Non-clinical Exposure Summary for Hyperpolarized Pyruvate (^{13}C) Injection

Contents

Introduction

This summary is of the pharmacology and toxicology studies filed to the US Food and Drug Administration with the initial IND (#71651) by General Electric. The ownership of this IND was transferred to the US National Cancer Institute in July 2015. For investigators in the US who wish to file their own IND, a letter of right of reference (a cross-file letter) can be requested from the NCI and it will not be necessary (nor advised) to file the actual data with the new IND filing although the summary or an Investigators Brochure will be helpful with the IRB approval.

Investigators outside of the US will need to follow their national regulatory policy with respect to investigational imaging agents and may need access to the complete reports for all or some

of the studies for national filing or for ethics committee approvals. The reports can be requested from the NCI.

As the product was developed, ten different test articles were used in the non-clinical studies with varying quantities, ratios, buffers and concentrations of:

- [1-13C] pyruvate, AH110896 or ^{13}C- AH110896
- Pyruvate, AH110896, ^{12}C-AH110896, or AH111710
- AH111501 (the trityl radical Tris{8-carboxyl-2,2,6,6-tetra[2-(1-methoxyethyl)]-benzo(1,2-d:4,5-d')bis(1,3)dithiole-4-yl}methyl acid),
- TRIS
- EDTA
- Potential Impurities
    - AH112623 (parapyruvate)
    - AH112615 (reaction product between TRIS and pyruvate)
    - AH113462 (lactone)

[Initially, one AH number covered both [1-^{13}C]pyruvic acid and pyruvic acid in its natural abundance. The terms ^{13}C- AH110896 and ^{12}C-AH110896 were then used.]

The final clinical formulation comprises: 250 mM [1-^{13}C] pyruvate, 0.43 mg/ml AH111501 in 100 mM TRIS with 0.1 mg/ml Na_2EDTA and 180 mM NaOH.

These data were provided by GE Healthcare and filed with FDA in 2010 in IND 70,651.

Summary of Non-Clinical Data

A range of non-clinical studies were undertaken by GE Healthcare as part of the development program, which were designed to support the planned early clinical studies. Margins of safety derived from the toxicology studies are given in Table 1.1, Table 1.2, Table 1.3 and Table 1.4 and from the safety pharmacology studies in Table 1.5 and Table 1.6. Table 1.7 provides a tabular summary of all non-clinical studies.

At the time the initial safety studies were carried out, it was expected that the clinical formulation would comprise: 500 mM [1-^{13}C] pyruvate, 0.85 mg/ml AH111501 in 200 mM TRIS with 0.1 mg/ml Na_2EDTA and 360 mM NaOH. In these early studies the injection rates employed were low compared to the suggested clinical injection rate of 5 ml/s. In order to study the effects of the injection rate, two non-GLP studies in anesthetized dog were undertaken. These studies were conducted with a test article comprising 500 mM pyruvate, in 200 mM TRIS with 0.1 mg/ml Na_2EDTA and 360 mM NaOH. Because [1-^{13}C] pyruvate and pyruvate have the same chemical characteristics they will have the same safety profile, when the impurity profiles of the two test articles are similar. The non-GLP studies demonstrated that the high osmolality (approximately 1,000 mOsmol/kg) of the test article contributed to effects on the cardiovascular system. As a consequence, it was decided to make a less concentrated formulation.

The clinical formulation now comprises: 250 mM [1-^{13}C] pyruvate, 0.43 mg/ml AH111501 in 100 mM TRIS with 0.1 mg/ml Na_2EDTA and 180 mM NaOH. This formulation has a lower osmolality of approximately 500 mOsm/kg. The maximum human dose (MHD) in the Phase 1 study was 0.43 ml/kg bw. This equals maximum doses of 9.6 mg/kg for [1-^{13}C] pyruvate and 1.97 µg/kg for AH111501.

In total, ten different test articles were used in the safety pharmacology and toxicology studies. These test items are:

(1) 500 mM [1-^{13}C] pyruvate and 0.85 mg/ml AH111501 in 200 mM TRIS with 0.1 mg/ml Na_2EDTA and 360 mM NaOH

(2) 500 mM [1-^{13}C] pyruvate in 200 mM TRIS with 0.1 mg/ml Na_2EDTA and 360 mM NaOH where AH111501 was removed by solid-phase extraction (filtered test item)

(3) 500 mM pyruvate and 0.85 mg/ml AH111501 in 200 mM TRIS with 0.1 mg/ml Na_2EDTA and 360 mM NaOH

(4) 500 mM pyruvate in 200 mM TRIS with 0.1 mg/ml Na_2EDTA and 360 mM NaOH where AH111501 was removed by solid-phase extraction (filtered test item)

(5) 500 mM pyruvate in 200 mM TRIS with 0.1 mg/ml Na_2EDTA and 360 mM NaOH

(6) 250 mM [1-^{13}C] pyruvate and 0.43 mg/ml AH111501 in 100 mM TRIS with 0.1 mg/ml Na_2EDTA and 180 mM NaOH

(7) 250 mM pyruvate and 0.43 mg/ml AH111501 in 100 mM TRIS with 0.1 mg/ml Na_2EDTA and 180 mM NaOH

(8) 250 mM pyruvate in 100 mM TRIS with 0.1 mg/ml Na_2EDTA and 180 mM NaOH where AH111501 was removed by solid-phase extraction (filtered test item)

(9) In the hERG assay pyruvate and AH111501 were tested separately. The two compounds were dissolved in HEPES buffer.

(10) AH111501 was also tested separately in the Ames and MLA assays. For these studies AH111501 was dissolved in sterile water.

(11) AH112623 (parapyruvate)

(12) AH112615 (reaction product between TRIS and pyruvate)

(13) AH113462 (lactone)

Note: The names of the test items have changed over time from when the initial studies were conducted. The original test item name remains in the study titles and full reports.

Table 1.1 Summary of NOAEL, HED and Margin of Safety for expanded acute toxicology studies for the [1-13C] pyruvate test articles

Study Type	Study No. (GE Ref. No.)	Test Item	NOAEL [1-^{13}C]pyruvate/AH111501 (mg/kg bw)	HED* Pyruvate/AH111501 (mg/kg bw)	Margin of Safety based on HED (HED/MHD)**
Expanded acute dose *-rat*	B101020	(1)	[1-^{13}C] pyruvate: 1784 AH111501: 34	288 5.5	30 2783
Expanded acute dose *-rat*	B101040	(6)	[1-^{13}C] pyruvate: 892 AH111501:17	144 2.8	15 1408
Expanded acute dose *-dog*	5887 (B101017)	(1)	[1-^{13}C] pyruvate: 446 AH111501: 8.5	248 4.7	26 2397

*HED Human Equivalent Dose: Dose adjusted for body surface area
Rat scaling factor to Human for surface area = 6.2
Dog scaling factor to Human for surface area = 1.8
**Margin of Safety, i.e., Human Equivalent Dose as multiples of the Maximum Human Dose
For the calculations, rounding has only taken place at the end of the calculation.

Table 1.2 Summary of genetic toxicology studies for the [1-^{13}C] pyruvate test articles and AH111501

Study Type	Study No. (GE Ref. No.)	Test Item	NOAEL [1-^{13}C]pyruvate/AH111501	HED* Pyruvate/AH111501 (mg/kg bw/day)	Margin of Safety based on HED (HED/MHD)**
Ames	5605/15 (B101014)	(1)	[1-^{13}C] pyruvate: 5000 µg/plate AH111501: 97 µg/plate	-	
Ames	2570/3 (B101043)	(1)	[1-^{13}C] pyruvate: 5352 µg/plate AH111501: 102 µg/plate	-	
Ames	2570/10 (B101055)	(10)	AH111501: 5000 µg/plate	-	
MLA	5603/08 (B101015)	(1)	Increased observed in mutation frequency, but similar increases were seen for the vehicle and TRIS/EDTA dissolution medium controls	-	
MLA	2570/4 (B101045)	(1)	Positive	-	
MLA	2570/11 (B101056)	(10)	Positive only for high concentrations after 24 h exposure without the addition of S-9	-	
Micronucleus *-rat*	5604/04 (B101016)	(1)	[1-^{13}C] pyruvate: 892 mg/kg bw/day AH111501: 17 mg/kg bw/day	144 5.5	15 1408
Comet *-rat*	2789/6 (B101057)	(1)	[1-^{13}C] pyruvate: 1784 mg/kg bw/day AH111501: 34 mg/kg bw/day	288 5.5	30 2783

*HED Human Equivalent Dose: Dose adjusted for body surface area
Rat scaling factor to Human for surface area = 6.2
** Margin of Safety, i.e., Human Equivalent Dose as multiples of the Maximum Human Dose
For the calculations, rounding has only taken place at the end of the calculation.

Table 1.3 Summary of NOAEL, HED and Margin of Safety for expanded acute toxicology studies with potential impurities

Study Type	Study No. (GE Ref. No.)	Impurity	NOAEL Impurity (mg/kg bw)	HED* Impurity (mg/kg bw)	Margin of Safety based on (HED/MHD)**
Expanded acute dose -*rat*	B101044	(11)	AH112623: 1216	196	1698
Expanded acute dose -*rat*	B101046	(12)	AH112615: 1400^	226	8176
Expanded acute dose -*rat*	B101065	(13)	AH113462 K: 28.1 AH113462 E: 135.4 AH112623	4.5 21.8 27.6	178 306 239

*HED Human Equivalent Dose: Dose adjusted for body surface area

Rat scaling factor to Human for surface area = 6.2

**Margin of Safety, i.e., Human Equivalent Dose as multiples of the Maximum Human Dose of 0.43 ml/kg bw of a formulation containing the respective impurities in the maximum measured concentration (by (^{13}C)NMR) at 24s after compounding. For the calculations, rounding has only taken place at the end of the calculation.

^Nominal concentration

(11) AH112623 (parapyruvate)

(12) AH112615 (reaction product between TRIS and pyruvate)

(13) AH113462 (lactone)

Table 1.4 Summary of genetic toxicology studies with potential impurities

Study Type	Study No. (GE Ref. No.)	Test Item	NOAEL [1-^{13}C]pyruvate/ AH111501	HED* Pyruvate/AH111501 (mg/kg bw/day)	Margin of Safety based on HED (HED/MHD)**
Ames	2570/5 (B101042)	(11)	AH112623: 4490 µg/plate	-	
Ames	2570/7 (B101047)	(12)	AH112615: 5000 µg/plate^	-	
Ames	2789/8 (B101062)	(13)	AH113462 K+E: 4078 µg/plate# AH112623: 3953 µg/plate	-	
MLA	2570/6 (B101041)	(11)	Positive only for high concentration after 24 h exposure without the addition of S-9. At these concentrations marked changes in osmolality occurred	-	
MLA	2570/4 (B101048)	(12)	AH112615: 5000 µg/ml^	-	
MLA	2789/9 (B101063)	(13)	Evidence of mutagenic activity, but the observations were considered of highly questionable biological relevance.	-	
Micronucleus *-rat*	2789/10 (B101064)	(13)	AH113462 K: 28.1 AH113462 E: 135.4 Ah112623: 170.9	4.5 21.8 27.6	178 306 239

*HED Human Equivalent Dose: Dose adjusted for body surface area

Rat scaling factor to Human for surface area = 6.2

** Margin of Safety, i.e., Human Equivalent Dose as multiples of a human dose of 0.43 ml/kg bw of a formulation containing the respective impurities in the maximum measured concentration (by (^{13}C)NMR) at 24 s after compounding. For the calculations, rounding has only taken place at the end of the calculation.

^ Nominal concentration

Release data for the acidic formulation of AH112623 at 48 h after dissolution give the concentration of the sum of AH113462K and AH113462E, but the concentrations of the two individual compounds are not specified.

Table 1.5 Summary of NOAEL, HED and Margin of Safety for safety pharmacology studies, with low injection rate for in vivo studies

Study Type	Study No. (GE Ref. No.)	Test Item	NOAEL pyruvate/AH111501	HED* Pyruvate/AH111501 (mg/kg bw)	Margin of Safety based on HED (HED/MHD)**
hERG	858776 (B101021)	(9)	Pyruvate 4 mg/ml AH111501 7.35 mg/ml	-	143^ 159898^
Telemetry -*Conscious dog*	855869 (B101019)	(1&2)	[1-^{13}C]Pyruvate 129 mg/kg bw AH111501 2.5 mg/kg bw	71.9 1.37	7.5 692
Irwin -*Conscious rat*	855869 (B101011)	(3&4)	Pyruvate 220 mg/kg bw AH111501 4.25 mg/kg bw	35.5 0.69	3.8§ 347

*HED Human Equivalent Dose: Dose adjusted for body surface area
Rat scaling factor to Human for surface area = 6.2
Dog scaling factor to Human for surface area = 1.8
** Margin of Safety, i.e., Human Equivalent Dose as multiples of the Maximum Human Dose
For the calculations, rounding has only taken place at the end of the calculation.
^ For hERG assay, the safety factor is based on the molar concentration in the cell bath divided by the measured C_{max} in the 0.43 ml/ kg bw cohort in study GE-101-003 (253±30 µM) for pyruvate.
For AH111501 the safety factor is based on the cell bath concentration divided with a calculated C_{max} (the MHD of 1.97 µg AH111501/kg administered to a 70-kg person and distributed in 3000 ml plasma equal to 0.046 µg/ml)
§ For the Irwin study the MHD was calculated as pyruvate (22 mg/ml) and not [1-^{13}C]pyruvate (22.3 mg/ml) in order to avoid bias caused by the weight differences of the isotopes.

Table 1.6 Summary of NOAEL, Cmax and Margin of Safety for safety pharmacology studies, with the clinical injection rate (5 ml/s)

Study Type	Study No. (GE Ref. No.)	Test Item	NOEL Pyruvate	Cmax* Pyruvate (µM)	Margin of Safety Based on Cmax (Cmax/Cmax)**
Cardiovascular effects -*anesthetized dog* Non-GLP	B101026	(5)	0.71 ml/kg bw *355 µmol/kg bw*	2086±771 *(1 min)*	8.2
Mechanism -*anesthetized dog* Non-GLP	B101031	(5)	NA	-	-
Cardiovascular effects -*anesthetized dog*	06.076/6 (B101037)	(7&8)	1.4 ml/kg bw *350 µmol/kg bw*	258±19 (3 min)	-
Dose-response -*anesthetized dog*	06.434/4 (B101049)	(7&8)	1.4 ml/kg bw *350 µmol/kg bw*	377±116 (3 min)	-
Telemetry -*conscious dog*	06.075/5 (B101036)	(7&8)	1.4 ml/kg bw *350 µmol/kg bw*	305±78 (1 min)	1.2
CNS effects (EEG) -*conscious dog* Non-GLP	06.210/5 (B101039)	(7&8)	5.7 ml/kg bw *1425 µmol/kg bw*	670±234 (3 min)	-

*C_{max} Maximum measure blood concentration of pyruvate adjusted for predose concentration

**Margin of Safety, i.e., C_{max} in animal study as multiples of C_{max} for study GE-101-003 (253±30 µM)

Table 1.7: Tabulated Summary of Non-clinical Studies

Study Title	Study No.	Results Summary
Primary Pharmacodynamic Studies		
Metabolic Imaging with hyperpolarized [1-^{13}C]-Pyruvate in Prostate of Healthy Dogs Using a Second- Generation Endorectal Coil and Reduced Dose	B101059	The injection rate was scaled to cardiac output to make it comparable to the clinical injection rate of 5 ml/s. The doses of 0.18 and 0.36 ml/kg were administered manually due to the low dose volume (2.0-4.7 ml). The dose of 1.4 ml/kg bw was administered by power injector. The study demonstrated pyruvate and lactate signal-to-noise ratios of ~20:1 for both the 0.18 and 0.36 ml/kg bw doses. The choice of dose appears to be limited by tissue contrast-to-noise and the highest acceptable dose from a safety perspective is recommended for clinical studies. Based on the results it is clear that the pulse sequence must be tailored to suit the human biology and this can only be accomplished in clinical studies.
Safety Pharmacology Studies		
Na-Pyruvate and AH111501: Effects on HERG-1 tail currents recorded from stably transfected HEK 293 cells.	RCC 858776 (B101021)	Neither AH111501 nor Na-pyruvate affected the amplitude of the hERG-1 tail current at the tested concentrations. Exposure was demonstrated for Na-pyruvate at a concentration of 4.0 mg/ml.
AH111501 dissolved in ^{12}C-pyruvate: Modified Irwin screen test in the rat.	RCC 855869 (B101011)	Intravenous administration of test item containing AH111501 and pyruvate up to dose volumes of 5 ml/kg bw had no significant effects on the behavior of male Wistar rats when evaluated in a modified Irwin screen test.
Cardiovascular assessment in anaesthetised dogs of pyruvate administered at high injection rates.	B101026	One female dog died after receiving the first dose of 126 mg/kg bw pyruvate. This animal had received ~4.5 times as much pentobarbital and more than 1.6 times as much fentanyl per hour compared to the average given to the other dogs. The heavy dose of anesthetic was thought to have abolished all cardiovascular compensatory mechanisms. Pyruvate caused an acute, short-lasting, dose-dependent decrease in arterial blood pressure, immediately followed by a compensatory increase in heart rate and femoral arterial flow. Pulmonary arterial pressure [PAP] gradually increased with peak effect at 2-3 min. The increase in PAP was either a passive effect, caused by an increase in cardiac output, or by an increase in pulmonary resistance. A short-lasting, dose-dependent, prolongation of the QT/QTcV-interval was observed. No evidence of treatment-related arrhythmias was recorded. Administration of TRIS/EDTA vehicle control caused an acute short-lasting decrease in blood pressure. Osmotically-matched glucose solution caused effects similar to administration of pyruvate. A reduction in injection rate appeared to diminish the effects on systemic arterial pressure and heart rate and to a lesser degree the effect on pulmonary arterial pressure. Pyruvate was rapidly metabolized to lactate following intravenous injection, with high concentrations observed as early as 1 min post dosing. Tmax was in the range of 1 to 10 min.

Study Title	Study No.	Results Summary
		The pharmacokinetics of pyruvate and lactate were calculated using noncompartmental analysis. The blood concentrations of pyruvate and lactate were corrected for pre-dose levels before pharmacokinetic analysis. There was no statistically significant difference in Cmax or AUCtot between filtered and unfiltered test substance in any of the dose groups, suggesting that the presence of AH111501 in the test article did not influence the blood concentration of pyruvate. Pyruvate was eliminated from blood with an average elimination half life of 18±11 min. Dose-normalized pyruvate data indicated that Cmax deviated negatively from proportionality in the 1.4 ml/kg bw dose group. AUCtot increased proportionally with dose. Dose-normalized lactate data indicated that Cmax and AUCtot deviated positively from proportionality in the 1.4 ml/kg bw dose group. Due to the individual variation in data, the biological significance of these findings is not known.
Mechanistic study of effects of 500 mM pyruvate on the cardiovascular system in anaesthetised male dogs.	B101031	A drop in arterial blood pressure was observed after pyruvate injections. Data from administration of hypertonic and isotonic test item indicate that the drop was caused by peripheral vasodilation and that the vasodilation was caused in part by the osmolarity of the test article and in part by a metabolite of pyruvate, probably lactic acid. Pyruvate caused no negative effect on the heart's pumping activity. There was no direct effect of pyruvate on the pulmonary circulation, the observed increase in pulmonary arterial pressure was a reflex of an increased cardiac output. Pyruvate was accompanied by a bi-phasic response in cardiac workload, the maximum increase (40% for 2 ml/kg) was of the same magnitude as variations seen during everyday activities (meals, postural changes, walking). A small increase (2 mmHg) in pulmonary venous pressure was observed after 2.0 ml/kg bw pyruvate injections. This was too small to have any effects on pulmonary tissue fluid balance and will not induce lung edema.
Effects of rapid intravenous administration of a mixture of natural abundant pyruvic acid and 15 mM AH111501 dissolved in TRIS/EDTA dissolution medium on the cardiovascular function in the telemetered dog.	Porsolt 06.075/5 (B101036)	Administration of test item and filtered test item caused similar effects. In doses of 4.3 and 5.7 ml/kg the test articles caused a non-significant, transient and slight increase in arterial blood pressure. At 5.7 ml/kg, the increase was preceded by a non-significant, weak and transient reduction in arterial blood pressure immediately after administration. Heart rate was increased compared to baseline by the test articles when administered in doses of 4.3 and 5.7 ml/kg, The increases for 5.7 ml/kg of test item and 4.3 ml/kg of filtered test article were also significant when compared to saline. No substantial effect on the PR- or QT-intervals was observed after administration of the pyruvate test articles. The test articles did not significantly modify the QTc interval (Fridericia's and van de Water's formulae) as compared with physiological saline. When compared with baseline, a significant lengthening was observed (Fridericia's) at 4.3 and 5.7 ml/kg and for the test article and at 4.3 ml/kg for the filtered test article. This lengthening was mainly ascribed to the delay in the QT-interval adaptation to the sudden variation of heart rate.

Study Title	Study No.	Results Summary
Safety pharmacology study of hemodynamic effects of a mixture of natural abundant pyruvic acid and 15 mM AH111501 dissolved in TRIS/EDTA dissolution medium after intravenous administration in the anesthetized dog.	Porsolt 06.076/6 (B101037)	Pyruvate caused an acute dose dependent peripheral vasodilation triggering a reduction in systemic arterial blood pressure, reflex tachycardia and an increase in cardiac output. dP/dt_{max} was decreased at the same time as the arterial blood pressure was reduced. Left cardiac work was unchanged for about the first minute after dosing with test item but increased acutely after injection of filtered test item. Left-ventricular end diastolic pressure and pulmonary artery blood pressure increased. After the acute phase, systemic arterial blood pressure, dP/dt_{max}, left cardiac work and stroke volume was increased. The maintained increase in cardiac output was due to the increase in myocardial contractility together with remaining tachycardia. Cerebral blood flow increased moderately. The PR interval was transiently shortened, at the same time as heart rate peaked. The QTc (van de Water's formula) interval was transiently lengthened after administration.
Evaluation of a mixture of natural abundance pyruvic acid and AH111501 dissolved in TRIS/EDTA dissolution medium on EEG and behaviour in the conscious dog.	Porsolt 06.210/5 (B101039)	Rapid bolus injections of the test item did not provoke pathological clinical symptoms or pathological EEG activity. The occasional symptoms observed were of a non-specific nature, transient or appeared before and after substance administrations and could not be attributed to either the substance or the composition of the vehicle. Convulsions and other pathologic signs were not observed following administration of test item in any of the 3 dogs tested. However, convulsions were observed following infusion of positive control in all 3 dogs confirming the sensitivity of the model.
Safety pharmacology study of hemodynamic effects after intravenous administration in the anesthetized dog of a mixture of natural abundant pyruvic acid and 15 mM AH111501 dissolved in TRIS/EDTA dissolution medium with AH111501 subsequently removed by solid phase extraction	Porsolt 06.434/4 (B101049)	As seen in study 06.076/6, rapid bolus injections of the pyruvate test article caused an acute dose dependent peripheral vasodilation, triggering dose dependent acute decreases in systemic arterial blood pressure and compensatory increases in heart rate. An acute onset and prolonged increase in cardiac output was observed. Left cardiac work increased immediately after administration. The initial increase (at 30 s) for this parameter was similar to the increase observed for the respective saline controls. When considering the measured effects as change from baseline, peak effects compared to the appropriate saline control and AUC for the duration of the peak (where available) compared to the appropriate saline control the effect level for this study was 2.1 ml/kg bw. The dose of 1.4 ml/kg bw caused only minor, though statistically significant, peak effects in systemic arterial blood pressure, left-ventricular end-diastolic pressure dP/dt_{max}, heart rate and QTcV when compared to the saline volume control. The effects were considered to be within normal physiological variation and the dose was considered to be the No-Observed-Adverse-Effect–Level for the study.
Pharmacokinetic Studies		
Study of Distribution and Excretion of [1-14C]Pyruvate in Male Sprague-Dawley Rat	B101003	After iv injection of sodium [1-^{14}C] pyruvate, radioactivity was rapidly distributed throughout the body. A dose of 56 mg sodium pyruvate/kg (including both [1-^{13}C] and [1-^{14}C] pyruvate) resulted in a blood concentration of radioactivity corresponding to 8.9% id (85.5 µg pyruvate equivalents/g blood) at 30 s post dosing. The initial volume of distribution was estimated to

Study Title	Study No.	Results Summary
		677 ml/kg, indicating distribution of pyruvate to a volume comparable to total body water. The highest concentration of radioactivity 30 s post dosing was found in the pancreas. Relatively high concentrations were also found in the blood, liver, adrenals, heart muscle and the small and large intestine wall. The lowest concentrations of radioactivity were found in white fat, testes, brain and spinal cord. The concentration of radioactivity in the prostate at 30 s post dosing was 1.87 kBq/g. This corresponds to a pyruvate equivalent concentration of 34.5 µg/g. The elimination rate in prostate during the first 2 min post dosing corresponded to a half-life of 1.5 min. The highest recovery of radioactivity was found in muscle and skin tissue. At 1 min post dosing, the recovery in muscle and skin was 55% and 19% id, respectively. At 60 min post dosing, the recovery in muscle and skin was 13% and 7% id, respectively. The recovery of radioactivity in whole body cryosections was rapidly decreasing. The autoradiograms indicate some elimination of radioactivity through the kidneys, but no visible amounts of urine were collected in the time-frame of the study. The major elimination route was through exhaled air. At 120 min post dosing, radioactivity corresponding to 63% id was recovered in exhaled air, as a result of the formation of $^{14}CO_2$.
Study of Distribution and Excretion of [1-14C] pyruvate in Male Sprague-Dawley Rats - follow-up of study B101003	B101018	At 15 min post dosing, the highest radioactivity concentrations were found in urinary bladder contents, bone mineral tissue and pancreas, whereas relatively high concentrations were found in liver, small and large intestine wall, bone marrow and salivary glands. The lowest radioactivity concentrations were found in brain, spinal cord, white fat and testes. The radioactivity concentrations decreased with time in all organs and tissues. At 24 h post dosing, the highest concentrations were found in urinary bladder contents, bone marrow, large intestine wall and skin. The lowest concentrations were found in brain, white fat and eye. The highest recovery was found in muscle, skin and bone tissue. At both 15 min and 24 h post dosing, these tissues accounted for approximately 70% of the whole body recovery. The total recovery determined in whole body sections decreased from 55% of the injected dose (% id) at 15 min to 7.9% id at 24 h. The major elimination route was through exhaled air. At 24 h post dosing, radioactivity corresponding to 56% id was recovered in exhaled air, as a result of the formation of $^{14}CO_2$. The elimination rate decreased biexponentially with time. Only minor amounts of radioactivity were excreted in urine and faeces. 2.1% id was found in voided urine 24 h post dosing (including cage wash water). The amount of radioactivity in the urine bladder was included in the whole body recovery calculations, assuming a residual urine volume of approximately 1 ml/205 gram body weight. The recovery in faeces was 0.26% id at 24 h post dosing. The total recovery was in the range of 53 to 67% id after 24 h. Despite the relatively low total recovery, the study is considered to give a satisfactory description of the biological fate of [1-^{14}C]pyruvate following intravenous injection in rats.

Study Title	Study No.	Results Summary
Toxicology Studies		
An expanded acute dose toxicity study with intravenously injected AH111501 dissolved in Na-pyruvate in male and female rats	B101020	There were no treatment-related adverse effects in male or female rats receiving acute intravenous injection of AH111501 in 500 mM pyruvate at 1784 mg pyruvate/kg. NOAEL 1784 mg/kg body weight.
Expanded acute dose intravenous toxicity study with AH111501 dissolved in ^{13}C-pyruvate in Beagle dogs	TNO 5887 (B101017)	There were no treatment-related adverse effects in male or female Beagle dogs receiving acute intravenous injections of these dose levels of AH111501 dissolved in ^{13}C-pyruvate. NOAEL 446 mg/kg body weight. Toxicokinetic analysis showed rapid transformation of ^{13}C-pyruvate to ^{13}C-lactate, with a peak concentration of ^{13}C-lactate at 3 minutes post dosing. The elimination half-life of ^{13}C-pyruvate and ^{13}C-lactate was 6.4±0.9 and 6.4±0.6 minutes, respectively. No biologically significant differences in systemic exposure between male and female animals were observed.
An expanded acute dose toxicity study with intravenously injected AH111501 in 250 mM pyruvate dissolved in 100 mM TRIS/EDTA in Sprague Dawley rats	B101040	There were no treatment-related adverse effects in male or female rats receiving acute intravenous injection of 250 mM [1-^{13}C]pyruvate and 0.43 mg/ml AH111501 in 100 mM TRIS/EDTA dissolution medium at 892 mg [1-^{13}C]pyruvate/kg. NOAEL 892 mg/kg bw
An expanded acute dose toxicity study with intravenously injected Parapyruvate in male and female rats	B101044	There were no treatment related adverse effects in male or female rats receiving acute intravenous injection of AH112623 at 1216 mg/kg. NOAEL 1216 mg/kg body weight.
An expanded acute dose toxicity study with Intravenously injected AH112615 (PA-TRIS) in male and female rats	B101046	There were no treatment related adverse effects in male or female rats receiving acute intravenous injection of AH112615 at 1400 mg/kg. NOAEL 1400 mg/kg body weight
Parapyruvate/Lactone in TRIS Solution: Expanded Acute Dose Toxicity Study in the rat	B101065	There were no treatment related adverse effects in male or female rats receiving single intravenous injection of AH112623/AH113462 at 100 or 200 mg/kg. NOAEL was 200 mg/kg body weight.
Local tolerance study with AH111501 dissolved in ^{12}C-pyruvate in Rabbits	TNO 5612/08 (B101013)	**Intra-arterial:** No signs of intolerance were observed at the injection site in any of the rabbits during the 14-days observation period. **Subcutaneous**: Injection in the subcutis induced a minimal local inflammatory response in one out of four cases, consisting of scattered single cell necrosis and infiltration with granulocytes, macrophages and monocytes. **Paravenous**: Injection in the paravenous area induced a moderate local inflammatory response in two out of four cases, generally consisting of hemorrhages, scattered single cell necrosis and infiltration with granulocytes, macrophages and monocytes. Intra-muscular: No signs of test item-related local adverse effects **Ocular**: No signs of eye irritation were observed. **Dermal application**: No signs of skin irritation

Study Title	Study No.	Results Summary
Micronucleus test in bone marrow cells of rats treated intravenously with AH111501 dissolved in ^{13}C pyruvate	TNO 5604/04 (B101016)	500 mM [1-^{13}C] pyruvate and 0.85 mg/ml AH111501 in 200 mM TRIS/EDTA dissolution medium did not produce chromosomal damage or damage to the mitotic spindle apparatus in the bone marrow indicator cells in male rats.
Mixture of 500 mM [1-^{13}C] pyruvic acid and AH111501 sodium salt dissolved in TRIS/EDTA dissolution medium: Detection of DNA damage in the blood of treated rats using the Comet assay	Covance 2789/6 (B101057)	500 mM [1-^{13}C] pyruvate and 0.85 mg/ml AH111501 in 200 mM TRIS/EDTA dissolution medium did not induce DNA damage in the blood of rats treated with up to 1784 mg pyruvate/kg/day on two consecutive days, when analysed 3 h after the last dose administration.
Parapyruvate/ lactone in TRIS solution: induction of micronuclei in the bone marrow of treated rats (GLP)	Covance 2789/10/(B101064)	Parapyruvate/lactone in TRIS solution did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of male rats treated up to the dose of 200 mg/kg (the maximum practicable dose) following both 24 and 48 hour sampling.
Formulation containing 557 µM AH111501 and 500 mM ^{13}C Pyruvate (AH110896) in 200 mM TRIS: Reverse Mutation in five Histidine requiring strains of *Salmonella typhimurium*	Covance 2570/03 (B101043)	500 mM [1-^{13}C] pyruvate and 0.85 mg/ml AH111501 in 200 mM TRIS/EDTA dissolution medium did not induce mutation in five histidine requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at amounts up to 5352 µg/plate, in the absence and in the presence of a rat liver metabolic activation system (S- 9).
Formulation containing 557µM AH111501 and 500 mµ ^{13}C Pyruvate (AH110896 in 200 mM TRIS): Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique	Covance 2570/04 (B101045)	500 mM [1-^{13}C] pyruvate and 0.85 mg/ml AH111501 in 200 mM TRIS/EDTA dissolution medium induced mutation at the tk locus of L5178Y mouse lymphoma cells when tested under the conditions employed in this study. These conditions included treatments up to toxic concentrations for 24 h in the absence of a rat liver metabolic activation system (S-9) and up to approximately 5000 µg/ml for 3 h in the absence and presence of S-9. The mutagenic activity following 3 hour treatments in the absence and presence of S-9 was observed at concentrations at which marked increases in osmolality were also seen.
Parapyruvate: Reverse mutation in five histidine requiring strains of *Salmonella typhimurium*	Covance 2570/05 (B101042)	AH112623 did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at amounts up to 5000 µg/plate, in the absence and in the presence of a rat liver metabolic activation system (S-9).
Parapyruvate: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique	Covance 2570/06 (B101041)	AH112623 did not induce mutation at the *tk* locus of L5178Y mouse lymphoma cells when tested under the 3 hour treatment conditions employed in this study. These conditions included treatments up to 5000 µg/mL in two independent experiments, in the absence and presence of a rat liver metabolic activation system (S-9). Parapyruvate may induce mutation at the *tk* locus of L5178Y mouse lymphoma cells when tested under 24 hour treatment conditions in the absence of S-9 up to toxic concentrations. However, as increases in mutant frequency were only noted a concentrations where marked changes in osmolality occurred, it cannot be determined from this study data whether these results are due to true mutation induction or physiological effects of increases in osmolality.

Study Title	Study No.	Results Summary
AH112615: Reverse mutation in five histidine requiring strains of *Salmonella typhimurium*	Covance 2570/07 (B101047)	AH112615 did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at amounts up to 5000 µg/plate, in the absence and in the presence of a rat liver metabolic activation system (S-9).
AH112615: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique	Covance 2570/08 (B101048)	AH112615 did not induce mutation at the tk locus of L5178Y mouse lymphoma cells when tested under the conditions employed in this study. These conditions included treatments up to 5000 µg/ml in two independent experiments in the absence and presence of a rat liver metabolic activation system (S-9).
AH111501: Reverse mutation in five histidine requiring strains of *Salmonella typhimurium*	Covance 2570/10 (B101055)	AH111501 did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatment at amounts up to 5000 µg/plate in the absence and in the presence of a rat liver metabolic activation system (S-9).
AH111501: Mutation at the Thymidine Kinase (*tk*) Locus of Mouse Lymphoma L5178Y Cells (MLA) using the Microtitre^{R} Fluctuation Technique	Covance 2570/11 (B101056)	AH111501 did not induce mutation at the tk locus of L5178Y mouse lymphoma cells when tested under the 3 hour treatment conditions employed in this study. These conditions included treatments up to 5000 µg/ml in two independent experiments, in the absence and presence of a rat liver metabolic activation system (S-9). AH111501 induce mutation at the tk locus of L5178Y mouse lymphoma cells when tested under 24 hour treatment conditions in the absence of S-9, up to toxic concentrations. The effect was found to be very weak.
Parapyruvate/ lactone in HCl solution: Reverse mutation in five histidine requiring strains of *Salmonella typhimurium*	Covance 2789/8 (B101062)	Parapyruvate/lactone in HCl solution did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations up to 5000 µg/plate, in the absence and in the presence of a rat liver metabolic activation system (S-9).
Parapyruvate/ lactone in HCl solution: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre Fluctuation Technique	Covance 2789/9 (B101063)	Parapyruvate/lactone in HCl solution showed evidence of mutagenic activity when tested in the absence of S-9 in this test system. However, following 3-hour treatment, the increase was associated only with extreme toxicity (<10% RTG), a decrease in pH of >1 unit and an increase in osmolality of >50 mOsm/kg. Furthermore, following 24 hour treatments in the absence of S-9, no marked increases in mutant frequency (exceeding the GEF) were observed at any concentration analysed, although there was a statistically significant linear trend. Overall, these observations were considered of highly questionable biological relevance. In the presence of S-9, Parapyruvate/lactone in HCl solution did not show reproducible evidence of mutagenic activity when tested up to highly toxic concentrations.

Study Title	Study No.	Results Summary
Bacterial reverse mutation test with AH111501 dissolved in ^{13}C pyruvate	TNO V5605/15 (B101014)	the test item containing [1-^{13}C] pyruvate and AH111501 was not mutagenic in *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and TA102 when tested under the conditions of this study. These conditions included treatment at amounts up to 5000 µg/plate in the absence and in the presence of a rat liver metabolic activation system (S-9).
Gene mutation test at the *t*k locus of L5178Y cells with AH111501 dissolved in ^{13}C pyruvate	TNO V5603/08 (B101015)	In the presence of S-9 the test item ([1-^{13}C] pyruvate formulation) was not mutagenic in this assay. In the absence of S-9, there was an increase in mutation frequency with the test item, but also with the vehicle and dissolution medium controls. In the cells treated with the test item this was only seen after exposure for 24 h in the absence of an S9 metabolizing system.