CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203137Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 203137
Submission date: 10/26/2012
Drug: Flutemetamol F 18 Injection
Sponsor: GE Healthcare, Inc.
Indication: Radioactive diagnostic agent developed for use with positron emission tomography (PET) for visual detection of fibrillar amyloid-β in brain

Reviewing Division: Division of Medical Imaging Products

Background Comments:
The pharmacology/toxicology reviewer and team leader in the Division of Medical Imaging Products reviewed the nonclinical information for Flutemetamol F 18 Injection and found it adequate to support approval from a pharmacology/toxicology perspective for the indication listed above.

Carcinogenicity and developmental and reproductive toxicity studies have not been conducted with Flutemetamol F 18 Injection. This is acceptable because the product is a radiopharmaceutical that is used acutely.

Conclusions:
I concur with the Division pharmacology/toxicology recommendation that this NDA can be approved. Calling Flutemetamol F 18 Injection a radioactive diagnostic agent for its Established Pharmacologic Class is consistent with other drugs in the class. I agree with the labeling recommendations outlined in the primary pharmacology/toxicology review.

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/s/

PAUL C BROWN
10/10/2013

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	203137
Supporting document/s:	NDA 203137; IND 101866
Applicant's letter date:	10/26/2012
CDER stamp date:	10/26/2012
Product:	Vizamyl™; Flutemetamol F 18 Injection
Indication:	Radioactive diagnostic agent developed for use with positron emission tomography (PET) (b)(4)
Applicant:	GE Healthcare, Inc. 101 Carnegie Center Princeton, NJ 08540-6231
Review Division:	Division of Medical Imaging Products (DMIP) ODE IV, OND, CDER
Reviewer:	Sally Hargus PhD
Supervisor/Team Leader:	Adebayo Laniyonu, PhD
Division Director:	Libero (Louis) Marzella, MD (Acting)
Project Manager:	Sharon Thomas, BSc, RHIT, CCRP

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 203137 are owned by GE Healthcare, Inc. or are data for which GE Healthcare, Inc. has obtained a written right of reference.
Any information or data necessary for approval of NDA 203137 that GE Healthcare, Inc. 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 203137.

Drug Substance is referred to as [^{18}F]Flutemetamol or Flutemetamol F 18..

Non-radiolabeled Flutemetamol was used in some nonclinical studies.

Synonyms for Flutemetamol used in nonclinical studies are GE-067 and AH110690.

Final Drug Product (FDP) is referred to as Flutemetamol F 18 Injection or Vizamyl™.

TABLE OF ABBREVIATIONS, ACRONYMS, AND CODES

ABBREVIATION, ACRONYM OR CODE	TERM
AD	Alzheimer's disease
ADME	Absorption, metabolism, distribution, and excretion
AH110690	Flutemetamol
AS	Amersham
$AUC_{0-\infty}$ or AUC_{0-INF}	Area under the curve (from zero to infinity)
Aβ; β-Amyloid	Amyloid-Beta; Beta-Amyloid
B_{max}	Maximal binding
BSA	Body surface area
bw	Body weight
C	Carbon
C_{max}	Maximum concentration
Cpm	Counts per minute
CT	Computed tomography
CTM	Clinical trial material
EC_{50}	Concentration yielding a 50% response in efficacy
ECG or EKG	Electrocardiography
F	Fluorine
FDP	Final Drug Product
fmol	Femtomole
GE-067	Flutemetamol
GEHC	General Electric Health Care
GLP	Good laboratory practice
H	Hydrogen
hERG	Human ether-a-go-go
I	Iodine
ICF	Informed consent form
id	Injected dose
IND	Investigational New Drug
K_d	Dissociation constant
M	Molar
MHD	Maximum human dose
µg	Microgram
NA	Not applicable
NaCl	Sodium chloride
NDA	New Drug Application
nM	Nanomolar
NOAEL	No observable adverse effect level
NOEL	No observable effect level
NPH	Normal pressure hydrocephalus

OECD	Organization for Economic Cooperation and Development
PBS	Phosphate-buffered saline
PET	Positron emission tomography
PET/CT	Positron emission tomography/ Computed tomography
p.i.	Post-injection
PIB or PiB	Pittsburgh Compound B
PK	Pharmacokinetic
QA	Quality Assurance
RCP	Radiochemical purity
RDRC	Radioactive Drug Research Committee
S9 or S9 Fraction	Supernatant from 9000 x g centrifugation of liver homogenate
SD	Study day
$T_{1/2}$	Half-life
Tau	Tau Protein
TK	Toxicokinetic

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1 Executive Summary

1.1 Introduction

Flutemetamol F 18 Injection (trade name Vizamyl™) is a radioactive diagnostic agent proposed for use with positron emission tomography (PET), "… [redacted] β-amyloid (Aβ) neuritic plaques in [redacted] adult patients with cognitive impairment who are being evaluated for Alzheimer's disease (AD) or other cognitive complaints. A [redacted] Vizamyl™ scan indicates sparse to no neuritic plaques and is inconsistent with a neuropathological diagnosis of AD at the [redacted] of image acquisition; a [redacted] scan reduces the likelihood that a patient's cognitive impairment is due to AD. A [redacted] Vizamyl™ scan is indicat[redacted] moderate to frequent Aβ neuritic plaques. Neuropathological examination[redacted] shown this amount of neuritic plaques is present in patients with AD, but also other types of neurologic conditions as well as in older people with normal cognition. Vizamyl™ is to be used as an adjunct to other diagnostic evaluations[1]."

The definitive diagnosis of AD currently depends upon characteristic post-mortem histopathology staining of brain tissue with the dye, Thioflavin-T. Flutemetamol, the active ingredient in Vizamyl™, is a structural analog of Thioflavin-T that was modified chemically to facilitate transport across the blood-brain barrier, and was evaluated previously in humans as Pittsburgh Compound B. Vizamyl™ is intended to be used as an *in vivo* diagnostic imaging agent to rule out a diagnosis of AD in a living person.

1.2 Brief Discussion of Nonclinical Findings

Pharmacology and Mechanism of Action Studies:
The sponsor conducted primary pharmacology proof-of-concept studies for using Flutemetamol to selectively target and bind to Aβ deposits in brain. Results from autoradiography studies provided support in terms of Flutemetamol binding to known components that are associated with Alzheimer's pathology [e.g., Aβ deposits and neurofibrillary tangles (NFT)] in post-mortem brain slices of AD-versus-non-AD decedents. Co-localization of Aβ antibody binding and autoradiography signal from Flutemetamol-bound protein showed Flutemetamol detected Aβ protein, but not Tau protein (Figure 1).

[1] NDA 203137, Proposed indication.

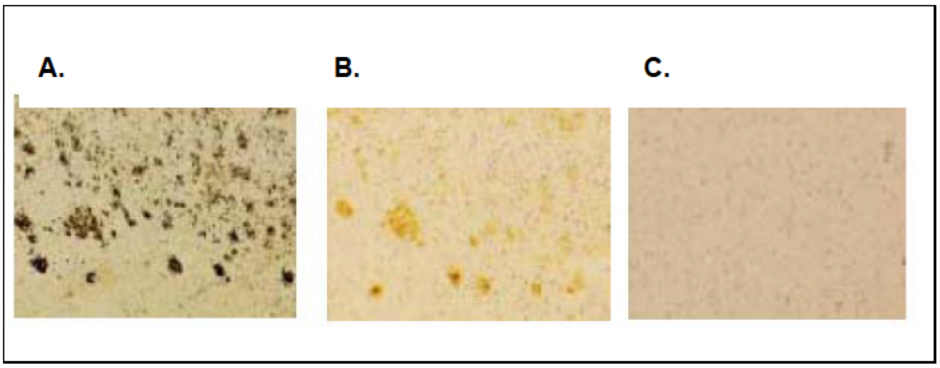


Figure 1. Microscopic Distribution of Tritiated Flutemetamol in the Temporal Cortex of a Representative Post-Mortem Brain Slice from an Alzheimer's Disease Patient.

Panels A, B, and C are magnified images of an area in adjacent brain slices from an AD decedent. Panel A is an image of Flutemetamol-positive area in which the slice was subsequently exposed to Bielschowsky silver stain to visualize protein. Adjacent sections also were stained using immunohistochemistry for Aβ (Panel B) and Tau (Panel C).

Furthermore, results of Flutemetamol studies using a combination of 1) saturation binding assays, 2) competition assays, 3) brain homogenate uptake assays of human brain tissue (obtained post-mortem), and 4) commercially-available Aβ fibrils, all provided support for Flutemetamol development as a diagnostic imaging agent.

Safety Pharmacology:
In an Irwin test in male rats, the results were negative for Flutemetamol effects at a dose multiple of 7-times the maximum human dose (MHD), adjusted for body surface area (BSA).

The cardiovascular screen in telemetered dogs showed that Flutemetamol had no effects on heart rate, Lead II ECG parameters, or blood pressure at 10-times the MHD. Results were negative for inhibition of hERG-1 at Flutemetamol concentrations up to 2.4 nM. Concentrations in the micromolar range would have been more appropriate for the hERG-1 assay, although Flutemetamol insolubility was a major limitation that precluded testing at higher concentrations.

Pulmonary effects:
The Respiratory Function sub-study, conducted during the 14-day Repeat Dose Toxicity Study in Dogs, showed that no differences were observed between Flutemetamol-treated and saline-treated dogs at a dose 23-times the MHD.

Pharmacokinetic (PK), Absorption, Distribution, Metabolism and Elimination (ADME) and Toxicokinetic (TK) studies:
Biodistribution studies in rats and in two baboons showed that Flutemetamol was rapidly distributed throughout the animal at 2 minutes post-injection (pi). The organs exposed to the highest dose of radiation were muscle and liver. More than 50% of radioactivity

was eliminated by 2 hour pi, and approximately 80% was eliminated by 4 hours pi, predominantly by the fecal route.

The sponsor obtained TK data from within the repeated dose toxicity studies in rats and dogs. In rats, the $AUC_{0-\infty}$ and C_{max} were proportional to the dose. The half-life of Flutemetamol on study day (SD) 1 ranged from 7 to 12 minutes, and from 15 to 19 minutes on SD 14. In dogs, the $AUC_{0-\infty}$ and C_{max} were proportional to the dose. The half-life of Flutemetamol was 6.4 to 9.1 minutes between 1 and 14 days. There was no difference between males and females, and the drug did not accumulate in either rats or dogs.

Toxicology studies:
The sponsor conducted two single-dose and two repeated-dose (7-day and 14-day) toxicity studies in rats and in dogs. The outcomes of the toxicity studies were mostly unremarkable, with no overt toxicities or adverse effects attributed to Flutemetamol, based on antemortem (clinical observations, clinical pathology, feed consumption and body weights) and postmortem (gross and microscopic examinations, organ weights) parameters that were monitored. Daily doses of Flutemetamol administered for 1, 7, or 14 consecutive days did not induce adverse effects in animals at doses 38-times (rat, single dose), 7-times (rats, 7- and 14-d studies) or 23-times (dogs, 1-, 7- and 14-d studies) higher than the MHD of 20 µg (assuming 60-Kg human body weight). The animal dose multiples of the MHD were much lower than typically expected for a PET imaging agent, due to the limited solubility of Flutemetamol in the Vehicle and consequent limitations in doses administered.

The sponsor conducted an additional single-dose toxicity study in rats during Phase III clinical development to provide nonclinical safety data with the Final Drug Product (FDP), which contained Polysorbate 80. The results of the study showed no Flutemetamol-dependent toxicity in either antemortem (clinical observations, clinical pathology, feed consumption and body weights) or postmortem (gross and microscopic examinations, organ weights) parameters. The results of the study supported the safety of the FDP at a Flutemetamol dose more than 93-fold the MHD. The higher dose multiple of the MHD obtained in the single-dose rat toxicity study using the FDP was attributed to addition of Polysorbate 80 as a solubilizer/detergent formulation change during clinical development. Polysorbate 80 in the FDP allowed higher doses of Flutemetamol to be administered, compared with doses administered in previous toxicity studies.

Flutemetamol was evaluated in local tolerance studies (un-occluded and occluded) in rabbits. The findings were unremarkable. A test for ocular irritation in rabbits was negative, as was an *in vitro* blood hemolysis test.

Overall, the results of the toxicity studies supported the safety of the proposed clinical dose of 20 µg of Flutemetamol.

Genotoxicity:
The sponsor conducted *in vivo* genotoxicity tests of Flutemetamol as a sub-study in the rat 14-day repeated dose toxicity study, in a stand alone micronucleus assay in rats dosed over a 2-day period, and an unscheduled DNA synthesis assay in hepatocytes from rats treated with Flutemetamol *in vivo*. Results from all of the *in vivo*–based genotoxicity assays of Flutemetamol were negative.

The results of *in vitro* assays (Bacterial Mutagenicity and Mouse Lymphoma Assay) with Flutemetamol were positive. The sponsor conducted studies to provide support for their hypothesis that only Aroclor-induced rat S9 fractions could generate the mutagenic metabolite(s). The sponsor asserted that no human would be exposed to the mutagenic substance. I agreed with the sponsor that the positive result in rat Aroclor-induced S9 fraction incubations is unlikely to be a safety issue for a microdose of a diagnostic imaging agent.

The sponsor conducted a risk assessment to examine and theoretically quantify the potential risks of genotoxicity from exposure to Flutemetamol. The sponsor concluded that the potential risk of genotoxicity from Flutemetamol (non-radiolabeled) is negligible, and I agreed.

Reproductive and Developmental Toxicity:
Reproductive and developmental toxicity (DART) studies were not conducted. Gonads were examined in 14-day repeat dose toxicity studies in rats and in dogs. There were no adverse findings in gonads (macroscopic or microscopic) or gonad weights associated with Flutemetamol. DART studies were not required for the product, based on the results of the rat and dog toxicity studies, the microdose of Flutemetamol administered for a single PET evaluation, and the proposed patient population adult patients with suspected AD or other dementia.

Carcinogenicity:
Carcinogenicity studies are not required for radioactive diagnostic imaging agents, and therefore, were not conducted.

1.3 Recommendations

1.3.1 Approvability

NDA 203137 is recommended for Approval, from the Pharmacology and Toxicology perspectives.

1.3.2 Additional Nonclinical Recommendations

None.

1.3.3 Labeling

The following label recommendations reflect the findings from nonclinical studies or the absence of nonclinical data. Sections are organized with the Sponsor's proposed Vizamyl™ language first (with a single line strike-through where text was deleted),

followed by DMIP P/T recommended statements for the Vizamyl™ label (underlined text).

12.1 Mechanism of Action

(b) (4)

-Flutemetamol F 18 binds to β-amyloid plaques in the brain and the F-18 isotope produces a positron signal that is detected by a PET scanner. The dissociation constant (K_d) for flutemetamol was 6.7 nM.

(b) (4)

Selectivity of [H]flutemetamol binding in post-mortem human brain sections was demonstrated using autoradiography, (b) (4) silver-stained protein, and immunohistochemistry (monoclonal antibody to β-amyloid) correlation studies.

(b) (4)

13.1. Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4) -Animal studies have not been performed to evaluate the carcinogenicity potential of flutemetamol. (b) (4)

lutemetamol was positive for mutagenicity in two *in vitro* assays: the bacterial reverse mutation assay (Ames test) and the mouse lymphoma assay. (b) (4)

-Flutemetamol was negative for genotoxicity after *in vivo* exposure in rats to flutemetamol at the highest cumulative dose level tested (b) (4) as measured in bone marrow micronucleus assays.

(b) (4)

Use in Specific Populations

8.1 Pregnancy

Sponsor: (b) (4) Pregnancy Category C.- (b) (4)

(b) (4)

8.3 Nursing Mothers

(b) (4) t is not known whether (b) (4) is excreted in human milk. Because many drugs are excreted into human milk and because of the potential for radiation exposure to nursing infants from Vizamyl, (b) (4)

2 Drug Information

2.1 Drug

[^{18}F]Flutemetamol, the active drug substance in Flutemetamol (F18) Injection, is an ^{18}F-Fluorine-labeled substance. The radiolabel has a half-life of 110 minutes.

CAS Registry Number: NA

Generic Name: Flutemetamol (^{18}F) Injection or ^{18}F -Flutemetamol or [^{18}F]Flutemetamol; non-radiolabeled product is referred to as Flutemetamol or AH110690

Code Name: AH110690 (^{18}F) Injection; GE-067

Chemical Name: 2-(4)-1,3-benzothiazol-6-ol

Molecular Formula/Molecular Weight: $C_{14}H_{11}FN_2OS$ / 274.32

Structure:

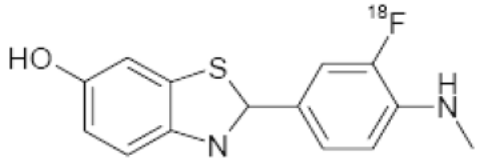


Figure 2. Structure of [^{18}F]Flutemetamol

Established Pharmacologic Class: Radioactive diagnostic agent for use in positron emission tomography

2.2 Relevant INDs, NDAs, BLAs and DMFs

Flutemetamol F 18 Injection was studied under IND 101866 (GE Healthcare, Inc.). A structural analogue of Flutemetamol F 18, Pittsburgh Compound B (PiB), was studied in humans under the Radioactive Drug Research Committee (RDRC), which is regulated according to 21 CFR 361.1.

2.3 Drug Formulation

The formulations of test article used in nonclinical GLP studies (Formulation A) and the Clinical Trial Material (CTM, Formulation B) and Final Drug Product (FDP, Formulation B) are shown below (Table 1). Differences in composition are highlighted.

Table 1. Comparison of Nonclinical and Clinical Formulations

Ingredient	Formulation A (GLP toxicity studies)	Formulation B (CTM and FDP)
Flutemetamol	2.1 µg/mL	2 µg/mL
[^{18}F]Flutemetamol	No radiolabel [^{19}F]	150 MBq/mL (10 mCi)
Radiochemical impurities present	0	Not more than (b) (4)
Ethanol (v/v)	7 %	7%
Polysorbate 80 (w/v)	None	(b) (4) %
Sodium chloride (NaCl)	(b) (4) %	(b) (4) %
Phosphate-buffered saline (PBS)	0.014 M	0.014 M
pH	6-8.5	6-8.5

Three issues related to nonclinical test article were identified and resolved during development:

1. The sponsor over-estimated the active ingredient concentration administered during *in vivo* studies. The issue was resolved based on results of dosing solution analysis and the conduct of Study B067058 (reviewed below). Subsequently, the sponsor amended study reports to reflect actual doses delivered. Throughout this review, actual (recalculated from nominal by the sponsor) concentrations are reported.
2. A formulation change, addition of Polysorbate 80, was made after the nonclinical studies were completed. A bridging biodistribution study (B067060, reviewed in the PK/ADME section) was conducted and showed similar biodistribution and excretion of Formulation A and Formulation B.
3. A manufacturing change from the TracerLab process to the FastLab process resulted in higher levels of impurities in the final clinical product. The sponsor conducted two nonclinical studies to investigate possible effects of the radiochemical impurities on safety and biodistribution of the product:
   a. A single dose toxicity study in rats (B067069, reviewed in the Toxicology section of this review) was conducted using the FDP (including impurities). Flutemetamol F 18 Injection was shown to be safe at a dose greater than 100-times the maximum human dose.
   b. A biodistribution and elimination study was conducted with *a priori* fixed levels of known impurities to determine if there was an effect on

biodistribution (Study B067062, reviewed in Section 5 of this review). The presence of the radiochemical impurities resulted in slightly lower brain uptake and slightly longer blood retention and slower excretion rate, resulting in a minimal reduction in the Effective Dose of Flutemetamol.

Study title: AH110690 Solution for Injection: Adsorption to Dosing Equipment Used in Preclinical Studies.

Study no.:	B067058
Study report location:	4.2.2.2.1
Conducting laboratory and location:	GE Healthcare AS Nycoveien 2 P.O. Box 4220 Nydalen N-0401 Oslo Norway
Date of study initiation:	26 April 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	AH110690 Test Item Stock (45 µg/mL in ethanol) volume of 7 mL was added to PBS (93 mL) for the assay. Batch FFA 093/048-601; purity not stated.

Key Study Findings: The results showed that there was a marked decrease in test article concentration in the solution after passage through infusion equipment. Based upon the results from this study, the NOAEL for each pivotal *in vivo* study was recalculated and the study reports were amended to state the re-calculated concentration of test article administered. Study summaries were revised with re-calculated dose multiples.

Method: The sponsor made up solutions of AH110690 in a range of concentrations used in the nonclinical studies. The solutions were passed through the same infusion equipment used in each specific in vivo nonclinical study. Concentrations of the solution were measured using HPLC analysis (using a validated method) before and after passage through the infusion equipment.

Results and report conclusion: The amount of test article loss was variable for different types of infusion system equipment. Test article recovery was 64% to 91% across studies. The dose concentrations and dose multiple calculations were revised, taking into account the actual dose delivered to animals.

Reviewer comments: I agree with the sponsor's conclusion and the action taken to re-calculate the NOAELs for the nonclinical toxicity and safety pharmacology studies. In the study reviews within this document, actual concentrations of Flutemetamol are reported and used in calculations of safety margins in relation to maximum clinical dose.

2.4 Comments on Novel Excipients

None.

2.5 Comments on Impurities/Degradants of Concern

There are no outstanding issues. The sponsor satisfactorily addressed impurity issues.

2.6 Clinical Population and Dosing Regimen

The clinical population is adults with probable AD. Patients will be administered a single dose of Flutemetamol F 18 Injection by the intravenous route. The maximum dose of Flutemetamol F 18 Injection is 370 MBq (10 mCi; 20 µg), in 10 mL volume.

2.7 Regulatory Background

Prior to the IND submission, several clinical trials and clinical research studies were conducted using Flutemetamol F 18 Injection in Finland, Europe, and in the United States under the aegis of the Radioactive Drug Research Committee(1) (RDRC) (The Pittsburgh Study, 2006).

On October 23, 2008, GE Healthcare presented the development plan for Flutemetamol to the FDA Peripheral and Central Nervous System Drugs Advisory Committee Meeting on β-Amyloid imaging agents.

In March of 2009, GE Healthcare requested a Type C meeting with the Division, during which the sponsor's overall development plan for Flutemetamol F 18 Injection was discussed.

3 Studies Submitted

3.1 Studies Reviewed

MODULE LOCATION	STUDY TITLE	GEHC STUDY NUMBER
4.2.1 PRIMARY PHARMACOLOGY STUDIES		
4.2.1.1	In Vitro Screening of Alzheimer's Disease Targeted Compound AH-110690.	B067050
4.2.1.1	Comparison of Alzheimer's Disease Imaging Agent Lead [^{3}H] AH110690 with [^{3}H]NC102245 Using Quantitative Autoradiography in Human Post-Mortem Brain.	B067051
4.2.1.1	Flutemetamol Histopathology Correlation Study	B067078

4.2.1.3 SAFETY PHARMACOLOGY STUDIES		
4.2.1.3.1	Effects On The Respiratory System In Dog - 14-Day Repeat Dose Study With Ah110690 Solution For Injection In The Beagle Dog.	B067040
4.2.1.3.1	Cardiovascular Effects Of Ah110690 In Conscious, Telemetered Male Beagle Dogs.	B067003
4.2.1.3.1	Ah110690 Effect On Herg-1 Tail Currents Recorded From Stably Transfected HEK 293 Cells.	B067043
4.2.1.3.1	Effects Of Ah110690 In The Irwin Test In Rats.	B067004

4.2.2 PHARMACOKINETICS STUDIES		
4.2.2.3 DISTRIBUTION		
4.2.2.3.1	AH110690 Solution for Injection: Adsorption to Dosing Equipment Used in Preclinical Studies.	B067058
4.2.2.3.1	The Biodistribution of Radioactivity After Intravenous Administration of a Formulation of [^{18}F]AH110690 in Wistar Rats.	B067059
4.2.2.3.1	A Study to Investigate whether the Distribution of Radioactivity after Administration of [^{18}F]AH110690 is Affected by the Inclusion of Polysorbate 80 in the Formulation.	B067060
4.2.2.3.1	A Study to Investigate whether the Distribution of Radioactivity after Administration of [^{18}F]AH110690 is Affected by the presence of radioactive impurities in the Formulation.	B067062
4.2.2.3.1	Human Internal Radiation Dosimetry Estimates for Intravenously Administered AH110690 [^{18}F] Injection Based Upon Biodistribution and Excretion Data from Male and Female Wistar Rats	B067065
4.2.2.3.1	Estimation of the Influence of Radiochemical Impurities in AH110690 (L8f) Injection upon the Effective Dose	B067066

4.2.2.4.1 Study contained distribution and metabolism results	Pilot Investigation of Plasma, Brain and Bile Samples Post [^{18}F]Flutemetamol Injection in the Rat	B067070
4.2.2.4.1 Study contained distribution and metabolism results	Investigation of the *In Vivo* Metabolism of [^{18}F]Flutemetamol in Plasma and Brain Samples in the Rat Including the Availability of any Metabolites to the Brain	B067071
4.2.2.3.1	Biodistribution of Fluorine-18 After Administration of a Formulation of [^{18}F]Flutemetamol Prepared Using The FASTlab SPE Process	B067075
4.2.2.4 METABOLISM		
4.2.2.4.1 Study contained distribution and metabolism results	Investigation of the *In Vivo* Metabolism of [^{18}F]Flutemetamol in Plasma and Brain Samples in the Rat Including the Availability of any Metabolites to the Brain	B067071
4.2.2.4.1	An Investigation of the Metabolic Profile of [^{14}C]AH110690 in the Presence of Aroclor 1254 Induced and Non-Induced Rat Hepatic S9 Fraction.	B067013
4.2.2.4.1	A Study to Investigate the *In Vivo* Metabolic Stability of [^{11}C]AH110690 in Rat.	B067018
4.2.2.4.1	A Study to Investigate the *In Vivo* Metabolic Stability of [^{11}C]AH110690 Following Intravenous Administration to Baboon.	B067019
4.2.2.4.1	Metabolic Profile of [^{14}C]AH110690 in Human Hepatic S9 Incubates.	B067023
4.2.2.4.1	The *In Vitro* Metabolism of [^{19}F]AH110690 in Mouse, Rat (Aroclor 1254), Dog and Human Hepatic S9.	B067045
4.2.2.4.1	The *In Vitro* Metabolic Profile of [^{18}F]AH110690 in Mouse and Dog Hepatic S9	B067046
4.2.2.4.1	*In Vitro* Stability of [^{3}H]AH110690 in Rat, Dog and Human Plasma, Human Whole Blood and Phosphate Buffered Saline	B067048

4.2.2.4.1	Human Plasma Protein Binding of [^{3}H]AH110690 using Equilibrium Dialysis.	B067049
4.2.2.4.1	Single Dose Intravenous Kinetics Study with AH-110690 Solution for Injection in the Wistar Rat	B067055
4.2.2.4.1	Rat and Dog Plasma Protein Binding of [^{3}H]AH110690 using Equilibrium Dialysis.	B067057
4.2.2.4.1	Pilot Investigation of Plasma, Brain and Bile Samples Post [^{18}F]Flutemetamol Injection in the Rat	B067070
4.2.2.4.1	Investigation of the *In Vivo* Metabolism of [^{18}F]Flutemetamol in Plasma and Brain Samples in the Rat Including the Availability of any Metabolites to the Brain	B067071
4.2.2.6 PHARMACOKINETIC DRUG INTERACTIONS		
4.2.2.6.1	A Study to Establish Whether the Distribution of Radioactivity after Administration of [^{18}F]AH110690 To Male Wistar Rats is Affected by MAB31 Pre-Dosing	B967064

4.2.3 TOXICOLOGY STUDIES		
4.2.3.1 SINGLE-DOSE TOXICITY		
4.2.3.1.1	Flutemetamol: One Day (Twice a Day) Intravenous (Bolus) Administration Toxicity Study in the Rat Following a 13-Day Treatment Free Period	B067069
4.2.3.1.1	An expanded single dose toxicity study of intravenously administered AH110690 in male and female rats.	B067001
4.2.3.2 REPEAT-DOSE TOXICITY		
4.2.3.2.1	7-Day Dose Range Finding Intravenous Toxicity Study with AH110690 Solution for Injection in the Wistar Rat.	B067039
4.2.3.2.1	Combined Single and 7-day Repeated Dose Study with AH110690 Solution for Injection in the Beagle Dog	B067038

4.2.3.2.1	14-Day Repeated Dose Intravenous Toxicity Study with AH110690 Solution for Injection in the Wistar Rat, (includes micronucleus).	B067056
4.2.3.2.1	14-Day Repeat Dose Study with AH110690 Solution for Injection in the Beagle Dog.	B067040
4.2.3.3 GENOTOXICITY STUDIES		
4.2.3.3.1 IN VITRO		
4.2.3.3.1	AH110690: Reverse mutation in five Histidine-requiring strains of *Salmonella typhimurium*.	B067005
4.2.3.3.1	L5178Y cells (MLA) using the Microtitre R Fluctuation Technique.	B067006
4.2.3.3.1	Assessment of the Mutagenic Potential of Flutemetamol (F18) Injection	B067072
4.2.3.3.2 IN VIVO		
4.2.3.3.2	AH110690 Solution for Injection: Induction of micronuclei in the bone marrow of treated rats.	B067017
4.2.3.3.2	AH110690 Solution for Injection: Measurement of unscheduled DNA synthesis in rat liver using an in vivo/in vitro procedure.	B067016
4.2.3.6 LOCAL TOLERANCE STUDIES		
4.2.3.6.1	AH110690 Solution for Injection: Local Perivenous, Intraarterial, Intramuscular and Intravenous Tolerability Study in Rabbits.	B067052
4.2.3.6.1	AH110690 Solution for Injection: Primary Eye Irritation Study in Rabbits.	067044
4.2.3.6.1	AH110690 Solution for Injection: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application).	B067042
4.2.3.6.1	A study to investigate the potential for the ALZ103 Phase I clinical vehicle formulation to hemolyze human red blood cells in vitro.	B067061

3.2 Studies Not Reviewed

GEHC STUDY NUMBER	STUDIES NOT REVIEWED
BPMEM067003-LCFLPLASMA_3F	Quantitation Of Ah-110690 In Plasma Samples Using Liquid Chromatography With Fluorescence Detection.
BPREV067003	Validation Of The Bioanalytical Method M067003: "Quantitation Of Ah-110690 In Plasma Samples Using Liquid Chromatography With Fluorescence Detection".
BPMEM067004HERG_3F	M067004: Determination Of Ah-110690 In hERG Assay Samples By Liquid Chromatography With Fluorescence Detection.
BPMEM067005SOL_FOR_INJ_3F	M067005: Determination Of Ah-110690 In Samples Of Solution For Injection By Liquid Chromatography With Fluorescence Detection.
BPREV067004	Validation Of M067005: Determination Of Ah-110690 In hERG Assay Samples By Liquid Chromatography With Fluorescence Detection.
B067070	Pilot Investigation of Plasma, Brain and Bile Samples Post [^{18}F]Flutemetamol Injection in the Rat*

*Pilot, range-finding study used few animals and abbreviated endpoints.

3.3 Previous Reviews Referenced

IND 101866, Pharmacology and Toxicology Review 1 (S. Hargus, DMIP).

4 Pharmacology

4.1 Primary Pharmacology

Brief Summary

Alzheimer's disease (AD) is associated mainly with two distinctive pathological changes in the brain: amyloid plaques and neurofibrillary tangles. The definitive diagnosis of AD is made using post-mortem staining of the brain, which reveals a distinct pattern of histopathology. One of the primary mechanisms associated with the etiology of AD is the deposition of an insoluble protein, β-amyloid (Aβ). The protein is produced by the abnormal processing of the membrane-bound amyloid precursor protein (APP), yielding a precipitation of protein (senile plaques) that aggregates extracellularly in frontal, temporal and parietal cortices, which are the brain regions responsible for cognitive functioning.

The investigational drug substance [^{18}F]Flutemetamol (also referred to as [^{18}F]AH110690 or GE-067) is a benzothiazole derived from two compounds previously tested for detection of Aβ:

- Thioflavin-T, a dye used to stain Aβ deposits in post-mortem neurohistological evaluations, and
- Pittsburg Compound B (PiB), a PET tracer that is nearly identical in structure to [^{18}F]Flutemetamol, except it does not contain a Fluorine atom; instead, the radiolabeled atom is Carbon-11.

The sponsor conducted two *in vitro* primary pharmacology studies early in product development. The study results demonstrated:

- Flutemetamol binding selectively to the target Aβ protein in human brain tissue slices and
- Flutemetamol binding to commercially-available Aβ provided a general pharmacological basis to support the proof of concept.

A third Pharmacology study, "Flutemetamol Histopathology Correlation Study (B067078)", was conducted in 2010. The main goal of the study was to determine if binding in regions of dense Aβ plaques in brain were quantitatively similar between [^{3}H]Flutemetamol and [^{3}H]PiB, based on *in vitro* analyses (post-mortem binding in brain slices and binding to regions of dense Aβ plaque load) and [^{11}C]PiB/PET *in vivo.* Post-mortem brain samples were obtained from subjects with histologically-confirmed AD (n=8) who previously had undergone PiB/PET imaging. Based on the results, the sponsor concluded that the *in vitro* binding and plaque load of [^{3}H]PiB and [^{3}H]Flutemetamol were similar. The sponsor then concluded that the *in vivo* binding patterns and plaque load of Flutemetamol also would be correlated in individual subjects. However, no post-mortem brain samples from AD-confirmed decedents who underwent Vizamyl™/PET imaging were available for analysis. The results of post-mortem plaque density analyses from Vizamyl™/PET-imaged patients are needed for valid conclusions to be drawn regarding quantitative detection of Aβ by Vizamyl™/PET and comparison with PiB/PET.

Study title: Comparison of Alzheimer's Disease Imaging Agent Lead [H3]AH-110690 with [H3]NC102245 Using Quantitative Autoradiography in Human Post-Mortem Brain

Study no.:	B067050
Study report location:	4.2.1.1.1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	April 2002
GLP compliance:	No
QA statement:	No
Drug ("Test Item"), lot #, and % purity:	[^{3}H]AH110690; Lot # not provided; radiochemical purity >90%
Reference Item "Positive Control Item":	[^{3}H]NC12245; Lot # not provided; radiochemical purity >90%

Key Study Findings:

Both Tritiated Flutemetamol ([^{3}H]AH110690) and tritium-labeled Positive Control Item showed:

- Specific binding to regions of the brain corresponding to regions associated with AD, such as the temporal cortex and the hippocampus in AD patients;
- No specific binding in temporal cortex and hippocampus of brain slices of non-AD patients;
- No specific binding in areas of the brain not associated with AD, such as cerebellum, in AD patients;
- Binding of [^{3}H]AH110690 at the microscopic level to protein that co-localized with Aβ, but not tau protein, in regions with neurofibrillary tangles (NFT).

Study Design
Brain slices were obtained post-mortem from subjects diagnosed with AD, or subjects diagnosed with dementia unrelated to AD, or subjects with no clinical symptoms of dementia. The uptake of tritiated Flutemetamol and positive control item were measured via autoradiography, following incubation of slices with either compound. Four brain areas were evaluated macroscopically: temporal cortex, cerebellum, hippocampus, and pons.

Following macroscopic examinations of the autoradiographs, Flutemetamol-positive slices were evaluated microscopically, using the techniques of silver staining (directly on slices that underwent autoradiography) and immunohistochemistry of slices with anti-Aβ or anti-tau sera.

Results
Macroscopic examination of autoradiographs showed localization of [^{3}H]AH110690 to Aβ and neurofibrillary tangles (NFT) in the temporal cortex and hippocampus regions of brain slices from AD-decedents, whereas tissue slices from the same regions of non-AD decedents did not show localization of signal from tritiated Flutemetamol. The temporal cortex and hippocampus are severely affected in AD. The cerebellum and pons regions, which are not affected in AD, were similar in signal intensity from tritiated Flutemetamol from all groups of decedents.

Brain sections from the tritiated Flutemetamol-positive macroscopic studies subsequently were exposed to silver stain (Panel A of Figure 4) to visualize protein. Adjacent sections also were stained using immunohistochemistry for Aβ (Panel B of Figure 4) and Tau (Panel C of Figure 4). The results showed the co-localization of tritiated Flutemetamol signal protein and with Aβ pathology, but not with Tau protein.

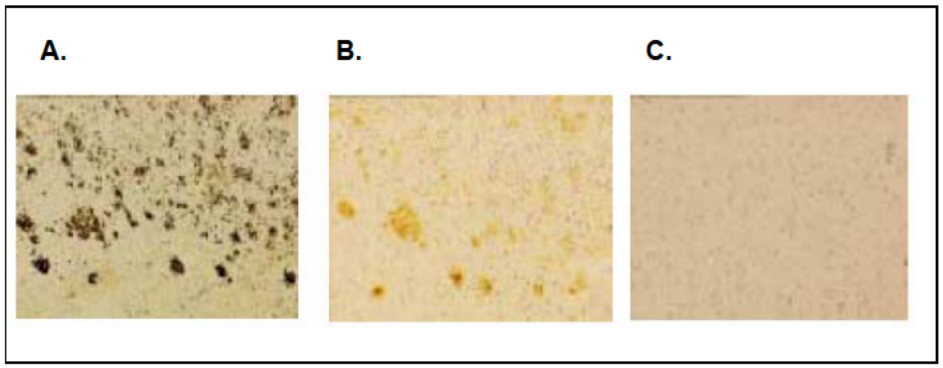


Figure 3. Microscopic Distribution Of Tritiated Flutemetamol In The Temporal Cortex Of A Representative Post-Mortem Brain Slice From An Alzheimer's Disease Patient.

Source: Study B067050, Sponsor's Figure 9.

Report conclusion
The sponsor concluded that tritiated Flutemetamol was selectively bound to the regions in the cerebral cortex and hippocampus that are associated with Aβ pathology in AD, which provided support for the development of Flutemetamol ([^{18}F]AH110690) as a candidate for *in vivo* detection of Aβ.

Reviewer comment: The sponsor showed that Flutemetamol binding was specific for Aβ, but not Tau protein, in Figure 3. Tau is a microtubule-associated protein and is a major constituent of neurofibrillary tangles (NFT), which are intracellular.. The sponsor did not indicate if the tau protein was associated with NFT. The pathways linking Aβ and tau protein in Alzheimer's Disease are not clearly understood, according to recent reviews (3; 6). The ability to selectively bind Aβ in extracellular neuritic plaques, but not intracellular Tau in NFT, may be important in the future diagnosis, monitoring of disease progression or therapeutic intervention, and the study of AD etiology.

Study title: In Vitro Screening of Alzheimer's Disease Targeted Compound: AH110690.

Study no.:	B067051
Study report location:	4.2.1.1.1
Conducting laboratory and location:	GE Healthcare Ltd The Grove Centre White Lion Road Amersham Buckinghamshire HP7 9LL England
Date of study initiation:	April 2002
GLP compliance:	No
QA statement:	No
Drug ("Test Item"), lot #, and % purity:	[^{3}H]AH-110690; Lot # not provided; radiochemical purity >90%
Reference Items-Positive Control and Negative Controls:	Positive Control- [^{125}I]NC102201, a benzothiazole; Negative Control- [^{125}I]AH111014, a benzimidazole; Lot # not provided; radiochemical purity >90%

Key Study Findings:

Results showed that Flutemetamol had *in vitro* binding properties that provided a pharmacological basis for development as an *in vivo* PET diagnostic imaging agent, based upon:

- Specificity of binding to Aβ sites in human brain slices (post-mortem) from Alzheimer's Disease patients;
- *In vitro* binding, affinity, and specificity characteristics in incubations of Aβ fibrils obtained commercially;
- Specific *in vitro* binding, in incubations consisting of Aβ fibrils + human brain homogenates of grey or white matter or both.

Study Design

The purpose of the study was to investigate the binding of [^{3}H]AH110690 (Test Item; Tritiated Flutemetamol) to known components associated with Alzheimer's pathology [Aβ and neurofibrillary tangles (NFT)] using a combination of saturation binding assays, competition assays, and brain homogenate uptake assays in:

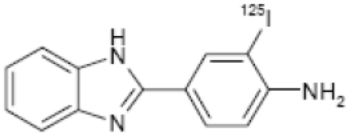
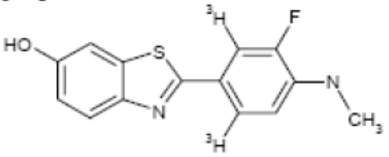
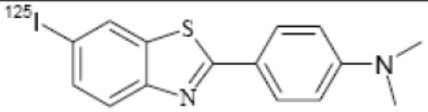
- Human brain tissue (obtained post-mortem);
- Commercially-available Aβ fibrils;
- Human brain homogenates containing either grey or white matter.

Saturation binding was determined in incubations containing Aβ fibrils + [^{3}H]AH110690 (in pM to μM amounts) +/- unlabeled Positive Control (for non-specific binding). The equilibrium constant at which 50% of the drug is bound to protein (EC_{50} or K_d) was calculated.

Competitive binding was determined in incubations containing Aβ fibrils + unlabeled competitor [Test and Reference Items (Table 2)] + labeled [^{125}I]NC102201.

Binding to Aβ-spiked Control Human Brain Homogenates was measured in incubations containing equimolar concentrations of Test and/or Reference Item (Table 2).

Table 2. Structures Of Test And Reference Items Used In Study B067051

[^{125}I]AH-111014 (BTA negative control) [^{125}I]Negative Control	 (b) (4) (IMQ2122, specific activity of 74 TBq/mmol)
[^{3}H]AH-110690 [^{3}H]Test Item = [^{3}H]Flutemetamol	[^{3}H]6-OH-3'F-BTA-1  (b) (4) (TRQ10700, specific activity of 555 GBq/mmol)
[^{125}I]NC102201 [^{125}I]Positive Control "Benchmark"	 (b) (4) (IMQ 2140, specific activity of 74 TBq/mmol).

Results

Saturation Binding (Table 3; Figure 4): The results showed that [^{3}H]Flutemetamol had a K_d of 6.7 nM when incubated with Aβ fibrils. A maximum binding value (B_{max}) of 4 fmol of Flutemetamol per 0.5 µg fibrillar Aβ was calculated. The EC_{50} of Flutemetamol was 118 nM.

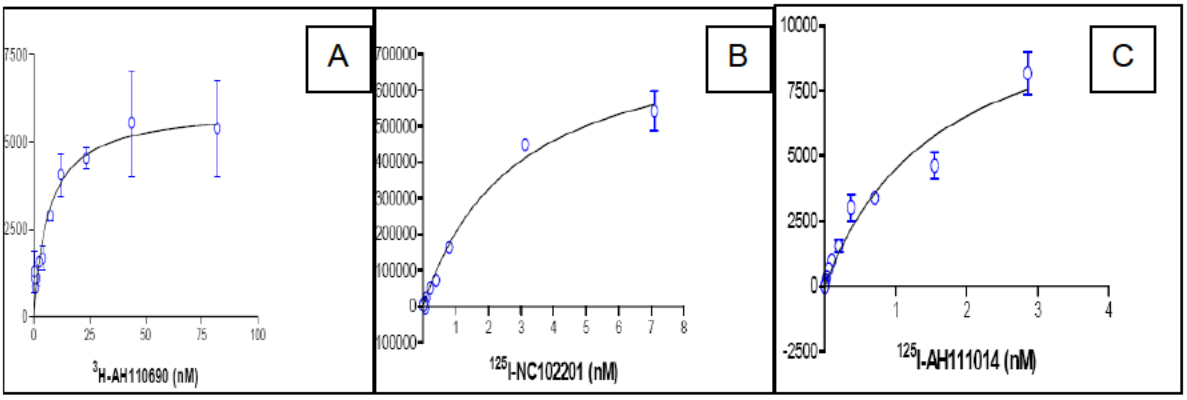


Figure 4. Saturation Binding Curves Used for Calculation of Dissociation Constants (K_d) For Flutemetamol (A), Positive Control (B), and Negative Control (C). Vertical Axis: Cpm.

Flutemetamol K_d =6.7 nM; Positive Control K_d =3.4 nM; Negative Control K_d =1.6 nM
Source: Study B067051, Sponsor's Figures 2, 1, and 3, respectively.

Competition Binding (Table 3 and Figure 5): Competition binding assays were performed to determine the EC_{50} values in incubations containing fibrillar amyloid.

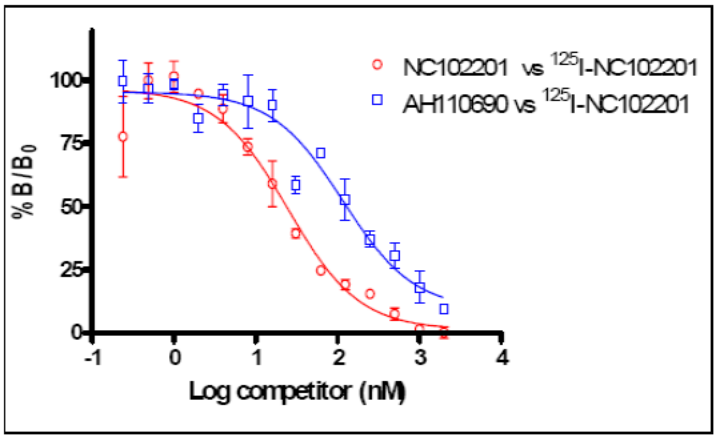


Figure 5. Competition Binding Curve Used To Determine EC50 In Fibrillar Amyloid Incubations Of Flutemetamol Versus Positive Or Negative Control Items.

Source: Study B067051, Sponsor's Figure 4.

Specific to Non-Specific Binding (Table 3): The amyloid binding properties of [^{3}H]AH110690 were tested in human brain homogenates in order to simulate an *in vivo* situation in which many lipophilic, non-specific binding sites may be present. Specific to non-specific binding ratios were determined in incubations of [^{3}H]AH110690 + amyloid fibrils, which then were added at known amounts (spiked) into either the grey or white matter from human brain homogenates. The results showed that spiked incubations with Flutemetamol ([^{3}H]AH110690) contained higher specific (spiked grey)-to-non-

Summary results of all *in vitro* pharmacology assays are shown in Table 3.

Table 3. Overall *In Vitro* Binding Results From Study B067051.

Binding dissociation, Bmax, EC_{50} and brain homogenate data

		K_d (nM)	B_{max} (fmol/0.5µg amyloid)	EC_{50} (nM)	Brain homogenate assay	
					spiked grey:grey	spiked grey:white
Negative Control	NC102201	3.4	176	14	2.3:1	2.2:1
Flutemetamol	AH-110690	6.7	246	118	5.3:1	9.3:1
Positive Control	AH-111014	1.6	4	N/A	5.5:1	6.3:1

Source: Study Report B067051, page 28, Table 2 (modified).

Report conclusion: Results of these *in vitro* binding assays supported the pharmacological basis for Flutemetamol as a PET tracer for detection of AD plaques.

Reviewer comment:
These studies were conducted early in the development of Flutemetamol, and the sponsor's interpretation of the data and the conclusions drawn are very general, and thus provide a reasonable pharmacological basis to support clinical studies.

Study title: Flutemetamol Histopathology Correlation Study

Study no.:	B067078
Study report location:	Module 4.1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	01 May 2010
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Not stated

Key Study Findings:

The main goal of the study was to determine if Flutemetamol binding patterns to amyloid-beta peptide deposits (Aβ) in the brain correlated with those of Pittsburgh Compound B (PiB) in post-mortem brain samples from Alzheimer's disease (AD) decedents and non-AD decedents. Ante-mortem PET images and post-mortem brain tissue from the same patients were available only for [^{11}C]PiB-imaged subjects. A positive correlation between PiB and Flutemetamol binding to Aβ *in vitro*, qualitatively

and quantitatively, provided support for the assertion that the binding and localization are similar *in vivo*. The results showed a positive correlation between PiB/PET Aβ distribution in PET images and post-mortem PiB binding to Aβ in brain homogenates and slices. No data were available from subjects who were evaluated with Vizamyl™/PET.

Methods
Tritium (^{3}H)-labeled PiB and Flutemetamol were synthesized for use in the study to overcome limitations due to the short half-lives of [^{11}C]PiB and [^{18}F]Flutemetamol. Highly fluorescent analogs of Flutemetamol (6-CN-Flutemetamol) and PiB (6-CN-PiB) also were synthesized to enable fluoroscence imaging and quantitation of Aβ binding for each tracer.

The study samples were obtained from autopsy cases who were participants in the Alzheimer's Disease Research Center (ADRC) (n=39) at the (b) (4). A subset of the cases (n=8) underwent [^{11}C]PiB PET imaging prior to death, which allowed the sponsor to compare ante-mortem [^{11}C]PiB PET images with post-mortem measures of PiB binding in brain tissue from each patient, i.e., ante- and post- mortem tests were conducted in brain from each of 8 patients. Samples were prepared for each type of analysis:
1) Histology and immunohistochemistry in frozen or paraffin-embedded tissue slices,
2) Homogenates of selected brain regions for biochemical assays and plaque load analyses, and
3) PiB/PET retention levels *in vivo* were determined using the Logan(5) method.

Results
The chemical structures and Ki values of PiB and Flutemetamol are shown in Figure 6. The addition of the 6-CN functional group increased the Ki 50% and 63% for PiB and Flutemetamol, respectively.

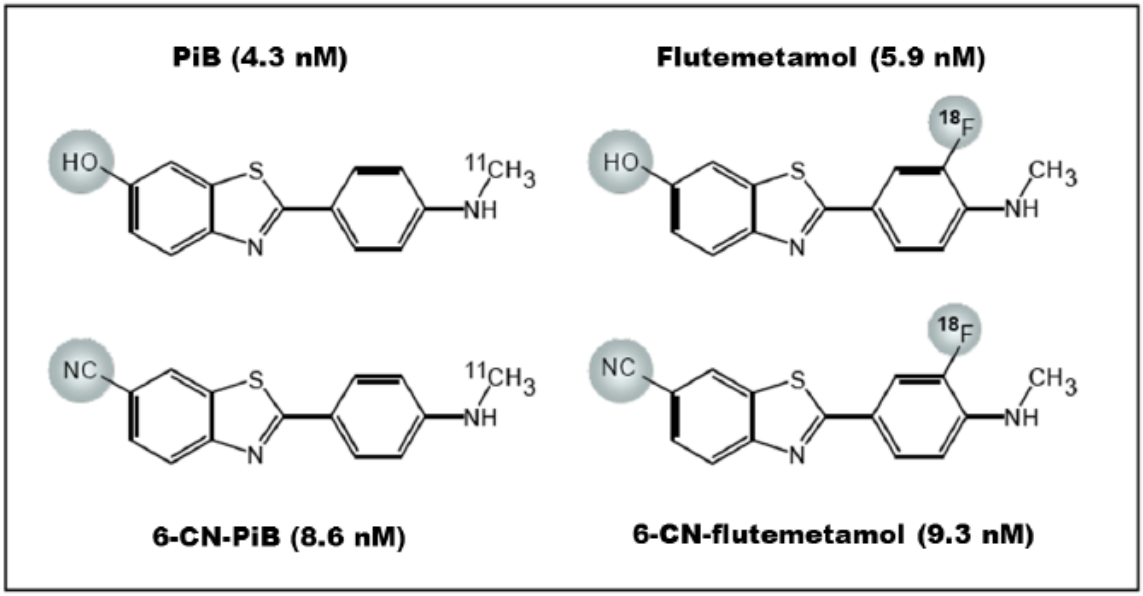


Figure 6. Chemical Structures and Ki values of [^{11}C]PiB and [^{18}F]Flutemetamol and Their Fluorescent 6-CN Derivatives.

Source: Study Report B067078

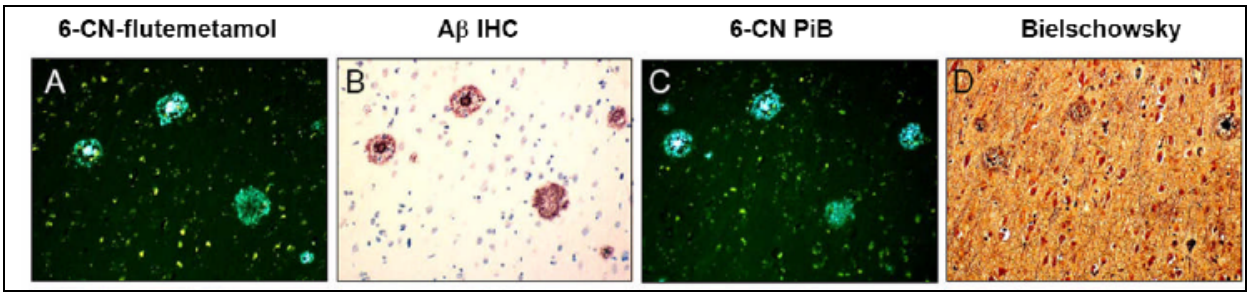


Figure 7. Tissue Sections from the Superior Temporal Cortex of an AD Decedent.

High magnification of four consecutive paraffin sections, stained using 6-CN-Flutemetamol (A), 4G8 (Aβ antibody) (B), 6-CN-PiB (C), and Bielschowsky silver stain (D). Source: Study Report B067078

The results showed that the Aβ-immunoreactive staining pattern (Panel B) was similar when stained with 6-CN-Flutemetamol and 6-CN-PiB, indicating that the tracers recognized the same Aβ epitopes in tissue slices from AD brain. Panel D, in which Bielschowsky stain was utilized, shows brown to dark brown plaques and vascular amyloid against a yellow to brown background. Axons, plaque neurites and tangles were visualized as black-stained areas. The slices used in the study were adjacent slices from an AD decedent.

Sponsor's Conclusion
The sponsor concluded that the *in vivo* PET retention of [^{18}F]Flutemetamol in AD brains reflected neocortical Aβ plaque load in a manner similar to [^{11}C]PiB. Furthermore, these data underscored "…the validity of using [^{18}F]Flutemetamol PET imaging not only to detect and follow the progression of Aβ-containing fibrillar amyloid deposits in clinically defined subject populations, but also to assess effectiveness of therapies aimed at clearing these potentially toxic deposits from the brain."

Reviewer's comments:
The sponsor averred that results were similar between PiB and Flutemetamol in vitro, and thus, the similarity observed in vitro may be extrapolated to the in vivo clinical situation. The sponsor's assertion seems reasonable although not supported in this study, due to the absence of data from post-mortem brain tissue from Vizamyl™/PET imaged subjects (AD and non-AD patients) for the following analyses: 1) post-mortem brain slice histopathology, and 2) samples for brain homogenate Aβ binding. The sponsor needs additional data in order to provide support for the assertion that in vitro and in vivo binding of Flutemetamol are correlated. Intuitively, one could assume there is a correlation, although the sponsor is striving for a quantitative and specific regional pattern and density of Aβ binding that isn't supported by the available data. For these reasons, I disagree with the sponsor's statement that the data "…underscores the validity of using [^{18}F]Flutemetamol PET imaging not only to detect and follow the progression of Aβ-containing fibrillar amyloid deposits in clinically defined subject populations, but also to assess effectiveness of therapies aimed at clearing these potentially toxic deposits from the brain." Furthermore, the assertion that [^{18}F]Flutemetamol/PET imaging could be used to monitor disease progression or regression is best evaluated by the clinical reviewer.

4.2 Secondary Pharmacology

Study title: In Vitro Interaction Study of GE's Amyloid PET-Ligands And Roche's Anti-Amyloid-beta Antibody (R04909832)

Study no.:	B067077
Study report location:	Module 4.1 of IND 101866
Conducting laboratory and location:	(b) (4)
Date of study initiation:	14 November 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[3H]AH110690; no lot #; 100% RCP

Key Study Findings:
Results showed that Roche's anti-Aβ therapeutic agent (R04909832) had no affect on binding of [3H]AH110690 or [3H]PiB to amyloid plaques in human brain tissue slices from AD decedents.

Experimental Design and Methods
The study was an *in vitro* investigation to determine if Roche's anti-Aβ therapeutic agent (R04909832) had an affect on binding of [^{18}F]AH110690 to amyloid plaques in human brain tissue slices from AD decedents. Tissue slice incubations were conducted under the following conditions:

- R04909832 binding to amyloid plaques in the presence and absence of tritium-labeled AH110690 or PiB;
- R04909832 binding to amyloid plaques in the presence and absence of non-radiolabeled-AH110690 or PiB.

Results and Conclusion
Results showed no interaction of RO4909832 with either AH110690 or PiB in binding to amyloid plaques in brain tissue slices from AD decedents. The sponsor concluded that the *in vivo* binding of AH110690 would be similar.

Reviewer's comment: Agree that results showed RO4909832 did not change the specific binding of AH110690 in brain tissue. I don't agree that the results would be the same in vivo, due to differences between available binding sites in vitro versus in vivo. Although I don't completely agree with the sponsor's conclusions regarding binding in vivo, the results of this binding experiment do not have an impact on the nonclinical or clinical safety profile of Flutemetamol. This study was submitted to the IND, although I could not find it in the NDA submission. I include it here for completeness.

4.3 Safety Pharmacology

Brief Summary
Results of the safety pharmacology studies conducted with Flutemetamol are summarized in Table 4, below. The No Observed Adverse Effect Level (NOAEL) was calculated using actual doses administered. The Maximum Human Dose (MHD) is based on 20 µg maximum dose to a human and assuming a human body weight of 60 kg. The results and dose multiples supported the safety and tolerability of Flutemetamol F 18 Injection in the clinical trials, with dose multiples of at least 7-fold.

Table 4. Safety Pharmacology Studies Conducted in Support of [^{18}F]Flutemetamol.

Study Type/Study Number	NOAEL		Maximum Human Dose for 60 Kg Human		Dose Multiple** (BSA-Corrected Animal NOAEL*/ MHD)
	µg/kg	µg/m^2	µg/kg	µg/m^2	
Irwin Screen in Rat/B067004	15	90	0.33	12.33	7
Cardiovascular in Dog (telemetry)/B067003	6.0	120	0.33	12.33	10
Respiratory Function in Beagle Dogs/B067040	14	280	0.33	12.33	23
In Vitro hERG Assay	Negative for inhibition up to 2.4 nM (0.65 µg/mL), the highest dose tested due to limited solubility of Flutemetamol				

*Correction for Body Surface Area (BSA; µg/m^2) is the animal NOAEL in µg/kg bw multiplied by 6.2 for rat or 20 for dog.
**Dose multiple is the BSA-adjusted NOAEL (in µg/m^2) divided by the Maximum Human Dose (MHD) in µg/m^2.

Neurological effects

Study Title: Effects of AH110690 in the Irwin Test in Rats.

Study no.: (b) (4) No. DIEL1005 / GE Healthcare AS Reference No. B067004
Study report location: 4.2.1.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 17 July 2003
GLP compliance: Yes (OECD)
QA statement: Yes
Drug, lot #, and % purity: AH110690 pre-formulated stock solution of 45 µg/mL in 96% ethanol, Batch FFA049/118-307, 94.5% purity by HPLC

Key Study Findings:
Except for uncoordinated movement (3/6 at 5 min) in the high-dose Flutemetamol group, vehicle and saline effects were very similar to AH110690. The positive control effects were as expected. The NOAEL was 15 µg/kg bw. The NOEL was 5 µg/kg due to uncoordinated movement in animals administered 15 µg/kg dose.

Methods

Dose:	1.5 (low), 4.6 (mid) and 14.72 (high) µg/kg based on body weight (bw)
Frequency of dosing:	Single injection
Route of administration:	IV bolus (tail vein)
Dose volume:	5mL/kg
Formulation/Vehicle:	7% Ethanol in PBS / 7% Ethanol in PBS
Control:	Saline
Reference item:	Chlorpromazine 0.4 mg/mL
Species/Strain:	Rat, Sprague-Dawley [Bkl:SD]
Number/Sex/Group:	6 males/group; animals were dosed on SD 1 or SD 2, not both days
Age:	6-7 weeks of age
Weight:	188-230 g
Dose groups:	Saline, vehicle, AH110690 low-, mid-, high-dose, chlorpromazine 4 mg/kg
Deviation from study protocol:	The dosing solutions were not analyzed post-injection. Study impact: no explanation for observed effects in saline-treated control animals.

Procedures

The test was administered to animals on two successive days at similar times on each of two days. Rats were dosed and observed: pre-dose, then at 5- and 30-minutes post-dose on day one. On day 2, rats were dosed and observed pre-dose, then 60- and 120-minutes post-dose. Animals were observed blinded (observers did not know what treatment was administered) and scored systematically.

Results

Rats treated with sterile saline (5 mL/kg) were observed to exhibit the following effects: exophthalmos (2/6 at 5 min and 30 min post-dose), abnormal gait-waddling (2/6 at 5 min and 30 min post-dose), piloerection (6/6 at 5 min post-dose).

Rats treated with vehicle (7 % ethanol in PBS 5 mL/kg) were observed to exhibit the following treatment-related effects: exophthalmos (2/6 at 5 min and 30 min post-dose), abnormal gait-waddling (4/6 at 5 min post-dose and 2/6 at 30 min post-dose), piloerection (6/6 at 5 min post-dose).

Rats treated with AH 110690 were observed to exhibit the following treatment-related effects, with no apparent dose dependency: exophthalmos (2/6 at 30 min post-dose), abnormal gait-waddling (2/6 at 30 min post-dose), piloerection (6/6 at 5 min post-dose), and uncoordinated movement (3/6 at 5 min post dose).

Chlorpromazine treatment elicited the expected pharmacologic effects consistent with CNS depression.

Report conclusion
Except for uncoordinated movement in high-dose Flutemetamol animals at 5 minutes, vehicle and saline effects were the same as AH110690. AH110690 and vehicle (both containing 7% ethanol) had similar effects. The only effect that appeared to be specific to AH110690 was the uncoordination in 3/6 high-dose animals at 5 minutes. The positive control effects were as expected.

Reviewer comments:
The sponsor stated that the saline-treated group was observed to have exophthalmos (2/6 at 5 min and 30 min post-dose), abnormal gait (2/6 at 5 min and 30 min post-dose), piloerection (6/6 at 5 min post-dose), at similar incidences compared with Flutemetamol- and vehicle-treated groups. Although I found this observation surprising, it is possible that the rats were somewhat traumatized by the treatment, even though it was only saline. No dose verification analysis, such as measurement of plasma flutemetamol amount, was conducted post-dosing. Therefore, one cannot rule out dosing errors to explain the similar findings between saline, vehicle, and Flutemetamol-treated groups. Even so, the effects of all dose levels of AH110690, vehicle, and saline were minimal and reversible. The uncoordinated movement observed in 3/6 animals was not considered "adverse" by the sponsor, although I assert that it would be a question of the degree of impairment. The NOEL was 5 µg/kg.

No female rats were evaluated in the rat modified Irwin test. The sponsor was asked to justify the exclusion of females in the studies, in an email message sent on 8/03/09. The sponsor responded that their repeat-dose study in rats (B067056) showed no consistent differences in toxicokinetic parameters; the sponsor concluded animals of both sexes were unnecessary. However, a rat biodistribution study (B067059) in both sexes showed differences at 2 minutes post-injection between brain tissue and bone: female uptake was ~2X greater than male uptake. At later time points (20 minutes onward) the distribution patterns were similar in both sexes.

Overall, the effects observed in Flutemetamol-treated groups were minimal and reversible.

<u>Cardiovascular effects</u>

Brief summary
<u>In vivo studies</u>: No adverse effects were observed on blood pressure, heart rate, or ECG (lead II) parameters in dogs. The NOAEL in dogs was 19-times the MHD (BSA adjusted).

Study title: Cardiovascular Effects of AH110690 in Conscious, Telemetered Male Beagle Dogs

Study no.:	B06003
Study report location:	4.2.1.3.1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	17 July 2003
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	AH-110690 stock solution in Ethanol (45 µg/mL); batch FFA 0049/118-307; 94.5% by HPLC

Key Study Findings:

One of four animals administered saline had clinical signs of salivation and tremor. Administration of vehicle and Flutemetamol (AH-110690) were associated with clinical observations of ataxia, lip-licking, tremors, and salivation. Sedation and one instance of agitation were observed after administration of Flutemetamol, although incidence and severity were not dose-dependent. Flutemetamol or vehicle administration did not affect arterial blood pressure, heart rate, or lead II ECG parameters examined. Observations following treatment with the positive control item were consistent with expected pharmacological effects. The NOAEL was 6.0 µg/kg bw.

Methods

Doses:	0.6, 1.9, 6.0 µg/kg	
Schedule and treatment:	Day 1	Saline
	Day 4	Vehicle
	Day 7	AH 110690 0.6 µg/kg
	Day 11	AH 110690 1.9 µg/kg
	Day 14	AH 110690 6.0 µg/kg
	Day 18	Noradrenaline 5 µg/kg
Route of administration:	IV, manual bolus over 2 minutes	
Dose volume:	2.9 mL/kg bw	
Formulation/Vehicle:	7% Ethanol in PBS / 7% Ethanol in PBS	
Control:	Saline	
Reference substance:	Noradrenaline bitartrate	
Species/Strain:	Pure-bred Beagle dogs	
Number/Sex/Group:	4 males for telemetry; 2 males for dose range finding	
Age:	Approximately 14-20 months	
Weight:	10.7-15.6	
Unique study design:	Two dogs were utilized in a range finding pilot, prior to telemetry of other dogs. Telemetry monitors were implanted in 4 dogs prior to the study. No anesthesia was needed during the dosing and monitoring period.	
Deviation from study protocol:	Minor with no impact to study outcome.	

Parameters monitored:
All clinical signs were observed and documented for each treatment. Telemetry recordings were made from animals placed in a sling, from 0.5 h pre-dose until approximately 2h post-dose. Thereafter, the animals were removed from the slings and allowed to move freely in the individual dog pens, while recording continued up to at least 6h post-dose. The following variables were measured, calculated, and reported:

- Systolic arterial blood pressure (SBP);
- Diastolic arterial blood pressure (DBP);
- Heart rate (HR);
- Mean arterial blood pressure (MAP);
- Variables from the lead II ECG: RR interval, PR interval QRS duration, QT interval, QTcF interval, QTcQ interval.

Results
Salivation and tremors were noted in 1/4 prior to and up to 4 minutes after administration of saline.

Lip-licking (4/4) and salivation (2/4) was noted after administration of vehicle.

Lip licking was noted in all animals at all doses of AH110690. Low dose AH110690 was associated with salivation and tremors (1/4) and agitation (1/4). The mid dose was

associated with sedation (1/4) and salivation (1/4). The highest dose of AH110690 produced tremors (2/4), salivation (2/4) and sedation (1/4).

Administration of the positive control, noradrenaline, induced clinical signs of lip licking, cold ears and pale gums in 2/4 animals.

No significant changes in arterial blood pressure (systolic, diastolic, and mean) were observed following administration of vehicle, or any dose level of AH 110960. A significant increase in arterial blood pressure (systolic, diastolic, and mean) was observed following administration of 5 µg/kg noradrenaline 0.5-3 min post-dose, and a significant decrease in heart rate occurred 0.5-5 min post-dose. Heart rate was not affected by administration of vehicle or AH110690 at any dose level, nor were there marked effects on the RR, PR, QT, QTcF or QTcQ intervals, or QRS duration.

Report conclusion
AH110690 was not associated with changes in heart rate, blood pressure, respiratory rate, nor electrocardiograph Lead II parameters (intervals of PR, QT, QTcF or QTcQ, or QRS duration). The NOAEL in the report was stated as 6 µg/kg.

Reviewer's Comments: The sponsor was asked why it did not evaluate female dogs; the sponsor responded that it was because no consistent differences were observed in toxicokinetic parameters in the repeat dose toxicity study. However, the sponsor assumed that sex differences in dynamic responses to drugs always are reflected by differences in PK or TK parameters. Similar PK or TK profiles between sexes don't necessarily imply similarity of pharmacodynamic responses. The sex-based difference in dynamic response was illustrated in the PK bridging study using a Polysorbate-80-containing formulation of Flutemetamol (B067060). The sponsor should have evaluated animals of both sexes in the safety pharmacology study to get a complete profile.

The sponsor measured HR, SBP, SAP, MAP, RR and Lead II ECG parameters in Flutemetamol-treated animals. However, in animals treated with the positive control item (noradrenaline), only HR, SBP, SAP, MAP, and RR were measured. The results were consistent with expected effects of noradrenaline, which led the sponsor to conclude that the study was valid. I agree.

The dosing solution contained ethanol (7% v/v), and the observed agitation (1/4 at the low dose), ataxia (all Flutemetamol- and vehicle-treated animals), lip licking (all Flutemetamol- and vehicle-treated animals), and sedation (1/4 mid and high dose) were attributed to the ethanol in the dosing solution. The behaviors were observed after vehicle- or Flutemetamol-administration, but not after saline administration. The salivation and tremor observed in one of four saline-treated dogs was unexpected; it may have been due to stress, although a dosing error cannot be ruled out.

I agree with the sponsor that the observed effects in vehicle- and Flutemetamol-treated dogs likely were due to the ethanol in the formulation, and not Flutemetamol.

Brief summary:
In Vitro Studies: The sponsor also conducted an *in vitro* study of the potential effects of Flutemetamol on human ether a-go-go (hERG) potassium channels expressed in human embryonic cells. The result of the hERG assay (4 fibers) was negative according to the sponsor; however, the highest concentration tested was only 2.4 nM. Concentrations of test article in the micromolar range would have provided data within the range typically tested in the hERG assay, although it was not feasible due to Flutemetamol insolubility in Vehicle.

Study title: AH110690 Effect on HERG-1 Tail Currents Recorded from Stably Transfected HEK 293 Cells

Study no.:	B067043
Study report location:	4.2.1.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	08 September 2005
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	AH 110690 Batch FKJ0168/031-02 powder; 91.3% purity

Key Study Findings: AH110690 was negative for HERG-1 inhibition at actual concentrations of 1.6-2.4 nM (0.43-0.65 µg/mL).

Methods

Test System:	Stably transfected HEK 293 cells expressing HERG-1 potassium channels
Concentrations in definitive study:	1.6-2.4 nM (0.43-0.65 µg/mL)
Basis of concentration selection:	Solubility in 0.5% DMSO and bath solution
Negative control:	0.5 % Dimethylsulfoxide (DMSO)
Positive control:	Potassium channel (I_{Kr}) blocker E-403, 100 nM
Formulation/Vehicle:	AH-110690 first solublized in DMSO, then diluted with bath solution for incubation/Vehicle 0.5% DMSO in bath solution [sodium chloride (137 mM), Potassium chloride (4 mM), calcium chloride (1.8 mM), Magnesium chloride (1mM), HEPES (10mM), D-glucose (10 mM); pH 7.4]

Incubations: AH110690 was evaluated for the potential effect of potassium channel inhibition of HERG-1 currents in a perfusion system containing HEK 293 cells stably expressing HERG-1 potassium channels. HERG-1 currents elicited by a voltage

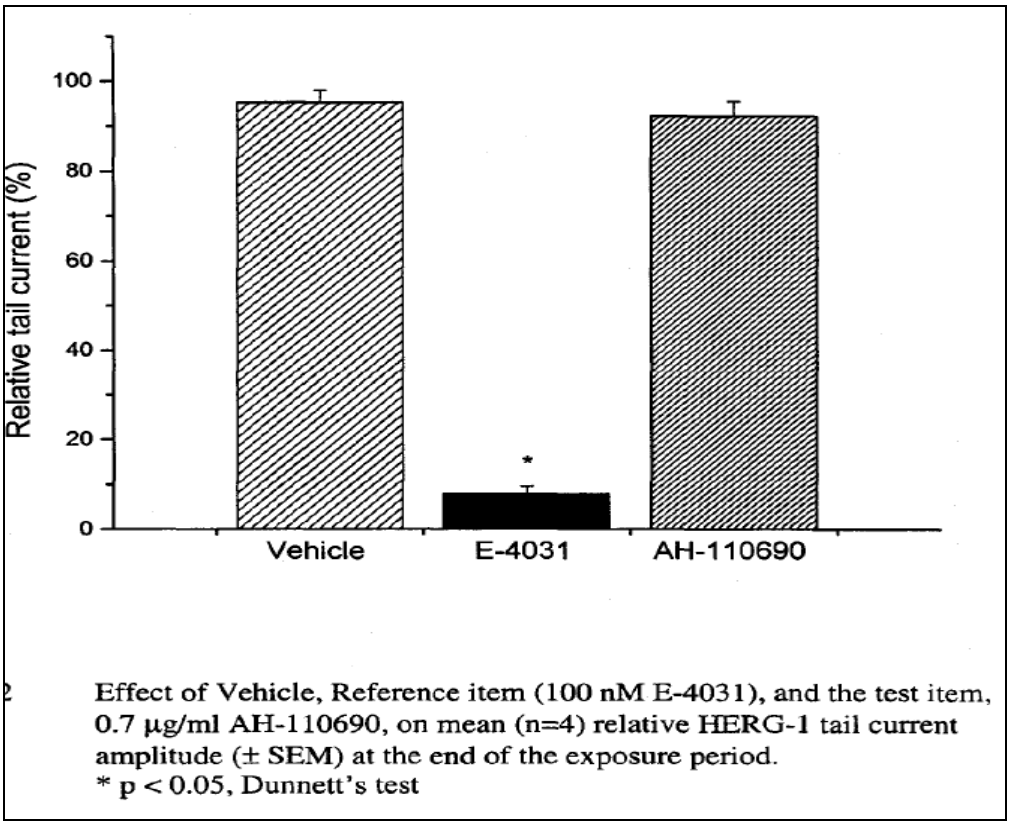
protocol were measured using the whole-cell patch-clamp technique. All recordings were performed at room temperature.

Current inhibition was determined as a decrease in the tail current amplitude after the drug application compared to the control level of the same cell in the pre-treatment phase (relative tail current).

Results: AH110690 at the highest achievable concentration did not cause inhibition of potassium channel currents, compared with vehicle control treatment. The positive control article E-4031, caused inhibition of the HERG-1 currents, as expected (Figure 8).

Report Conclusion
AH110690 was negative for HERG-1 inhibition at concentrations of 1.6-2.4 nM (0.43-0.65 µg/mL).

Reviewer comment: Agree. The concentration of test article that was soluble in incubation medium was a limiting factor in the study. Concentrations of test article in the micromolar range would have provided data within the range typically tested in the hERG assay (e.g., 0.1 µM E4031 positive control), although it was not feasible due to Flutemetamol insolubility in Vehicle. The sponsor stated that the highest possible Flutemetamol concentration was tested, which is acceptable.



2 Effect of Vehicle, Reference item (100 nM E-4031), and the test item, 0.7 µg/ml AH-110690, on mean (n=4) relative HERG-1 tail current amplitude (± SEM) at the end of the exposure period.
* $p < 0.05$, Dunnett's test

Figure 8. HERG Assay Results for AH110690.

Source: Study Report B067043, Page 31.

Pulmonary effects (Respiratory Function)
Brief summary
The effects of Flutemetamol on respiratory function, evaluated on SD10 during the 14-day Repeat Dose Toxicity Study in Dogs (Study B067040). The reader is referred to the review of Study B067040, "14-Day Repeat Dose Study with AH110690 Solution for Injection in the Beagle Dog". There were no Flutemetamol-dependent effects on monitored respiration parameters (respiration rate, tidal volume, and minute volume) in Beagle dogs at a dose multiple of 45X.

Pharmacodynamic drug interactions: None conducted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Brief Summary
The sponsor obtained TK data from within the repeated dose toxicity studies in rats and dogs. In rats, the AUC and Cmax were proportional to the dose. The half-life at day 1

ranged from 7 to 12 minutes, and from 15 to 19 on day 14. In dogs, the AUC and Cmax were proportional to the dose. The half-life of Flutemetamol was 6.4 to 9.1 minutes between 1 and 14 days. There was no difference between males and females, and the drug did not accumulate in either study. The biodistribution profile in rats showed that Flutemetamol was excreted mostly by the fecal route, and that muscle and liver received the highest radiation exposure during the first 20 minutes, and the gastrointestinal and urinary tracts received highest exposure to radiation during the entire 4 hour monitoring period. Protein binding studies in human, dog, and rat plasma indicated a high level of protein binding (greater than 95%) in vitro, although in vivo studies showed Flutemetamol is rapidly eliminated after IV administration.

In addition to the typical battery of studies, the sponsor conducted investigational studies to evaluate the impact of unanticipated factors on the nonclinical program, listed below:

- The sponsor showed that the positive results for mutagenicity in the Bacterial Mutagenicity Test and the *in vitro* Mouse Lymphoma Assay were due to the Aroclor-induced hepatic metabolism pathway found only in rats, and not found in other species. The suspected mutagenic metabolite was observed only in Aroclor-induced rat hepatic S9 incubations.
- A biodistribution study was undertaken to evaluate the impact of a potential increase in the amount of radiolabeled impurities in the product, after a manufacturing scale-up (B067062); the results showed that the presence of relatively high amounts of radioactivity ((b) (4) % and (b) (4) % of 2 major impurities) radiochemical impurities 1) slowed the brain uptake of [^{18}F]Flutemetamol during the first 20 minutes post-injection; 2) had longer residence time in the blood compartment; and 3) the presence of impurities resulted in a slightly delayed excretion/elimination, in comparison with 100% RCP Flutemetamol.
- A biodistribution bridging study was undertaken to evaluate the potential effects of the addition of Polysorbate 80 to the final formulation of Flutemetamol F 18 Injection (B067060); no changes to biodistribution occurred that would have an adverse affect on exposure in clinical settings.

Biodistribution Studies

Brief summary

Biodistribution studies in rats showed that Flutemetamol was rapidly distributed throughout the animal at 2 minutes post-injection. The organs exposed to the highest dose of radiation were small and large intestine, and bladder. More than 50% of radioactivity was eliminated by 2 hour pi, and approximately 80% was eliminated by 4 hours pi, predominantly by the fecal route, and to a lesser extent via the urinary route. The inclusion of Polysorbate 80 in the formulation did not affect the biodistribution of Flutemetamol. The presence of radiochemical impurities prolonged the residence time of Flutemetamol F 18 Injection in the blood compartment and slowed the penetration of radioactivity into the brain compartment during the first 20 minutes post-injection. Thereafter, the amount of radioactivity in the brain was similar to results from previous distribution studies.

Study title: The Biodistribution of Radioactivity After Intravenous Administration of a Formulation of [^{18}F]AH110690 in Wistar Rats

Study no.:	B067059
Study report location:	4.2.2.3.1
Conducting laboratory and location:	GE Healthcare Ltd. The Grove Center White Lion Road Amersham, Buckinghamshire HP7 9LL England
Date of study initiation:	03 May 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{18}F]AH-110690 was supplied as a formulation in phosphate buffer (0.01M, pH 7.4), 10% ethanol. Radiochemical purity was 100%.

Key Study Findings:

[^{18}F]Flutemetamol was rapidly distributed throughout the body at two minutes post-injection. Most of the radioactivity was in small intestine and contents of small intestine, liver, bladder and urine, and skin at 20 minutes post-injection. The compound was excreted via the feces (~80%) and, to a lesser extent, urine (~10%) by four hours post-injection. Differences between male and female distribution of radioactivity was observed in brain at 2 minutes post-injection (female 5.7 $\pm$ 1.4% id; male 3 $\pm$ 0.5% id), but not at other time points.

Methods

Dose:	Radiolabeled Test Item was used. Specific Activity was calculated for each batch, before and after administration to the animal. Specific activity of Test Item was 17-34 MBq/µg; 10-100 ng/animal was injected.
Frequency of dosing:	Single
Route of administration:	IV
Dose volume:	Selected based on specific activity of Test Item on day of dosing
Formulation/Vehicle:	7% Ethanol in PBS / 7% Ethanol in PBS
Control:	NA
Species/Strain:	Rat, Wistar (Crl: (WI))BR)
Number/Sex/Group:	6/sex/group
Age:	Not provided
Weight:	144-237 g
Deviation from study protocol:	• Due to the numerous time points, the study was conducted over a period of 4 separate study days (males) or 5 separate study days (females), and it was necessary to make fresh test article each day • Test article batches varied in terms of specific activity. • A few animals died under anesthesia and were replaced. • No deviation impacted study interpretation

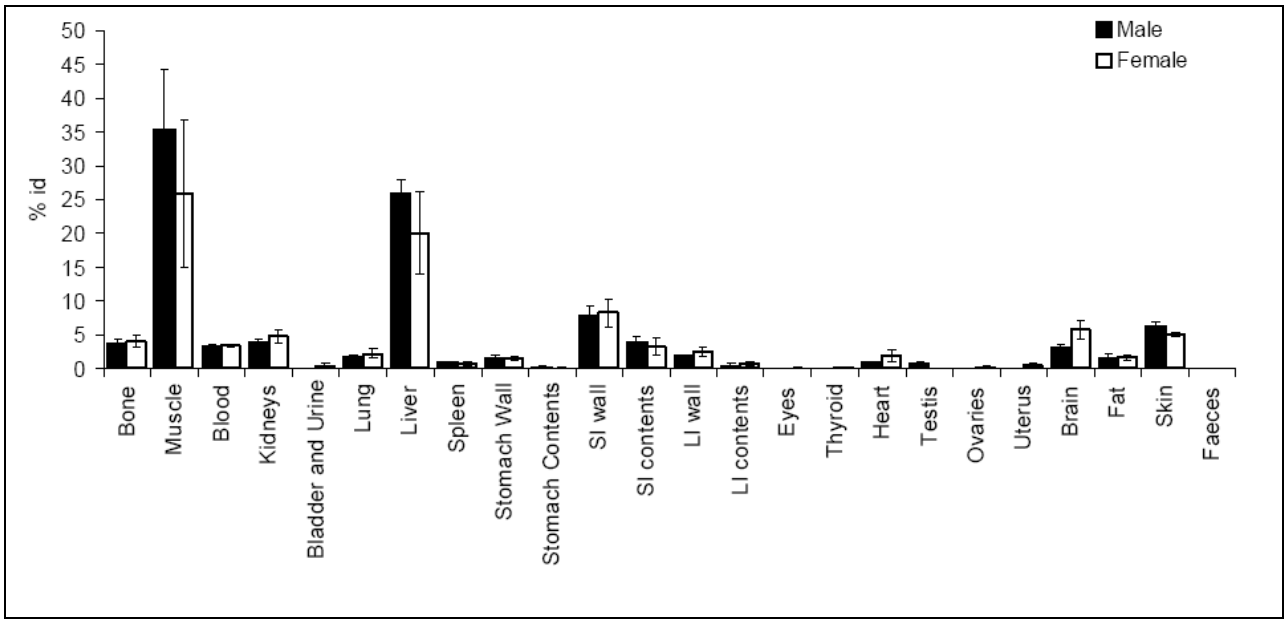
Study Design:
The study was designed to investigate the biodistribution, retention and elimination of radioactivity, from injection time up to 4 hours after intravenous (IV) administration of a formulation of [^{18}F] AH110690 in Wistar rats. Due to the size of the study (72 rats in total) the study was conducted over several days, and new test article was prepared each day.

Method:
Animals were injected with test article, and at appropriate time points the following samples were collected: Bone, muscle, blood, left and right kidneys, bladder and urine, lung, liver, spleen, stomach and contents, small intestine and contents, large intestine and contents, eyes, thyroid, heart, testes or ovaries and uterus, brain, fat, skin, feces, carcass, injection site (whole tail). Calculations were made for the % id in organ or tissue.

Results:
The results showed that Flutemetamol was distributed in high amounts per gram of tissue or organ at two minutes post-injection in the muscle > liver > small intestinal wall,

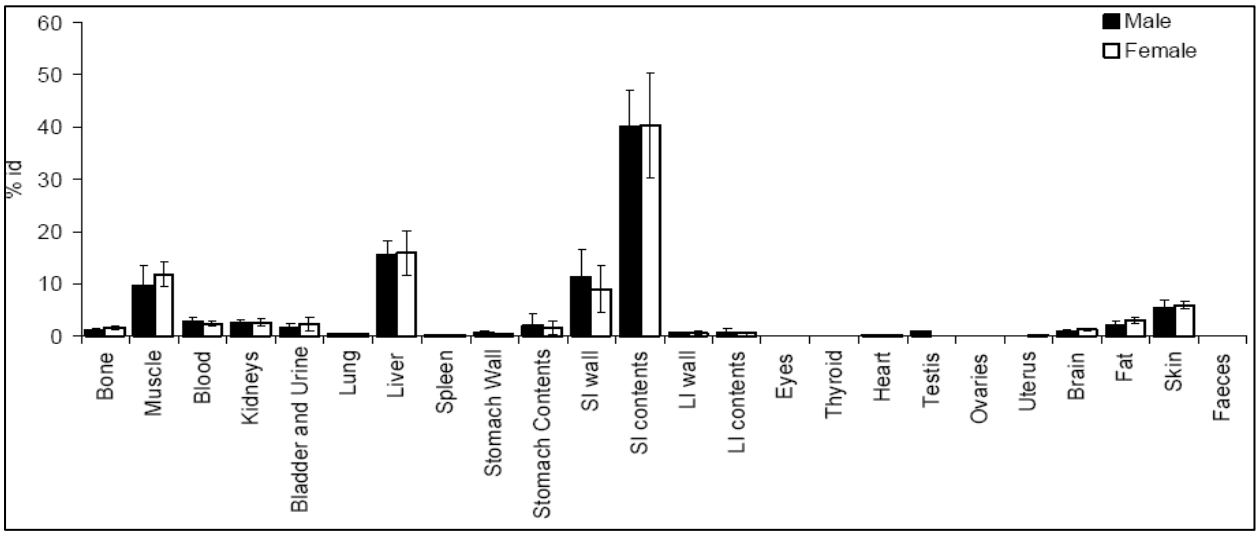
brain, and skin. Bone, blood, kidneys, and small intestine contents also contained appreciable, though lower, amounts (Figure 9).

Figure 9. Biodistribution of Flutemetamol in Wistar Rats 2 Minutes Post-Injection. Source of Figure: Study B067059, Sponsor's Figure 1.



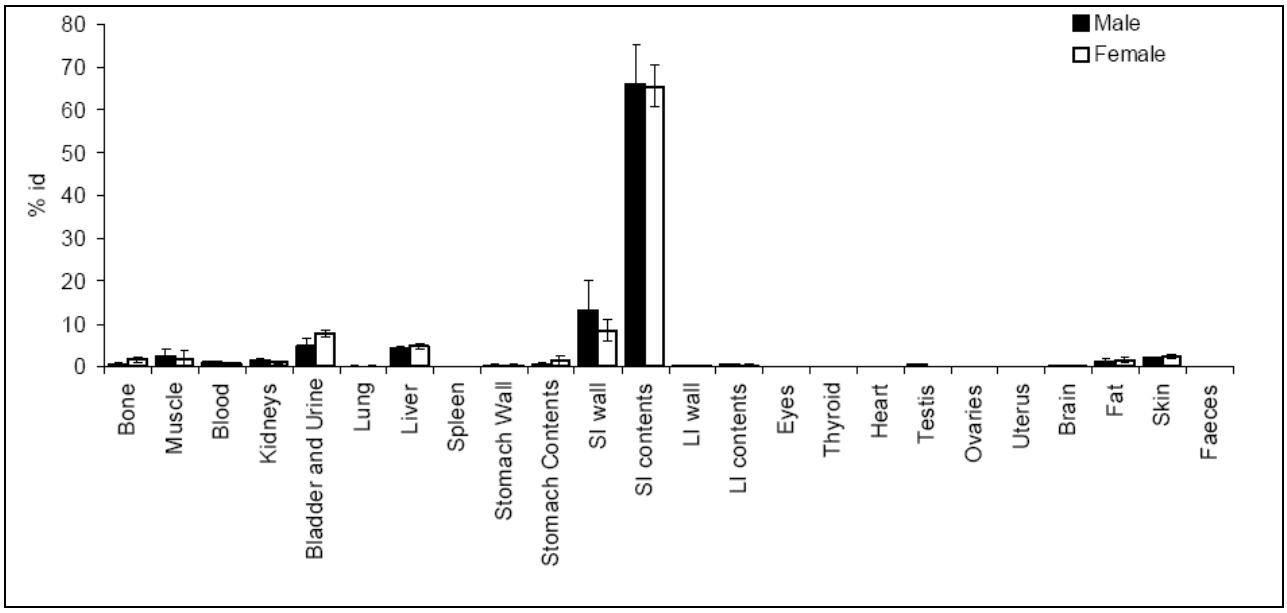
Twenty minutes after Flutemetamol injection, the distribution was greatest in small intestinal contents >> liver > muscle > small intestinal wall > skin > fat (Figure 10).

Figure 10. Biodistribution of Flutemetamol in Wistar Rats 20 Minutes Post-Injection. Source of Figure: Study B067059, Sponsor's Figure 2.



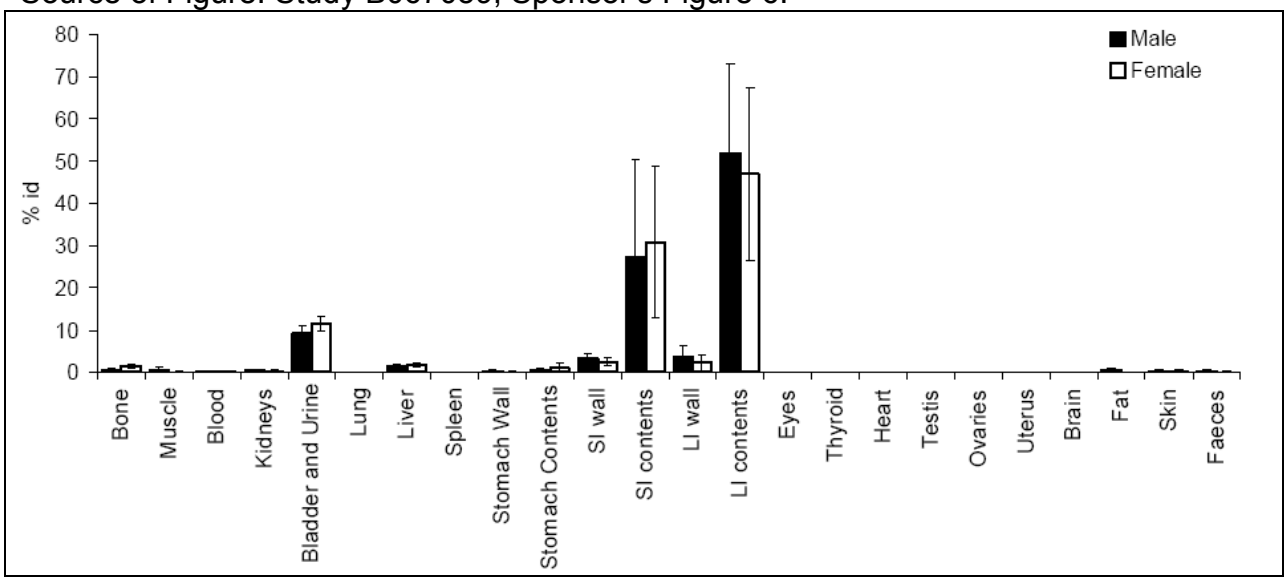
Sixty minutes post-injection, most of the radioactivity was localized to the small intestine (Figure 11).

Figure 11. Biodistribution of Flutemetamol in Wistar Rats 60 Minutes Post-Injection. Source of Figure: Study B067059, Sponsor's Figure 3.



The final time point evaluated, 4 hours post-injection, showed that small intestine contents and large intestine contents contained most of the radioactivity, with bladder and urine retaining a small portion (Figure 12).

Figure 12. Biodistribution of Flutemetamol in Wistar Rats 4 Hours Post-Injection. Source of Figure: Study B067059, Sponsor's Figure 6.



[^{18}F]Flutemetamol was rapidly distributed throughout the body at two minutes post-injection, including brain tissue, the target organ of Flutemetamol for imaging. Most of the radioactivity was in small intestine and contents of small intestine, liver, bladder and urine, and skin at 20 minutes post-injection. The amount of radioactivity had decreased by ~50% compared with the 2 minute time point in brain. Later time points showed that the compound was undergoing excretion via the feces and, to a lesser extent, in urine.

Report conclusion: According to the sponsor, the test article did not accumulate in organs or tissues for a prolonged time, and is excreted mostly via the fecal route.

Reviewer comment: I agree with the sponsor's conclusion. The biodistribution profile appeared favorable for the intended use of Flutemetamol as a brain imaging agent, i.e., there was rapid uptake and washout in brain tissue. Most of the product was excreted in feces (~80%), with a small amount found in the bladder and urine (~10%), by 4 hours post-injection.

Differences between male and female distribution of Flutemetamol were observed in brain tissue and bone (female uptake was ~2X greater than male) at 2 minutes post-injection. At later time points the distribution patterns were similar in both sexes.

Study title: A Study to Investigate whether the Distribution of Radioactivity after Administration of [^{18}F]AH110690 is Affected by the Inclusion of Polysorbate 80 in the Formulation

Study no.:	B067060
Study report location:	4.2.2.3.1
Conducting laboratory and location:	GE Healthcare Ltd. The Grove Center White Lion Road Amersham, Buckinghamshire HP7 9LL England
Date of study initiation:	08 September 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Test Item A (similar to 'Formulation A' in Table 1) was [^{18}F]AH110690 (Lot # not specified; 100% radiochemical purity) in 0.01 M phosphate buffer, 10% ethanol. Test Item B (the same as 'Formulation B' in Table 1) was [^{18}F]AH110690 (Lot # not specified; 100% radiochemical purity) in 0.01 M phosphate buffer, 7% ethanol, and 1% Polysorbate 80.

Key Study Findings: There were no biologically significant differences between the biodistribution of Test Item A (without Polysorbate 80) and Test Item B (with Polysorbate 80) in male Wistar rats.

Methods

Dose:	Radiolabeled Test Item was used. Test Item A was 0.5 MBq; Test Item B was 1.0 MBq. Specific Activity was calculated for each batch, before and after administration to the animal.
Frequency of dosing:	Single
Route of administration:	IV
Dose volume:	0.75 mL
Formulation:	Test Item A and Test Item B (see above) were compared
Control:	NA
Species/Strain:	Rat, Wistar (Crl: (WI))BR)
Number/Sex/Group:	3/group, males only
Age:	Not provided
Weight:	178-212 g
Deviation from study protocol:	None

Study Design:
The purpose of the study comparing two Flutemetamol formulations was to bridge the study results from the GLP safety pharmacology, GLP toxicology, and PK/PD studies, which were conducted using Formulation A (Table 1) without Polysorbate 80, to the Clinical Trial Material containing Polysorbate 80, Formulation B.

Procedures:
Animals were injected with test article, and at appropriate time points the following samples were collected: Bone, muscle, blood, left and right kidneys, bladder and urine, lung, liver, spleen, stomach and contents, small intestine and contents, large intestine and contents, eyes, thyroid, heart, testes or ovaries and uterus, brain, fat, skin, feces, carcass, injection site (whole tail). Calculations were made for the % id in organ or tissue.

Results:
There were no appreciable statistically significant differences in biodistribution of the two Test Items, A and B. Similar results were shown by the sponsor for distribution of both Test Items A and B at time points between 2 minutes and 1 hour in brain, blood, liver, and small intestine of rats, following a single administration of [^{18}F]Flutemetamol. [^{18}F]Flutemetamol was excreted predominantly via the gastrointestinal tract in rats (approximately 80% injected dose by 4 hours post-injection) with minor excretion in urine (approximately 10% injected dose by 4 hours post-injection).

Report conclusion: There were no biologically significant differences between the biodistribution of Test Item A and Test Item B in male Wistar rats.

Reviewer comment: I agree with the sponsor's conclusion. Test Item A in the current study contained 10% ethanol, while Formulation A (the nonclinical Test Items used in toxicity studies) contained 7% ethanol. It is unclear why the sponsor chose to use a 10% ethanol-containing formulation instead of the 7% ethanol-containing formulation, although I think it's unlikely that the 3% difference in ethanol in the formulation would have an effect on the distribution of test article; when it is injected IV, the test article is diluted by the large blood compartment volume.

*No female animals were evaluated in the study. There was a slight, but statistically significant, difference between males and females evaluated in a previous biodistribution study (B067059) in terms of the distribution of Flutemetamol (Formulation A, containing 7% ethanol) in brain. Uptake in brain was higher in females at 2 minutes post-injection (*Figure 9*). The clinical implications of the observation are unknown, but it supports the view that animals of both sexes should have been used for the safety pharmacology studies. Overall, the results of study B067060 support bridging of nonclinical studies using Formulation A (the nonclinical formulation in 7% ethanol), to the Clinical Formulation, Formulation B, (containing ethanol and Polysorbate 80) in males. The sponsor should have evaluated both sexes to get a complete distribution profile with the PS-80-containing formulation. Although the lack of data in females is not a major safety issue due to the microdose administered in clinical trials, it would have been important to know if the PS-80 had an effect on Flutemetamol distribution to brain, in consideration of sex-based differences in brain distribution as shown in B067059.*

Study title: A Study to Investigate whether the Distribution of Radioactivity after Administration of [^{18}F]AH110690 is Affected by the presence of radioactive impurities in the Formulation

Study no.:	B067062
Study report location:	4.2.2.3.1
Conducting laboratory and location:	GE Healthcare Ltd. The Grove Center White Lion Road Amersham, Buckinghamshire HP7 9LL England
Date of study initiation:	10 July 2007
GLP compliance:	No
QA statement:	No
Drug and % purity:	Test Item 75*: [^{18}F]AH110690 Drug Product (Table 1) with 75% radiochemical purity (RCP) Test Item 83*: Drug product (Table 1) with 83% RCP

*Test Item 75 and Test Item 83 also appeared to be the unique identifier (Lot/Batch designation) for each Test Item.

Key Study Findings:
Overall, the presence of radiochemical impurities increased the amount and residence time of radioactivity in the blood, delayed uptake into brain, and slowed the excretion rate through the gastrointestinal route. At the 20-minute time point, the brain tissue had similar amounts of Flutemetamol from each injected dose (100% RCP, 83 % RCP, or 75% RCP).

Methods

Dose:	2.4 Mbq was injected. Specific Activity was calculated for each Test Item, before and after administration to the animal.
Frequency of dosing:	Single
Route of administration:	IV
Dose volume:	0.6 mL
Formulation:	[^{18}F]AH110690 in Phosphate Buffer (0.01M) containing 7% ethanol and 0.5% Polysorbate 80. Specific activity was 4 MBq/mL. Test Item 75 contained (b) (4)% radiochemical impurities ((b) (4)% of which was Impurity (b) (4)). Test Item 83 contained (b) (4)% radiochemical impurities (b) (4)% of which was Impurity (b) (4)).
Control:	NA
Species/Strain:	Rat, Wistar (Crl: (WI))BR)
Number/Sex/Group:	3/group and time point, males only
Age:	Not provided
Weight:	210-280 g
Unique study design:	Scheduled terminations occurred at 2, 20, 60 and 120 minutes post-treatment
Deviation from study protocol:	Due to an unanticipated delay in Test Item availability, the rats were heavier than specified in the protocol. Study impact was that older, fatter rats have more Test Item residing in the fat, and a resulting prolongation of clearance (discussed in the report).

Study Design
The purpose of the study was to compare the biodistribution of Test Items containing [^{18}F]AH110690 of varying radiochemical purity (RCP) with results from a previous study (B067060, above) in which the Test Item had 100% RCP. The sponsor scaled up production of Flutemetamol for Injection, and recognized that the scaled up batches would contain up to (b) (4)% radiochemical impurities (radiolysis products of Flutemetamol). The Test Items were designated 'Test Item 75', which was 75% RCP [^{18}F]AH110690, and Test Item 83, which was 83% RCP [^{18}F]AH110690. The formulation used was the clinical formulation, 'Formulation B' in Table 1 of this review, which contained 7% ethanol and 0.5 % Polysorbate 80 in Phosphate Buffered Saline, pH 7.4.

The specific radiochemical impurity that has the highest abundance in the scaled up Flutemetamol For Injection product is “Radioactive Impurity (b) (4)”, which is designated as such because the relative retention time in high performance liquid chromatography (HPLC) analysis is 2.4 minutes. The Test Items were deliberately modified such that Test Item 75 contained Radioactive Impurity (b) (4) at (b) (4)% of total radioactivity and Test Item 83 had an Impurity (b) (4) level of (b) (4)% of total radioactivity.

Procedures
Animals were injected with Test Item as specified in the protocol, and at appropriate time points the following samples were collected: : Bone, muscle, blood, left and right kidneys, bladder and urine, lung, liver, spleen, stomach+contents, small intestine+contents, large intestine+contents, eyes, thyroid, heart, testes or ovaries and uterus, brain, fat, skin, feces, carcass, injection site (whole tail). Calculations were made for the % id in organ or tissue, with corrections for decay.

Results
According to the sponsor, results showed the presence of radioactive impurities in the Test Item did have a significant effect on the biodistribution profile of both Test Items containing impurities, compared with results of a previous study that utilized 100% radiochemically pure Flutemetamol for Injection.

The main effect on biodistribution of the high-radiochemical impurity samples was an elevated retention of radioactivity in blood, with resulting delayed distribution to brain, muscle, liver, and small intestine. The dose of radioactivity to bone was slightly higher for the high-impurity formulations. The impurities are thought to be radiolysis products of Flutemetamol, and the sponsor posits that the impurities do not cross the blood brain barrier.

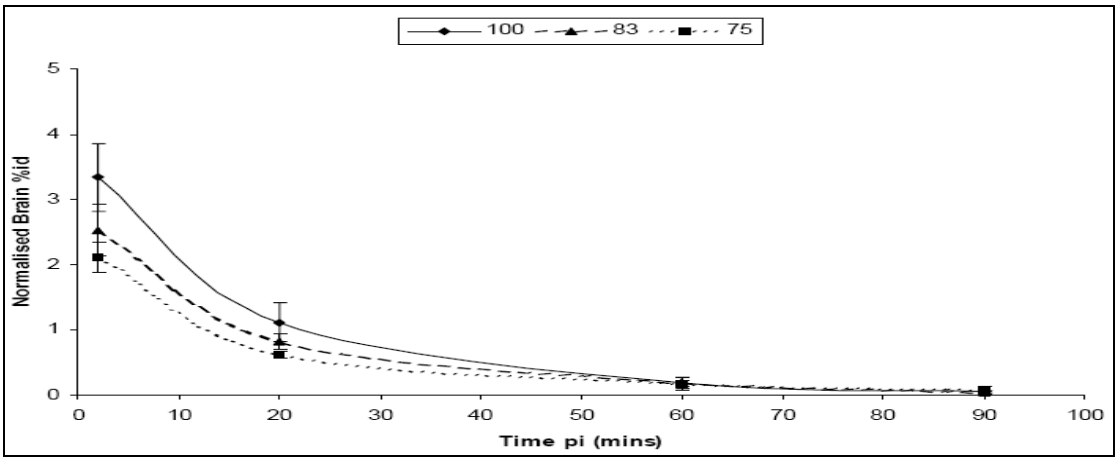


Figure 13. Comparison of Normalized Brain Uptake of Flutemetamol for Injection in Test Items with 75%, 83%, and 100% RCP in Wistar Rats.

Report conclusion
The sponsor stated that the clinical impact of the results is minimal in terms of the effective dose of Flutemetamol to the brain for the purposes of imaging (see Figure 13). By 20 minutes, the levels of radioactivity in brain were similar for all Test Items, including 100% RCP Flutemetamol for Injection. The sponsor stated that a proportional increase in the total dose administered in clinical trials may be needed and should be considered, based on the results of the study. There was no accumulation of radioactivity in the brain or in any radiosensitive organ, according to the sponsor. The sponsor asserted that the impurities do not cross the blood-brain barrier. The sponsor stated that the results support an RCP purity specification of "not less than 90%".

Reviewer comment: Agree with sponsor's interpretation and conclusion.

Study title: Estimation of the Influence of Radiochemical Impurities in AH110690 (^{18}F) Injection upon the Effective Dose

Study no.:	B067066
Study report location:	Module 4.2.2.3
Conducting laboratory and location:	Not applicable; Internal GE Healthcare Report

Key Study Findings
The sponsor concluded that the effect of a (b) (4)% reduction in radiochemical purity (RCP) of [^{18}F]Flutemetamol would reduce the Effective Dose (ED) by (b) (4)%, from 51.7 µSv per MBq to (b) (4) µSv per MBq. The reduction in RCP would have minimal impact on the ED.

Summary
The sponsor stated that the specification for [^{18}F]Flutemetamol Clinical Product is not less than 90% radiochemical purity (RCP). This report summarized the sponsor's theoretical analysis of the impact of radiochemically impure (not less than 90% RCP)

[^{18}F]Flutemetamol on the ED compared with 100% RCP [^{18}F]Flutemetamol. The study data from B067062 was used for the ED calculations of radiochemically impure [^{18}F]Flutemetamol (Test Articles were 75% or 83% RCP [^{18}F]Flutemetamol), and then compared with estimated ED from Study B067065 (reported above) which used 100% RCP [^{18}F]Flutemetamol. Assumptions underlying the calculations and normalization of cumulated activity were described.

Study Title: Investigation of the *In Vivo* Metabolism of [^{18}F]Flutemetamol in Plasma and Brain Samples in the Rat Including the Availability of any Metabolites to the Brain

Study no.:	B067071
Study report location:	Module 4.2.2.4
Conducting laboratory and location:	GE Healthcare Ltd The Grove Center, White Lion Road Amersham Buckinghamshire HP79LL England
Date of study initiation:	08 February 2010
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	[^{18}F]AH-110690 (lot # not stated); 100% purity

Key Study Findings: Flutemetamol rapidly partitioned to the brain compartment at the two minute time point, with negligible metabolism to M1, M2, or M3. At 20 and 60 minutes p.i.,, the parent Flutemetamol was detected at 75% of injected dose in the brain. In contrast, plasma Flutemetamol had decreased 40% via metabolism to M2 and M3 within the first two minutes p.i., and was metabolized to M1, M2, and M3 (more than 85% of injected dose) by 60 minutes p.i.

Experimental Design and Methods
The objective of the study was to determine the distribution of [^{18}F]Flutemetamol in plasma and brain in three rats per time point at 2, 20, and 60 minutes post-injection.

Methods

Doses:	Approximately 20 MBq/kg
Frequency of dosing:	Single injection
Route of administration:	Intravenous
Dose volume:	1 mL
Formulation	19-24 MBq/mL [^{18}F]Flutemetamol formulated in Saline (0.9% NaCl w/w) containing 18 mM Phosphate buffer, 7% Ethanol (v/v) and 0.5% Polysorbate 80.
Species/Strain:	Rat/Sprague Dawley
Number/Group (time point):	3; females only
Weight:	Approximately 210 g

Study Design

Rats were anesthetized and then injected IV with [^{18}F]Flutemetamol. At the designated time points (2, 20, and 60 min. p.i.), animals were terminated and brain and blood samples were collected. Samples were processed and then analyzed for radioactivity content and metabolites using HPLC with UV and radiochemical detection.

Summary of Results

Three metabolites of [^{18}F]Flutemetamol were detected and quantified in the study, and were designated M1, M2, and M3. The metabolites were less lipophilic than the parent compound, Flutemetamol. Table 5 shows the summary results from each group of 3 rats per time point.

Table 5. Percent Activity Recovered as Parent or Metabolite in Brain And Plasma, based on Area Percent.

Samples	**Plasma**			**Brain**		
Time pi (minutes)	**2 n=3**	**20 n=3**	**60 n=3**	**2 n=3**	**20 n=3**	**60 n=3**
Mean % M1 ± SD	<LOQ	3 ± 3.5	3± 3.1	ND	ND	ND
Mean % M2 ± SD	15 ± 3.0	46 ± 6.1	59 ± 18.1	ND	2 ± 1.7	4 ± 1.7
Mean % M3 ± SD	27 ± 6.0	30 ± 3.5	25 ± 9.6	3 ± 0.6	13 ± 3.0	21 ± 8.5
Mean % [^{18}F]flutemetamol ± SD	58 ± 2.6	21 ± 4.6	13 ± 6.8	97 ± 0.6	85 ± 2.1	75 ± 8.9

ND Not detected
LOQ Limit of quantification

Source of Table: Report B067071, Table 8.

Study title: Biodistribution of Fluorine-18 after Administration of a Formulation of [^{18}F]Flutemetamol Prepared using the Fastlab SPE Process

Study no.:	B067075
Study report location:	Module 4.2.2.3
Conducting laboratory and location:	GE Healthcare, The Grove Centre White Lion Road, Amersham Buckinghamshire HP7 9LL England
Date of study initiation:	22 March 2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{18}F]Flutemetamol, Batches 70 (22 March 2011) and 74 (18 May 2011), synthesized on the FASTlab with integrated SPE purification, was supplied in a formulation of 0.9% NaCl (w/v) containing 7% Ethanol (v/v), 0.5% Polysorbate 80, and 0.015M phosphate. Test items were used approximately 7h after End of Synthesis. Certificates of Analysis for each test item, including amounts of specified impurities, were included in the report.

Key Study Findings

The tissue distribution of [^{18}F]Flutemetamol purified using the SPE method compared with [^{18}F]Flutemetamol purified using the HPLC method was similar in male Wistar rats. Based on the study results, the sponsor concluded that the modified radiochemical impurity profile of SPE-purified [^{18}F]Flutemetamol did not have an impact on the distribution and elimination of radioactivity, and therefore, is not expected to affect the safety of the product.

Experimental Design and Methods

The sponsor implemented a change in the purification method of [^{18}F]Flutemetamol for Injection Final Product, which resulted in a modified radiochemical impurity profile in the to-be-marketed product versus HPLC-purified product. The objective of this nonclinical study was to determine if the changed radiochemical impurity profile of [^{18}F]Flutemetamol for Injection (synthesized using the FASTlab-SPE purification process) affected the biodistribution profile of [^{18}F]Flutemetamol for Injection produced using the previous HPLC purification method. The results of the current study were compared with Study B067059, which used Final Product that was purified using the previous HPLC method.

Twenty one male Wistar outbred rats (Crl: (WI)BR) (210-280 g at sacrifice) were used in the study. The distribution of intravenously injected test item amongst 24 tissues and

organs from each rat was studied at 5 time points post-injection (pi) (2, 20 or 30, 60 and 120 minutes pi) with 3 or 4 rats at each sacrifice time (Table 6).

Table 6. Study Design in B067075

Study Date	Test Item	Number Of Rats Per Group	Time Points (Minutes)	Approximate Activity Administered (MBq)*	Volume Administered Per Animal (mL)
22 March 2011	FASTlab-SPE-purified [^{18}F]Flutemetamol; Batch 70	3	2, 20, 60, 120	2-4	0.06
18 May 2011	FASTlab-SPE-purified [^{18}F]Flutemetamol; Batch 74	3	2, 30, 60	3-4	0.06

*Approximate amount of radioactivity was dependent on the time of injection. The shortest timepoint was injected last; therefore, the estimated level of radioactivity received was lower due to decay.

The organs and tissues from each rat were weighed per protocol and then were assayed for radioactivity in a gamma counter. Data were analyzed for statistically significant differences between groups (where $p<0.05$ was considered significant) using appropriate statistical analysis. Biodistribution data from the current experiment were compared with data from Study B067075, and results were presented in tabular and graphical formats.

Results

The distribution of radioactivity from SPE-purified and HPLC-purified [^{18}F]Flutemetamol was similar. Figure 14 shows the distribution of radioactivity in brain following administration of SPE-Purified [^{18}F]Flutemetamol in male Wistar rats from the current study compared with tissue distribution of HPLC-Purified [^{18}F]Flutemetamol in Male Rats from Study B067059.

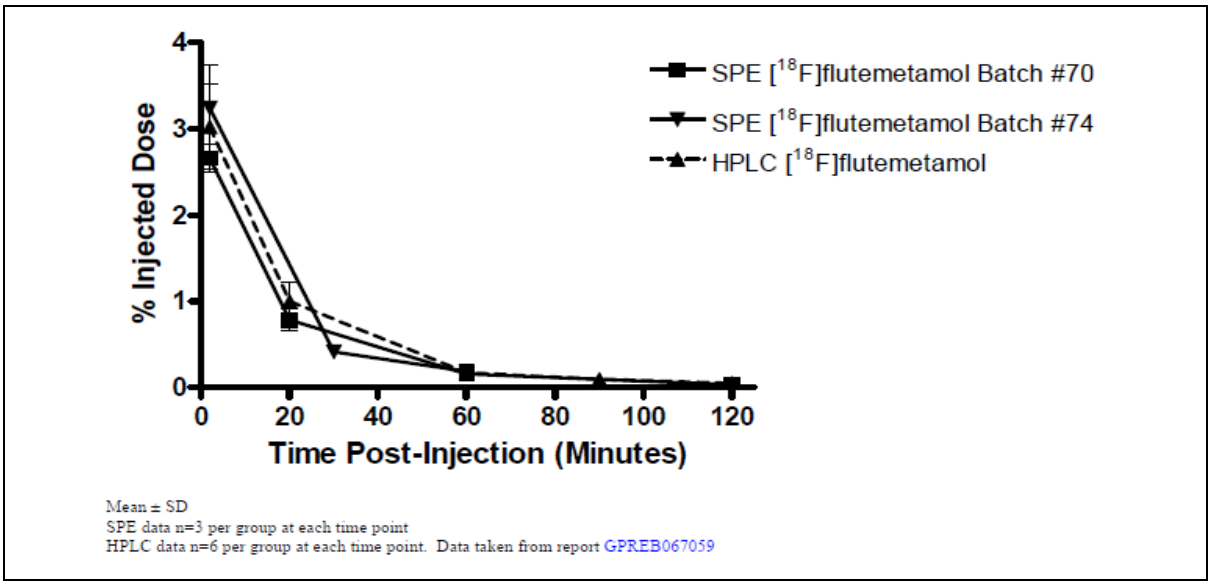


Figure 14. Radioactivity in Brain Following Administration of SPE-Purified and HPLC-Purified [^{18}F]Flutemetamol in Male Wistar Rats.

Source of Figure: Study B067075.

The sponsor also showed results for muscle, skin, bone, kidney, liver, gut contents+feces, and bladder+urine, all of which indicated there was no significant difference in distribution of radioactivity amongst the Test Items used.

Sponsor's Conclusion- The distribution of radioactivity in tissues from animals given SPE-purified [^{18}F]Flutemetamol was comparable to that previously reported for a preparation of HPLC purified [^{18}F]Flutemetamol in male Wistar rats. Therefore, the use of SPE purified product is not expected "…to have a biologically significant impact on the biodistribution or elimination of radioactivity following the administration of [^{18}F]Flutemetamol".

Reviewer's comment: Agree that the current data showed no remarkable differences in biodistribution of [^{18}F]Flutemetamol in male rats at the time points tested.

Metabolism

In vivo Metabolism of Flutemetamol

Brief Summary

Flutemetamol is rapidly metabolized in rat, baboon and humans resulting in at least 2 radioactive hydrophilic metabolites. HPLC profiles of C11-labeled products present in rat and baboon plasma after intravenous administration of C11-Flutemetamol were studied. In both rat and baboon, C11-Flutemetamol was rapidly metabolized and at least 2 hydrophilic products were observed and the retention times of these hydrophilic products were comparable in both rat and baboon plasma.

Study title: An Investigation of the Metabolic Profile of [14C]AH110690 in the Presence of Aroclor 1254 Induced and Non-Induced Rat Hepatic S9 Fraction.

Study no.:	B067013
Study report location:	4.2.2.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	09Feb 2004
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	14C-labeled AH-110690 batch CFQ13532 (480 µg/mL (3.7 MBq/mL); chemical purity 99.1%; radiochemical purity 99.3%.

Key Study Findings: Incubations (*in vitro*) in the presence of Aroclor-induced S9 fraction contained 3 additional radioactive species that were not present in incubations with non-Aroclor-induced S9 fraction.

Study design:
The purpose of the study was to determine if the *in vitro* metabolic profile of Flutemetamol incubated with rat S9 fraction from Aroclor-induced rats was similar to the metabolic profile of Flutemetamol incubated with S9 from non-Aroclor-induced rats.

Results:
The study results showed that the metabolic profiles were dissimilar in incubations of 14C-Flutemetamol in Aroclor-induced and non-induced rat S9 fractions. Incubations in the presence of Aroclor-induced S9 fraction contained 3 additional radioactive species that were not present in incubations with non-Aroclor-induced S9 fraction.

Report conclusion:
The sponsor hypothesized that the positive signal from the genotoxicity study (reviewed in Section 7.1) was due to metabolites that are only produced after incubation with Aroclor-induced rat liver S9 fraction, and that patients would not be exposed to such metabolites because human liver enzymes would not be induced with Aroclor. The sponsor concluded that the results of this study lent support to the hypothesis.

Reviewer comment: It is possible that the sponsor's assertion is valid. However, the logical definitive study that could have been conducted is to have tested the putative genotoxic metabolites produced from the Aroclor-induced incubations. The sponsor provided supportive evidence for their hypothesis, but not definitive evidence. They also could have identified the structure of the metabolites, which would have provided

information on the strength of electrophilicity of the metabolite(s) as related to DNA binding.

Study title: AH110690 A Study to Investigate the *In Vivo* Metabolic Stability of [^{11}C]AH110690 in Rat.

Study no.:	B067018
Study report location:	4.2.2.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	08 January 2004
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{11}C]AH110690 Injection in saline containing 5% ethanol; 99.1 RCP

Key Study Findings

Two major metabolites of Flutemetamol were observed in the two female rats on study. Only females were used in the study.

Methods

^{11}C-Labeled AH110690 was synthesized in the (b) (4) using a no-carrier-added method. The product was injected IV into each of two female Sprague-Dawley rats 0.5 to 0.8 ml, approximately 74 to185 MBq [^{11}C]AH110690. Plasma samples were collected at 2, 10, 30, and 60 minutes post-injection, and then were processed and analyzed using HPLC (scintillation detection).

Results

Three major peaks were observed in the HPLC chromatograms: Parent compound, ([^{11}C]AH110690 [^{11}C]Flutemetamol, Retention Time (RT) of ~13 min.), and two metabolites, designated Species 1 (RT of 2.8 min.) and Species 2 (RT of 4.9 min.). A profile was generated to show the change in metabolite amount over time in the two rats tested.

Report conclusion

Two major metabolites were formed in the two female rats studied.

Reviewer comment: Agree with sponsor's conclusion.

Study title: A Study to Investigate the *In Vivo* Metabolic Stability of [^{11}C]AH110690 Following Intravenous Administration to Baboon

Study No:	B067019
Study report location:	4.2.2.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	08 January 2004
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{11}C]AH110690 Injection in saline containing 5% ethanol; 98.9 RCP

Key Study Findings

Two major metabolic products of Flutemetamol were observed in adult male baboons (N=2).

Methods

^{11}C-Labeled AH110690 was synthesized in the (b) (4) using a no-carrier-added method. The product was injected IV into each of two male baboons (9 years and 13 years of age), in a volume of 10 mL, approximately 296 to555 MBq [^{11}C]AH110690. Plasma samples were collected at 2, 10, 30, 60, and 90 minutes post-injection, and then were processed and analyzed using HPLC (scintillation detection).

Results

Three major peaks were observed in the HPLC chromatograms: Parent compound, [^{11}C]AH110690 [^{11}C]-Flutemetamol, Retention Time (RT) of ~13 min.)], and two metabolites, designated Product 1 (RT of 2.8 min.) and Product 2 (RT of 4.9 min.). Summary tables of the amounts of radioactivity in each sample peak over time, for each animal (animals were referenced according to Injection Date) are shown in Table 7. The metabolite profiles for each animal differed over time, but similar metabolite products were generated.

Table 7. *In Vivo* Metabolite Profile of [^{11}C]Flutemetamol in Two Baboons

Table 4 Carbon-11 labelled products in baboon plasma following intravenous administration of [C11]AH110690 Injection to baboon-04 March 2003

Carbon-11 labelled products	2 minutes pi	10 minutes pi	30 minutes pi	60 minutes pi	90 minutes pi
1	5.9	29.1	45.2	59.5	100.0
2	12.7	52.0	49.4	37.8	0.0
[^{11}C]AH110690	81.4	18.9	5.4	2.7	0.0

Each data point is based on a single sample.

Table 5 Carbon-11 labelled products in baboon plasma following intravenous administration of [C11]AH110690 Injection to baboon-27 January 2004

Carbon-11 labelled products	2 minutes pi	10 minutes pi	30 minutes pi	60 minutes pi	90 minutes pi
1	4.3	18.6	33.6	50.0	63.0
2	9.2	54.1	57.7	43.5	31.2
[^{11}C]AH110690	86.4	27.3	8.7	6.5	5.8

Each data point is based on a single sample.

Source: Study Report B067019.

Report conclusion: Two major metabolic products of Flutemetamol were observed in adult male baboons (N=2).

Reviewer comment: Agree with sponsor's report conclusion. The metabolites were not identified.

It appears that the same two metabolites were generated in the rat study (B067018) and in this baboon study (B067019).

In vitro Metabolism and Plasma Protein Binding of Flutemetamol

Brief Summary
Studies were performed to investigate the *in vitro* metabolism of Flutemetamol by incubation with hepatic S9 fraction obtained from man, dog, mouse and rat (where the rats had either been not treated or pre-treated with Aroclor 1254, a potent inducer of liver metabolizing enzymes). The major metabolite in these studies, irrespective of the origin of the hepatic S9, was the N-demethylation product of Flutemetamol. Based on these results, the sponsor concluded that metabolism in human in vitro incubations is similar to that of the rat and dog, and gives support for these two species being appropriate for non-clinical studies.

No metabolism or degradation was observed following incubation of [14C]Flutemetamol with citrated rat, dog or human plasma for up to 3 hours. Low levels of 2 metabolites were seen in citrated human whole blood at 3 hours.

The binding of [^{3}H]Flutemetamol to human, dog and rat plasma proteins was shown to be greater than 95% by equilibrium dialysis. Despite this high protein binding, Flutemetamol is rapidly eliminated from blood after intravenous administration of [^{18}H]Flutemetamol to male and female Wistar rats.

Study title: Metabolic Profile of [14C]AH110690 in Human Hepatic S9 Incubates

Study no.:	B067023
Study report location:	4.2.2.4.1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	24 May 2004
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[14C]AH-110690 batch CFQ13532; 99.3 RCP and 99.1% chemical purity

Key Study Findings: The in vitro incubations of Flutemetamol with human S9 liver fraction or rat Aroclor-induced S9 liver fraction yielded different metabolite profiles. The desmethyl-metabolite of Flutemetamol was the only product observed in human S9 incubations. In contrast, the rat Aroclor-induced metabolites were formed very rapidly (visible peaks in chromatograms at 4 minutes), in addition to the desmethyl metabolite.

Study design
The study was conducted to investigate the *in vitro* metabolic profile of Flutemetamol in human hepatic S9, and to compare the human S9 profile with the metabolic profiles of Flutemetamol in rat hepatic S9 from untreated or Aroclor-induced rats. The Test Item, [14C]AH110690, was incubated in the presence of human liver S9 fraction using appropriate incubation conditions for Phase I, Phase II, or both Phases I and II biotransformation reactions to occur. Incubation times were 4, 20, 160, and 180 minutes. Control incubations were conducted in the presence of heat-inactivated S9. At the end of each incubation time, reactions were stopped and then analyzed for metabolites using HPLC with radiochemical and fluorescence detection.

Results
Results using fluorescence detection showed formation of one major metabolite in incubations with human S9.

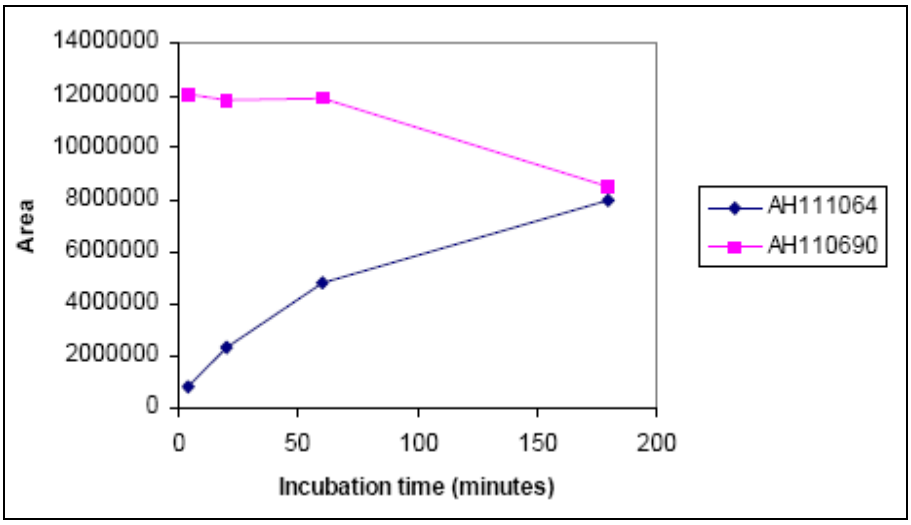


Figure 15. Decrease of Parent Compound and Increase of N-Demethylated Metabolite Following Incubation of [^{14}C]Flutemetamol Human S9 Incubations.

Source: Study Report B067023, Sponsor's Figure 5.

Results also showed that one major metabolite was formed in both human hepatic S9 and Aroclor-induced rat hepatic S9 (Figure 16, Peak A), which co-eluted with a synthetic standard of the N-demethylated species of Flutemetamol. The sponsor stated that the peak with RT of 1 minute (Peak A) was the major N-demethylation metabolite in all systems tested, including non-induced rat S9. Additional peaks were apparent in the Aroclor-induced rat S9 incubates that were not observed in Human or non-induced rat S9 incubations, according to the sponsor.

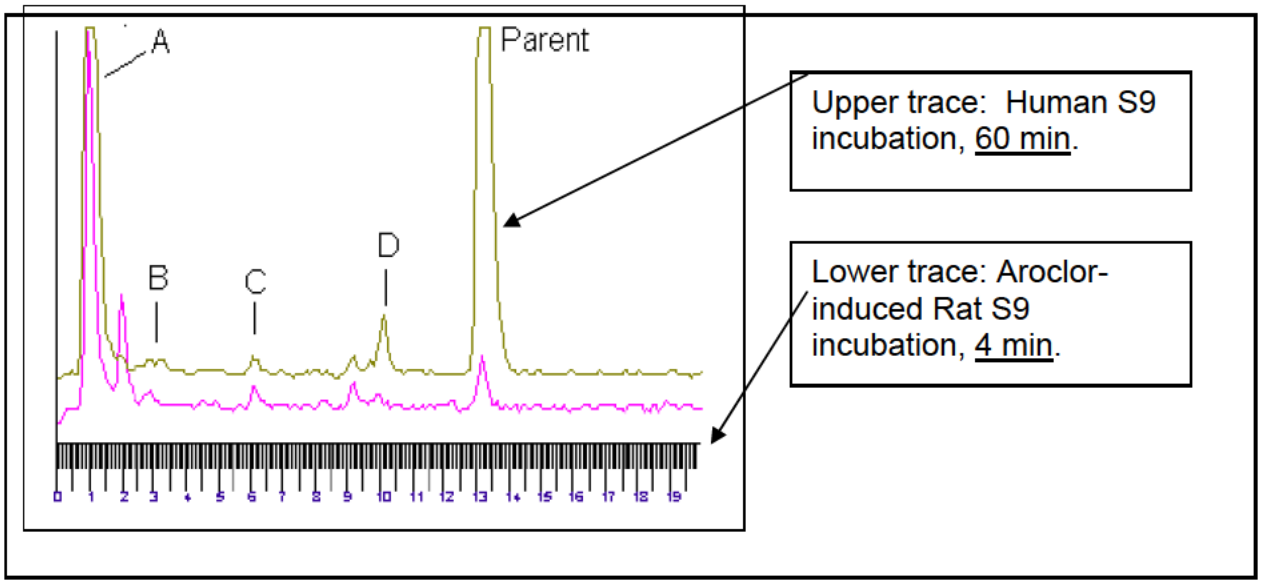


Figure 16. Typical Chromatograms of Flutemetamol Metabolite Profiles in Human and Rat Hepatic S9 using Fluorescence Detection.

Source: Study Report B067023.

Report conclusion: The sponsor concluded that the products of biotransformation were formed by cytochrome P450-dependent mechanisms, and that the major metabolite in human and rat incubations was desmethyl-Flutemetamol. Also, S9 liver fraction from Aroclor-induced rats generated unique metabolites (see Study B067013 results), which may have explained the positive and equivocal results in the Bacterial Mutagenicity Test (Study B067005).

Reviewer comment: The Aroclor-induced rat S9 incubations generated a major radiolabeled metabolite peak (Peak A, identified as the desmethyl metabolite of Flutemetamol), which was abundant at the 4-minute time point. In contrast, the 1-hour human S9 incubation showed the major peak was the one containing parent compound. The largest radiolabeled metabolite peak from human S9 incubation at 1-hour appeared to be the desmethyl metabolite, which co-eluted with Peak A.

The products of the in vitro incubations tested in this study were generated on different days. The rat incubation products were generated in Study B067013. This practice may have introduced some variability, due to differences in sample prep and stability of metabolites over time. Despite the possible impact of not conducting incubations in parallel on the same days, I agree with the sponsor that the major labeled metabolite appears to be desmethyl-Flutemetamol, in incubations of human S9 and rat S9 (induced and non-induced with Aroclor).

Study title: The *In Vitro* Metabolism of [19F]AH110690 in Mouse, Rat (Aroclor 1254), Dog and Human Hepatic S9

Study no.:	B067045
Study report location:	4.2.2.4.1
Conducting laboratory and location:	GE Healthcare Ltd. The Grove Center White Lion Road Amersham, Buckinghamshire HP7 9LL England
Date of study initiation:	29 July 2005
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	AH-110690; batch FKJ 0139/111-04 (3 mg/mL nominal stock solution concentration); purity not reported.

Key Study Findings: Metabolite profiles generated *in vitro* were similar across species, and generally supported the sponsor's choices of nonclinical toxicity species to be used in the Flutemetamol testing program.

Study design
The study was undertaken to characterize the *in vitro* metabolite profile of Flutemetamol (non-radiolabeled) in human, dog, rat, and mouse hepatic S9 incubations. A combination of HPLC and mass spectrometry were used to analyze the metabolites after specified incubation time periods.

Results
Results showed that metabolite profiles generated across species were broadly similar. The major metabolites produced were de-methylated Flutemetamol (m/z ratio of 261) and hydroxylated Flutemetamol (m/z ratio of 291). The metabolite with m/z of 291 was only observed in incubations with rat Aroclor-induced S9 preparations (Table 8). The findings lend support to the sponsor's hypothesis that S9 from Aroclor-induced rats produced a unique metabolite that was not observed in incubations with S9 from human, dog, or mouse. Results are summarized below (Table 8).

Report Conclusion
The sponsor concluded that the chosen test species for toxicology studies were appropriate, based on the similarity of metabolite profiles generated in all species tested, with the exception of Aroclor-induced hepatic S9 from rat.

Reviewer comment: Agree.

Table 8. Summary of Metabolite Profiles From Incubations with Flutemetamol and S9 from Human, Dog, Rat (Aroclor-Induced), Mouse, And Control (Water), *In Vitro*.

Incubation time and conditions	Human	Dog	Rat	Mouse	Water
m/z 261, retention time approximately 17.1 minutes					
-1 minutes	x	x	x	x	x
2 minutes	x	x	✓	x	x
5 minutes	✓	✓	✓	✓	x
30 minutes	✓	x	✓	✓	x
β-NADPH deficient (30 minutes)	✓	x	x	✓	N/A
Boiled S9 (30 minutes)	x	x	x	x	N/A
m/z 291, retention time approximately 17.7 minutes					
-1 minutes	x	x	x	x	x
2 minutes	x	x	✓	x	x
5 minutes	x	x	✓	x	x
30 minutes	x	x	✓	x	x
β-NADPH deficient (30 minutes)	x	x	x	x	N/A
Boiled S9 (30 minutes)	x	x	x	x	N/A
m/z 275 (AH-110690), retention time approximately 19.1 minutes					
-1 minutes	x	x	x	x	x
2 minutes	✓	✓	✓	✓	✓
5 minutes	✓	✓	✓	✓	✓
30 minutes	✓	✓	✓	✓	✓
β-NADPH deficient(30 minutes)	✓	✓	✓	✓	N/A
Boiled S9 (30 minutes)	✓	✓	✓	✓	N/A
m/z 289, retention time approximately 20.3 minutes					
-1 minutes	x	x	x	x	x
2 minutes	✓	✓	✓	✓	✓
5 minutes	✓	✓	✓	✓	✓
30 minutes	✓	x	x	✓	✓
β-NADPH deficient (30 minutes)	✓	x	✓	✓	N/A
Boiled S9 (30 minutes)	✓	✓	✓	✓	N/A
m/z 547, retention time approximately 20.8 minutes					
-1 minutes	x	x	x	x	x
2 minutes	✓	✓	✓	✓	✓
5 minutes	✓	✓	✓	✓	✓
30 minutes	✓	x	✓	✓	✓
β-NADPH deficient(30 minutes)	✓	x	✓	x	N/A
Boiled S9 (30 minutes)	✓	✓	✓	✓	N/A

✓, denotes the presence of a particular species at the relevant time point and experimental conditions.
x, denotes the absence of a particular species at the relevant time point and experimental conditions.
N/A, not applicable.

Metabolite m/z 291 is Unique to Aroclor-induced Rat S9

Source of Table 8: Study Report B067045.

Study title: The In vitro Metabolic Profile of [^{18}F]AH110690 in Mouse and Dog Hepatic S9

Study no.:	B067046
Study report location:	Module 4.2.2.4.1
Conducting laboratory and location:	GE Healthcare Ltd The Grove Center, White Lion Road Amersham Buckinghamshire HP79LL England
Date of study initiation:	16 July 2005
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	[^{18}F]AH-110690 (lot #, purity not stated)

Key Study Findings

The sponsor concluded that [^{18}F]Flutemetamol was not metabolized significantly in the presence of dog or mouse hepatic S9 after 30 minutes.

Summary

The study was conducted to investigate the metabolic profile of [^{18}F]Flutemetamol in the presence of dog and mouse hepatic S9. Incubations of hepatic S9 samples from mouse and dog, each containing [^{18}F]Flutemetamol and β-NADPH, were analyzed for [^{18}F]Flutemetamol metabolites after 2, 5, and 30 minutes. Results showed that 90% of radioactivity was associated with [^{18}F]Flutemetamol after 30 minutes in dog S9 incubations. In mouse hepatic S9 incubations, 72% of the radioactivity was associated with [^{18}F]Flutemetamol after 30 minutes. The sponsor concluded that the [^{18}F]Flutemetamol is not significantly metabolized in dog or mouse hepatic S9 fraction.

Study title: *In Vitro* Stability of [^{3}H]AH110690 in Rat, Dog and Human Plasma, Human Whole Blood and Phosphate Buffered Saline

Study no.:	B067048
Study report location:	4.2.2.4.1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	24 August 2005
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{3}H]AH-110690 batch TRQ11157 Supplied as a 1 mL ethanol solution containing 18.33 µg [^{3}H]Flutemetamol; 98.9% RCP; chemical purity not specified

Key Study Findings:
[^{3}H]Flutemetamol was stable at 37^{0}C for up to 3 hours in PBS and plasma from rat, dog, and human samples. However, human whole blood incubations with [^{3}H]Flutemetamol yielded 2 metabolites after 3 hours at 37^{0}C.

Study Design
The *in vitro* stability of [^{3}H]Flutemetamol was investigated in vitro for 1 and 3 hours in rat plasma, dog plasma, human plasma, human whole blood, and phosphate buffered saline (PBS), at 37^{0}C, with HPLC and radiochemical detection.

Results
Results showed that [^{3}H]Flutemetamol was stable at 37^{0}C for up to 3 hours in PBS and plasma from rat, dog, and human samples. However, human whole blood incubations with [^{3}H]Flutemetamol yielded 2 metabolites after 3 hours at 37^{0}C.

Reviewer comment: Agree.

Study title: Human Plasma Protein Binding of [^{3}H]AH110690 using Equilibrium Dialysis

Study no.:	B067049
Study report location:	4.2.2.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	08 September 2004
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{3}H]AH-110690 (tritiated Flutemetamol) batch TRQ11157; supplied as a 1 mL ethanol solution containing 18.33 µg (15 mCi/mmol, 1 mCi/mL); RCP 99.5%; chemical purity not reported.

Key Study Findings:
The study was undertaken to determine the human plasma protein binding level of tritiated Flutemetamol. Tritiated Flutemetamol was incubated in dialysis chambers containing human plasma or PBS solution, and separated by a dialysis membrane. Incubation times of 3h were required to reach equilibrium. Results showed that Flutemetamol was 97.9% bound to plasma protein after 3h.

Reviewer comment: Agree with sponsor's interpretation of results.

Study title: Rat and Dog Plasma Protein Binding of [^{3}H]AH110690 using Equilibrium Dialysis

Study no.:	B067057
Study report location:	4.2.2.4.1
Conducting laboratory and location:	GE Healthcare AS Nycoveien 2 P.O. Box 4220 Nydalen 0401 Oslo Norway
Date of study initiation:	04 May 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{3}H]AH-110690 (tritiated Flutemetamol) batch TRQ11157; supplied as a 1 mL ethanol solution containing 18.33 µg (15 mCi/mmol, 1 mCi/mL); RCP 99.5%; chemical purity not reported.

Key Study Findings:
Flutemetamol was highly bound to plasma protein from rat (97.3%) and dog (95.3%).

Methods
The study was undertaken to determine the rat plasma protein and dog plasma protein binding level of tritiated Flutemetamol. Tritiated Flutemetamol was incubated in dialysis chambers containing rat or dog plasma, or PBS solution, with separation between plasma and PBS utilizing a dialysis membrane. Incubation times of 3h were required to reach equilibrium.

Results
Flutemetamol was 97.3% bound to rat plasma protein, and 95.3% bound to dog plasma protein, after 3h incubations.

Reviewer comment: Agree with sponsor's interpretation of results.

Study title: Single Dose Intravenous Kinetics Study with AH-110690 Solution for Injection in the Wistar Rat

Study no.:	B067055
Study report location:	4.2.2.4.1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	27 January 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{18}F]AH-110690 was supplied as a formulation in phosphate buffer (0.01M, pH 7.4), 10% ethanol. Radiochemical purity was 100%.

Key Study Findings
The study was an exploratory kinetics study to show the test item could be detected in peripheral blood (sampled from the orbital sinus) of 3 male rats at 3 and 10 minutes post-infusion (p.i.) of AH-110690 (60 µg/kg) into the tail vein. The maximum concentration was measured at 3 minutes p.i. (8.9 ± 0.8 ng AH-110690/mL).

Reviewer comment: The study was a pilot study, and therefore, was not described in detail.

Pharmacokinetic Drug Interactions

Study title: A Study to Establish Whether the Distribution of Radioactivity after Administration of [F I8]AH110690 To Male Wistar Rats is Affected by MAB31 Pre-Dosing

Study no.:	B067064
Study report location:	Module 4.2.2.6.1
Conducting laboratory and location:	GE Healthcare Ltd The Grove Centre, White Lion Road Amersham, Buckinghamshire, HP7 9LL England
Date of study initiation:	30 May 2007
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[^{18}F]AH110690; no lot #; 100% RCP

Key Study Findings:

Results showed that the presence of MAB31, a therapeutic anti-Aβ agent, in the circulation did not affect biodistribution of [^{18}F]AH110690 in male Wistar rats.

Experimental Design and Method
The study was conducted to determine if pre-dosing with MAB31, a therapeutic anti-Aβ investigational drug, affected the biodistribution of [^{18}F]AH110690. Male rats (n=3 per time point) were injected with MAB31 (10 mg/kg) or vehicle control. After a 24h interval, animals were injected with [^{18}F]AH110690 intravenously and subsequently were terminated at the designated time points of 2, 20, or 60 minutes. Animals were weighed, organs were harvested, and blood and urine were collected. Samples were prepared and assayed for radioactivity.

Results and Sponsor's Conclusion
Pre-dosing of male rats with MAB31 did not significantly affect the biodistribution of radioactivity observed. Similar plasma concentrations of MAB31 were achieved in each of the MAB31 pre-dosed rats and did not affect delivery to and clearance from the brain. The distribution of [^{18}F]AH110690 radioactivity to peripheral tissues and the excretion profile of radioactivity were not affected by MAB31 circulating antibodies.

Reviewer comment: Agree with the sponsor's conclusion.

5.2 Toxicokinetics

Brief Summary
The sponsor conducted a toxicokinetic (TK) study during the 14-day repeat dose toxicity study in rats (B067056). The average maximum plasma concentrations (C_{max}) were 1.27, 2.95, and 4.98 ng/mL for low, mid, and high dose groups, respectively, at 5 minutes post dosing. The elimination half-life was 7 to 19 minutes. No sex differences in TK parameters were observed. Systemic exposure to Flutemetamol increased proportionally with dose.

The sponsor also conducted a TK study during the 14-day repeat dose toxicity study in dogs (B067040): Flutemetamol was eliminated from the plasma in the high dose animals with an elimination half-life of 7.5 minutes on average (range was 6.4-9.1 minutes). The C_{max} and $AUC_{0\text{-}inf}$ were the same in males and females, on days 1 and 14. There was no systemic accumulation of Flutemetamol during the study, and systemic exposure was proportional with dose.

Details of the TK study designs are described in the rat (B067056) and dog (B067040) 14-day repeated dose toxicology studies.

6 General Toxicology

Brief Summary
The toxicology studies conducted in support of Flutemetamol F 18 Injection are summarized in Table 9, below.

Table 9. Toxicology Studies Conducted In Support Of [^{18}F]Flutemetamol

Study Type/Study Number	NOAEL		Maximum Human Dose (MHD) for 60 Kg Human		Dose Multiple** (BSA-Corrected Animal NOAEL*/ MHD)
	µg/kg	µg/m^2	µg/kg	µg/m^2	
Flutemetamol: One Day (Twice a Day) Intravenous (Bolus) Administration Toxicity Study in the Rat Following a 13-Day Treatment Free Period/B067069	192	1152	0.33	12.33	93
Expanded Single Dose in Rat/B067001	78	468	0.33	12.33	38
7-Day Dose Range Finding in the Wistar Rat/B067039	13.5	162	0.33	12.33	7
14-Day Repeated Dose in Rat/B067056	13.5	81	0.33	12.33	7
Single and 7-Day Repeated Dose in Dog/ B067038	15	300	0.33	12.33	24
14-Day Repeated Dose in Dog/B067040	14	280	0.33	12.33	23

*Correction for Body Surface Area (BSA; µg/m^2) is the animal NOAEL (µg/kg) multiplied by 6.2 for rat or 20 for dog.
**Dose multiple is the BSA-adjusted NOAEL (in µg/m^2) divided by the Maximum Human Dose (MHD) in µg/m^2.

6.1 Single-Dose Toxicity

Brief Summary
The sponsor conducted two expanded single-dose toxicity studies in rats and a combined single/repeated-dose study in dogs (reviewed under 6.2). Study B067001, which was conducted in rats early in the product development program, utilized a Flutemetamol formulation containing 7% ethanol in PBS (Formulation A in Table 1). During Phases 2 and 3 of clinical development, the formulation was modified with the

addition of (b) (4) Polysorbate 80; this formulation became the Final Drug Product (Formulation B in Table 1). The sponsor conducted an additional expanded single dose toxicity study in rats (B067069) to bridge the nonclinical study results using the early Flutemetamol formulation (Formulation A in Table 1) to studies using the Final Drug Product (Formulation B in Table 1) intended for NDA approval and subsequent marketing.

Results of the studies showed that a single dose of Flutemetamol in rats did not cause adverse effects at 192 µg/kg, which was the highest dose tested and the NOAEL. Administration of vehicle (7% ethanol in PBS) was associated with ataxia in both vehicle and Flutemetamol groups.

Study title: Flutemetamol: One Day (Twice a Day) Intravenous (Bolus) Administration Toxicity Study in the Rat Following a 13-Day Treatment Free Period

Study no.:	B067069
Study report location:	4.2.3.1.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07 September 2010
GLP compliance:	Yes, except formulation analysis, which was conducted by the sponsor
QA statement:	Yes
Drug, lot #, and % purity:	Flutemetamol and related impurities, Lot FFA093/192-009 ((b) (4) lot number 1).

Key Study Findings

The purpose of the study was to evaluate potential toxicities of the Final Drug Product (FDP) of Flutemetamol F 18 Injection, which differed from previous nonclinical test items regarding the presence of Polysorbate 80 and the amounts and composition of radiochemical impurities. The NOAEL was 192 µg/kg.

Methods

Doses:	0, 38.4, 192 µg/kg
Frequency of dosing:	Two injections on SD 1, 4 h apart
Route of administration:	Intravenous
Dose volume:	20 mL/kg
Formulation/Vehicle:	Concentration of Flutemetamol: 68.6 µg/mL Concentration of Flutemetamol and related impurities: 253.3 µg/mL Vehicle: Saline (0.9% NaCl w/w) containing 18 mM Phosphate buffer, 7% Ethanol (v/v) and 0.5% Polysorbate 80.
Species/Strain:	Rat/Crl:WI(Han)
Number/Sex/Group:	5/sex/group
Age:	7 to 8 weeks
Weight:	Males: 195.5 to 271.0 g Females: 142.8 to 179.4 g
Deviation from study protocol:	None with impact on study outcome

Observations and Monitoring

Mortality-Animals were observed at the beginning and the end of each day.

Clinical Signs-All animals were observed immediately after dosing and approximately 0.5, 1, 2, and 4 hours post-dose on SD1. All animals were observed daily for signs of ill health or overt toxicity. Each animal was given a detailed physical examination at weekly intervals.

Body Weights-Body weights were recorded on SD -7, SD1, prior to necropsy (SD 2 or 14) and on SD 8.

Feed Consumption-Amounts of food consumed were determined over SD 1-2 for animals killed on SD 2, and weekly for animals killed on SD 14.

Ophthalmoscopy-Not conducted.

ECG-Not conducted.

Clinical Pathology-Blood samples were withdrawn from the abdominal aorta of fasted animals prior to scheduled terminations on SD 2 or SD 14, and then processed for hematology or clinical chemistry measurements. Standard hematology and clinical chemistry parameters were measured for each animal.

Urinalysis-Not conducted.

Gross Pathology- A full macroscopic examination was performed under the general supervision of a pathologist and all lesions were recorded.

Tissues were collected and processed.

Organ Weights- All animals were weighed prior to necropsy. Specified organs were weighed, and then fixed.

Histopathology-All tissues from control and high-dose groups (specified in Appendix 2), and the liver, spleen, kidney, adrenals, and gross lesions from low-dose groups, were examined by the study pathologist.

Adequate Battery-Yes

Peer Review-Not stated

Toxicokinetics-Not conducted.

Dosing Solution Analysis-A Certificate of Analysis was provided (GLP-compliant). Post-study analysis was performed by the sponsor under non-GLP conditions.

Results
Mortality-All animals survived to scheduled necropsy.

Clinical Signs-Ataxia and decreased activity (males only) were observed after administration of both doses on SD 1 in all groups.

Body Weights-No notable findings (NNF).

Feed Consumption-NNF.

Ophthalmoscopy-Not conducted.

ECG-Not conducted.

Hematology and Clinical Chemistry-On SD 2, statistically significantly differences between control and treated animals were observed in lymphocyte counts, prothrombin time, and chloride levels. The differences were minimal and were not related to dose or sex, and were not considered adverse. Otherwise, NNF.

Urinalysis-Not conducted.

Gross Pathology-NNF.

Organ Weights-NNF.

Histopathology-NNF. Adequate Battery-Yes. Peer Review-No
Histological Findings-NNF.

Special Evaluation-NA

Toxicokinetics-NA

Dosing Solution Analysis-The sponsor provided a Certificate of Analysis of the Test Item (release date (b) (4)). A non-GLP analysis of test item was performed 28 September 2010. I conferred with the CMC reviewer, Dr. Ravindra Kasliwal, to verify that the Test Items used in the study were representative of the Final Drug Product. In terms of Flutemetamol plus total radiochemical impurities, the Test Item met specifications for FDP.

Sponsor's conclusion-There were no adverse effects on male or female adult rats associated with test article exposure (197 µg/kg of Flutemetamol and related impurities). Observations of ataxia (all animals; all groups) and decreased activity (males only) were observed immediately following administration, and were attributed to ethanol contained in the formulation. The highest dose tested, 197 µg/kg, was the NOAEL.

Reviewer's comments: Agree with sponsor's conclusion.

The purpose of the study was to evaluate potential toxicities of the Final Clinical Product formulation, which differed from previous nonclinical toxicity test items regarding the presence of Polysorbate 80 and the amounts and composition of radiochemical impurities.

The results of the study supported a NOAEL of 192 µg/kg.

Study title: An Expanded Single Dose Toxicity Study Of Intravenously Administered AH110690 In Male And Female Rats

Study no.:	B067001
Study report location:	4.2.3.1.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	18 June 2003
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	AH-110690 batch FFA 049/112-306 (2.7 µg/mL; purity 91% by HPLC) in 7% Ethanol and PBS; AH-110690 batch FFA 049/116-306 (2.5 µg/mL; purity 95% by HPLC) in 7% Ethanol and PBS; provided ready-for-use

Key Study Findings: All animals survived until the scheduled termination. When compared to saline-treated rats, the effects of vehicle (7% ethanol in PBS) and Flutemetamol were similar: all Vehicle- and Flutemetamol-treated animals were observed to have ataxia after dosing. The NOAEL was 78 µg/kg bw.

Methods

Dose:	78 µg/kg (bw) per day (given in 2 separate IV infusions on same day)
Frequency of dosing:	Single day (2 separate infusions, 4 h apart)
Route of administration:	IV
Dose volume:	20 mL/kg bw per day divided between two dosing sessions, about 4 h apart
Formulation/Vehicle:	7% Ethanol in PBS / 7% Ethanol in PBS
Control:	Saline
Species/Strain:	Rat, Sprague-Dawley [Bkl:SD]
Number/Sex/Group:	6/sex/group
Age:	7 (Males) or 8 (Females) weeks of age
Weight:	280-290 g (Males); 191-197 g (Females)
Unique study design:	Scheduled terminations were 1 day post- and 14 days post-treatment
Deviation from study protocol:	None with impact

Observations and procedures:

Mortality-Animals were monitored for mortality during acclimatization; before, during, and after each of two infusions on Study Day 1 (dosing day), and at least once daily thereafter.

Clinical Signs-Animals were monitored for clinical signs during acclimatization; before, during, and after each of two infusions on Study Day 1 (dosing day), and at least once daily thereafter.

Body Weights-Body weight was recorded pre-study, on SD 1, and then every other day until termination.

Feed Consumption-Feed consumption was recorded pre study, on SD 1, and then every other day until termination.

Ophthalmoscopy-Not conducted.

ECG-Not conducted.
Hematology-Blood samples were collected on SD 2 and SD 15. The following hematology parameters were measured: WBC, NEU, LYM, MONO, EOS, BASO, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, and MPV.

Clinical Chemistry-Blood samples were collected on SD 2 and SD 15. The following clinical chemistry parameters were measured: AST, ALT, ALP, CK, 5'ND, GLDH, TBIL, TG, CHOL, Urea, PO_4, Na, K, Cl, Ca, TP, ALBG.

Urinalysis-Not conducted.

Gross Pathology-Conducted 1 day post- and 14 days post-treatment during scheduled necropsy.

Organ Weights-collected during each scheduled necropsy.

Histopathology-Adequate battery conducted for SD2 necropsy; the saline, vehicle, and high dose groups were evaluated. Not conducted for SD14 necropsy.

Toxicokinetics-Not conducted.

Dosing Solution Analysis-Not conducted and not noted as a deviation.

Results

Mortality-There were no mortalities in any group

Clinical signs-Ataxia was noted for all animals that were administered vehicle (7% ethanol in PBS) or test article, within 10 minutes of dosing. There were no other notable findings that were definitely related to Flutemetamol in the test article.

Hematology and clinical chemistry-No notable findings.

Organ weights- No notable findings.

Gross pathology-There were gross findings of venous/perivenous necrosis at the injection site in animals terminated 1-day post-treatment, which were present in both vehicle and test article groups.

Microscopic pathology-Glycogen depletion in liver of females administered either vehicle or test article, compared with saline-control animals, at the scheduled termination on 1-day post-treatment. According to the sponsor, no meaningful differences were observed between animals in any group on the day 14 scheduled termination.

Report conclusion
There were no adverse effects following administration of 78 µg/kg Flutemetamol in male or female rats. The sponsor designated a No-effect-level (NOEL) of 78 µg/kg in a single day for Flutemetamol.

Reviewer comment: Agree.

6.2 Repeat-Dose Toxicity

Brief Summary
The sponsor conducted 7-day (range finding) and 14-day repeated-dose toxicity studies in rats and dogs.

Rat Studies
A Flutemetamol-dependent finding in the 14-day repeated-dose toxicity study was slightly increased severity of irritation around injection sites in high-dose males. There were no serious adverse effects on in-life or post-mortem assessments associated with Flutemetamol at doses of 13.5 µg/kg/day, which was the NOAEL in both studies. The safety margin was 7-fold relative to the MHD.

In the 14-day repeated dose toxicity study, unexpected mortality occurred in Vehicle and Flutemetamol groups. Two of twenty rats in the Vehicle group and 5 of 60 animals in the Flutemetamol groups died immediately after injection of Vehicle or Flutemetamol. The deaths occurred on several different study days: SD 1, SD 2, SD 4, SD 9, and SD 14, without an association to Flutemetamol dose or treatment, i.e., Vehicle-treated animals died also, and the number of repeated injections did not appear to be a factor. The common factor in the deaths was the presence of 7% ethanol administered by tail vein injection. The deaths were not considered to be a Flutemetamol-dependent effect.

In the 7-day repeated-dose toxicity study in rats, Flutemetamol was well-tolerated. No deaths occurred and there were no Flutemetamol-dependent adverse findings. The dose multiples based on the Maximum Human Dose (MHD) of 3.33 µg/m^2 (assuming 20 µg Flutemetamol in a 60 kg person) were 7X (BSA-corrected) in rats administered Flutemetamol daily for 7 days.

Study title: 7-Day Dose Range Finding Intravenous Toxicity Study with AH110690 Solution for Injection in the Wistar Rat

Study no.:	B067039
Study report location:	4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	03 June 2005
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	AH-110690 stock solution in Ethanol (45 µg/mL); batch FFA 093/039-504; 97% by HPLC

Key Study Findings: Males and females in the high dose group (27 µg/kg/day) and the Vehicle (7% ethanol) group had clinical signs of dizziness and uncoordination on study days 1 through 4. The high dose males had statistically significantly (P>.05) increased epididymus-to-body-weight ratios compared to saline and vehicle groups. The sponsor

chose to keep the same dose levels for the definitive study as were tested in the current study. All animals survived the entire study period.

Methods

Doses:	7, 14 or 27 µg/kg bw/day
Frequency of dosing:	Daily for 7 days
Route of administration:	IV
Dose volume:	5, 10, 20 mL/kg bw
Formulation/Vehicle:	7% Ethanol in PBS / 7% Ethanol in PBS
Control:	Saline
Species/Strain:	Rat, Han (b) (4):WIST (SPF)
Number/Sex/Group:	3
Age:	7-8 weeks
Weight:	186-206 g (Males); 138-155 g (Females)
Unique study design:	None
Deviation from study protocol:	None

Observations and procedures
Mortality-Viability and mortality were checked and recorded twice daily.

Clinical signs-Clinical signs were checked once prior to each administration and approximately 1, 2, and 4 hours post-administration.

Body weights-Recorded on days 1 and 7.

Food consumption-Not conducted.
Ophthalmoscopy-Not conducted.
ECG-Not conducted.
Clinical chemistry-Not conducted.
Hematology -Not conducted.
Urinalysis -Not conducted.

Termination-On study day 8, animals were weighed, anesthetized and then terminated by exsanguination.

Macroscopic (Gross) examination-all animals were examined for gross abnormalities.

Tissues were collected, weighed and then saved for histopathology.

Microscopic evaluation-Not conducted.
Toxicokinetics-Not conducted.
Dose formulation analysis-Not found in report.

Table 10. Tissue List from Study B067039.

Organs	Examine	Weigh	Save Tissues	Routine Histology
Adrenals	X	X	X	
Brain	X	X	X	
Epididymides	X	X	X	
Gross lesions	X		X	
Heart	X	X	X	
Kidneys	X	X	X	
Liver	X	X	X	
Lungs [1)]	X	X	X	
Lymph nodes	X		X	
Cervical	X	X	X	
Mesenteric	X	X	X	
Ovaries/Oviducts	X	X	X	
Spleen	X	X	X	
Testes [1)]	X	X	X	
Thymus	X	X	X	

[1)] filled with formalin at necropsy

Results
All animals survived to the scheduled termination day.

The animals in the high dose group and the vehicle (7% ethanol) group had clinical signs of dizziness / uncoordinated movements on treatment days 1 to 4.

In males, epididymus-to-body weight ratio was statistically significantly increased (P>.05) in the high dose group, compared with saline controls (+18%) and vehicle controls (+26%).

No other notable findings.

Report conclusion: The sponsor concluded that the test article was well tolerated, and the dose levels for the 14-day repeat dose toxicity study were selected to be the same dose levels used in this study.

Reviewer comment: All animals survived the 7-day repeated dosing regimen. The sponsor stated that no histopathology evaluations were conducted because there were no abnormalities observed during the gross examination. However, there was a statistically significant increase (18%) in epididymus-to-body-weight ratios of high-dose males compared with saline controls and vehicle controls (+26%). No histopathology assessments were conducted in this range-finding study. The NOAEL was 13.5 µg/kg due to the increased epididymus-to-body-weight ratios in males.

Study title: 14-Day Repeated Dose Intravenous Toxicity Study with AH110690 Solution for Injection in the Wistar Rat

Study no.:	B067056
Study report location:	4.2.3.2.1
Conducting laboratory and location:	(b) (4) (Toxicology Study) CH-4414 (b) (4) (Clinical Pathology and Micronucleus Assay)
Date of study initiation:	21 February 2006
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	AH-110690 stock solution in Ethanol (45 µg/mL); batch FFA 093/048-601; 97% by HPLC

Key Study Findings

Two of twenty rats in the Vehicle group and 5 of 60 animals in the Flutemetamol groups died immediately after injection of Vehicle or Flutemetamol. The deaths occurred immediately after the tail vein injection on Study Days 1, 2, 4, 9, and 14, without a trend in terms of Flutemetamol dose. Mortality occurred in Vehicle-injected animals, in both males and females, and without a trend in the number of repeated administrations. The common factor in the deaths was the presence of 7% ethanol administered by tail vein injection. The deaths were not considered to be a Flutemetamol-dependent effect.

A consistently noted effect in the Vehicle group and the low-, mid- and high-dose Flutemetamol groups was apparent intoxication due to the 7% ethanol vehicle. The only finding that was related to Flutemetamol itself was an area of irritation located at the injection site (around the tail vein). The high-dose Flutemetamol group appeared to have slightly increased severity in vasculitis and perivasculitis in the area of the injection. In all study groups, including the saline control group, there were findings of minimal to mild thrombosis, vasculitis, perivasculitis, focal tissue necrosis, intimal proliferation, and fibrosis at the injection sites, which indicated that the daily IV injections caused some trauma, irrespective of the type of solution injected. No other changes in parameters monitored antemortem or postmortem were associated with Flutemetamol treatment.

The NOAEL stated by the sponsor was 27 µg/kg. I didn't agree, because of the increased vasculitis and perivasculitis in the high-dose Flutemetamol males. The data supported a NOAEL of 13.5 µg/kg.

Methods

Doses:	6.7 (low), 13.5 (mid), 27 (high) µg/kg bw/day
Frequency of dosing:	Daily for 14 or 15 days
Route of administration:	IV
Dose volume:	5, 10, 20 mL/kg bw
Formulation/Vehicle:	7% Ethanol in PBS / 7% Ethanol in PBS
Control:	Saline
Species/Strain:	Rat, Han (b) (4):WIST (SPF)
Number/Sex/Group:	10/sex/group (main study)
Age:	7 weeks
Weight:	181-208 g (Males); 159-193 g (Females)
Satellite groups:	• Micronucleus positive control (5 per sex); • TK groups 6.7, 13.5, 27 µg/kg Flutemetamol (5/sex/group SD1; 6/sex/group SD14)
Unique study design:	Included micronucleus assay in main study animals
Deviation from study protocol:	None with impact on study integrity

Table 11: Study Design Groups

Study Group Number	Description	Dose volume	Flutemetamol dose (µg/kg)
1	Saline Control	20 mL/kg/day	0
2	7% Ethanol Vehicle Control	20 mL/kg/day	0
3	Low Dose Flutemetamol	5 mL/kg/day	6.725
4	Mid Dose Flutemetamol	10 mL/kg/day	13.5
5	High Dose Flutemetamol	20 mL/kg/day	27

Study dose groups are referred to as low, mid, and high dose groups. Prior to dosing, each rat was incubated in a warming cabinet to facilitate tail vein injections.

Observations and Procedures
Mortality- Animals were checked twice daily.

Clinical signs-Checked at least twice daily during the treatment period.

Body weights-Recorded once weekly throughout the study, and prior to necropsy.

Feed consumption-Recorded weekly throughout the study.

Ophthalmoscopy-Conducted in both eyes of all animals on Main Study during acclimatization and during treatment week 2.

ECG-Not conducted.

Hematology-Parameters were evaluated in all Main Study animals on the final day of treatment (Day 14 or 15) after an 18-hour fasting period. Parameters measured were erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, red cell volume distribution width, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, hemoglobin concentration distribution width, reticulocytes count, reticulocytes maturity index, methemoglobin, Heinz bodies, total leukocyte count, differential leukocyte count, coagulation (thromboplastin time and activated partial thromboplastin time).

Clinical chemistry-Parameters were evaluated in all Main Study animals on the final day of treatment (Day 14 or 15) after an 18-hour fasting period. Parameters measured were glucose, urea, creatinine, total bilirubin, total cholesterol, triglycerides, phospholipids, aspartate aminotransferase, alanine aminotransferase lactate dehydrogenase, glutamate dehydrogenase, alkaline phosphatase, gamma-glutamyl transferase, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, albumin, globulin, and albumin-to-globulin ratio.

Urinalysis-Urine was collected during the 18-h fasting period into a specimen vial after the final treatment (Day 14 or 15). Samples were evaluated for volume, specific gravity, color, appearance, pH, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes, and leukocytes.

Gross Pathology-All animals in the main study group were weighed and necropsied (Males-Day 15, Females- Day 16). Descriptions of all macroscopic abnormalities were recorded.

Organ Weights-The following organ weights were recorded on the scheduled dates of necropsy: brain, heart, liver, lungs, thymus, kidneys, adrenals, testes and epididymides, ovaries, and spleen.

Histopathology-Samples of all major organs and tissues were collected and fixed in neutral phosphate buffered 4% formaldehyde solution (unless otherwise indicated), and then were processed for microscopic examination. Samples from animals in groups 1, 2, and 5 were examined, and well as injection sites from animals of groups 3 and 4.

Adequate Battery: Yes

Peer Review: Yes

Special Evaluation-A micronucleus assay was conducted on bone marrow samples from animals in the main study group, and an additional positive control group.

Toxicokinetics-Blood samples for toxicokinetic (TK) analyses were drawn from 3 male and 3 female animals per dose group on treatment days 1 and 14. TK parameters were determined using pooled measurements from all individuals in each treatment group,

using software for non-compartmental analysis for bolus injection. Nominal dose and exact sampling time points were used in the calculations.

Dose formulation analysis-Certificate of Analysis included

Results
Mortality: Seven animals died while on study. The deaths are summarized below in Table 12. The deaths occurred immediately after the IV injection in the tails vein. The cause of death was not determined, although the sponsor conducted a necropsy on each decedent. All other animals survived until scheduled necropsy.

Table 12. Mortality in Repeat-Dose Rat Toxicity Study.

Treatment Group	Males (Study Day of death)	Females (Study Day of death)
Saline control	0	0
Low	1 (14)	1 (1)*
Mid	1 (1)*	1 (9)
High	0	1 (2)
7% Ethanol vehicle	1 (4)	1 (9)

*Animal was replaced.

Clinical Signs: Signs of dizziness and uncoordinated movements were observed just after administration with a duration of 1-2 hours in most rats given 7% Ethanol in PBS (group 2) or Flutemetamol Solution for Injection (groups 3, 4, 5), during days 1 to 8.

Sporadic clinical signs were noted in a few animals, such as ruffled fur, crusts, ear fissures, hair loss, scars, and kinked tail.

Body Weights: No statistically significant findings occurred in males. In females, statistically significant decreases in mean body weights were noted on Day 14 in the high dose group, compared to the saline control females. In the high dose groups of both males and females, lower body-weight-gains were observed when compared with saline controls. The differences were not statistically significant.

Feed Consumption: The high dose group was observed to have reduced relative feed consumption over one of the two-week measurement intervals (males during week 2; females during week 1), when compared with saline controls.

Ophthalmoscopy: Females (7/10) in the Ethanol (7%) vehicle control group were observed to have corneal opacity. The finding was not observed in males of the ethanol vehicle control group.

ECG: Not conducted.

Hematology: Sporadic differences between control groups and ethanol vehicle or Flutemetamol groups were observed, although values were within normal historical reference ranges and no dose dependency was observed.

Clinical Chemistry: Sporadic differences between control groups and ethanol vehicle or Flutemetamol groups were observed, although values were within normal historical reference ranges and no dose dependency was observed.

Urinalysis: There were no notable findings.

Gross Pathology: There were no findings that were associated with test article. The only findings were those typically seen in rats of the strain, age, and study type, and were considered incidental.

Organ Weights: In males, there were statistically significantly decreased testes-to-body weight ratios, in the low- and mid-dose Flutemetamol groups, and in the 7% ethanol vehicle group, when compared with saline control animals. There was no microscopic pathology finding in the testes, however. The absence of a histologic correlate and no indication of a Flutemetamol dose-relationship indicated the finding is likely incidental.

No other statistically significant differences between treatment groups and controls were noted.

Histological Findings: Findings related to test article were located at the injection site. Thrombosis, vasculitis, perivasculitis, focal tissue necrosis, intimal proliferation, and fibrosis were observed in all study groups, including controls. Only the high-dose Flutemetamol group appeared to have slightly increased severity in vasculitis and perivasculitis.

Special Evaluation: Results of the Micronucleus Assay are discussed in the Genetic Toxicology Section.

Toxicokinetics: The sponsor noted that the dosing solution concentrations were 67% of the nominal concentrations; however, the TK report did not include an adjustment for the differences. The report stated that plasma profiles suggested biphasic elimination of Flutemetamol on day 14. The average plasma concentrations were 1.27, 2.95, and 4.98 ng/mL for low, mid, and high dose groups, respectively, at 5 minutes post dosing. The elimination half-life was 7 to 19 minutes. No sex differences in TK parameters were observed. Systemic exposure to Flutemetamol increased proportionally with dose.

Dosing Formulation Analysis: The dose formulation analysis showed that the dose preparations were 67% of the nominal dose. Additionally, a portion of the injected drug was adsorbed to the infusion equipment. The sponsor stated that the reported safety margins included adjustments for lower-than-expected concentrations of test article and also the test article adsorption to infusion equipment.

Sponsor's Conclusion
The results of the study showed that daily injection of doses of 6.7, 13.5, or 27 µg/kg of Flutemetamol for Injection did not result in adverse effects to males or females that survived the 14-day dosing period. The unscheduled deaths of animals immediately following injection of vehicle or Flutemetamol were not attributed to Flutemetamol by the sponsor.

Reviewer comments:
Two of twenty rats in the Vehicle group and 5 of 60 animals in the Flutemetamol groups died immediately after injection of Vehicle or Flutemetamol (see Table 13*). The deaths occurred on different dosing days, in male and female animals, and in Vehicle and low, mid, and high Flutemetamol dose groups. The temporal relationship between the tail vein injection and immediate death thereafter was consistent with injection and animal handling errors. The common factor in the mortalities was injection of 7% ethanol in PBS into the tail vein.*

Table 13. Mortality in Repeat-Dose Rat Toxicity Study.

Treatment Group	Males (Study Day of death)	Females (Study Day of death)
Saline control	0	0
Low	1 (14)	1 (1)
Mid	1 (1)	1 (9)
High	0	1 (2)
7% Ethanol vehicle	1 (4)	1 (9)

A consistently noted effect in the Vehicle group and the low-, mid- and high-dose Flutemetamol groups was apparent intoxication due to 7% ethanol in the Vehicle. The only finding that was related to Flutemetamol itself was an area of irritation located at the injection site (around the tail vein). The high-dose Flutemetamol group appeared to have slightly increased severity in vasculitis and perivasculitis in the area of the injection. In all study groups, including the saline control group, there was a finding of thrombosis, vasculitis, perivasculitis, focal tissue necrosis, intimal proliferation, and fibrosis at the injection site, which indicated that the daily IV injections caused some trauma, irrespective of the type of solution injected.

I agreed with the sponsor that unexpected deaths were not due to Flutemetamol for the following reasons:

- *The deaths in the vehicle control group were inconsistent with Flutemetamol-dependent mortality;*
- *The only common treatment in all rats that died was an injection of ethanol-containing solution (the Vehicle) in the tail vein, followed by immediate death;*
- *The deaths were not dependent on the dose of Flutemetamol, the number of days on study, or the sex of the rat.*

- *No unscheduled deaths occurred in any other nonclinical study, including two single-dose studies and a 7-day repeated-dose toxicity study in rats, and single-dose, 7-day and 14-day repeated-dose toxicity studies in dogs.*

Dog Studies
Flutemetamol was well-tolerated in dogs at doses up to 23X (BSA-corrected) the MHD in the 7- and 14-day repeated-dose toxicity studies in dogs,. There were no mortalities or adverse findings associated with Flutemetamol administration in either study.

Study title: Combined Single and 7-Day Repeated Dose Intravenous Toxicity Study with AH110690 Solution for Injection in the Beagle Dog

Study no.:	B067038
Study report location:	4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	17 May 2005
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	Drug Batch # FKJ 0162/147-05; 97% by HPLC AH-110690 stock solution in Ethanol (nominal 45 µg/mL; actual 43 µg/mL), batch FFA093/039-504; 97% purity (by area)

Key Study findings: In this range-finding study, 15 µg/kg/day of Flutemetamol was well tolerated for 7 days, with daily dosing, in both dogs. Therefore, 15 µg/kg/day was chosen as the high dose level for the 15-day repeat-dose study in dogs.

Methods

Doses:	15 µg/kg/day
Frequency of dosing:	Daily for 7 days
Route of administration:	IV
Dose volume:	5.0 mL/kg
Formulation/Vehicle:	7% Ethanol in PBS / 7% Ethanol in PBS
Control:	Saline
Species/Strain:	Pure-bred Beagle dogs
Number/Sex/Group:	1
Age:	Approximately 8 months
Weight:	8.7 kg (M); 5.8 kg (F)
11111	Animals were administered saline on SD1, vehicle on SD2, and test article on SD 3-9. On SD 10, animals were terminated.
Deviation from study protocol:	None with impact ton study integrity

Results:
Both animals survived to scheduled termination. No clinical signs were seen. There were no apparent effects on food intake, body weights, clinical chemistry, hematology, coagulation factors, cardiac effects (ECGs), gross examinations at necropsy, or organ weights at necropsy.

Report conclusion: The dose tested was well-tolerated.

Reviewer comment: These dogs appeared unaffected by the 7% ethanol contained in the vehicle. Note that this was a study with few animals (only 1/sex), to determine if dose levels were tolerated with repeat dosing. A 14-day repeat dose study in dogs followed, based on the results of this study.

Study title: 14-Day Repeated Dose Intravenous Toxicity Study with AH110690 Solution for Injection in the Beagle Dog

Study no.:	B067040
Study report location:	4.2.3.2.1
Conducting laboratory and location:	(b) (4) (Toxicology Study) CH-4414 (b) (4) (Clinical Pathology)
Date of study initiation:	22 June 2005
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	Drug Batch # FKJ 0162/147-05; 97% by HPLC AH-110690 stock solution in Ethanol (nominal 45 µg/mL; actual 43 µg/mL), batch FFA093/039-504; 97% purity (by area)

Key Study Findings: Flutemetamol was well-tolerated at all dose levels. The NOAEL was 14 µg/kg/day. The effects observed and attributed to ethanol in the vehicle could not be distinguished from those due to Flutemetamol, i.e., the observed effects were consistent with ethanol intoxication.

Methods

Doses:	7.0 or 14 µg/kg/day
Frequency of dosing:	Daily for 14 or 15 days
Route of administration:	IV
Dose volume:	2.5 or 5.0 mL/kg
Formulation/Vehicle:	7% Ethanol in PBS / 7% Ethanol in PBS
Control:	Saline
Species/Strain:	Pure-bred Beagle dogs
Number/Sex/Group:	4
Age:	Approximately 9 months
Weight:	6.5-11.4 (M); 5.6-10.7 (F)
Satellite groups:	NA
Unique study design:	NA
Deviations:	Minor, with no impact to study integrity

Table 14: Study Design Groups in Dog 14-Day Toxicity Study

Study Group Number	Description	Dose volume	Dose Flutemetamol (µg/kg) daily
1	Saline Control	5.0 mL/kg/day	0
2	7% Ethanol Vehicle Control	5.0 mL/kg/day	0
3	Low Dose Flutemetamol	2.5 mL/kg/day	7.0
4	High Dose Flutemetamol	5.0 mL/kg/day	14

Observations and Procedures

Mortality-Animals were checked twice daily for mortality/morbidity.

Clinical Signs-Checked at least twice daily during the treatment period.

Body Weights-Body weights were recorded once weekly throughout the study, and prior to necropsy.

Feed Consumption-Recorded daily throughout the study.

Ophthalmoscopy-Both eyes of all animals on Main Study were examined during acclimatization and during treatment week 2.

Electrocardiograms (ECG)-Recorded at pretest, Day 2, and Day 13. ECG were obtained using Einthoven (I, II, III) and Goldberger (aVR), aVL, aVF) leads. The heart rate, P wave duration and amplitude and P-Q, QRS, and Q-T intervals were measured using a representative section of the ECG from lead II and traces were assessed for arrhythmias.

Respiratory Function-Measured on Day 10 immediately before and immediately after dose administration. Respiration rate, tidal volume and minute volume were recorded.

Hematology-Standard hematology parameters were evaluated in all animals pretest and on Day 13, after an 18-hour fasting period. Parameters measured were erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, red cell volume distribution width, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, hemoglobin concentration distribution width, reticulocytes count, reticulocytes maturity index, methemoglobin, Heinz bodies, total leukocyte count, differential leukocyte count, coagulation (thromboplastin time and activated partial thromboplastin time).

Clinical Chemistry-Standard hematology parameters were evaluated in all animals pretest and on Day 13, after an 18-hour fasting period. Parameters measured were glucose, urea, creatinine, total bilirubin, total cholesterol, triglycerides, phospholipids,

aspartate aminotransferase, alanine aminotransferase lactate dehydrogenase, glutamate dehydrogenase, alkaline phosphatase, gamma-glutamyl transferase, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, albumin, globulin, and albumin-to-globulin ratio.

Urinalysis-Urine was collected using a catheter in all animals pretest and on Day 13, after an 18-h fasting period. Samples were evaluated for volume, specific gravity, color, appearance, pH, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes, and leukocytes.

Gross Pathology All animals were weighed and necropsied after 14 days of dosing. All macroscopic abnormalities were recorded.

Organ Weights The following organ weights were recorded on the scheduled dates of necropsy: brain, heart, liver, lungs, thymus, kidneys, spleen, adrenals, pituitary; testes, prostate and epididymides; ovaries and uterus.

Histopathology Samples of all organs and tissues listed in Necropsy Tissue List (Table 15) were collected and fixed in neutral phosphate buffered 4% formaldehyde solution (unless otherwise indicated), and then were processed for microscopic examination. Samples from all animals were examined.

Table 15. Necropsy Tissue List for Dog 14-d Toxicity Study.

Adrenal glands	Pancreas
Aorta	Pituitary gland
Bone (femur)	Prostate gland (males only)
Bone marrow (sternum)	Rectum
Brain	Salivary glands-mandibular, parotid, sublingual
Cecum	Sciatic nerve
Colon	Seminal vesicles
Duodenum	Skeletal muscle
Epididymides (males only) (fixed in Bouin's Solution)	Skin
Esophagus	Spinal cord-cervical, thoracic, lumbar
Eyes w/optic nerve (fixed in Davidson's Solution)	Spleen
Harderian gland (fixed in Davidson's Solution)	Stomach
Heart	Testes (fixed in Bouin's Solution)
Ileum, with Peyer's patches	Thymus
Kidneys	Thyroid (incl. parathyroid gland, if possible)
Larynx	Tongue

Lacrimal gland, exorbital	Trachea
Liver	Urinary bladder, filled w/formalin at necropsy
Lungs w/bronchi and bronchioles, filled w/formalin at necropsy	Uterus and cervix (females only)
Lymph nodes, mesenteric, mandibular	Vagina
Mammary gland area	Injection site
Nasal cavity	Gross lesions
Ovaries (females only)	

Adequate Battery Yes

Peer Review Yes

Toxicokinetics (TK)-Blood samples for TK analysis were collected from all animals on Days 1 and 14 of dosing. Serial blood samples were collected from each animal in Groups 3 and 4 on Days 1 and 14 of dosing (pre-dose, 3, 10, 20, 40, and 60 minutes post-dose). In groups 1 and 2, samples were collected pre-injection and 3 minutes after to verify that correct dosing was performed. Samples also were collected on Day 10, the day of the respiration assessment.

Results

Mortality: All animals survived to scheduled necropsy.

Clinical Signs: There were no notable findings.

Body Weights: There were no notable findings.

Feed Consumption: There were no notable findings.

Ophthalmoscopy: There were no notable findings.

Electrocardiograms (ECG): There were no notable findings that could be related to test article.

Respiratory Function: There were no notable findings.

Hematology: There were no notable findings.

Clinical Chemistry: There were no notable findings.

Urinalysis: There were no notable findings.

Gross Pathology: There were no findings that were associated with test article.

Organ Weights: There were no findings that were associated with test article.

Histological Findings: None related to Flutemetamol.

Toxicokinetics (TK): Flutemetamol was eliminated from the plasma in the high dose animals with an elimination half-life of 7.5 minutes on average (range was 6.4-9.1 minutes). The C_{max} and $AUC_{0\text{-}inf}$ were similar in males and females, on days 1 and 14 (Table 16). There was no systemic accumulation of Flutemetamol during the study, and systemic exposure was proportional to dose.

Table 16. Toxicokinetic Results from Study B067040.

Daily Dose (mg/kg)	Group 1 * (Saline control)		Group 2 ** (Vehicle control) 7% Ethanol in PBS		Group 3 7.5 µg/kg		Group 4 15 µg/kg	
Number of Animals Toxicokinetics	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
AUC (min x ng/ml) Day 1	NA	NA	NA	NA	16.4±1.9[2]	NC[3]	38.0±4.4	38.0±6.5
AUC (min x ng/ml) Day 14	NA	NA	NA	NA	NC	NC	36.2±4.8	42.1±7.0
Cmax (ng/ml) Day 1	NA	NA	NA	NA	1.1±0.3	1.1±0.5	2.8±0.4	2.5±0.3
Cmax (ng/ml) Day 14	NA	NA	NA	NA	1.1±0.2	1.3±0.5	2.7±0.4	3.1±0.3
T½ (min) Day 1	NA	NA	NA	NA	8.5±1.4	NC	7.8±0.5	8.4±0.6
T½ (min) Day 14	NA	NA	NA	NA	NC	NC	7.5±0.2	6.5±0.2

* - Control group 1 was treated with physiological saline (phosphate buffered saline, PBS) only.
** - Control group 2 was treated with the vehicle (7% Ethanol in phosphate buffered saline) only.
[1] N=3 due to insufficient data in one animal
[1] NC = Not calculated (due to insufficient data)

Dosing Formulation Analysis
The sponsor reported that animals were administered the correct control article, vehicle, or test article as scheduled, except on Day 3, when two animals in the saline control group were administered vehicle, in error. The test article was acceptable in terms of stability, identity, and purity. The actual concentration of test article stock solution was lower than nominal; Study B067058 documented the test article adsorption to dosing equipment. The actual dose delivered to animals was documented and reported in the amended Final Report, and is reported in this review.

Report conclusions
Sporadic differences between control groups and ethanol-containing vehicle or Flutemetamol groups were observed, although differences were within normal historical reference ranges and thus, were attributed to typical differences between individual animals. Also, no dose dependency was observed for any of the findings.

Flutemetamol was eliminated from the plasma in the high dose animals with an elimination half-life of 7.5 minutes on average (range was 6.4-9.1 minutes). The C_{max} and $AUC_{0\text{-}inf}$ were the same in males and females, on days 1 and 14. There was no systemic accumulation of Flutemetamol during the study, and systemic exposure was proportional to dose.

Reviewer comment: Agree with Sponsor's interpretation of results.

The sponsor reported the NOEL as 14 µg/kg, which is also the NOAEL. In this study, the dogs did not seem to be affected by the ethanol. The dose levels in dogs (maximum of 14 µg/kg per day) were lower than doses in rat studies (maximum of 27 µg/kg per day), and consequently, the amount of ethanol delivered was also lower. The dose volume for dogs was 5 mL/kg, compared with 20 mL/kg in the rat 14-day toxicity study.

7 Genetic Toxicology

Brief Summary

The results of the In Vitro Reverse Mutation Assay (Ames Test) and the In Vitro Mouse Lymphoma Assay For Mutation At The Tk Locus were both positive for mutagenicity. In vivo exposure to Flutemetamol in rats, and subsequent assays for genetic toxicity in bone marrow and hepatocytes were negative for clastogenicity or mutagenicity. Considering the overall weight of evidence, the potential for genetic toxicity due to the Flutemetamol exposure at the proposed clinical dose of 20 µg is negligible. The sponsor's genetic toxicology studies conducted are summarized in Table 17.

Table 17. Summary of Genetic Toxicology Studies

Study Type/Study Number	NOAEL		Maximum Human Dose (MHD)		Dose Multiple** (BSA-Corrected Animal NOAEL*/MHD)
	µg/kg	µg/m^2	µg/kg	µg/m^2	
In Vivo Micronucleus Assay Using Bone Marrow Cells From Rats After 14-Days Of Repeat Dosing With Flutemetamol/ B067056	27	162	0.33	12.33	13
In Vivo Micronucleus Assay In Male Rats 24 And 48h After Flutemetamol Infusion/B067017	120	720	0.33	12.33	156
In Vivo/In Vitro Unscheduled DNA Synthesis In Rat / B067016	39		0.33	12.33	17
In Vitro Reverse Mutation Assay (Ames Test)/ B067005	Positive in Strain TA98 In The Presence of S9 Fraction.				
In Vitro Mouse Lymphoma Assay for Mutation at the TK Locus/ B067006	Positive in The Presence of S9 Fraction; Equivocal In The Absence Of S9.				

*Correction for Body Surface Area (BSA; µg/m^2) is the animal NOAEL in µg/kg bw multiplied by 6.2 for rat or 20 for dog.
**Dose multiple is the BSA-adjusted NOAEL (in µg/m^2) divided by the Maximum Human Dose (MHD) in µg/m^2.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: AH 110690: Reverse Mutation in Five Histidine-requiring Strains of *Salmonella typhimurium*

Study no.:	B067005
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	7 July 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AH 110690 (Flutemetamol), Batch 0305013 CLJ-1 (powder); 95.5% (HPLC, UV detection)

Key Study Findings

Under the conditions employed in the study, Strain TA98 was positive for mutagenicity in the presence of S-9, but was negative in the absence of S-9. Strains TA100, TA1535, TA1537 and TA102 were not mutagenic in the absence or presence of S-9.

Methods

Strains:	TA98, TA100, TA1535, TA1537, TA102
Concentrations in definitive study:	1.5-50 µg/plate
Basis of concentration selection:	Solubility; lack of toxicity
Negative control:	DMF
Positive control:	See Table 18, below
Formulation/Vehicle:	0.5 mg/mL in dimethylformamide (DMF)
Incubation & sampling time:	10 h & 11-12 h
Metabolic activation system included in designated incubations	Rat liver S-9 fraction (S-9)

Table 18 Positive control treatments (Study B067005)

Chemical	Final concentration (µg/plate)	Use strain(s)	S-9
2-nitrofluorene (2NF)	5.0	TA98	-
Sodium Azide (NaN3)	2.0	TA100, TA1535	-
9-aminoacridine (AAC)	50.0	A1537	-
Glutaraldehyde (GLU)	25.0	TA102	-
Benzo[a]pyrene (B[a]P)	10.0	TA98	+
2-aminoanthracene (AAN)	5.0 and 20.0	TA100, TA1535, TA1537, TA102	+

Study Validity for B067005
The study was considered valid, based on the following:

- The mean negative control counts fell within the normal ranges
- The positive control chemicals induced clear increases in revertant number, confirming discrimination between different strains, and an active S-9 preparation
- No more that 5% of the plates were lost through contamination or some other unforeseen event.

Results
The test article was considered to be mutagenic if the following criteria were met:
The study was valid as defined in Study Validity for B067005

- Dunnett's test gave a significant response ($p \leq 0.01$) and the data set(s) showed a significant dose correlation
- The positive responses described above were reproducible.

Under the conditions employed in the study, Strain TA98 was positive for mutagenicity in the presence of S-9, but was negative in the absence of S-9. Strains TA100, TA1535, TA1537 and TA102 were not mutagenic in the absence or presence of S-9.

Conclusion: Flutemetamol was mutagenic in *Salmonella typhimurium*, Strain TA98, when a rat metabolic activation system (S-9) was included in the incubation.

Reviewer comment: Agreed.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: AH110690: Mutation at the Thymidine Kinase (tk) locus of Mouse Lymphoma L5178Y cells (MLA) using the Microtitre R Fluctuation Technique

Study no.:	B067006
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07 July 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AH 110690, batch # CLJ-1 (LIMS ID 0305013); 95.5% (HPLC, UV detection)

Key Study Findings

Under the conditions of the study, Flutemetamol is positive for mutagenic activity in the presence of S-9, and equivocal for mutagenicity in the absence of S-9.

Methods

Cell line:	L5178Y TK $^{+/-}$ mouse lymphoma cells
Concentrations in definitive study:	1, 2, 3, 4, 4.5, 5 µg/mL
Basis of concentration selection:	Solubility limitation; lack of toxicity
Negative control:	DMF
Positive control:	4-niroquinolone 1-oxide (NQO), without S-9 benzo[a]pyrene (BP), with S-9
Formulation/Vehicle:	0.5 mg/mL in DMF
Incubation & sampling time:	3 h or 24 h incubation, then sampling 48 h later
Metabolic activation system included in designated incubations	Rat liver S-9 fraction (S-9)

Study Validity-B067006
The study was considered valid if all the following criteria were met:

- The mutant frequencies in negative control culture fell within the normal range (above 60 mutants per 10^6 viable cells but not more than three times the historical mean value)
- At least one concentration of each of the positive control chemicals induced a clear increase in mutant frequency (the difference between the positive and negative control mutant frequencies was greater than half the historical mean value)
- The plating efficiencies of the negative controls from the mutation experiments were between the range of 60% to 140% on Day 0 and 70% to 130% on Day 2.

According to the validity criteria (above), the assay was valid.

Results
The test article was considered to be mutagenic if all the following criteria were met:

- The study was valid according to criteria stated above for Study Validity B067006.
- The mutant frequency at one or more doses was significantly greater than that of the negative control ($p<0.05$).
- There was a significant dose-relationship as indicated by the linear trend analysis ($p<0.05$).

In accordance with the criteria for a positive result, the test article showed positive evidence of mutagenic activity in the presence of S-9. The test article showed equivocal evidence of mutagenic activity in the absence of S-9. Three replicate experiments were performed, and the stated results were shown in two of three experiments.

Sponsor's Conclusion
Under the conditions of the assay, Flutemetamol is positive for mutagenic activity in the presence of S-9, and equivocal for mutagenicity in the absence of S-9.

Reviewer comment: Agreed.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Micronucleus Assay in Male Rats: AH110690 Solution for Injection: Induction of Micronuclei in the Bone Marrow of Treated Rats

Study no:	B067017
Study report location:	4.2.3.3.2.1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	17 February 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AH110690 in Ethanol stock solution (45µg/mL) batch FFA093/024-401; prepared using AH110690 (powder) batch FKJ0129/101-01; 99.8% purity by HPLC (UV detection)

Key Study Findings

Flutemetamol was negative for induction of micronuclei in polychromatic erythrocytes of male Wistar rats at approximately 24 and 48 hours after treatment.

Methods

Doses in definitive study:	19, 39, 78 µg/kg/day
Frequency of dosing:	Daily dose was divided into two infusions each day, 4h apart; animals were dosed on 2 consecutive days
Route of administration:	IV
Dose volume:	5, 10, or 20 mL/kg bw
Formulation/Vehicle:	3 µg/mL Flutemetamol / 7% Ethanol in PBS (pH 7.4); Saline control group also included
Species/Strain:	Rat/Wistar
Number/Sex/Group:	6 males per group; no females
Satellite groups:	NA
Basis of dose selection:	Doses were used in Expanded Single Dose Toxicity Study (B067001)
Negative control:	Vehicle or saline
Positive control:	Cyclophosphamide (CMA) 40 mg/kg given IV on 2nd day
Sampling time:	Day 3 (24 h after 1st injection on Day 2)
Protocol Deviation(s)	None

Study Validity

The study was considered valid if the following criteria were met:

1. The frequency and distribution of micronucleated PCE in vehicle control group were consistent with the historical vehicle control range for the conducting laboratory, and
2. At least five animals (males) of each group are available for analysis, and
3. The positive control chemical (CPA) induced a statistically significant increase in the frequency of micronucleated PCE.

Criteria for a Positive Result

1. Statistically significant increase in the frequency of micronucleated PCE occurred in at least one test article dose group, and
2. The frequency of micronucleated PCE at such a point exceeds the historical vehicle control range.

Results

The study was valid, according to validity criteria.

Flutemetamol (AH110690) was found to be negative for clastogenicity (according to the criteria for a positive assay). There was no evidence of cytotoxicity to bone marrow. The NOAEL after the cumulative 2-day exposure to Flutemetamol was 156 µg/kg bw.

Reviewer comment: Agree with sponsor's interpretation.

Study title: Micronucleus Assay from Rats dosed in 14-day Repeat Dose Toxicity Study (B067056), 14-Day Repeated Dose Intravenous Toxicity Study with AH110690 Solution for Injection in the Wistar Rat

Subtitle: Micronucleus Assay Using Bone Marrow Cells

Study no.:	B067056
Study report location:	4.2.3.3.2.1
Conducting laboratory and location:	(b) (4) (Toxicology Study) CH-4414 (b) (4) (Clinical Pathology and Micronucleus Assay)
Date of study initiation:	March 16, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AH-110690 stock solution in Ethanol (45 µg/mL); batch FFA 093/048-601; 97% by HPLC

Key Study Findings

Flutemetamol was negative for the induction of micronucleus formation in polychromatic erythrocytes at all doses of Flutemetamol after 14 days of daily IV administration. Treatment with positive control (CPA) resulted in induction of polychromatic erythrocytes according to the sponsor, although no historical range for positive control in rat was provided. The sponsor reported the NOAEL at 27 µg/kg. I agreed.

Methods

Doses in definitive study:	0, 6.7, 13.5, 27 µg/kg daily (see also Table 11)
Frequency of dosing:	Daily for 14 days
Route of administration:	IV
Dose volume:	5, 10, or 20 mL/kg
Formulation/Vehicle:	7% Ethanol in PBS
Species/Strain:	Rat/Wistar
Number/Sex/Group:	10
Satellite groups:	NA
Basis of dose selection:	Doses were used in 14-Day toxicity study
Negative control:	Saline group; Vehicle group
Positive control:	Cyclophosphamide (CPA)
Protocol Deviation(s)	None with study impact

Study Validity

The study is considered valid if the positive control yields the expected result. The sponsor did not state if the test was considered valid or not. No historical control range for the positive control item was provided.

Results

The sponsor stated that the test item is considered mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group.

There was no cytotoxic effect due to treatment with any dose level of Flutemetamol compared to saline or vehicle controls, based on the ratio between polychromatic and total erythrocytes in the same sample.

There was no increase in the frequency of micronuclei in polychromatic erythrocytes at any dose level of Flutemetamol, in comparison to vehicle control.

The sponsor reported a significant increase in micronuclei of normochromatic erythrocytes in the high dose group of Flutemetamol, in comparison to vehicle control group. The sponsor stated it was not biologically relevant, and was due to “very low numbers”.

The positive control group (single dose of 40 mg/kg CPA) demonstrated a significant increase in the frequency of micronuclei in polychromatic erythrocytes, compared with

the frequency in the saline control group. No historical positive control range was provided.

The sponsor used the non-parametric Mann-Whitney statistical test with significance at the 5% level ($p \leq 0.05$) to compare groups. The data is summarized below.

Table 19. Summary Of Micronucleus Assay In Repeat-Dose Toxicity Study (B067056)

Vehicle Control versus Flutemetamol Group	**Micronuclei in Polychromatic Erythrocytes (PCE)**	
	Significance (Yes or No)	p (based on Mann-Whitney Test)
Low (15 µg/kg/day)	No	0.2043
Mid (30 µg/kg/day)	Not tested	-
High (60 µg/kg/day)	No	0.2100
Positive Control versus Saline Group		
Cyclophosphamide (CPA)	Yes	< 0.0001

Reviewer comment: Usually only the polychromatic erythrocytes are the focus of the micronucleus test, not normochromatic erythrocytes. The biological relevance of increased micronuclei frequency in normochromatic erythrocytes is unknown.

The sponsor concluded the test was negative, with a NOAEL of 27 µg/kg/day for 14 days. I agree.

Other Genetic Toxicity Studies

Study title: AH110690 Solution For Injection: Measurement of Unscheduled DNA Synthesis In Rat Liver Using an *In Vivo/In Vitro* Procedure

Study no:	B067016
Study report location:	4.2.3.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	13 February 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AH110690 in Ethanol stock solution (45 µg/mL) batch FFA093/024-401; prepared using AH110690 (powder) batch FKJ0129/101-01; 99.8% purity by HPLC (UV detection)

Key Study Findings

Lethargy was observed in the Flutemetamol and Vehicle groups. The assay results were negative for induction of UDS, at early (2-4 hours) and later (12-14 hours) time points after injection. The assay was deemed valid according to historical and published assay criteria.

Methods

Doses in definitive study:	19.5, 39 µg/kg bw
Frequency of dosing:	Single
Route of administration:	IV
Dose volume:	10 or 20 mL/kg bw
Formulation/Vehicle:	3 µg/mL Flutemetamol / 7% Ethanol in PBS; Saline control group also included
Species/Strain:	Rat/ Sprague-Dawley Crl:CD®(SD) IGS BR
Number/Sex/Group:	4 males/group and sampling time
Sampling times:	12-14 h and 2-4 h
Basis of dose selection:	Dose based on results from expanded single dose toxicity study in rats (B067001)
Negative control:	Vehicle group and saline group
Positive controls:	2-acetamidofluorine (2-AAF; 75 mg/kg) and dimethylnitrosamine (DMN; 10 mg/kg); dosed via oral gavage
Protocol deviations:	None with study impact

Study Validity

The negative and positive control preparations yielded results that were consistent with both published and historical control data, according to the sponsor, and the assay was accepted as valid.

Results

A clinical sign of lethargy was observed in the Flutemetamol group and the Vehicle group. Results of the unscheduled DNA synthesis assay showed that no induction of UDS in hepatocytes isolated ex vivo approximately 12-14 or 2-4 hours after dosing.

Report conclusion: Flutemetamol had no detectable genotoxic activity in the test system. The NOAEL was 39 µg/kg.

Reviewer comment: Agree with sponsor's conclusion.

Study title: Assessment of the Mutagenic Potential of Flutemetamol (F18) Injection

Study no: B067072
Study report location: 4.2.3.3.1
Author: (b) (4)

Key Study Findings

The report is a risk assessment of the mutagenic potential of Flutemetamol F 18 Injection. The author examined each component of the FDP: Flutemetamol F 18 plus radiolabeled impurities (including metabolites) and excipients in the formulation. The specification for Flutemetamol F 18 plus related impurities is (b) (4), with a maximum injection volume of 10 mL, or 60 µg total. The amount of potentially mutagenic ingredients was below the Toxicologic Threshold of Concern[2] (TTC) of 120 µg/day for up to 14 days. The FDP also contains up to (b) (4) of (b) (4) as an impurity from the Polysorbate 80 and ethanol excipients. The level of total (b) (4) is below the TTC 0f 120 µg/day for 14 days. The author concluded that the greatest risk of potential genotoxicity is attributed to the ethanol component of Vizamyl™, which is higher than permitted daily exposure for industrial use, but lower compared to recreational use. Consideration of the one- or few-times of exposure to Vizamyl™ in a lifetime supports the assertion that the risk of genetic toxicity from Vizamyl is low and the potential benefit in the patient population outweighs the potential risk. The sponsor asserted that no further genotoxicity evaluations should be required, nor further tightening of the specifications, from the P/T perspective.

Reviewer comment: Agree with the sponsor that risk of genotoxicity from Vizamyl™ exposure is negligible, and is acceptable in the target population.

8 Carcinogenicity

Waived.

9 Reproductive and Developmental Toxicology

Waived.

10 Special Toxicology Studies

Brief Summary:
The sponsor conducted several local tolerance studies with Flutemetamol Solution for Injection. An *in vitro* blood hemolysis study also was conducted. The studies are summarized in Table 20.

[2] Food and Drug Administration. Guidance for Industry Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), December 2008, Pharmacology and Toxicology

Table 20. Other Toxicity Studies Conducted With Flutemetamol

Study Type/Study Number	Route of Exposure	µg/mL	Volume (mL)	Results
Local Tolerance Study in Rabbits/ B067052	Intraarterial	1.9	0.3	Well-tolerated
	Intravenous	1.9	0.3	
	Perivenous	1.9	0.3	
	Intramuscular	2.8	0.3	
	Subcutaneous	2.8	0.3	
Local Tolerance Study in Rabbits/ B067044	Ocular	2.8	0.1	
Local Tolerance Study in Rabbits/ B067042	Cutaneous	2.8	0.5	
In vitro Hemolysis of Blood from Healthy Human Volunteers/ B067061	In vitro incubation			Low hemolysis potential

Study title: AH110690 Solution for Injection: Local Perivenous, Intraarterial, Intramuscular and Intravenous Tolerability Study in Rabbits

Study no.: B067052
Study report location: 4.2.3.6.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 05 July 2005
GLP compliance: Yes (OECD)
QA statement: Yes
Drug, lot #, and % purity: AH-110690 stock solution in Ethanol (45 µg/mL); batch FFA 093/039-504; 97% by HPLC

Key study findings

AH110690 solution for injection was well-tolerated in male and female rabbits when injected once intravenously, intraarterially, perivenously, or intramuscularly.

Methods

Doses in definitive study:	1.9-2.8 µg/mL
Frequency of dosing:	Single injection
Route of administration:	See Table 21 below
Dose volume:	0.3 mL
Formulation/Vehicle:	7% Ethanol in PBS
Species/Strain:	Rabbit/New Zealand White [Specific pathogen free, (SPF)]
Number/Sex/Group:	1 male, 2 females; 12 animals per regimen (see Table 21)
Test groups:	See Table 21 below
Basis of dose selection:	Solubility
Age:	13-16 weeks (M); 12-14 weeks (F)
Body weight range:	2497-2612 g (M); 2408-2687 g (F)
Protocol Deviation(s)	None with study impact

Table 21. Study Design Regimen For Rabbit Local Tolerance Study B067052

	Group 1	Group 2	Group 3	Group 4
Route of administration	Intravenous (IV)	Perivenous (PV)	Intramuscular (IM)	Intraarterial (IA)
Site of injection	Marginal ear vein (retrograde)	Adjacent to marginal ear vein	Thigh muscle	Central ear artery (retrograde)
Rationale	Intended clinical route	Inadvertent route	Inadvertent route	Inadvertent route
Treatment (right side)	Test item formulation: AH-110690 Solution for Injection (2.8 µg/mL AH-110690 in ethanol (7%) and saline (0.9%)			
Test item concentration	2.8 µg/mL AH-110690			
Dosage volume	0.3 mL			
Treatment (left side)	Control item: Physiological saline			

Procedures

Mortality and morbidity were monitored daily.

Clinical observations were conducted on study day (SD) 1 immediately after injections and at approximately 3 and 6 hours later, and twice daily on SD 2, 3, 4, and 5. Thereafter, signs were noted daily on SD 6 to 8.

Body weights were measured pre-test, on the day of injections and on the day of necropsy.

Necropsy was conducted on SD 8. Gross findings at each injection site were recorded, and all injection sites were removed with adjacent tissue, and processed for histopathology.

Results:
There was no mortality or morbidity during the in-life phase.

No signs of systemic toxicity were observed throughout the study period. No local signs of irritation were noted after intravenous, intraarterial, or intramuscular injection of test item. Perivenous injection of test item or control item resulted in slight erythema post-injection (0-3 hours).

All body weights were considered to be within the normal range.

At necropsy, no gross lesions were noted, and no consistent test-item-related findings were noted upon microscopic examination of sites.

Sponsor's Conclusion
There were no consistent differences between AH-110690-treated sites and saline-treated sites under the conditions of the study. Any findings observed were within normal limits. AH-110690 solution for injection was well-tolerated in male and female rabbits when injected once intravenously, intraarterially, perivenously, or intramuscularly.
Reviewer comment: I agree with the sponsor's conclusion.

Study title: AH110690 Solution for Injection: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)

Study no.:	B067042
Study report location:	4.2.3.6.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	28 June 2005
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	AH-110690 stock solution in Ethanol (45 µg/mL); batch FFA 093/039-504; 97% by HPLC

Key study findings
The test item was not irritating following a 4 h exposure period under semi- occlusive conditions.

Methods

Dose:	2.8 µg/mL
Frequency of dosing:	Single exposure, 4h
Route of administration:	Topical on skin of left flank, semi-occluded
Dose volume:	0.5 mL
Formulation/Vehicle:	AH-110690 Solution for Injection/7% Ethanol in PBS
Species/Strain:	Rabbit/New Zealand White [Specific pathogen free, (SPF)]
Number/Sex/Group:	1 male, 2 females
Basis of dose selection:	Solubility
Age:	15 weeks (M); 12-13 weeks (F)
Body weight range:	2835 g (M); 2717-2836 g (F)
Protocol Deviation(s)	None with study impact

Procedures
Animals were monitored daily for mortality, clinical signs, and body weights.

Local signs and irritation scores were noted at 1h, 24 h, 48 h, and 72 h post-removal of semi-occlusive dressing and test item.

Results
There were no notable findings regarding mortality, clinical signs or body weights.

Well-defined to very slight erythema was observed at 1 h and 24 h post-treatment, respectively, in the male and 1 of 2 females. One female was observed to have very slight edema at 24 hours. No other abnormal findings were observed at any time point.

Report conclusion: The test item was not irritating following a 4 h exposure period under semi- occlusive conditions.

Reviewer comment: Agreed.

Study title: AH110690 Solution for Injection: Primary Eye Irritation Study in Rabbits

Study no.:	B067044
Study report location:	4.2.3.6.1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	12 July 2005
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	AH-110690 stock solution in Ethanol (45 µg/mL); batch FFA 093/039-504; 97% by HPLC

Key study findings

There were no test article-related effects.

Methods

Dose:	2.8 µg/mL
Frequency of dosing:	Single
Route of administration:	Instillation-conjunctival sac of left eye
Dose volume:	0.1 mL
Formulation/Vehicle:	AH-110690 Solution for Injection/7% Ethanol in PBS
Species/Strain:	Rabbit/New Zealand White [Specific pathogen free, (SPF)]
Number/Sex/Group:	1 male, 2 females
Test groups:	Each animal was untreated in one eye to serve as a negative control
Basis of dose selection:	Solubility
Age:	13-16 weeks (M); 12-14 weeks (F)
Body weight range:	4165 g (M); 2418-2729 g (F)
Protocol Deviation(s)	None with study impact

Procedures

Clinical observations, body weights, and irritation scores were assigned at 1, 24, 48, and 72 h post-treatment, according to prospectively defined evaluation criteria (Section 14.3 of the Final Report). The right eye was untreated and served as a negative control.

Results:

There were no test article-related effects, and no ocular irritation was observed.

Sponsor's Conclusion

Flutemetamol solution for injection was not an ocular irritant under the conditions of the study.

Reviewer comment: I agree.

Study title: A Study To Investigate The Potential For The ALZ103 Phase I Clinical Vehicle Formulation To Hemolyze Human Red Blood Cells In Vitro

Study no.:	B067061
Study report location:	4.2.3.6.1
Conducting laboratory and location:	Not stated
Date of study initiation:	Not stated
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	ALZ 103 Phase I Vehicle (FFF150/082-708) containing sodium phosphate (14 mM) pH 7.2 with 7% ethanol and 0.5% Tween 80

Key study findings: In vitro incubations of human whole blood cells with ALZ103 Phase I vehicle (same as Formulation B in Table 1) showed a low potential for hemolysis under the conditions of the assay. The assay was considered valid because the control incubations resulted in expected hemolysis levels.

Methods

The sponsor referenced two articles in the method, study design and interpretation of results(2; 7).

Two mL of whole blood containing citrate as an anticoagulant was collected from six human volunteers. Various amounts of the ALZ 103 Phase I Vehicle solution (Table 1) (0.002 mL to 0.2 mL) in the range 1:10 to 1:1000 (v/v) were added to whole blood. Control treatments were addition of 0.9% saline, addition of water, or freeze and thaw. Samples were processed, and then absorbance of supernatants at 540 nm was measured. Percent hemolysis (relative to controls) was calculated.

Results
No signs of hemolysis were detected after exposure of whole blood to the ALZ103 Phase I vehicle. Saline treatment did not result in any hemolysis. Treatment with water resulted in 10% and 25% hemolysis when incubated with 30% (v/v) or 50% (v/v) water in whole blood, respectively. The freeze/thaw treatment resulted in 100% hemolysis.

Sponsor's Conclusion
Flutemetamol for Injection did not cause hemolysis in whole blood samples under the conditions of the assay, which was interpreted as "low hemolysis potential".

Reviewer comment: Agreed.

11 Integrated Summary and Safety Evaluation

The sponsor conducted a comprehensive battery of nonclinical studies, consistent with ICH guidelines and FDA guidance, to characterize the Pharmacology and Toxicology profiles of Flutemetamol F 18 Injection (Vizamyl™). The nonclinical studies were adequate and sufficient to support the recommendation of "Approval" for NDA 203137 from the Pharmacology and Toxicology perspectives. The Safety Pharmacology, Pharmacokinetic, and Toxicity studies provided adequate support for the safety of Flutemetamol for Injection under the conditions specified in NDA 203137. There were no safety concerns identified in the nonclinical studies. Major components of the nonclinical program that supported the recommendation are discussed below.

Comprehensive safety pharmacology evaluations included *in vivo* neurological effects, cardiovascular effects, respiratory function, and *in vitro* cardiac cell function. Clinical observations in dog studies and in rat studies were consistent with ethanol exposure, and were noted in animals administered Vehicle only (containing 7% ethanol) and those administered Flutemetamol, with no increased incidence or severity at increasing doses of Flutemetamol. The results indicated that the observations were due to the Vehicle, not Flutemetamol.

Pharmacokinetic studies demonstrated that Flutemetamol was rapidly distributed throughout the body, and that muscle and liver were the organs receiving the greatest radiologic exposure. Flutemetamol was rapidly eliminated via the hepatobiliary route, although renal clearance accounted for approximately 20% of the dose. Flutemetamol did not accumulate in any organs, and the PK profile was similar in both male and female animals.

Flutemetamol was tested in single dose toxicity studies in rats and dogs. Results showed that no toxicity was observed based on antemortem or postmortem assessments at doses 38-fold (rat) or 24-fold (dog) the MHD. A single-dose toxicity study in rats, conducted concurrently with clinical Phase III studies, evaluated the Final Drug Product (FDP). The FDP consisted of decayed (^{18}F to ^{19}F) Vizamyl™, containing (b) (4) Polysorbate 80 and the same impurity profile as FDP. There were no adverse outcomes in antemortem or postmortem assessments, and the safety factor was 93-fold compared to MHD.

Flutemetamol was well tolerated in 7-day and 14-day repeated dose toxicity studies in rats and dogs. A finding of increased epididymus-to-body weight ratio was observed in high dose males in the 7-day repeated-dose rat toxicity study; however, the finding was not observed in the 14-day rat toxicity or any dog toxicity studies. The only apparent Flutemetamol-dependent finding was a slight increase in local irritation and inflammation (minimal to mild) observed at injection sites of high-dose male rats in the 14-day repeated dose toxicity study. No other Flutemetamol-dependent findings were evident from the toxicity studies. Safety factors of 7-fold (rats, 7- and 14-d studies) to 23-fold (dog studies, 7- and 14-d) the MHD were obtained in the studies.

Local toxicity studies in rabbits yielded unremarkable outcomes.

Genotoxicity studies of Flutemetamol conducted in *in vitro* systems yielded positive results, although studies conducted *in vivo* yielded negative results. The potential risk of genotoxicity due to Flutemetamol exposure at the MHD is negligible because of the single microdose administration. Moreover, genotoxicity studies are not usually required for radioactive diagnostic agents administered in microdose amounts [ICH M3(R2)]. There is a risk of genotoxicity due to the ^{18}F radionuclide in Vizamyl™; however, the benefit of a diagnosis likely outweighs the risk of exposure to ^{18}F, which has a relatively short half life of 110 minutes.

In conclusion, NDA 203137 is recommended for Approval from the Pharmacology and Toxicology perspectives.

12 Appendix/Attachments

Reference List

1. US Code of Federal Regulations. Radioactive Drugs for Certain Research Uses. Title 21 Section 361. 2013.

2. Amin K and Dannenfelser RM. In vitro hemolysis: Guidance for the pharmaceutical scientist. *Journal of Pharmaceutical Sciences* 95: 1173-1176, 2006.

3. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D and Jones E. Alzheimer's disease. *Lancet* 377: 1019-1031, 2011.

4. Cheung WK, Natarajan J, Sanders M and Vercammen E. Comparative pharmacokinetics, safety, and tolerability after subcutaneous administration of recombinant human erythropoietin formulated with different stabilizers. *Biopharm Drug Dispos* 21: 211-219, 2000.

5. Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS and Alexoff DL. Distribution volume ratios without blood sampling from graphical analysis of PET data. *J Cereb Blood Flow Metab* 16: 834-840, 1996.

6. Mondragan-Rodriguez S, Basurto-Islas G, Lee Hg, Perry G, Zhu X, Castellani RJ and Smith MA. Causes versus effects: the increasing complexities of Alzheimer's disease pathogenesis. *Expert Rev Neurotherapeutics* 10: 683-691, 2010.

7. Reed KW and Yalkowsky SH. Lysis of human red blood cells in the presence of various cosolvents. *J Parenter Sci Technol* 39: 64-69, 1985.

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/s/

SALLY J HARGUS
06/27/2013

ADEBAYO A LANIYONU
06/27/2013
I concur

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 203137 **Applicant: GE Healthcare** **Stamp Date: 10/26/2012**

Drug Name: Flutemetamol (18F) for Injection **NDA Type: 505 (b)(1) - NME** **Filing Meeting Date: 12/03/2012**

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		Organized in the Electronic Common Technical Document (eCTD) format.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
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 submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	NA		Not applicable-no studies requested.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
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		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		X	To be discussed during Filing Meeting.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	NA		Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? _Yes_____

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

The NDA is fileable.

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

None.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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

/s/

SALLY J HARGUS
11/30/2012

ADEBAYO A LANIYONU
12/01/2012