
25	M	25	329	Discoloration of the skin on foreleg White spots in lungs, nodule in skeletal muscle on leg Urinary bladder enlarged with a reddish content Urinary bladder enlarged
		28	307	
		30	273	
		33	294	
	F	40	322	Nodules in head (mandible) and in clitoral gland region
50	M	49	294	Nodule in second thoracic mammary gland Pale pituitary, liver and parathyroid discolored orange, spleen and heart enlarged Nodule on upper leg Urinary bladder enlarged and contents cloudy Edema in prostate gland, attachment to bladder Nodules in lung, enlarged pituitary, diminished testes, haemorrhage in thymus, dilated urinary bladder Nodule in vertebrae, dilated urinary bladder Nodule in mandibular region
		51	283	
		52	310	
		53	282	
		54	322	
		55	224	
		59	223	
	F	63	330	Sore in head region
		65	322	Nodule on foreleg
		69	328	Foreleg sampled due to clinical observation
100	M	77	271	Nodule in spinal cord, enlarged urinary bladder Nodule in spinal cord, red focus in lung, enlarged thyroid gland
		82	331	
		83	282	

				Nodule in joint
	F	86	269	Unremarkable
		87	260	Unremarkable
		88	329	Nodule on leg, dilated urinary bladder, red discoloration of vagina and lung
		89	330	Ovaries: Cyst
		90	281	Nodule on lower leg
		92	293	Nodule on lower leg
		95	293	Nodule on lower leg

Terminal sacrifice: unremarkable

Organ Weights:

Male: Mean weight of spleens, both absolute (↑up to 39%) and relative to body weight (↑up to 60%) or to brain weight (↑up to 44%), was significantly higher in all dose groups.

Female: Mean weight of spleens, both absolute (↑25%) and relative to body weight (↑27%) or to brain weight (↑25%), was significantly higher in female rats in group 3.

Note: The findings were consistent with high level of hematopoiesis observed in hematological tests.

Histopathology

Unscheduled deaths

Dose (kBq/kg)	Sex	Animal #	Days of death	Findings
25	M	25	329	Fracture w. repair
		28	307	Osteosarcoma in lung and in nodule on leg
		30	273	unremarkable
		33	294	unremarkable
	F	40	322	Osteosarcoma in skeletal muscle in head. Carcinoma in mammary gland region
50	M	49	294	Osteosarcoma
		51	283	Lymphocyte lymphoma in brain, liver, spleen and heart
		52	310	Osteosarcoma
		53	282	Interstitial diffuse edema in prostate gland
		54	322	Osteosarcoma in lungs, cyst in pituitary, tubular atrophy in testes
		55	224	Osteosarcoma in cervical vertebrae region
		59	223	Osteosarcoma
	F	63	330	Focal fibrosis
		65	322	Osteosarcoma
69		328	Osteosarcoma	

100	M	77	271	Osteosarcoma in spinal cord region Osteosarcoma in lungs and spinal cord region. Follicular cell adenoma (benign neoplasm) in thyroid gland Osteosarcoma
		82	331	
		83	282	
	F	86	269	Unremarkable
		87	260	Unremarkable
		88	329	Osteosarcoma in skeletal muscle on leg, perivaginal hemorrhage, Congestion in the lung, associated with alveolar hemorrhage
		89	330	Unremarkable
		90	281	Osteosarcoma
		92	293	Osteosarcoma
		95	293	Osteosarcoma

Terminal sacrifices:*Non-neoplastic changes*

In femur, a decrease in bone mass (occasionally associated with focal fibrosis) and the presence of abnormal/disorganized growth lines was reported in all treated animals evaluated. This finding was characterized by necrosis/loss of chondrocytes, mineralization of the growth line and in several animals accompanied by a decrease in the bone mass/ fibrosis (often at the endosteal surface of the diaphysis). This disorganisation was also observed in cartilage elements of other organs eg. sternum and vertebrae.

In addition, depletion of osteoblasts/osteocytes was reported in the HD females.

In sternum, minimal to slight decreased cellularity of the bone marrow was reported in HD males.

One male at 50 kBq/kg had marked tubular atrophy in the testes and oligospermia in the epididymides.

*Neoplastic changes: Osteosarcomas*

The following table is the summary of neoplastic changes and non-neoplastic changes in all animals (unscheduled and scheduled deaths). The tables are copied from the Applicant's submission; the data was verified by the reviewer.

*Neoplastic changes*

## The incidences of osteosarcomas

Dose group	Group 1		Group 2		Group 3		Group 4	
	Control		25 kBq/kg		50 kBq/kg		100 kBq/kg	
Sex	M	F	M	F	M	F	M	F
BONE FEMUR/BONE No. animals examined	12	10	4	1	7	3	12	12
<b>Osteosarcoma, primary tumor, No. animals</b>	0	0	0	0	2	3	2	1
LUNG No. animals examined	12	12	4	1	7	3	12	12
<b>Osteosarcoma, metastasis, No. animals</b>	0	0	2	0	3	0	2	4
SKELETAL MUSCLE No. animals examined	12	12	4	1	7	3	12	12
<b>Osteosarcoma, metastasis, No. animals</b>	0	0	1	1	2	0	1	4
SKIN/SUBCUTIS No. animals examined	12	12	4	2	8	3	12	12
<b>Osteosarcoma, metastasis, No. animals</b>	0	0	0	0	1	1	0	0
ADRENAL GLAND No. animals examined	12	12	4	1	7	3	11	12
<b>Osteosarcoma, metastasis, No. animals</b>	0	0	0	0	0	0	0	1

*Summary:* Osteosarcomas were demonstrated in 19 (18 decedents + 1 non-decedent) treated animals (Group 2: 3 animals, Group 3: 8 animals, Group 4: 8 animals), with several of the animals having tumours in more than one organ.

Osteosarcomas were observed in bone tissue and skeletal muscle at different body locations and metastases of osteosarcoma origin were observed in the lungs and the adrenal gland.

non-neoplastic

The following tables are copied from the Applicant's submission; the data was verified by the reviewer.

## Bone femur

Dose group	Group 1		Group 2		Group 3		Group 4	
	Control		25 kBq/kg		50 kBq/kg		100 kBq/kg	
Sex	M	F	M	F	M	F	M	F
Number of animals examined	12	10	4	1	7	3	12	12
<b>BONE FEMUR</b>								
Depletion osteoblasts/osteocytes (No. animals, decedents)			(1)		(2)			(6)
No. animals, total	0	0	1	0	2	0	1	10
Grade 1 – minimal	-	-	1	-	2	-	1	10
Bone depletion/fibrosis With disorganized growth line, No. animals, total	0	0	4	1	7	3	12	12
Grade 1, minimal	-	-	3	0	5	1	0	0
Grade 2, slight	-	-	1	1	2	2	2	5
Grade 3, moderate	-	-	0	0	0	0	10	7
Hyperostosis (No. animals decedents)							(0)	
No. animals	0	0	0	0	0	0	1	2
Grade 2 – slight	-	-	-	-	-	-	0	1
Grade 3- moderate	-	-	-	-	-	-	1	0
Grade 4, marked	-	-	-	-	-	-	0	1

*Summary:* In femur, a decrease in bone mass (occasionally associated with focal fibrosis) and the presence of abnormal/disorganized growth lines was reported in all treated animals evaluated. This finding was characterized by necrosis/loss of chondrocytes, mineralization of the growth line and in several animals accompanied by a decrease in the bone mass/fibrosis (often at the endosteal surface of the diaphysis).

## Sternum

Dose group	Group 1		Group 2		Group 3		Group 4	
	Control		25 kBq/kg		50 kBq/kg		100 kBq/kg	
Sex	M	F	M	F	M	F	M	F
Number of animals examined	12	12	4	1	7	3	12	12
<i>STERNUM</i>								
Decreased cellularity Bone marrow (No. animals decedents)					(3)		(3)	(1)
No. animals, total	0	0	0	0	3	0	10	2
Grade 1 - minimal	-	-	-	-	3	-	4	0
Grade 2 – slight	-	-	-	-	0	-	6	2
Bone depletion/fibrosis Occ. with disorganized growth line.								
No. animals, total	0	0	0	0	0	0	12	12
Grade 2- slight	-	-	-	-	-	-	12	12

*Summary:* In sternum, minimal to slight decreased cellularity of the bone marrow was reported in the majority (10/12) of high-dose males. In addition a decrease in bone mass often associated with the presence of fibrous tissue in the marrow cavity/endosteal surface of bone was demonstrated in all high-dose animals.

Toxicokinetics: refer to PK section of this review

**STUDY SUMMARY:**

Treatment related clinical signs started to appear after 6 months of monthly intravenous injection. Treatment-related mortalities were observed in all dose groups, and osteosarcoma was the main cause of death. Dose-dependent body weight and food consumption were observed, possibly due to non-functional teeth or weak physical condition resulting from osteosarcoma. Hematologic changes included decreased RBC counts accompanied by an increase in reticulocytes starting from the 1st or the 2nd month of treatment, and decreased WBC counts starting after the first month of treatment. These hematological changes were most pronounced during the first 6 months of the study, were relatively stable thereafter, but were not fully recovered after a 4-week recovery period. Increases in ALP levels occurred in some of the Group 4 animals (both males and females) at the end of the study period. The osteosarcomas observed are considered related to the radiation treatment. Additional neoplastic lesions observed in this study included mammary gland carcinoma in one 25 kBq/kg female and lymphoma in multiple organs in one 50 kBq/kg male. Non-neoplastic lesions included bone depletion/fibrosis associated with disorganized growth lines, osteocyte depletion and decreased cellularity of bone marrow in femur and sternum. The increased severity of extramedullary haematopoiesis in the white pulp of the spleen

correlated with the observed increase in spleen weight and is considered to be secondarily related to the treatment.

**Study title: Long term radiotoxicity study of repeat dose intravenous radium-223 in normal dogs**

Study no.: MS RA2

Study report location: M4.2.3.2, page 1-145

Conducting laboratory and location: (b) (4)

Date of study initiation: March 1, 2008

GLP compliance: yes

QA statement: yes ( X ) no ( )

Drug, batch #, and % purity: Radium-223, A803004, >99% (radiochemical purity)

**Key study findings:**

- Dogs were well tolerated to radium-223 dichloride at a dose of 50 kBq/kg given monthly for a total of 6 treatments;
- A decrease in the granulocyte and platelet counts were observed with a maximum decrease after the 2nd injection and with gradual recovery over the remainder of the study period;
- The mean myeloid:erythroid (M:E) ratio of bone marrow aspirates was lower in the radium-223 dichloride treated dogs after the third test article injection; however, there was no difference in the mean M:E ratio between control and radium-223 dichloride treatment groups after the sixth test article injection;
- There was a general decline in the sternal and/or vertebral bone marrow hematopoietic cellularity in the radium-223 dichloride treatment group.

**METHODS:**

<b>Doses:</b>	50 kBq/kg
<b>Dose Justification:</b>	The dose level was determined based on doses used in nonclinical studies and human phase I and II trials and the results of the biodistribution and acute toxicity trial in normal beagle dogs (Study MSRA1).
<b>Controls:</b>	2ml 0.9% sodium chloride
<b>Species/strain:</b>	Beagle dog
<b>Age</b>	1.4-3.5 years old
<b>Weight</b>	7-12 kg
<b>Number/sex/group</b>	2/sex (control) 4/sex (treatment group)

<b>Route, formulation, volume</b>	IV at a dose volume of 2 mL with 0.9% sodium chloride, Control vehicle: 2ml 0.9% sodium chloride
<b>Satellite groups used for toxicokinetics:</b>	None
<b>Study design:</b>	Male and female dogs were dosed once every 4 weeks for 6 treatments Note: only one dose level was included in the study.

**OBSERVATIONS AND TIMES:**

<u>Mortality</u>	Daily
<u>Clinical examinations</u>	Daily
<u>Detailed physical examinations</u>	Not performed
<u>Body weights</u>	on arrival, during the acclimation period, each day of test article administration, weekly throughout the study and immediately prior to euthanasia
<u>Food consumption</u>	Not recorded
<u>Hematology and coagulation</u>	14 days and 25 days after each test article injection for hematology testing; and on the day of the 4th, 5th and 6th test article injections and immediately prior to euthanasia
Bone marrow evaluation	Once prior to test article administration, and 25 days after the 3rd test article administration and again 25 days after the 6th test article administration.
<u>Serum chemistry</u>	at baseline and once per month on day 25 post test article injection
<u>Urinalysis</u>	at baseline and 25 days after each test article injection
<u>Urine metabolite profiling</u>	on study days -8, 0 and 176
<u>Ophthalmoscopy</u>	prior to starting the study and during the week prior to euthanasia and necropsy
<u>EKG</u>	weeks -2, 25 and 31
<u>Gross pathology:</u>	All animals sacrifice, approximately 35 days after the 6th test article administration
<u>Organ weights:</u>	All animals sacrifice, approximately 35 days after the 6th test article administration
<u>Histopathology:</u>	All animals sacrifice, approximately 35 days after the 6th test article administration Adequate Battery: yes (x), no ( ) Peer review: yes (x), no ( )
<u>Toxicokinetics:</u>	Radioactivity levels of 1ml whole blood samples were measured immediately before

	<p>and 5, 15, 30, 45 minutes and 1, 2, 4, 6, 8 and 12 and 24, 48 and 72 hours after test article administration.</p> <p>Radioactivity levels of a 1ml urine sample were measured before and 1, 2, 4, 8, 12, 24, 48 and 72 hours after test article administration.</p> <p>Fecal radioactivity was measured on a 1cm<sup>3</sup> fecal sample at 12, 24, 48, 72, 96 and 168 hours after test article administration.</p>
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## RESULTS:

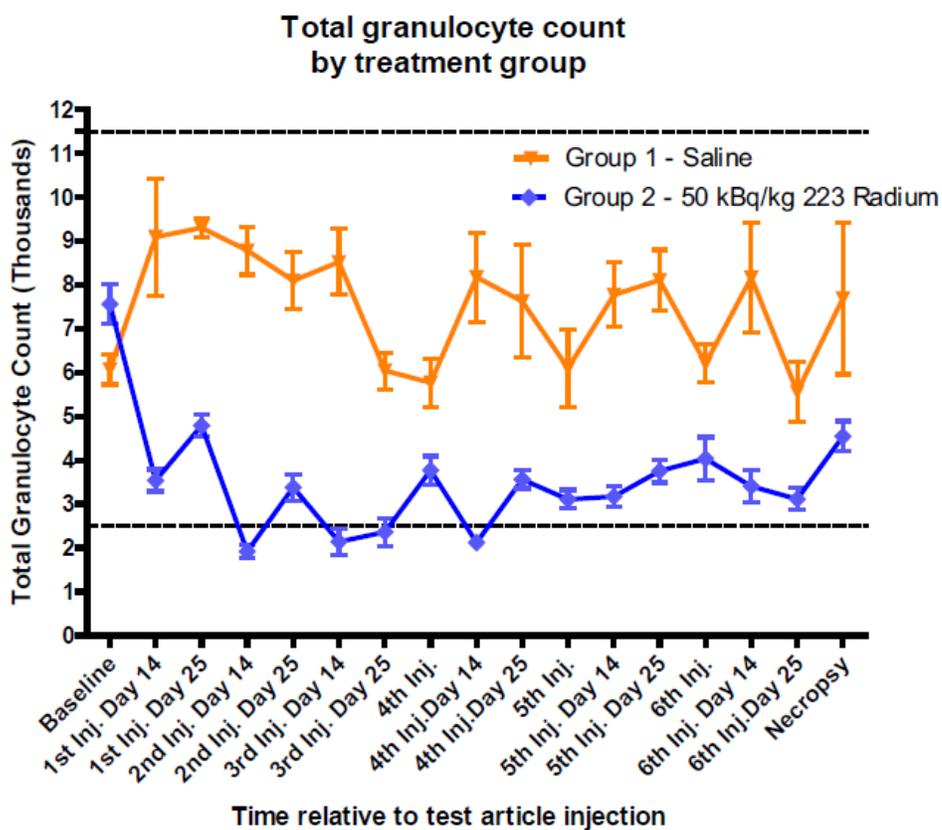
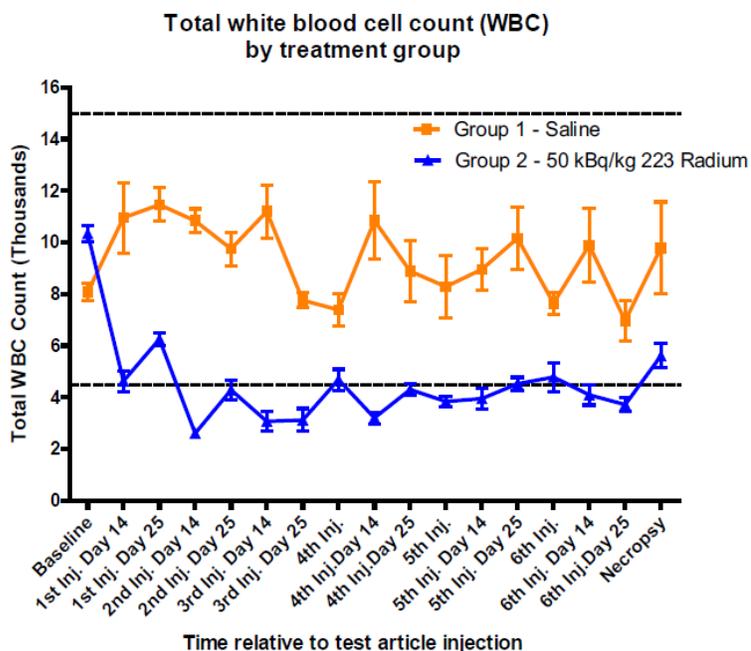
Mortality: none

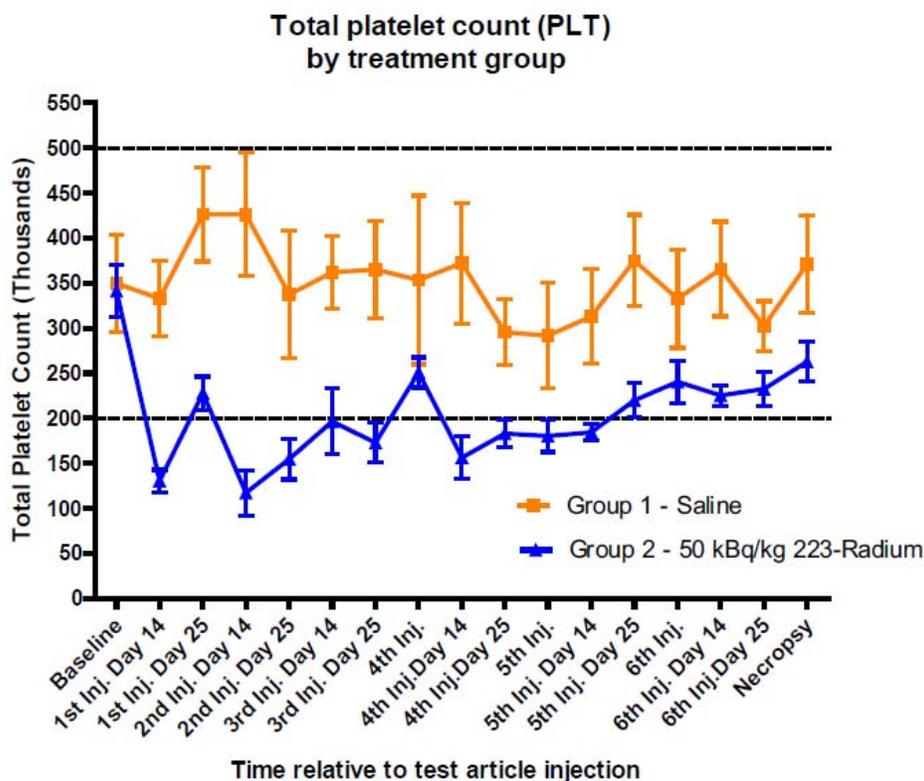
Clinical signs: Two females in the radium-223 dichloride treatment group (housed together) developed pelvic fractures 14 and 28 days after the 5th test article administration, respectively.

Note: Dual energy X-Ray absorption (DEXA) scans of hemipelves, proximal femur and the lumbar vertebral unit were included as a study protocol amendment. No difference in bone mineral density (BMD) of the bones was detected between the control and the treatment group. The Applicant concluded that the dogs most likely suffered a traumatic incident that resulted in the fractures.

Bodyweight: unremarkable

Hematology: The following figures are copied from the Applicant's submission; the data was verified by the reviewer. Note that there is no significant gender difference with the observed changes.



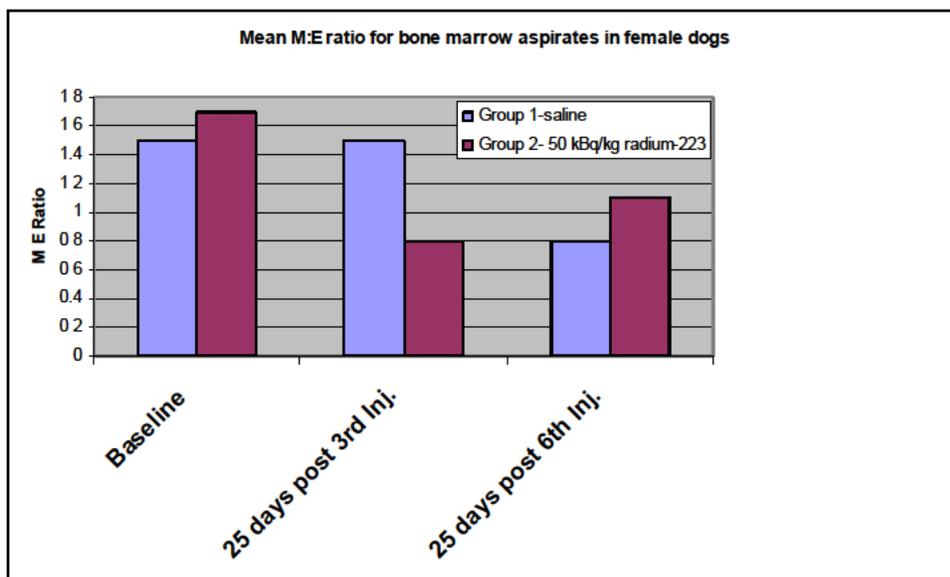
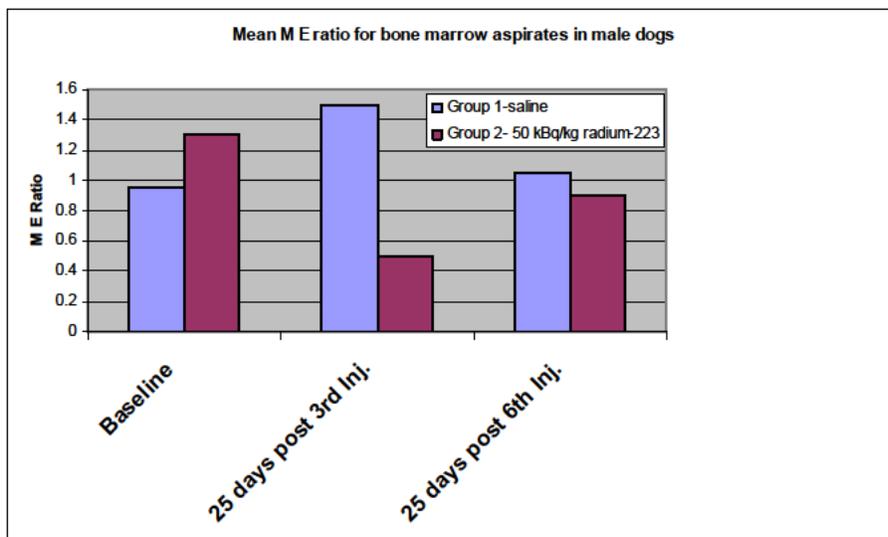


*Summary:* The mean total white cell and granulocyte counts were decreased in the treated dogs after the first dose, and stabilized at lower levels (↓up to 73% compared to control group) by the 3rd test article administration. Cell count recovery trends were observed between day 14 and 25 after each test article administration. There was a recovery trend were observed after a recovery period of 35 days following the 6th test article injection.

The mean platelet count was lower in Group 2 compared to Group 1 with a maximum decrease after the 2<sup>nd</sup> injection (↓up to 73%). A recovery trend for the decreased platelet count was observed from the 25th day after the 5th test article injection to the end of the study period.

Note: 14 days after the 3rd test article injection was eighty-two (82) days after the first test article administration, approximately 7 half lives of the radium-233.

Bone marrow evaluation:



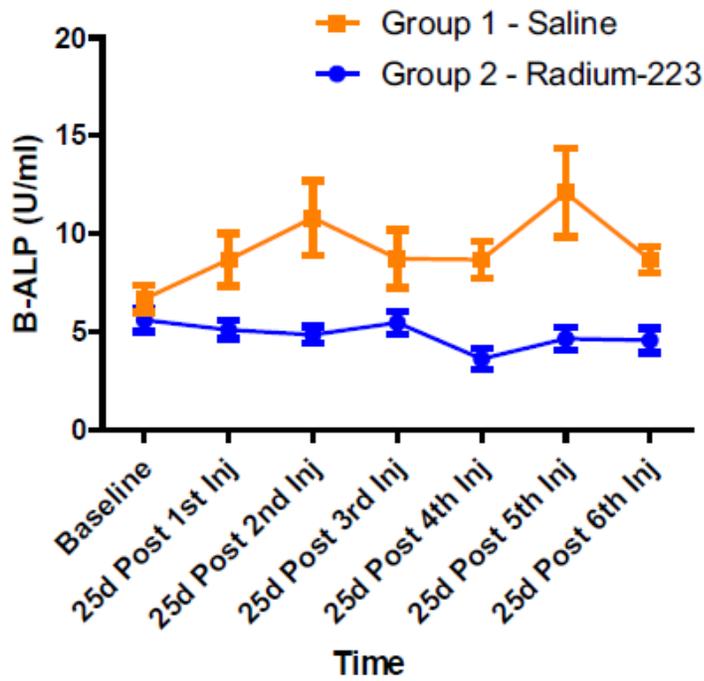
Note: Analysis of all the bone marrow aspirate cytology slides was performed with standardized 300 cell counts and M:E ratio was calculated (b) (4)

**Summary:** A significant difference for the mean M:E ratio was observed on the 25 day after the 3rd test article administration time point between the treatment group and control group (↓67% in males; ↓47% in females). The M:E ratio change was recovered by 25 days following the last dose.

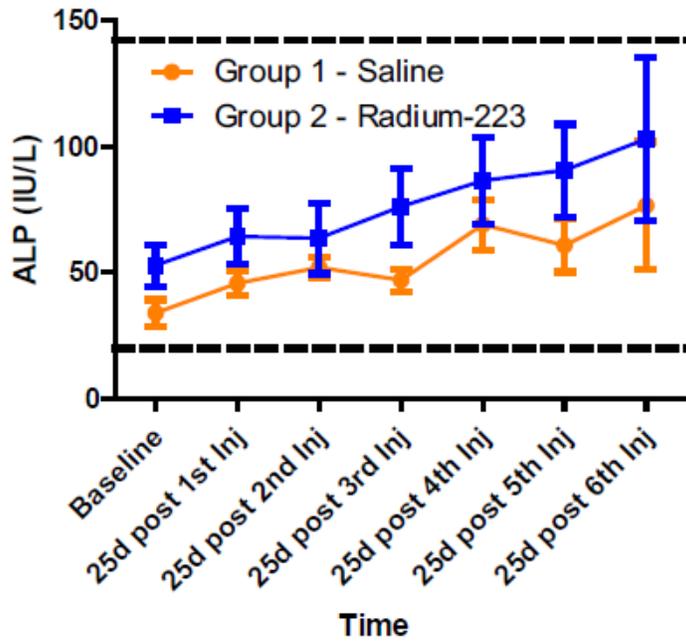
Clinical Chemistry:

The following figures are copied from the Applicant’s submission; the data was verified by the reviewer. Note that there is no significant gender different with the observed changes.

### Bone specific ALP



### Alkaline Phosphatase (ALP)



*Summary:* Serum bone specific alkaline phosphatase (B-ALP) values were decreased in the radium-223 dichloride treatment group dogs compared to the control group at all time points after the first test article injection, which may suggest a decrease in osteoblast activity. Total ALP values were higher in the treatment group; however, the changes were not statistically significant.

Note: radium-223 dichloride treatment resulted in ALP increases in rats possibly associated with changes in liver function.

Urinalysis: unremarkable

Ophthalmoscopy: unremarkable

Organ Weight: unremarkable

Gross Pathology: unremarkable

Histopathology:

A general decline in sternal and/or vertebral bone marrow hematopoietic cellularity was observed in the radium-223 dichloride treatment group. Increased splenic and hepatic extramedullary hematopoiesis and pigment accumulation was observed in dogs with radium-223 dichloride treatment. Fracture callus with inflammation and necrosis was present in the ilial bones of the two dogs that had pelvic fractures during the study.

Comment: Increased splenic and hepatic extramedullary hematopoiesis can be interpreted to be a compensatory response to marrow hypocellularity.

Toxicokinetics: refer to PK section of this review

#### **STUDY SUMMARY:**

Radium-223 dichloride was administered monthly to male and female dogs at a dose of 50 kBq/kg for a total of 6 doses. A decrease in white blood cells and platelet counts were observed with a maximum decrease after the second injection with gradual recovery over the remainder of the study period. The mean myeloid:erythroid (M:E) ratio of bone marrow aspirates was lower in the radium-223 dichloride treatment group dogs after the third test article injection. There was no difference in the mean M:E ratio between control and radium-223 dichloride treatment groups after the sixth test article injection. A general decline in the sternal and/or vertebral bone marrow hematopoietic cellularity was seen in radium-223 dichloride treatment group dogs. The findings of this study demonstrate that target organs of radium-223 dichloride treatment are the bone and bone marrow which accounts for the observed changes in bone marrow and hematopoietic cells.

**Histopathology inventory**

<b>Study</b>	<b>62985</b>	<b>67071</b>	<b>MS RA2</b>
<b>Species</b>	<b>Rat</b>	<b>Rat</b>	<b>Dog</b>
Adrenals	X*	X*	X*
Aorta	X	X	X
Bone Marrow smear	X	X	X
Bone (femur)	X	X	X
Brain	X*	X*	X*
Cecum	X	X	X
Cervix		X	
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X*	X*	X*
Esophagus	X	X	X
Eye	X	X	X
Fallopian tube			
Gall bladder			X
Gross lesions	X	X	X
Harderian gland			
Head (including lower jaw and teeth)	X	X	
Heart	X*	X*	X*
Ileum	X	X	
Injection site	X	X	X
Jejunum	X	X	X
Joint (knee)	X	X	
Kidneys	X*	X*	X*
Lachrymal gland			
Larynx	X	X	X
Liver	X*	X*	X*
Lungs	X	X	X*
Lymph nodes, cervical			
Lymph nodes mandibular	X	X	X
Lymph nodes, mesenteric	X	X	X
Mammary Gland	X	X	X
Nasal cavity			
Optic nerves	X	X	
Ovaries	X*	X	X
oviducts			

Pancreas	X	X	X
Parathyroid	X	X	
Peripheral nerve			
Peyer's patches			
Pharynx			X
Pituitary	X	X	X*
Prostate	X	X	X*
Rectum	X	X	
Salivary gland	X	X	X
Sciatic nerve	X	X	X
Seminal vesicles	X	X	
Skeletal muscle	X	X	X
Skin	X	X	X
Spinal cord	X	X	X
Spleen	X*	X*	X*
Sternum	X	X	X
Stomach	X	X	X
Teeth			
Testes	X*	X*	X*
Thymus	X*	X*	X*
Thyroid	X	X	X*
Tongue	X	X	X
Trachea	X	X	X
Ureters	X	X	X
Urinary bladder	X	X	X
Uterus	X*	X*	
Vagina	X	X	
Zymbal gland			

X, histopathology performed

\*, organ weight obtained

**7 Genetic Toxicology**

No study conducted

**8 Carcinogenicity**

No study conducted.

**8 Reproductive and Developmental Toxicology**

No study conducted

**10 Special Toxicology Studies**

None

**11 Other toxicology studies****Study title: Alpharadin223 Local Irritation Study in Rabbits**

Study no.: 69865

Study report location: M4.2.3.6, page 1-37

Conducting laboratory and location:

(b) (4)

Date of study initiation: March 24, 2009

GLP compliance: yes

QA statement: yes ( X ) no ( )

Drug, batch #, and % purity: Radium-223, A903008, >99.9%  
(radiochemical purity)**Key study findings:**

Perivenous injection of radium-223 dichloride resulted in local erythema only, edema or hemorrhage. The observed edema disappeared after 7 days post dosing. No systemic effects of treatment were observed.

**METHODS:**

<b>Doses:</b>	750 kBq
<b>Dose Justification:</b>	Not provided
<b>Controls:</b>	Citrate buffer
<b>Species/strain:</b>	female albino rabbits ~4 months old 3.4 – 4.1 kg
<b>Number/sex/group:</b>	3
<b>Route, formulation, volume</b>	perivenous injection at a dosing volume of 0.52mL (1.44 MBq/mL)

<b>Satellite groups used for toxicokinetics:</b>	None
<b>Study design:</b>	Female rabbits were given a single perivenous injection with an observation period of one week vehicle was injected at left ear

**OBSERVATIONS AND TIMES:**

- All visible signs of ill health and any behavioral changes were recorded daily.
- The animals were weighed on the day of allocation to the study, on Day 1, Day 4 and at necropsy.
- Injection sites were observed directly after dosing, one hour post dosing, and daily thereafter. Particular attention was paid to hemorrhage, erythema and swelling. Animals were necropsied 7 days post dosing, and injection sites were examined microscopically.

**Erythema and Eschar Formation****Score**

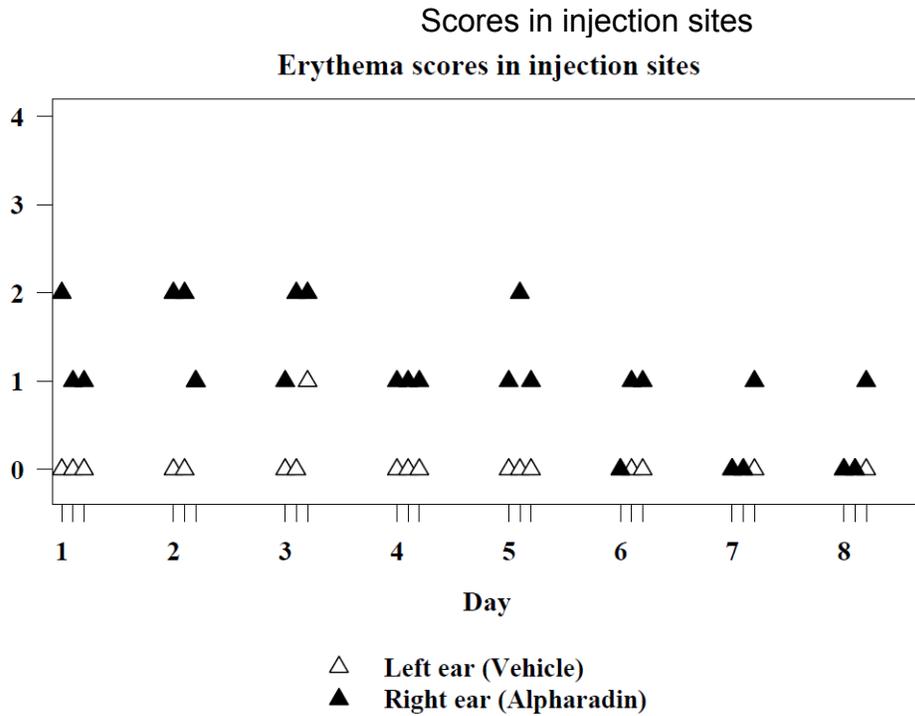
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar formation preventing grading of erythema	4

**Edema Formation****Score**

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm, extending beyond area of exposure)	4

**RESULTS:**

The following figure is copied from the Applicant's submission; the data was verified by the reviewer.



**Summary:** Erythema (scores 1-2) was observed at the injection sites of radium-223 dichloride dosing starting from one hour post dosing. Reaction diminished on Day 4, and erythema was barely perceptible on only one of the animals on Day 7.

## 12 Integrated Summary and Safety Evaluation

### TOXICOLOGY TABULATED SUMMARY

<i>Repeat Dose Toxicity Studies</i>															
Title	12-month GLP		6-month GLP												
Species	Rat		Dog												
Test System	IV		IV												
Schedule	Once every 4 weeks		Once every 4 weeks												
Dose (kBq/kg)	25, 50 100 kBq/kg/day		50 kBq/kg/day												
Dose (kBq/m <sup>2</sup> )	150, 300, 600 kBq/m <sup>2</sup>		1000 kBq/m <sup>2</sup>												
Mortality	Treatment-related death incidence <table border="1"> <thead> <tr> <th>Dose group</th> <th>LD</th> <th>MD</th> <th>HD</th> </tr> </thead> <tbody> <tr> <td>Male</td> <td>4/12</td> <td>7/12</td> <td>3/12</td> </tr> <tr> <td>Female</td> <td>1/12</td> <td>3/12</td> <td>7/12</td> </tr> </tbody> </table> Death cause: osteosarcoma		Dose group	LD	MD	HD	Male	4/12	7/12	3/12	Female	1/12	3/12	7/12	None
Dose group	LD	MD	HD												
Male	4/12	7/12	3/12												
Female	1/12	3/12	7/12												
Clinical sign	Most clinical signs associated with osteosarcoma -Piloerection ,Lame due to fracture, -swelling due to local nodule, -Passive, laboured respiration Changes in teeth after study month 6		Two females developed pelvic fractures between the 5 <sup>th</sup> and 6th test article injections *No difference in bone mineral density (BMD) of the bones was detected in the treatment group.												
Body weight	Male: ↓ up to 7% dose dependent	Female: ↓ up to 8% dose dependent	Unremarkable												
Food consumption	Male: ↓ after week 9, more prominent between week 15-29 (↓up to 59%), Dose dependent	Female: unremarkable	-												
Ophthalmoscopy	-		Unremarkable												
Hematology	Male: ↓ WBC (↓up to 54%)	Female: ↓ WBC (↓up to 36%)	↓WBC (↓ up to 73%) ; ↓platelet (↓up to 73%) ↓ after 1 <sup>st</sup> dosing, and stabilized by the 3rd administration.												

	↓RBC (↓up to 23%) ↑RETIC(↑up to 35%)  Dose dependent	↓RBC (↓up to 20%) ↑RETIC(↑up to 65%)  Dose dependent	Recovery trends between day 14 and 25 after each test article administration. No gender difference
Bone marrow	-		↓M:E ratio 25 days after 3 <sup>rd</sup> injection (↓67% in males; ↓47% in females). Recovered by 25 days following the last dose, no gender different
Clinical chemistry	Male: ↑ALP at HD after 10 <sup>th</sup> dosing (↑up to 98%) ↓Albumin (and Albumin/Globulin ratio) after 10 <sup>th</sup> dosing in all treatment groups (↓up to 12%)	Female: ↑ALAT at HD after 12 <sup>th</sup> dosing (↑up to 66%)	↓specific alkaline phosphatase (B-ALP) ↑ ALP No gender difference
Urilysis	-		Unremarkable
Organ weight	Male: ↓spleen in all groups (↑up to 39% for absolute weight, ↑up to 60% relative to body weight) Female: ↓spleen in MD (↑25% for absolute weight, ↑27% relative to body		Unremarkable
Gross Pathology	Nodules in various organs		Unremarkable
Histopathology	<u>neoplastic changes:</u> Osteosarcomas, mammary carcinoma (one LD female), lymphoma (one MD male) <u>Non-neoplastic changes:</u> <ul style="list-style-type: none"> <li>In femur, a decrease in bone mass and the presence of abnormal/disorganized growth lines characterized by mineralization and necrosis/loss of chondrocytes</li> <li>Depletion of osteoblats/osteocytes in the HD females</li> </ul>		Decline in sternal and/or vertebral bone marrow hematopoietic cellularity. Increased splenic and hepatic extremedullary hematopoiesis and pigment accumulation.

	<ul style="list-style-type: none"> <li>Decreased cellularity of the bone marrow in sternum in HD males</li> </ul>	
Review comment	<p>This study did not include recovery group. Late onset toxicity and recovery for the treatment-related toxicity were evaluated in stud#62985 (Repeated Dose Toxicity Study in Rats with an Extended Recovery Period), which showed recovery trends 6 month after treatment.</p>	<p>The study included only one dose level with no recovery groups. Recovery of the treatment-related toxicities was not studied in this study. Dose-response was studied in Study#MS RA1 (Biodistribution and acute radiotoxicity study of single dose intravenous radium-223 in normal dogs), in which MTD was reached, the profile of treatment-related toxicities were similar and showed in a dose-dependent manner.</p>
<b><i>Genetic Toxicology Studies</i></b>		
Not conducted		
<b><i>Carcinogenicity studies</i></b>		
Not conducted		
<b><i>Reproductive and Developmental Toxicology Studies</i></b>		
Not conducted		

## **OVERALL CONCLUSIONS AND RECOMMENDATIONS**

The nonclinical studies adequately support the safety of radium-223 dichloride by intravenous injection in castration-resistant prostate cancer with bone metastases. See the EXECUTVE SUMMARY, Page 4, for an overall summary of nonclinical findings.

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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WEI CHEN  
03/28/2013

TODD R PALMBY  
03/29/2013

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

**NDA Number:** 203,971

**Applicant:** Bayer

**Stamp Date:** December 14, 2012

**Drug Name:** Xofigo®  
(Radium-223 dichloride)

**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?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		*Appears acceptable.
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		*Appears acceptable
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		
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?	x		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			n/a

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement  
010908

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

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		x	Human dose multiples is expressed in kBq/kg in Sponsor proposed label.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	*x		* Issues generally identified during review
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 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.

Wei Chen, Ph.D	01/10/2013
_____ Reviewing Pharmacologist	_____ Date
Todd Palmby, Ph.D	01/10/2013
_____ Team Leader/Supervisor	_____ Date

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement 010908

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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WEI CHEN  
01/11/2013

TODD R PALMBY  
01/11/2013