NCT no.: NCT04756960

STATISTICAL ANALYSIS PLAN

STUDY CODE No.: CLI-06001AA1-02

EVALUATION OF THE ABSOLUTE BIOAVAILABILITY AND MASS BALANCE OF CHF6001 FOLLOWING A SINGLE INHALED DOSE CO-ADMINISTERED WITH AN INTRAVENOUS RADIOLABELLED MICROTRACER DOSE IN HEALTHY VOLUNTEERS

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Contents

List of Abbreviations

ADR	Adverse Drug Reaction
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical classification
AUC	Area Under the Curve
$AUC_{0-\infty}$	Area Under the Curve, from 0 to infinite
AUC_{0-t}	Area Under the Curve, from 0 to the last quantifiable concentration
BMI	Body Mass Index
BLQ	Below Limit of Quantification
CI	Confidence Interval
CL	Systemic Clearance
CL_{blood}	Total body blood clearance
CL_R	Renal plasma clearance
CL_{R_blood}	Renal blood clearance
CP	Clinical Pharmacologist
C_{max}	Maximum of concentration
C_{min}	Minimum of concentration
CSR	Clinical Study Report
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
DPI	Dry-Powder Inhaler
DRM	Data Review Meeting
DRR	Data Review Report
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
Fe	Cumulative percent of dose excreted
Fr	Fraction of the relevant metabolites
HR	Heart Rate
IV	Intravenous
LLQ	Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
PK	Pharmacokinetics
PT	Preferred Term
$R_{b/p}$	Whole blood to plasma ratio
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
SOC	System Organ Class
$t_{½}$	Elimination half-life
TEAE	Treatment Emergent Adverse Event
t_{max}	Time to reach the C_{max}
t_{min}	Time to reach the C_{min}
V_{dss}	Volume of distribution at steady-state
V_z	Volume of distribution during the terminal phase

WHO	World Health Organisation
WHO-DD	World Health Organisation Drug Dictionary

VERSION HISTORY

Version	Date	Change History
1.0	*15March2022*	*First version*

1 Introduction

This document presents the Statistical Analysis Plan (SAP) for Chiesi Farmaceutici S.p.A. protocol CLI-06001AA1-02: Evaluation of the absolute bioavailability and mass balance of CHF6001 following a single inhaled dose co-administered with an intravenous radiolabelled microtracer dose in healthy volunteers.

This analysis plan is based on the final protocol (version 1.0, 19 October 2020) and the final electronic case report form (eCRF) (version 2.0, 02Mar2021).

The SAP provides the description of the final analyses. In case of deviations from the SAP, explanations will be provided in the Clinical Study Report (CSR).

[redacted] will perform the statistical analyses and is responsible for the production and quality control of all outputs described in this document.

2 Study Design

This is a phase I, open-label, uncontrolled, non-randomized, single dose, single centre study in 8 healthy male subjects designed to evaluate absolute bioavailability and mass balance of CHF6001 following a single inhaled dose co-administered with an intravenous radiolabelled microtracer dose.

The study consists of two periods (Screening and Treatment) and Follow-up call.

Screening:

At Screening visit (31 to 3 days before first study treatment administration), subjects will be selected to enter the study according to the eligibility criteria. Study related procedures will be performed upon obtaining a signed informed consent.

Treatment Period:

During treatment period, subjects will remain at the clinical site from Day -1 until the morning of Day 11 and will be administered a single dose of CHF6001 on Day 1. 4 inhalations of CHF6001 800µg/20mg NEXThaler® DPI (total dose of 3200µg) co-administered with an intravenous microdose (18.5µg and 500nCi (18.5kBq)) of [^{14}C]-labelled CHF6001 will be administered. The subjects will be discharged after the 240h post-dose assessments are completed.

Follow-up:

A follow-up phone call (or visit, if necessary) will be performed between 7 to 10 days after discharge or premature discontinuation to check and record concomitant medications and the status of any unresolved adverse events or occurrence of any new adverse events (AEs).

The end of the trial is defined at execution of the Follow-up call (or visit) of the last subject of the trial.

The study plan and scheduled tests are summarised in the following flow-chart:

Table 1: Study Plan

	Screening	Treatment Period				Follow-up
		Day -1	Day 1	Day 2-10	Day 11	
Informed consent	X					
Residential period		X	X	X		
Discharge					X	
Treatment intake						
IMP administration			X[1]			
Training						
Training with In-Check Dial	X	X	X			
Training with Placebo	X	X	X			
Subject Health Evaluation						
In/Ex Criteria	X	X				
Medical History	X					
Demographic data	X					
Height and weight	X					
Alcohol breath test	X	X				
Physical examination	X	X			X[2]	
Adverse Events recording	X	X	X	X	X	X
Restrictions	X	X	X	X	X	
Concomitant Med	X	X	X	X	X	X
Safety assessment in blood						
Clinical Chemistry	X				X[2]	
Serology	X					
Haematology	X				X[2]	
Fasting glucose	X				X[2]	
Generic assessments in urine						
Urinalysis	X					
Drug panel	X	X				
Cotinine	X	X				
Samples for PK evaluations [a]						
Blood			X	X	X	
Urine		X	X	X	X	

	Screening	Treatment Period				Follow-up
		Day -1	Day 1	Day 2-10	Day 11	
Feces		X	X	X	X	
Cardiac assessments [b]						
Local ECG	X		X			
Vital Signs - Blood Pressure	X		X			
Pulmonary Assessment						
Spirometry	X					

Footnotes for Evaluation Schedule:

1. There will be 2 types of administration:
   - 4 inhalations of CHF6001 800µg/20mg DPI NEXThaler®
   - Intravenous infusion for 15min of the tracer starting 1h45min after the inhaled dose (time of the first inhalation) and ending at the expected T_{max} of 2 h for the inhaled dose
2. A full physical examination and collection of blood safety test will be performed at discharge. A follow-up visit will be done only if deemed necessary by the principal investigator.

a) Pharmacokinetic samples will be collected as:

- **Blood samples** for plasma non-radiolabeled CHF6001 to be collected at pre-dose (within 60 min from inhaled dosing) and at the following timepoints post inhaled dose**: 15, 30, 60, 90 min, 2, 3.75, 5.75, 7.75, 9.75, 11.75, 13.75, 25.75, 49.75, 73.75, 97.75, 121.75, 145.75, 169.75, 193.75, 217.75 and 241.75 hours.**
- **Blood samples** for plasma [^{14}C]-total, [^{14}C]-CHF6001, [^{14}C] of the relevant metabolites to be collected at pre-dose (within 60 min from inhaled dosing) and at the following timepoints relative to the start of the IV infusion: **5, 10, 15 [end of infusion], 20, 25, 30, 45, 60 min, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours.**
- **Blood samples** for whole blood [^{14}C]-total and [^{14}C]-CHF6001 at pre-dose (within 60 min from inhaled dosing) and **20 min after start of infusion**
- **Urine samples** for assessments of [^{14}C]-total, [^{14}C]-CHF6001 and [^{14}C] of the relevant metabolites to be collected at pre-dose from -12 hours to immediately before study drug inhaled administration (-12-0h) and at the following time frames relative to the start of the IV infusion: **0-4h, 4-8h, 8-12h, 12-24h, 24-48h, 48-72h, 72-96h, 96-120h, 120-144h, 144-168h, 168-192h, 192-216h and 216-240h**.
- **Fecal samples** for assessments of [^{14}C]-total, [^{14}C]-CHF6001 and [^{14}C] of the relevant metabolites to be collected at pre-dose from check-in (Day -1) to immediately before study drug inhaled administration (check-in -0h) and at the following time frames relative to the start of the IV infusion: **0-24h, 24-48h, 48-72h, 72-96h, 96-120h, 120-144h, 144-168h, 168-192h, 192-216h and 216-240h**.

b) Cardiac assessments will be performed at Screening and on Day 1 the treatment period as:

- **Local 12-lead ECG:** A triplicate ECG will be done for assessing eligibility at screening and a single ECG will be performed for general safety at predose and at 2,5 hours post dose (inhaled administration).
- **Vital Signs - BP**: BP measurement will be performed in triplicate at the following time points: at Screening and, for general safety, at predose and at 2,5 hours post dose (inhaled administration).

3 Study Objectives

The objectives of this study are:

- To determine the absolute bioavailability of CHF6001 following single inhaled dose co-administered with an IV microtracer dose
- To characterize the mass balance and routes of elimination of CHF6001 after an intravenous (IV) microtracer dose
- To characterize the relevant metabolites in plasma, urine and feces
- To assess the safety and tolerability of the study treatment

4 Study Variables

4.1 Pharmacokinetic Variables

Following are the pharmacokinetic (PK) variables of this study:

- Plasma [^{14}C]-CHF6001, $AUC_{0\text{-}t\ iv}$, C_{max_iv}, t_{max_iv}, $AUC_{0\text{-}\infty\_iv}$, $t_{1/2\ iv}$, V_z, Vd_{ss} and CL
- Plasma [^{14}C]-total $AUC_{0\text{-}t\ iv}$, C_{max_iv}, t_{max_iv}, $AUC_{0\text{-}\infty\_iv}$, $t_{1/2\ iv}$
- Plasma CHF6001 $AUC_{(0\text{-}t)\_inh}$, C_{max_inh}, t_{max_inh}, $AUC_{(0\text{-}\infty)\_inh}$, $t_{1/2\_inh}$, F_{inh}
- Urine and feces [^{14}C]-CHF6001and [^{14}C]-total excreted fraction (F_{eu}, F_{ef} and F_{eu+f})
- Systemic blood clearance [^{14}C]-CHF6001 CL_{blood}, renal plasma and blood clearance CL_R and CL_{R_blood}
- Blood to plasma ratio ($R_{b/p}$) [^{14}C]-CHF6001
- Blood to plasma ratio ($R_{bt/pt}$) [^{14}C]-total
- Metabolized fraction (Fe_{met})
- Hepatic extraction [^{14}C]-CHF6001 (E_{h_blood})

Characterization of the relevant metabolites

For metabolites in plasma accounting for ≥10% of total radioactivity recovered in this matrix, structural characterization may be performed, where possible. For metabolites in urine and feces (total excreta) accounting for ≥10% of total administered radioactivity, structural characterization may be performed, where possible:

- Fraction of the relevant metabolites in plasma (Fr_plasma), fraction of the relevant metabolites recovered in urine and feces (Fr_urine, Fr_feces, Fr_urine+feces)

Where possible, these data will be reported in a separate report together with structural characterization.

4.2 Safety Variables

Following are the safety variables for this study:

- AEs and ADRs
- Vital signs parameters (SBP and DBP)
- HR from 12-lead electrocardiogram (ECG)
- Laboratory parameters (haematology, chemistry and fasting glucose)

4.3 Other Variables

No other variables defined.

5 Sample Size

According to the exploratory nature of the study, no formal sample size calculation was performed. A total of 8 subjects will be included in the study to characterize the absolute bioavailability and the mass balance of CHF6001.

6 Analysis Sets

The definitions of the analysis sets are summarised below. A final agreement on the subjects be included in or excluded from each analysis set will be reached during the Data Review Meeting (DRM) before the database lock. Inclusions and exclusions from analysis sets will be fully documented in the Data Review Report (DRR).

6.1 Safety Population

Safety Population is defined as all enrolled subjects who start study treatment inhalation.

The Safety population will be used in the analysis of all safety variables.

6.2 Pharmacokinetic (PK) Population

PK population consists of all subjects from the safety population excluding subjects without any valid PK measurement or with major protocol deviations significantly affecting PK, for example: incorrect inhalation, change in subject condition (cold), failure in delivery of the device, use of non-permitted medications.

PK variables will be analysed in the PK population.

6.3 Other Sets Defined for Tables and Listings

For the purposes of tables and listings the following set is defined:

- Enrolled Set: all subjects who provided informed consent for the study.

7 General Considerations for Statistical Analysis

7.1 Statistical Significance

There is no hypothesis testing or confidence interval (CI) calculation performed hence statistical significance is not applicable.

7.2 Multiplicity

Since there is no hypothesis testing involved, multiplicity adjustment is not applicable.

7.3 Handling of Missing Data

The number of subjects with missing data will be presented under a “Missing” category. Unless otherwise stated, missing values will not be included in the denominator count when calculating percentages.

When quantitative variables are being summarised, only the non-missing values will be evaluated for calculating summary statistics.

7.3.1 Missing/Incomplete Dates:

Medications:

In case of missing or incomplete dates not directly allowing allocation to any category of medications, a worst-case allocation will be done according to the available parts of the start and the stop dates. The medications will be allocated to the first category allowed by the available data, according to the following order:

- Concomitant medication;
- Post-Study medication;
- Prior medication.

Procedures:

In case of missing or incomplete dates not directly allowing allocation to any category of procedure, a worst-case allocation will be done according to the available parts of the start and the stop dates. The procedure will be allocated to the first category allowed by the available data, according to the following order:

- Concomitant procedure;
- Post-Study procedure
- Prior procedure.

Adverse events:

In case of missing or incomplete date/time not directly allowing allocation to any of the category of AEs, a worst-case allocation will be done according to the available parts of the start and the stop dates/times. The AE will be allocated to the first category allowed by the available data, according to the following order:

- Treatment emergent;
- Post-study;
- Pre-treatment.

Missing severity of adverse events:

In case of missing severity, the severity will not be imputed and will be reported as “Missing”.

Other critical missing data, if any, will be discussed during the review of the data. Decisions will be fully documented in the DRR.

7.4 Covariates

Not applicable.

7.5 Interim Analyses

No interim analysis will be performed.

7.6 Examinations of Subgroups

No subgroup analysis will be performed.

7.7 Descriptive Statistics

PK variables (except for t_{max}, t_{max_inh} and t_{max_iv}) will be summarized by means of descriptive statistics including n (number of observed values), arithmetic mean, standard deviation (SD), coefficient of variation (CV), geometric mean, geometric CV, median, minimum and maximum. t_{max}, t_{max_inh}, t_{max_iv} will be summarized by using n, median, minimum and maximum.

Descriptive statistics for quantitative variables will include n (the number of non-missing values), mean, standard deviation (SD), median, minimum and maximum values.

Categorical variables will be summarised by using frequency count and percent distributions.

7.8 Definitions

7.8.1 Baseline and Change from Baseline

Baseline values are defined as the last evaluation before the first treatment dose (Inhalation).

For each of the above variable, change from baseline is defined at each post-dose as:

Post-dose value – baseline value.

7.8.2 Study Day

The study day relative to the date of study medication intake will be calculated as:

- date of event – date of first study drug inhalation + 1 (if date of event ≥ date of first study drug inhalation);

or

- date of event – date of first study drug inhalation (if date of event < date of first study drug inhalation).

7.9 Exclusion of Data from the Statistical Analyses

7.9.1 Exclusion of Data from All Statistical Analyses

There will not be any exclusions of the collected data from the statistical analyses.

7.9.2 Exclusion of Data from PK Analyses

Subjects may be excluded from the PK population and hence from the PK analysis if they fall in the cases as defined in the European Medicines Agency (EMA) "Guideline on the Investigation of Bioequivalence".

All data will be reviewed during the DRM in order to make decisions of exclusion from PK analyses and documented in the DRR.

If any data or subject is excluded from the tables or figures, a footnote will be added to notify the exclusions.

7.10 Listings

All data collected in the eCRF will be presented in the listings.

7.11 Coding

Medical and surgical history, concomitant diseases, procedures and adverse events will be coded according to Medical Dictionary for Regulatory Activities (MedDRA) version 24.0

Medications will be coded using the World Health Organization Drug Dictionary (WHO-DD) WHO Global B3 format Version -March 2021.

8 Study Population

8.1 Disposition of Subjects and Discontinuations

8.1.1 Disposition of Subjects

The number of subjects treated, who completed treatment period and who completed the follow-up will be summarized. All subjects will be included.

8.1.2 Discontinuation from the Study

The number and percentage of subjects who completed the study, withdrew from the study and the number and percentage of subjects with each reason for withdrawal from the study and from the treatment period will be presented on Enrolled Set.

8.1.3 Protocol Deviations and Analysis Sets

Deviations will be classified according to the following categories:

- Violation of Inclusion Criterion;
- Violation of Exclusion Criterion;
- Treatment Administration Deviation;
- Non-Permitted Medication;
- Assessment Performed Outside the Allowed Time Window;
- Visit Performed Outside the Allowed Time Window;
- Study Procedure Deviation;

Major and minor protocol deviations will be summarised using the Safety Set.

The number of subjects included in the Safety and PK Sets will be summarised.

All deviations, including significant GCP deviation, will be listed.

8.2 Demographic and Baseline Characteristics

8.2.1 Demographic Characteristics

Demographic characteristics will be summarised. These will include age (years) and race.

8.2.2 Physical Characteristics

Height (cm), weight (kg) and body mass index (BMI, kg/m^2) will be summarized.

Note:

- BMI (kg/m^2) will be calculated as: weight at the visit (kg) / height $(m)^2$ at Visit 1.

8.2.3 Alcohol Breath Test

Results of alcohol breath test will only be listed.

8.2.4 Spirometry at Screening

Spirometry results at Screening will only be listed.

8.3 Medical History and Concomitant Diseases

Medical/surgical history and concomitant diseases will be summarised by MedDRA system organ class (SOC) and preferred term (PT) using Safety Set.

Notes:

- medical/surgical history is defined as conditions in the medical/surgical history and concomitant diseases eCRF form which are not ongoing at Day 1;
- concomitant diseases are defined as conditions in the medical/surgical history and concomitant diseases eCRF form which are ongoing at Day 1.

8.4 Medications

Previous medications and concomitant medications will be summarised for the Safety Set through frequency distributions and percentages by Anatomical Main Group (1st level of the anatomical therapeutic chemical (ATC) classification), Therapeutic Subgroup (2nd level of the ATC classification), Chemical Subgroup (4th level of the ATC classification) and preferred name. Subjects with at least one prior/concomitant medications will also be summarized. Post-study medications will only be presented in a listing.

The medications will be classified according to the following rules:

- previous medication: medication start date < date of first study drug inhalation and stop medication date ≤ date of first study drug inhalation;
- concomitant medication: date of first study drug inhalation ≤ medication end date;
- post-study medication: medication start date ≥ date of discharge.

8.5 Procedures

Previous procedures and concomitant procedures will be summarised for the Safety Set through frequency distributions and percentages by MedDRA SOC and PT.

The procedures will be classified according to the following rules:

- previous procedures: procedure start date < date of first study drug inhalation and end procedure date ≤ date of first study drug inhalation;

- concomitant procedures: date of first study drug inhalation ≤ procedure end date;
- post-study procedures: procedure start date ≥ date of discharge.

8.6 Compliance

Treatment Compliance

In general, treatment compliance will be evaluated on the basis of the information recorded in the eCRF.
Number of correct and incorrect inhalations, number of interruptions during the infusion will be summarized using descriptive statistics on Safety population.
In addition, all variables related to the inhalation and to the IV infusion will be listed.

9 Pharmacokinetics Analyses

9.1 Pharmacokinetic Analysis

The concentration ratios of whole blood [^{14}C]-total and [^{14}C]-CHF6001 radioactivity to plasma [^{14}C]-total and [^{14}C]-CHF6001 radioactivity, individual and cumulative mass balance of [^{14}C]-total radioactivity (amount (Ae_u, cumulative Ae_u, Ae_f and cumulative Ae_u) and percentage of dose (fe_u, Fe_u, fe_f and Fe_f) recovered as [^{14}C]-total radioactivity in urine, feces and total excreta) will be calculated by radioanalysis laboratory ([redacted]).

In addition, metabolites in plasma accounting for ≥10% of total radioactivity recovered in this matrix, structural characterization may be performed, where possible. For metabolites in urine and feces (total excreta) accounting for ≥10% of total administered radioactivity, structural characterization may be performed, where possible.
If available, fraction of the relevant metabolites in plasma (Fr_plasma), fraction of the relevant metabolites recovered in urine and feces (Fr_urine, Fr_feces, Fr_urine+feces) will be reported in a separate report together with structural characterization performed by radioanalysis laboratory ([redacted]).

Urine and Feces [^{14}C]-CHF6001 and [^{14}C]-total radioactivity

Parameter	Units[a]	Definition
Ae_u	mg	amount excreted in urine (expressed in weight) over sampling interval
Cumulative Ae_u	mg	cumulative amount excreted in urine, calculated as the sum of the amount excreted in urine for each collection period
fe_u	%	percent of the dose excreted in urine over a sampling interval
Fe_u	%	cumulative percent of dose excreted in urine, calculated as the sum of the percent of dose excreted in urine for each collection period
Ae_f	mg	amount excreted in the feces over sampling interval
Cumulative Ae_f	mg	cumulative amount excreted in feces, calculated as the sum of the amount excreted in feces for each collection period
fe_f	%	percent of the dose excreted in feces over a sampling interval

Fe_f	%	cumulative percent of dose excreted in feces, calculated as the sum of the percent of dose excreted in feces for each collection period
CL_R	L/h	renal plasma clearance (urine [^{14}C]-CHF6001 only)
CL_{R_blood}	L/h	renal blood clearance where $CL_{R\_blood} = CL_R/R_{b/p}$ (urine [^{14}C]-CHF6001 only)
$E_{h\ blood}$		hepatic extraction calculated from intravenous systemic and renal blood clearance ($CL_{blood} = CL/R_{b/p}$) and $CL_{R\_blood} = CL_R/R_{b/p}$, respectively and human hepatic blood flow (Q_H; assumed to be 87 l/h). $E_{h\ blood} = (CL_{blood} - CL_{R\_blood})/ Q_H$
Cumulative Ae_{u+f}	mg	cumulative amount excreted in urine and feces, calculated as Cumulative Ae_u + Cumulative Ae_f
Fe_{tot}	%	cumulative amount excreted in urine and feces, calculated as $Fe_u + Fe_f$
Fe_{met}		metabolized fraction calculated as Fe_{met} = [cumulative Ae_{u+f} (^{14}C-total) – cumulative Ae_{u+f} ([^{14}C]-CHF6001)]/ cumulative Ae_{u+f} (^{14}C-total)

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.

The following PK parameters will be determined by [redacted] where possible from the plasma concentrations of CHF6001 following inhaled administration using noncompartmental methods in validated software program Phoenix WinNonlin (Phoenix WNL, Certara, Version 8.1 or higher):

Parameter	**Units[a]**	**Definition**
$AUC_{(0-t)\_inh}$	h*xg/mL	area under the concentration-time curve from 0 to the last quantifiable concentration (t_{last})[b]
$AUC_{(0-\infty)\_inh}$	h*xg/mL	area under the concentration-time curve extrapolated to infinity[c]
$\%AUC_{extrap}$	%	percentage of AUC due to extrapolation from the last quantifiable concentration to infinity
C_{max_inh}	xg/mL	the value of the maximum observed concentration
t_{max_inh}	h	the time of the maximum observed concentration
t_{last_inh}	h	the time of the last quantifiable concentration
$t_{1/2\_inh}$	h	terminal half-life
F_{inh}		inhaled absolute bioavailability based on $AUC_{(0-\infty)}$

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
[b] Area under the concentration-time curve will be calculated using the linear trapezoidal rule with linear interpolation
[c] Based on the last observed quantifiable concentration

The following PK parameters will be determined where possible from the plasma, whole blood, urine and feces [^{14}C]-CHF6001 concentrations, plasma whole blood, urine and feces [^{14}C]-total concentrations following intravenous (IV) infusion administration of [^{14}C]-CHF6001 in each subject using noncompartmental methods in validated software program Phoenix WNL (Certara, Version 8.1 or higher):

Plasma [^{14}C]-CHF6001 and [^{14}-C]-total radioactivity

	Parameter	Units[a]	Definition
[^{14}C]- CHF6001 and [^{14}C]- Total	$AUC_{(0-t)\_iv}$	h*xg/mL	area under the concentration-time curve from 0 to the last quantifiable concentration (t_{last})[b]
[^{14}C]- CHF6001 and [^{14}C]- Total	$AUC_{(0-\infty)\_iv}$	h*xg/mL	area under the concentration-time curve extrapolated to infinity[c]
[^{14}C]- CHF6001 and [^{14}C]- Total	$\%AUC_{extrap}$	%	percentage of AUC due to extrapolation from the last quantifiable concentration to infinity
[^{14}C]- CHF6001 and [^{14}C]- Total	C_{max_iv}	xg/mL	the value of the maximum observed concentration
[^{14}C]- CHF6001 and [^{14}C]- Total	t_{max_iv}	h	the time of the maximum observed concentration
	t_{last_iv}	h	the time of the last quantifiable concentration
[^{14}C]- CHF6001 and [14-C]-Total	$t_{1/2\_iv}$	h	terminal half-life
[^{14}C]- CHF6001	CL	L/h	total body plasma clearance (plasma [^{14}C]-CHF6001)
[^{14}C]- CHF6001	V_z	L	volume of distribution during the terminal phase (plasma [^{14}C]-CHF6001)
[^{14}C]- CHF6001	V_{dss}	L	volume of distribution at steady state (plasma [^{14}C]-CHF6001)
	Plasma concentration of [^{14}C]-CHF6001/Total Radioactivity Ratio	NA	ratio of plasma concentration of [^{14}C]-CHF6001 to plasma concentration of [^{14}C]-total
	$AUC_{(0-\infty)}$ Plasma [^{14}C]-CHF6001/Total Radioactivity Ratio	NA	$AUC_{(0-\infty)}$ of plasma [^{14}C]-CHF6001 relative to $AUC_{(0-\infty)}$ of plasma [^{14}C]-total radioactivity
	C_{max} Plasma [^{14}C]-CHF6001/Total Radioactivity Ratio	NA	C_{max_iv} of plasma [^{14}C]-CHF6001 relative to C_{max_iv} of plasma [^{14}C]-total radioactivity

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study. For total radioactivity, C_{max} and AUC units will be presented as mass equivalents (ng Eq/g and h*ng Eq/g, respectively).
[b] Area under the concentration-time curve will be calculated using the linear trapezoidal rule with linear interpolation
[c] Based on the last observed quantifiable concentration

Blood [^{14}C]-CHF6001 and [^{14}-C]-total radioactivity

	Parameter	Units	Definition
[^{14}C]-CHF6001	$R_{b/p}$	NA	whole blood to plasma ratio is to be determined on the [^{14}C]-CHF6001 whole blood and plasma concentration (C_b and C_p) 20 minutes after the start of infusion, calculated as $R_{b/p} = C_b/C_p$
[^{14}C]-total	$R_{bt/pt}$	NA	whole blood to plasma ratio is to be determined on the total-[^{14}C] whole blood and plasma concentration (C_{bt} and C_{pt}) 20 minutes after the start of infusion, calculated as $R_{bt/pt} = C_{bt}/C_{pt}$

Additional PK parameters may be determined where appropriate.

Concentration ratios of plasma [^{14}C]-CHF6001 to plasma [^{14}C]-total radioactivity will be listed by time point and summarized.

Pharmacokinetic analysis will be carried out using actual dose administered (mg) and actual blood sampling times post-dose. If an actual time is missing, the sample concentration result will be treated as missing unless there is scientific justification to include the result using the nominal time.

The parameters C_{max_inh}, C_{max_iv}, t_{last_inh}, t_{last_iv}, t_{max_inh} and t_{max_iv} will be obtained directly from the concentration-time profiles. If C_{max} occurs at more than 1 timepoint, t_{max} will be assigned to the first occurrence of C_{max}.

Inhaled absolute bioavailability (F_{inh}) based on $AUC_{(0-\infty)}$ will be calculated as follows:

$F_{inh} = (AUC_{(0-\infty)\_inh} \times Dose_{iv}) / (AUC_{(0-\infty)\_iv} \times Dose_{inh})$.

The parameter $AUC_{(0-t)}$ or other common partial area may be used to determine F_{inh}, metabolite and total radioactivity ratios if $AUC_{(0-\infty)}$ cannot be reliably calculated for the majority of subjects.

9.2 Criteria for the Calculation of Apparent Terminal Elimination Rate Constant and Half-life

The start of the terminal elimination phase for each subject will be defined by visual inspection and generally will be the first point at which there is no systematic deviation from the log-linear decline in concentrations.

The apparent terminal elimination rate constant (λ_z) will only be calculated when a reliable estimate can be obtained using at least 3 data points, preferably not including C_{max}, and the adjusted coefficient for determination of exponential fit (R^2-adj) of the regression line is ≥0.7.Parameters requiring λ_z for their calculation (e.g., $AUC_{(0-\infty)}$, $t_{1/2}$, CL, V_z and V_{dss}) will only be calculated if the R^2-adj value of the regression line is ≥0.7. If this rule of r²adj ≥0.7 is not respected, this should be well described in the Data Review Report (DRR).

The following regression-related diagnostic PK parameters will be determined, when possible:

Parameter	Units	Definition
λ_z	1/h	apparent terminal elimination rate constant
λ_z Upper	h	end of exponential fit
λ_z Lower	h	start of exponential fit
λ_z N		number of data points included in the log-linear regression
λ_z Span Ratio		time period over which λ_z was determined as a ratio of $t_{1/2}$
R^2-adj		adjusted coefficient for determination of exponential fit

Where possible, the span of time used in the determination of λ_z (i.e., the difference between λ_z Upper and λ_z Lower) should be ≥2 half-lives. If the λ_z Span Ratio is <2, the robustness of the $t_{1/2}$ values will be discussed in the Data Review Report (DRR).

9.3 Criteria for Calculation and Reporting of Area Under the Concentration-time Curve

The minimum requirement for the calculation of area under the concentration-time curve (AUC) will be the inclusion of at least 3 consecutive concentrations above the lower limit of quantification. If there are only 3 consecutive concentrations, at least 1 should follow C_{max}. An exception may be made for metabolites, where C_{max} may be the last timepoint.

If the extrapolated area is >20%, $AUC_{(0-\infty)}$ (and derived parameter i.e. Plasma [14C] - CHF6001) / Total Radioactivity Ratio) may be excluded from the summary statistics at the discretion of the sponsor or pharmacokineticist and should be well described in the Data Review Report (DRR).

9.4 Calculation of [^{14}C]-CHF6001 and [^{14}C]-total radioactivity Urine Parameters

The amount of the dose administered recovered (Ae_u) in urine as [^{14}C]-CHF6001 for each urine collection interval (t_1-t_2) will be calculated as the product of urine concentration and urine volume. Where only urine sample weight is supplied, a specific gravity of 1 g/mL will be assumed, and it will be considered equivalent to urine volume. A total cumulative $Ae_{u\ 0\text{-}x\ h}$ will be calculated by summing across collection intervals over the 0-x h interval, where x = end of the last collection time interval.

The percentage of the dose administered recovered in urine over the time interval t1 to t2 ($fe_{u\ t1\text{-}t2}$) as [^{14}C]-CHF6001 will be calculated for each urine collection interval as follows:

$$fe_{u\ t1\text{-}t2} = (Ae_{u\ t1\text{-}t2} / dose_{IV}) \times 100$$

Cumulative fe_u (Fe_u) will be calculated by summing the $fe_{u\ t1\text{-}t2}$ values across collection intervals in the same manner as cumulative Ae_u.

Renal plasma clearance (CL_R) will be calculated according to the following formula:

$$CL_R = \text{cumulative } Ae_u / AUC_{(0-\infty)\_iv}$$

Alternatively, $AUC_{(0-t)}$ may be used if $AUC_{(0-\infty)}$ cannot be reliably calculated following the formula cumulative $Ae_{(0\,t)}$ / $AUC_{(0-t)}$, where 't' is the longest common interval with quantifiable concentrations in plasma and urine.

The fraction of the relevant metabolites recovered in urine calculated as amount recovered (Fr_Urine) will be calculated as follows:

$Fr\_Urine = (Ar_u / Dose_{IV}) \times 100$

9.5 Calculation of [^{14}C]-CHF6001 and [^{14}C]-total radioactivity Feces Parameters

The amount of the dose administered recovered (Ae_f) in feces as [^{14}C]-CHF6001 for each faecal collection interval (t_1-t_2) will be calculated as concentration in faecal collected sample and faecal collected sample weight. A total cumulative $Ae_{f\ 0\text{-}x\ h}$ will be calculated by summing across collection intervals over the 0-x h interval, where x = end of the last collection time interval.

The percentage of the dose administered recovered in feces over the time interval t1 to t2 ($fe_{f\,t1\text{-}t2}$) as [^{14}C]-CHF6001 will be calculated for each faecal collection interval as follows:

$fe_{f\,t1\text{-}t2} = (Ae_{f\,t1\text{-}t2} / Dose_{IV}) \times 100$

Cumulative fe_f (Fe_f) will be calculated by summing the $fe_{f\,t1\text{-}t2}$ values across collection intervals in the same manner as cumulative Ae_f.

The fraction of the relevant metabolites recovered in feces calculated as amount recovered (Fr_feces) will be calculated as follows:

$Fr\_feces = (Ar_f / Dose_{IV}) \times 100$

9.6 Criteria for Handling Below the Limit of Quantification or Missing Concentrations for Pharmacokinetic Analysis

Plasma and whole blood concentrations below the limit of quantification will be reported as BLQ in the concentration and parameters tables. All BLQ values will be considered as 0.00 for pharmacokinetic and descriptive statistical analyses.

All concentration data-points with time deviations outside the permitted ranges (see Table in section 7.2.1.1 and 7.2.1.2 of study protocol) will be excluded from the descriptive statistics on plasma, whole blood and urine concentrations, explained by a footnote in the appropriate tables and figures, but kept in PK parameters estimation unless very large deviations not allowing for a proper calculation of PK parameters are found: these cases will be discussed during the Data Review Meeting and decision will be fully documented in the DRR.

General rules to handle missing PK data are detailed below, but a case by case evaluation will be performed during the DRM in order to properly handle every specific situation (e.g., missing values at time points close to expected t_{max}). Decisions will be fully documented in the DRR.

Rules on how to deal with missing values when deriving PK AUC:

- If intermediate values (not the pre-dose, and not more than 3 consecutive) are missing, they will be considered as missing for the PK analysis and automatically imputed by linear interpolation by Phoenix WNL software.

- If multiple (>1) adjacent values are missing or more than 3 values are missing, then the AUCs will be reported, flagged in the result tables and excluded from the statistical analyses. However, such situations will be discussed case by case at the discretion of the clinical pharmacologist (CP) during the review of the data and fully documented in the DRR.

- If the predose value is missing, then the predose value should be set to "0" for the estimation of the PK parameters. In case of missing predose sample, the Phoenix WNL software will automatically use the value of 0 for the estimation of the PK parameters;

- For an estimation of the urine and stool PK parameters, missing predose value do not affect the final parameter estimates because they are not used in the calculation of Ae.

The following rules apply with special situations defined below:

- If an entire concentration-time profile is BLQ, it will be excluded from PK analysis.

- Where 2 or more consecutive concentrations are BLQ at the end of a profile, the profile will be deemed to have terminated and any further quantifiable concentrations will be set to missing for the calculation of the PK parameters, unless they are considered to be a true characteristic of the profile of the drug.

- If a predose plasma and whole blood concentration is missing, it will be set to 0 by default within Phoenix WNL.

Urine and feces concentrations that are missing or BLQ will be set to zero for the calculation of $Ae_{u\ t1\text{-}t2}$ and $Ae_{f\ t1\text{-}t2}$.

9.7 Treatment of Outliers in Pharmacokinetic Analysis

If a value is considered to be anomalous due to being inconsistent with the expected PK profile, it may be appropriate to exclude the value from the PK analysis. However, the exclusion of any data be fully documented in the DRR and discussed in the CSR.

Any quantifiable predose concentration value will be considered anomalous and set to missing for the PK analysis. This will be set to 0 by default within Phoenix WNL.

9.8 Presentation of Pharmacokinetic Data

For plasma whole blood and urine concentration data, the following rules will apply:

- Values that are BLQ will be set to 0 for the calculation of summary statistics. In listings BLQ values will be reported as "<LLQ" where LLQ will be replaced with the value for the lower limit of quantification

- Arithmetic mean or median values that are BLQ will be presented as 0.

- If any BLQ results (treated as 0) are in a series of summarized data, geometric mean and CV% of geometric mean will be reported as not calculated (NC).

For PK parameters the following rule will apply:

- Geometric mean and coefficient of variation will not be calculated for t_{last} and t_{max}.

Concentration (mass equivalent in mL or mass in mL)/time curves for plasma total [^{14}C], [^{14}C]-CHF6001 and non-radiolabelled CHF6001 will be presented in linear/linear and log/linear scale. Plots will be presented based on arithmetic means and by subject i.e. all individual plasma concentration/time curves will be presented in one graph.

Arithmetic means (+ SD) showing [^{14}C]-total recovery (Fe_f, Fe_u, Fe_tot in the same graph) and 14C-CHF6001 recovery (Fe_f, Fe_u, Fe_tot in the same graph) over the time will be presented using line charts.

All individual concentration data (mass equivalent per mL or mass per mL) and PK parameters will be listed.

In addition, a listing of the actual sampling times relative to drug inhalation will be provided. Concentrations will be summarized by scheduled sampling time by using n, arithmetic mean, SD, CV, median, minimum and maximum.
For each applicable listing, appropriate flag will be added in case of a concentration reported as ND (i.e., not done) or NS (i.e., no sample) or of value BLQ or for PK parameters not estimated / Not calculated.

10 Safety Analyses

10.1 Extent of Exposure

Not applicable since this is a single dose study.

10.2 Adverse Events

An AE will be classified as pre-treatment AE if it starts after the informed consent signature and before the time of first inhalation of the study drug (date of informed consent < AE onset date and AE onset date-time < date-time of first study drug inhalation).

All adverse events starting on or after the time of first study drug inhalation will be classified as treatment emergent adverse event (TEAE) (date-time of first study drug inhalation ≤ AE onset date-time ≤ date of discharge).

An AE will be classified as a post-study AE if it starts after the date of discharge (AE onset date > date of discharge).

An adverse drug reaction (ADR) is an AE judged as related to the study medication.

A serious ADR is a serious AE (SAE) judged as related to the study medication.

A severe AE is an AE with severe intensity.

An AE leading to discontinuation will be identified based on the information collected in study termination form (i.e. reason for discontinuation =Adverse event).

An AE leading to death is an AE with outcome equal to “Fatal”.

Pre-treatment AEs, TEAEs and post-study AEs will be presented separately. Pre-treatment AEs and post-study AEs will be presented in the listings only.

The number of TEAEs, drug-related TEAE, serious TEAE and TEAE leading to study discontinuation and the number and the percentage of subjects experiencing TEAEs, drug-related TEAEs, serious TEAEs and TEAEs leading to study discontinuation will be summarised.

The MedDRA SOC and PT will also be used for tabulation. The number and percentage of subjects with at least one AE and the number of AEs will be presented by SOC and PT for the above categories of AEs.

Derived variables presented in the listings:

The relative day of AE onset will be calculated as follows:

- for pre-treatment AEs:
    - AE onset date - date of first inhalation of study drug (if AE onset date is completely known);
    - missing (if AE onset date is incomplete or unknown).
- For TEAEs and post-Study AEs:
    - AE onset date – date-time of first inhalation of study drug +1 (if AE onset date is completely known);
    - missing (if AE onset date is incomplete or unknown).

The duration of an AE (hours) will be calculated as follows:

- (AE end date-time – AE onset date-time) /3600 (when both date-time are completely known)

The duration of an AE (days) will be calculated as follows:

If duration in hours ≥ 24 hours:

- AE end date – AE onset date +1 (when both dates are completely known);
- date of completion/discontinuation – AE onset date + 1 (when the AE onset date is fully known but the AE is not resolved at the end of the trial): in this case the duration will be presented as ">x days" in the listing rather than "x days";
- missing (when the AE onset date is incomplete or unknown, or when the AE has resolved but with an incomplete or unknown end date, or when the AE onset date is > date of completion/discontinuation and the AE is not resolved).

If duration in hours <24 hours:

- duration (hours)/24

Duration will be displayed in days with values <1 days in case of short term AE.

10.3 Vital Signs

For the purpose of safety analyses, the mean of triplicate measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP) will be used. The mean SBP and DBP values at baseline and at 2.5 hours post- dose will be summarized using descriptive statistics. Change from baseline to 2.5 hours post-dose in mean SBP and DBP will also be summarized.

Number and percentage of subjects with clinically relevant changes from baseline to 2.5 hours post-dose in SBP and DBP will be summarized. Clinically relevant changes are defined as:

- Change from baseline in SBP > 10 mmHg
- Change from baseline in DBP > 20 mmHg

10.4 12-lead Safety Electrocardiogram

Triplicate ECG measurements will be averaged on available values and the average value will be used to calculate summary statistics for heart rate (HR), PR interval (PR), QRS interval (QRS) and QTcF interval (QTcF)..

Baseline value (i.e. pre dose value) and 2.5 hours post dose will be summarized.

Number and percentage of subjects with clinically relevant change in HR at 2.5 hours post-dose defined as change from baseline > 20 bpm, will also be summarized.

The number and the percentage of subjects with QTcF abnormalities defined as:

- QTcF >450 ms,
- QTcF>480 ms
- QTcF>500 ms;
- change from baseline to 2.5 Hours post dose in QTcF >30 ms
- change from baseline to 2.5 Hours post dose in QTcF >60 ms;

will be summarized.

10.5 Laboratory Data

Quantitative chemistry and haematology parameters and fasting glucose values will be summarized descriptively at screening and discharge. Shift tables from screening with reference to normal ranges will be presented.

Serology and urinalysis results will only be listed.

Abnormal laboratory data will be flagged in the listings.

11 Other Analyses

No other analyses planned.

12 Changes in the Planned Analyses from Study Protocol

There are no changes to the protocol defined analysis.

13 Output

13.1 Software

SAS version 9.4 will be used to perform all the statistical analyses. PK analyses will be carried out using WNL Phoenix 8.1 or higher.

13.2 Reporting Conventions

13.2.1 Treatment, Visit and Subgroup Descriptors

In the tables, listings and figures, the treatments and the visits will be identified as described below.

Treatment group	Descriptor for treatment
CHF6001 800 µg/ 20 µg, 4 inhalations + [^{14}C] CHF6001 IV Infusion 18.5 µg	CHF6001

Output	Descriptor for visits
Tables	Screening, Day -1, Day 1, Day 2–10, Day 11, Follow-up
Listings	Same as above.
Figures	Same as 'Tables'.

13.2.2 Decimal places

Quantitative variables except PK variables will be listed with the same number of decimal places as in the actual data.

In case of PK variables, plasma concentrations as well as calculated PK parameters will be presented with 3 decimal points except for t_{max}, t_{max_iv}, and t_{max_iv} which will be presented with 2 decimals. Values ≥1000 will be presented without the decimals and the whole number will be reported without roundings (i.e. 1237 is not rounded to 1240 and 12322 is not rounded to 12300).

The following rules on decimal places will be considered in the listings for the derived variables (in the analyses rounding will not be performed):

- BMI: whole numbers;
- average of triplicate measurements (ECG and Blood Pressure): same as the actual variable
- change from baseline: same as the variable considered.
- AE duration (days): whole number if duration (hours)≥24 and 2 decimals in case of AE duration (hours)<24 hours

The following rules on decimal places will be considered for the results of the analyses (if the analyses are performed on derived variables, the level of precision of the actual data is derived from the previous list):

- min, max: same as actual data;
- mean, SD, median, geometric mean: actual data + 1 decimal place;
- percentage, geometric CV%, CV%: 1 decimal place;

13.2.3 Other reporting conventions

Unless otherwise stated, listings will be sorted by Subject ID.

In a listing, in the case that a subject's record has been continued to the next page, an appropriate identification (e.g., the subject ID number) must be presented at the beginning of that page.

In general, dates will be presented on listings in the format ddmmmyyyy (date9.) and time in the format hh:mm (time5.). In case of partial dates or times, missing information will be replaced by dashes.

13.3 Format

In the top left portion of each table/listing, a table/listing number followed by the title of the table/listing will be presented. After the title line, optional sub-title or analysis set information can be presented. Horizontal lines will appear before and after the column heading of the table/listing. Footnotes will be put under the main body of text at the bottom of the page.

The sponsor name, protocol number, programmer's User ID, status of the table/listing (i.e. draft or final) and SAS program name will appear bottom left in a string and the page number will appear on the bottom right corner of each table/listing. The date and time of creation of table/listing will appear bottom left under the sponsor name. The source listing number will appear bottom left.

Tables and listings will be produced in rich text format (i.e., they will tabular in format).

A landscape layout will be used for both tables and listing.

The left and right margins of all tables and listings will be a minimum of 2.1 cm from the left and 1.9 cm from the right. The top and bottom margins will be a minimum 2.92 cm. Header and footer will be both 1.27 cm.

A 9-point font size for tables and 7 or 8-point font size for listings will be used using Courier New font. A maximum SAS line size=141 and page size=44 for 8-point font size, and line size=161 and page size=50 for 7-point will be used so as to fit on both UK and US paper sizes.

A portrait layout will be used for figures.

Titles and footnote will not be included in the body of figure.

The size of the figures (except forest plot) will be: width=16.3 cm height=12.2 cm. The size of forest plot figures will be: width=16.3 cm height=20 cm. The resolution will be set using the option IMAGE_DPI=400.

All tables, listings and figures will be collated into three Microsoft Word complete documents. If the listings are too large to be included in one file they will be separated into manageable sized files. The Microsoft Word documents will be subsequently converted in PDF format. Both, Word and PDF documents will include a table of contents with hyperlinks.

13.4 Quality Control

The following steps will be taken to ensure the quality of the outputs:

- The author of each table/listing/figure program will review the program and will verify that no error message is highlighted in the 'LOG' file.
- Tables and figures will be independently programmed form the raw datasets by a second statistician/programmer and outputs will be compared either electronically (by comparing the data being tabulated using PROC COMPARE) or by manually comparing the data in the two independent outputs.

- Listings will be checked either electronically (by comparing the data being listed using PROC COMPARE) or by manually comparing the data in the listings to the raw/derived data.
- All outputs will be compared to the shells.
- Related outputs will be compared for consistency.

14 SAS Code

14.1 Tables that need descriptive statistics

PROC UNIVARIATE DATA=dset NOPRINT;
VAR var1 var2 var3 …varn;
BY byvar; (optional)
OUTPUT OUT=outname
N=n MEAN=mean MIN=min MAX=max MEDIAN=median STD=std CV=cv;
RUN;

Notes:

- var1, var2 represent the variables for which summary statistics will be calculated;
- byvar represents by variables to be used if any
- out=outname specifies the output dataset that should be created

For tables that need also geometric mean and geometric CV, the original variable will be LOG transformed and the geometric mean will be calculated using exp (mean_Log_var) and Geometric CV will be calculated using sqrt{exp[std_Log_var ^2]-1}

14.2 Tables that need frequency counts

PROC FREQ DATA=dset NOPRINT;
BY byvar; (optional)
*TABLES var1*var2;(for two ways table)*
TABLES var1 var2; (for one way table)
OUTPUT OUT=outname;
RUN;

Notes:

- byvar represents by variables to be used for frequency tabulations, if any
- var1, var2 represent the variables to be tabulated;

15 References

NA