Document Title

Summary of the toxicological and metabolism studies for Fluopyram

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EU) No 283/2013

Document MCA

Section 5: Toxicological and metabolism studies – Part 1

According to the Guidance Document SANCO/10181/2013 for applicants on preparing dossiers for the approval of a chemical active substance

Date

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Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4, 'How to revise an Assessment Report'.

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CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

Fluopyram (AE C656948) was included in Annex I to Reg. (EC) No 1107/2009 in 2013 (Reg. (EU) No 802/2013, Entry into Force on August 22, 2013). This Supplementary Dossier contains only data which were not submitted at the time of the Annex I inclusion of fluopyram under Council Directive 91/414/EEC and which were therefore not evaluated during the first EU review. All data which were already submitted by Bayer AG (former Bayer CropScience) for the Annex I inclusion under Council Directive 91/414/EEC are contained in the Draft Assessment Report (DAR) and its Addenda and are included in the Baseline Dossier provided by Bayer AG.

Relevant information for classification as detailed in the "*Combined Draft (Renewal) Assessment Report prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008 – Volume 1, Level 2*" is provided in Document NJ, Sections 6.1.1 – 6.1.10 and 6.2, and highlighted in light grey.

CA 5.1 Studies on absorption, distribution, metabolism and excretion in mammals

Studies to address the data requirements for ADME for fluopyram were presented in the dossier submitted for first inclusion in Annex I and were deemed acceptable following evaluation and peer review at EU level (2013). New *in vitro* comparative metabolism studies have been conducted with fluopyram using rat, mouse, rabbit, dog and human liver microsomes. These studies are summarised in chapter CA 5.1.2.

Data Point:	KCA 5.1/01
Report Author:	[redacted]
Report Year:	2009
Report Title:	Fluopyram - Evaluation of OECD joint review dossier - Questions addressed from German BfR - Metabolism, toxicology, dated February 03, 2009
Report No:	M-345006-01-1
Document No:	M-345006-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	None
Previous evaluation:	Yes, responses to the February 23, 2009 questions from Germany BfR on metabolism and toxicology
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

This position paper was provided upon request of BfR. It contains responses to multiple questions. They are presented in the relevant sections throughout this document. For ADME studies, these are described below in 5.1.1.

Data Point:	KCA 5.1/02
Report Author:	[redacted]
Report Year:	2009
Report Title:	Fluopyram - Evaluation of OECD joint review dossier - Questions addressed from German BfR – Metabolism, toxicology, dated February 23, 2009
Report No:	M-345012-01-1
Document No:	M-345012-01-1
Guideline(s) followed in study:	
Deviations from current test guideline:	None
Previous evaluation:	Yes, responses to the February 23, 2009 questions from Germany BfR on metabolism and toxicology
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

This position paper was provided upon request of BfR. It contains responses to multiple questions. They are presented in the relevant sections throughout this document. For ADME studies, these are described below in 5.1.1.

Data Point:	KCA 5.1/03
Report Author:	[redacted]
Report Year:	2009
Report Title:	Fluopyram (AE C656948) - Responses to the February 23, 2009 questions from Germany BfR on metabolism and toxicology
Report No:	M-345743-01-1
Document No:	M-345743-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	Yes, responses to the February 23, 2009 questions from Germany BfR on metabolism and toxicology
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

This is a compilation of position papers provided upon request of BfR. It contains responses to multiple questions. They are presented in the relevant sections throughout this document. For ADME studies, it contains the 2 papers (M-345010-01-1 and M-345006-01-1 described below in 5.1.1).

CA 5.1.1 Absorption, distribution, metabolism and excretion by oral exposure

Data Point:	KCA 5.1.1/01
Report Author:	[redacted]
Report Year:	2011
Report Title:	[Phenyl-UL-14C]AE 656948: Absorption, distribution, excretion and metabolism in the rat
Report No:	MEF-07508
Document No:	M-298614-02-1
Guideline(s) followed in study:	US EPA OPPTS 870.7485; EU 91/414/EEC amended by 94/79/EC; Canadian PMRA Ref.: DACO 4.5.9; OECD 417; Japanese MAFF, 12 Nousan 8147
Deviations from current test guideline:	None
Previous evaluation:	Yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The absorption, distribution, excretion and metabolism were investigated with radiolabelled fluopyram, ^{14}C-uniformly-labelled in the phenyl ring (phenyl-UL-^{14}C), in male and female Wistar rats.

[Phenyl-UL-^{14}C]fluopyram was administered orally to four groups of four male or female rats at a low dose of 5 mg/kg bw or at a high dose of 250 mg/kg bw. In addition one group of four male rats received a daily oral dose of non-labelled fluopyram at 5 mg/kg bw for 14 consecutive days, and then one oral dose of [phenyl-UL-^{14}C]fluopyram at 5 mg/kg bw (multiple dose tests). In addition, [phenyl-UL-^{14}C]fluopyram was administered orally to one group of eight bile-duct cannulated male rats at a low dose of 5 mg/kg bw.

Animals were sacrificed 168 hours after dosing, except for the animals in the main bile-duct cannulation test, in which the animals were sacrificed 48 hours after dosing. Urine and faeces were collected from all rats at several intervals over the time period from dosing until sacrifice. Additionally, bile was collected from the bile-duct cannulated rats. Blood plasma samples were collected from the intact rats. After sacrifice, blood, tissues and organs were collected, except for the main bile-duct cannulation test, where only gastrointestinal tract, skin and carcass were collected. The radioactivity was determined in the collected samples. The metabolism was investigated in urine, faeces and bile.

At least approximately 93% of the administered radioactivity were recovered in all tests. The absorption of [phenyl-UL-^{14}C]fluopyram from the gastrointestinal tract started immediately after administration and was rapid and nearly quantitative, shown by short half-lives of absorption ($t_{1/2\ abs}$ ≤ 0.5 h) and the high rate of biliary excretion in the bile-duct cannulation experiment. The oral absorption was at least approximately. 94% of the administered dose. Plasma peak levels were observed within 0.8 – 41.9 hours after dosing.

From peak levels, the radioactivity concentrations in plasma declined rapidly and continuously. fluopyram and its metabolites were mostly eliminated of from the body. The calculated first elimination half-lives ranged from 3.9 – 16.2 hours and the second elimination half-lives ranged from 23.6 – 53.0 hours. Slightly oscillating time course of the plasma radioactivity in female rats of the high dose group indicated that the radioactivity was at least partially subjected to an enterohepatic circulation. After 168 hours, the highest dose normalised concentrations were detected in the organs and tissues responsible for metabolism and excretion: in liver (0.077 – 0.246) and in kidney (0.071 – 0.218) but also in the adrenal gland (0.016 – 0.185). In all other cases the radioactivity level was low (≤ 0.6).

No significant difference was observed in the excretion behaviour among the dose groups. The excretion was mostly completed 168 hours post administration (48 hours for the bile-duct cannulation experiment). In total, amounts of 89.5 – 99.3% of the administered radioactivity were excreted with urine, bile and faeces. The main excretion route was biliary with 78.5% of the administered radioactivity measured in the bile 48 hours after dosing in the test with the bile-duct cannulated rats. In the tests with the intact rats 46.6% to 63.6% of the administered radioactivity was excreted *via* faeces, while 35.1 to 45.3% of the administered radioactivity was excreted with the urine. Amounts of 2.9% to 5.9% of the administered radioactivity were found in the total bodies of the rats at sacrifice 168 hours after administration, and 10.9% in the bodies of the bile-duct cannulated rats at sacrifice 48 hours after administration. These results show that the excretion was mostly complete at sacrifice.

[Phenyl-UL-^{14}C]fluopyram was extensively metabolised. In total, 29 metabolites and the parent compound were identified in urine, faeces, and bile. The total identification rate in all tests was between *ca.* 73 and 87% of the administered dose. The unchanged parent compound was found at *ca.* 0.4% – 16.7% of the administered radioactivity, and it was found only in faeces.

The ethyl linking group of the molecule was the preferred site for degradation. The principal metabolic transformation was the molecular cleavage of the ethyl linking group yielding fluopyram-benzamide (M25) found up to 24.5% of the administered dose, which was subsequently hydrolysed to fluopyram-benzoic acid (M33) or hydroxylated and conjugated to form various fluopyram-benzamide and fluopyram-hydroxybenzamide conjugates. fluopyram-benzoic acid represented up to 6.65% of the dose administered.

Prior to the cleavage of the ethyl moiety, was the hydroxylation of the ethyl linking group and the phenyl ring followed by further hydroxylation and conjugation. Various hydroxylated metabolites conjugated mostly with glucuronic acid and to a lower extent sulphate were identified. Metabolites containing both intact ring moieties of the parent compound accounted in total for up to 72.05% of the given dose and were found in all matrices. Of these, the major metabolites were isomer 1 of fluopyram-7-OH-GA (M09) (up to 20.20% of the administered radioactivity), and isomer 2 of fluopyram-enol-GA (M04) (up to 19.95% of the administered radioactivity).

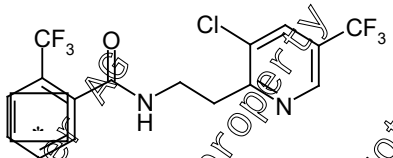
Conjugation of the phenyl ring moiety with glutathione followed by further degradation gave rise to fluopyram-7-OH-methyl-sulfone (M10), fluopyram-BA-methyl-sulfoxide (M29) (BA = benzamide) and fluopyram-BA-methyl-sulfone (M30). Further degradation of the phenyl ring was less prominent (reaction with methoxy and methylsulfone). All metabolites present in the total excreta at ≥ 5% of the administered dose and many other metabolites representing < 5% of the administered dose were identified.

I. Materials and methods

A. Materials

1. Test material: [Phenyl-UL-^{14}C]AE C656948

Chemical structure:



Description:	solid,
Batch no.:	KML3392-1
Specific radioactivity:	3.85 MBq/mg (104.15 Ci/mol) for the high dose tests (tests 4 and 5) dilution of radioactivity with non-labelled AE C656948 resulting in: 0.077 MBq/mg
Radiochemical purity:	> 98% (HPLC) > 99% (TLC)
Chemical purity:	> 99% (HPLC)
CAS no.:	658066-35-4 (non-labelled fluopyram)
Stability of test compound:	The administration suspensions of each test were analysed by HPLC with radioactivity detection. The radiochemical purity of the test compound was at least 99% for all analyses, demonstrating the stability of the test compound under the conditions of storage and handling prior to administration.

2. Vehicle: 0.5% aqueous Tragacanth solution

3. Test animals:

Species:	Rat, male and female rats derived from *Rattus norvegicus*
Strain:	Wistar Hsd/Cpb:Wu
Age:	7 – 8 weeks (males), 8 – 10 weeks (females)
Weight at dosing:	Approximately 200 g
Source:	[redacted]
Acclimation period:	The animals were acclimated to the laboratory conditions in Makrolon® cages on wood shavings for one week prior to the start of each experiment.
Identification:	Cage cards with study number, substance name, and animal number as well as coloured spots on the tail
Diet:	Rat/mice maintenance long life diet (no. 3883.0.15), Fa. Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland, *ad libitum* The animals were fed with approximately 16 g per day and animal. The animals were fed the last time ca. 16 h prior to administration of the dose and again 6 h after dosing
Water:	Tap water, *ad libitum*
Housing:	During the excretion studies the animals were kept in Makrolon® metabolism cages, which allowed for a separate and quantitative sampling of the excreta.

Environmental conditions

Temperature:	18 – 26 °C
Humidity:	40 – 72%
Air changes:	10 – 15 fold air change per hour
Photoperiod:	12 hours light/dark-cycle

4. Preparation of dosing solutions

Upon receipt the solid radiolabelled test compound was dissolved in acetonitrile. For the preparation of each administration suspension an adequate portion of the stock solution was pipetted and evaporated to near dryness. The nearly dry residue was suspended in a 0.5% aqueous Tragacanth solution by stirring overnight at approximately 4 °C on a magnetic stirrer. During administration the suspension was stirred at room temperature and an adequate volume of the test suspension was administered to each rat.

B. Study design and methods

All tests were performed according to the current EPA, PMRA, OECD and Japanese MAFF test guidelines for supporting the registration of chemical pesticides.

1. Dose regimen and design of tests

Test no.	Administered single dose of ^{14}C-fluopyram, route (experiment)	Number of rats and sex	Collection of samples during the test and at sacrifice	Duration
1	5 mg/kg bw, oral (bile duct cannulation)	5 male	Bile, urine, faeces, blood, GIT, skin, carcass	48 hours
2	5 mg/kg bw, oral (single low dose, EPA)	4 male	Microplasma, urine, faeces, blood, organs and tissues, GIT, skin, carcass	168 hours
3	5 mg/kg bw, oral (single low dose)	4 female	Microplasma, urine, faeces, blood, organs and tissues, GIT, skin, carcass	168 hours
4	250 mg/kg bw, oral (single high dose)	4 male	Microplasma, urine, faeces, blood, organs and tissues, GIT, skin, carcass	168 hours
5	250 mg/kg bw, oral (single high dose)	4 female	Microplasma, urine, faeces, blood, organs and tissues, GIT, skin, carcass	168 hours
6	5 mg/kg bw, oral, after daily non-labelled dose at 5 mg/kg bw for 14 days (multiple low dose)	4 male	Microplasma, urine, faeces, blood, organs and tissues, GIT, skin, carcass	168 hours

The rats of all test groups received a single dose of fluopyram radiolabelled in the phenyl ring (phenyl-UL-^{14}C).

[Phenyl-UL-^{14}C]fluopyram was administered orally to four groups of four male or female rats at a low dose of 5 mg/kg bw or at a high dose of 250 mg/kg bw (tests 2 to 5). One group of four male rats received a daily oral dose of non-labelled fluopyram at 5 mg/kg bw for 14 consecutive days, and on the next day one oral dose of [phenyl-UL-^{14}C]fluopyram at 5 mg/kg bw (test 6). Urine, faeces and plasma samples were collected for 168 hours until sacrifice, and blood, organs and tissues, GIT, skin and carcass were collected.

In addition, [phenyl-UL-^{14}C]fluopyram was administered orally to one group of eight bile-duct cannulated male rats at a low dose of 4 mg/kg bw (test 1). Of these, only five were used for analysis, as two animals died in the second night after administration and and one had to be sacrificed early due to lack of bile flow. Bile, urine and faeces were collected for 48 hours until sacrifice, and blood, GIT, skin and carcass were collected.

2. Cholangiostomy

The animals were anaesthetised for the cholangiostomy surgery. After shaving of the stomach area below the rib cage, a small incision was made. The portion of the small intestine with the pancreatic tissue containing the bile duct was carefully pulled out. Surgical thread was pulled through prior to puncturing the bile duct. Afterwards, the indwelling biliary duodenal catheter were implanted for collection of the bile. The surgical field was relocated into the body by lifting the skin. A small incision was made on the back at the basis of the tail of the animal to pull the bile catheter through. The muscle layer was closed by careful sewing and the use of surgical clamps. The incisions were sealed with all-purpose glue. The rats were placed onto warming pads while regaining consciousness. After coming out of the anaesthesia, they were kept individually in the metabolism cages and the cannula of each animal led through the roof of the cage to permit bile collection into an appropriate weighed container which could be removed and replaced without disturbing the animal. The animals received a diluted analgesic during and after surgery by intramuscular injection.

3. Dosing

Adequate volumes of the suspensions of the test compound in 0.5% aqueous Tragacanth were administered to each rat, 1 mL orally by gavage to the bile-duct cannulated rats and 2 mL to the rats of all other tests. Oral dosing was performed using a syringe attached to a special needle. The concentration of each administration suspension was calculated to reach an administered amount of 5 mg parent compound per kg body weight (bw) in tests 1, 2, 3 and 6 and 250 mg/kg bw in tests 4 and 5. As the animal weights varied slightly, the actual doses varied slightly with the body weight.

The concentration of the active substance in the administration suspensions was determined by LSC. The results of these measurements served as a basis for the calculation of the total radioactivity in the biological samples. The stability of the active substance in the administration suspensions for each test was checked by HPLC, showing a radiochemical purity of at least 99%.

4. Collection of excreta

After the administration of the radiolabelled test substance, the rats were kept individually in Makrolon® metabolism cages, which allowed for separate and quantitative collection of urine and faeces.

Urine was collected separately for each rat in a cryogenic trap cooled with dry ice at intervals of 8, 24, 32 and 48 hours for test 1 and 4, 8, 24, 48, 72, 96, 120, 144 and 168 hours for tests 2 to 6, and additionally 12 hours for test 2, after administration of the radiolabelled dose. The funnels for urine collection were rinsed with demineralised water at the end of each sampling period. The rinsing

solutions were drained into the same vial as the corresponding urine fraction. The radioactivity was determined by LSC.

Faeces were collected separately for each rat every 24 hours until 48 hours (test 1) or 168 hours (test 2 to 6) after administration of the radiolabelled dose. The faeces fractions were weighed, diluted with water (1:1) and homogenised. The radioactivity was determined by combustion/LSC.

In test 1, bile was collected separately for each cannulated rat at intervals of 4, 8, 24, 32 and 48 hours after administration, in test tubes cooled by crushed ice. The radioactivity was determined by LSC.

6. Plasma micro samples

Blood samples were collected in heparinised capillaries separately for each rat by puncture of the tail veins. The samples were taken at intervals of 0.16, 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 8, 24, 28, 32, 48, 52, 56, 72, 96, 120, 144, 152 and 168 hours after administration of the radiolabelled dose in tests 2 to 6. The wound was closed with adhesive tape. The capillaries were centrifuged at *ca.* 12000 G for 10 minutes using a haematocrit centrifuge to separate plasma from erythrocytes. After centrifugation, the capillary was broken at the border between plasma and erythrocytes and the plasma (*ca.* 10 – 80 mg) pressed onto a small metal dish for weighing. This dish was then placed into a scintillation vial for radioactivity measurement. This procedure allowed for the collection of blood samples at the different time points from the same animals and providing plasma curves from single animals. These show lower variability compared to curves that were calculated from whole blood samples of different animals and inter-animal variations are avoided.

7. Sacrifice

The rats were sacrificed in Pentobarbital-Na anaesthesia by transection of the cervical vessels and exsanguinated.

8. Blood, tissues and organs at sacrifice

At sacrifice of the rats the blood was collected and separated into plasma and erythrocytes by centrifugation, and the following organs and tissues were collected: spleen, gastrointestinal tract (GIT), liver, kidney, testis, uterus, ovaries, skeletal muscle, bone femur, heart, lung, skin, perirenal fat, adrenal gland, thyroid gland, brain and carcass for tests 2 to 6. At sacrifice of the rats in tests 1, skin, gastrointestinal tract (GIT) and carcass were collected.

The organs and tissues prepared at the end of the experiments were weighed immediately after dissection and again following lyophilisation. Finally, they were homogenised before aliquots were taken for the determination of radioactivity by combustion. The radioactivity remaining in the GIT, skin, and residual carcass was determined by combustion in order to establish the radioactivity balance. For the small organs and tissues (e.g. renal fat, uterus, harderian gland, pancreas, adrenal glands, thyroid, ovaries and eye), only the wet weight was determined before they were solubilized using BTS (Beckman Tissue Solubiliser) and radioassayed by LSC.

9. Sample handling and storage

All freeze-dried samples, such as organs, were stored in plastic vials at room temperature or at ca. +4 °C in a refrigerator. All liquid samples were kept frozen at ca. -18 °C at all times except during

aliquotation for analysis. During the analytical work, the samples were stored either at ca. +4 °C in a refrigerator or at ca. -18 °C in a freezer.

10. Measurement of radioactivity

The measurement of the radioactivity in liquid samples was carried out by liquid scintillation counting (LSC). All solid samples were solubilised by means of a tissue solubiliser. Portions of these solutions were mixed with a suitable scintillation cocktail and measured by LSC. All solid samples with the exception of those solubilised were weighed and combusted in an oxygen atmosphere. The released $^{14}CO_2$ was trapped in an alkaline absorber and the radioactivity was determined by LSC.

For all samples, the limit of detection (LOD) was established at 20 dpm measured per aliquot after correction for the background radioactivity. The limit of quantification (LOQ) for each individual measurement was established as 2 times the background radioactivity (dpm) of each instrument/method. This background counting rate was in a range between 10 – 35 dpm, it was automatically subtracted from the measured results.

11. Toxicokinetic analysis

In this study, the software TOPFIT (version 2.0) was used to calculate the pharmacokinetic parameters by plasma concentration-time curve analysis. The standard 3-compartment model (test 1 and 3) was applied for curve fitting computations. The calculations are based on the average values of plasma concentrations sampled from the rats of the test group.

12. Preparation of urine, faeces and bile for analysis

Generally, the excreta samples of the rats were combined to representative pools of each test group.

Pools of urine samples were prepared to represent the following excretion intervals:

0 – 48 hours for test 1

0 – 4, 4 – 8, 8 – 12, 12 – 24, 24 – 48, 48 – 72, 72 – 96, 96 – 120, 120 – 144 and 144 – 168 for test 2

0 – 120 and 120 – 168 test 3 to 6.

The bile pool samples were analysed by HPLC without further purification.

Pools of faeces samples were prepared to represent the following excretion intervals:

0 – 24, 24 – 48 and 48 –72 hours for test 2 (72 – 168 hours not analysed)

0 – 72 hours for tests 3 to 6 (72 – 168 hours not analysed).

Aliquots were extracted in six steps using acetonitrile, acetonitrile/water (both neutral), twice acetonitrile/water/formic acid (acidic) and finally for exhaustive extraction twice acetonitrile/water with addition of ammonia (alkaline). Each extraction step was performed by maceration using a Polytron or an Ultra-Turrax homogeniser. The solids were dried and measured by combustion followed by LSC.

Neutral, acidic and alkaline extracts were each combined. Acidic and alkaline extracts were each evaporated to the aqueous remainder and adjusted to pH 7. All extracts were adjusted to 400 mL with acetonitrile. 200 mL of each extract were taken and combined. The combined extract was evaporated

to the aqueous remainder. The volume of the concentrate was measured and aliquots radioassayed by LSC. The distillate of each faeces preparation was trapped, its volume measured and aliquots radioassayed by LSC. The distillates were discarded because they contained only negligible radioactivity.

The concentrates were subjected to HPLC analysis with the profiling method AE948-33.

Pools of bile samples were prepared to represent the excretion intervals of 0 – 48 hours for test 1. The bile samples of only 5 rats each were pooled, as two rats had died in the second night after dosing and one had to be sacrificed early due to lack of bile flow. The bile pool samples were analysed by HPLC without further purification.

13. Analytical methods

Urine, faeces and bile were analysed for parent compound and metabolites by HPLC with radioactivity detection, with LC-MS and with NMR methods for structure elucidation.

14. High performance liquid chromatography (HPLC)

The metabolic profiles in bile, urine and faeces extracts, and the purity of the stock solution and the administration suspensions were analysed by reversed phase radio-HPLC. HPLC analysis was performed on Hewlett Packard modular LC systems with radiometric detection supported by UV detection. The separation was carried out on a reversed phase column using a neutral water + ammonia solution + formic acid/ acetonitrile + methanol gradient (Method: AE948-33), using water + formic acid/ acetonitrile + methanol + formic acid gradient (Method: AE948-45) or using neutral water + ammonia solution + formic acid/ acetonitrile + methanol gradient (Method: BM1610). A radioactivity peak was regarded as relevant having a signal to noise ratio at least 2.5 (LOD). In order to check the completeness of the chromatographic elution, representative samples were injected, re-collected, and radioassayed by LSC. The chromatographic recoveries were equal to or greater than 97%.

For co-chromatography, the sample was mixed with the reference compound before injection. The detection was carried out either by UV absorbance of the non-radiolabelled or by ^{14}C-detection of the radiolabelled reference compound. Chromatographic matching with the non-radiolabelled reference compound was assessed by comparison of the UV-trace and the associated ^{14}C-trace. Chromatographic matching with the radiolabelled reference compound was assessed by comparison of the ^{14}C-chromatogram of the mixture with the ^{14}C-chromatogram of the sample without the reference compound. For identification, the HPLC fractions were subjected to spectroscopic methods (LC-MS/MS and ^{1}H-NMR).

15. Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was used to identify and confirm metabolites in isolated HPLC fractions. The absorbent was silica $60F_{254}$ and the solvent system was dichloromethane/methanol (90:10, v/v).

16. Mass spectroscopy (MS)

The electro-spray ionisation MS spectra (ESI) were obtained with a LTQ mass spectrometer by Finnigan. Agilent HP1100 LC systems were used for chromatography. The separation was carried out on a reversed phase column using an acidic water/acetonitrile gradient.

17. Nuclear magnetic resonance spectroscopy (NMR)

NMR spectra were recorded on a BRUKER AV 600 (600 MHz).

18. Enzymatic hydrolysis

Conjugates isolated from bile were incubated with a standardised solution of ß-glucuronidase/arylsulfatase from *Helix pomatia*.

Selected metabolites isolated from bile (test 1) were buffered to a pH value of approximately 6. A portion of enzyme suspension was added and the sample incubated for about 17 hours at 37 °C. For HPLC analysis further purification was not necessary.

20. Identification, characterisation and quantification

Urine and bile pools and extracts of faeces pools were analysed by HPLC using HPLC method AE948-33 and compared among each other and among the different matrices. Furthermore, selected profiles (urine, bile and faeces extract) of this study were compared to the profiles measured in the rat ADME conducted with [pyridyl-2,6-^{14}C]fluopyram ([redacted], KCA 5.1.1/02). The profile of urine in males, low dose test 2, was compared with urine from the rat QWBA study conducted with [phenyl-UL-^{14}C]fluopyram ([redacted], KCA 5.1.1/03).

Co-eluting metabolites in urine samples were fractionated using HPLC method AE948-33 and analysed using the acidic method AE948-45. One metabolite (fluopyram-benzamide-SA, isomer 1) was fractionated from urine and identified by LC-MS and LC-MS/MS. Other metabolites were identified by HPLC and TLC co-chromatography with reference items. Prominent metabolites in bile were purified by SPE, isolated and purified by HPLC and subjected to structure elucidation. Selected metabolites were furthermore subjected to enzymatic cleavage with ß-glucuronidase/arylsulfatase, which resulted in the aglyca fluopyram-7-OH-GA (isomer 1), fluopyram-8-OH-GA (isomer 1), fluopyram-8-OH-GA (isomer 2) and fluopyram-enol-GA (isomer 2). Other metabolites from bile and and from faeces were identified by HPLC co-chromatography with reference items.

All known and unknown metabolites and parent compound were determined quantitatively in composite samples of bile and extracts of composite faeces samples. For quantitation the radioactivity signals in the HPLC chromatograms were integrated and related to the amount of radioactivity present in the respective samples.

II. Results and discussion

A. Recovery

Over all tests the mean recoveries of radioactivity were at least approximately 93% of the administered radioactivity. A summary of the radioactivity in percent of the administered dose found in excreta and organs and tissues at sacrifice is presented in Table 5.1.1- 1.

Table 5.1.1- 1: Recovery of radioactivity in excreta, gastrointestinal tract and the body of rats following oral dosing of [phenyl-UL-[14]C]fluopyram, data presented as % of dose administered

Test no.	**Test 1**	**Test 2**	**Test 3**	**Test 4**	**Test 5**	**Test 6**
Dose, route	**5 mg/kg bw, p.o.**	**5 mg/kg bw, p.o.**	**5 mg/kg bw, p.o.**	**250 mg/kg bw, p.o.**	**250 mg/kg bw, p.o.**	**5 mg/kg bw, p.o.**
Experiment	**Bile duct cannulation**	**single low dose, EPA**	**single low dose**	**single high dose**	**single high dose**	**multiple low dose***
Duration, sex	**48 h, male**	**168 h, male**	**168 h, female**	**168 h, male**	**168 h, female**	**168 h, male**
Urine	7.29	38.25	45.27	35.70	35.53	35.14
Bile	78.54	----	----	----	----	----
Faeces	3.70	53.06	46.58	63.60	57.12	55.45
Sum excreta	89.53	91.31	91.85	99.30	92.65	90.59
Skin	1.34	0.47	0.76	0.55	0.68	0.30
Sum organs	6.38	2.85	4.73	2.00	2.71	1.91
Body w/o GIT	7.72	3.32	5.50	2.54	3.39	2.20
GIT	3.17	0.24	0.42	0.32	0.32	0.18
Total body	10.88	3.56	5.91	2.86	3.72	2.38
Balance	100.4	94.9	97.8	102.2	96.4	93.0

p.o. = per os, oral

* one radiolabelled dose after a daily non-labelled dose at 5 mg/kg bw for 14 days

B. Absorption

[Phenyl-UL-[14]C]fluopyram was rapidly absorbed from the gastrointestinal tract of male and female rats in all dose groups. The absorption started immediately after oral administration as shown by the time course of the plasma concentrations of radioactivity (Table 5.1.1- 2) and the pharmacokinetic calculations (Table 5.1.1- 4). In the pharmacokinetic model short half-lives of absorption ($t_{1/2\ abs}$ ≤ 0.5 h) were calculated.

Male rats in the single low dose experiment (test 2) reached peak plasma level after 15 hours. For females, absorption of the single low dose was slightly faster than males and reached peak plasma levels at 11.2 hours. Plasma curves of the high dose tests showed slower absorption with peak plasma levels apparent as a broad peak at 28 – 56 hours. Plasma curves of the single low dose and single low dose after 14 days after pre-treatment in males were very similar, while after pre-treatment the maximum was reached sooner than without pre-treatment (0.8 hours and 15 hours, respectively). Compared to males rats, the plasma curves of female rats reached lower peak levels. Plasma curves of the high dose groups were similar for male and female rats until 56 hours after administration, after which plasma levels oscillated in females, while they decreased continuously in males.

The oral absorption was at least approximately 94% of the administered dose at the low dose level of 5 mg/kg bw. This was calculated from the results of the bile-duct cannulation experiment with male rats (test 1) by adding the radioactivity excreted with the bile and urine and the radioactivity residues in the body without the gastrointestinal tract (Table 5.1.1- 1).

Table 5.1.1- 2: Time course of radioactivity in the plasma of male and female rats following an oral dose of [phenyl-UL-^{14}C]fluopyram expressed as parent equivalent concentration in µg/g

Test no.	Test 2	Test 3	Test 4	Test 5	Test 6
Dose, route	5 mg/kg bw, p.o.	5 mg/kg bw, p.o.	250 mg/kg bw, p.o.	250 mg/kg bw, p.o.	5 mg/kg bw, p.o.
Experiment	single low dose, EPA	single low dose	single high dose	single high dose	multiple low dose*
Duration, sex	168 h, male	168 h, female	168 h, male	168 h, female	168 h, male
0.16 h	0.462	0.330	1.433	0.807	0.494
0.33 h	0.898	0.622	4.458	2.165	1.186
0.66 h	1.027	1.036	8.839	3.827	1.436
1.0 h	0.959	1.321	11.394	5.413	1.156
1.5 h	0.835	1.559	13.484	7.604	0.850
2.0 h	0.731	1.641	16.377	8.815	0.686
3.0 h	0.779	1.743	17.052	11.271	0.843
4.0 h	0.975	1.869	16.500	12.798	0.884
6.0 h	1.245	1.995	18.510	14.487	0.972
8.0 h	1.445	2.114	22.705	17.492	1.248
24 h	1.450	1.843	50.446	45.854	1.047
28 h	1.359	2.653	52.341	55.812	0.972
32 h	1.263	1.521	56.348	61.453	0.944
48 h	0.821	1.106	59.938	63.089	0.672
52 h	0.755	1.011	59.300	61.262	0.623
56 h	0.694	0.932	57.506	59.175	0.594
72 h	0.500	0.683	41.714	46.965	0.402
96 h	0.299	0.459	25.913	12.495	0.242
120 h	0.184	0.317	16.032	8.700	0.149
144 h	0.123	0.226	10.076	18.297	0.101
152 h	0.103	0.192	8.750	31.125	0.085
168 h	0.081	0.167	6.398	19.894	0.066

p.o. = per os, oral

* one radiolabelled a dose after daily non-labelled dose at 1 mg/kg bw for 14 consecutive days

Table 5.1.1- 3: Time course of radioactivity in the plasma of male and female rats following an oral dose of [phenyl-UL-^{14}C]fluopyram expressed as dose normalised concentration

Test no.	Test 2	Test 3	Test 4	Test 5	Test 6
Dose, route	5 mg/kg bw, p.o.	5 mg/kg bw, p.o.	250 mg/kg bw, p.o.	250 mg/kg bw, p.o.	5 mg/kg bw, p.o.
Experiment	single low dose, EPA	single low dose	single high dose	single high dose	multiple low dose*
Duration, sex	168 h, male	168 h, female	168 h, male	168 h, female	168 h, male
0.16 h	0.096	0.066	0.006	0.004	0.105
0.33 h	0.184	0.125	0.019	0.009	0.253
0.66 h	0.211	0.209	0.038	0.017	0.307
1.0 h	0.197	0.266	0.049	0.023	0.247
1.5 h	0.172	0.314	0.058	0.033	0.182
2.0 h	0.151	0.331	0.070	0.038	0.146
3.0 h	0.161	0.352	0.073	0.049	0.130
4.0 h	0.202	0.378	0.071	0.055	0.189
6.0 h	0.259	0.404	0.079	0.063	0.208
8.0 h	0.300	0.428	0.097	0.076	0.266
24 h	0.302	0.373	0.216	0.198	0.223
28 h	0.283	0.335	0.225	0.242	0.208
32 h	0.263	0.308	0.242	0.267	0.202
48 h	0.171	0.224	0.257	0.274	0.143
52 h	0.157	0.205	0.254	0.266	0.133
56 h	0.144	0.189	0.247	0.257	0.127
72 h	0.104	0.138	0.179	0.204	0.086
96 h	0.062	0.093	0.111	0.054	0.052
120 h	0.038	0.064	0.069	0.038	0.032
144 h	0.026	0.046	0.043	0.079	0.022
152 h	0.022	0.039	0.038	0.134	0.018
168 h	0.017	0.034	0.028	0.086	0.014

p.o. – per os, oral

* one radiolabelled dose after a daily non-labelled dose at 5 mg/kg bw for 14 consecutive days

C. Distribution

In all low dose groups the plasma curves showed a rapid absorption. The plasma curve of female rats from the high dose test showed oscillation during the declining from t_{max} to 168 hours. Plasma concentrations of radioactivity reached peak values 11 – 15 hours after oral administration in low dose groups, while in the rats of the repeated low dose test, the maximum of the plasma concentration of radioactivity was reached distinctly faster approximately 0.8 h after dosage. The maximum of the plasma concentration of radioactivity in high dose tests was reached distinctively later than in low dose tests (approximately 35 – 42 hours after dosage; Table 5.1.1- 2, Table 5.1.1- 3 and Table 5.1.1-

4). Maximum measured and calculated plasma concentrations (C_{max} values) were in the range 1.5 – 2.2 µg/g for low dose and 61 – 62 µg/g for high dose tests. The maximum dose normalised concentration of the low dose tests were slightly higher in females compared to males and about 20 – 40% higher in low dose tests compared to high dose tests. Male rats of the low dose tests with and without pre-treatment had similar maximum dose normalised concentrations in plasma. AUC values for single and multiple low doses ranged from 80 – 148 µg/g*h, AUC values for single high doses were 5680 and 7060 µg/g*h. The mean residence time (MRT) of the total radioactivity was moderate in all dose groups with values varying between ca. 52 – 84 hours. The 3-compartment model yielded two elimination half-lives for each treatment regimen, the first elimination half-lives [$t_{1/2\ elim(1)}$] ranged from 3.9 – 16.2 hours and the second elimination half-lives [$t_{1/2\ elim(2)}$] ranged from 23.6 – 53.0 hours.

The amount of radioactivity remaining in the total body at sacrifice 168 hours after administration was low and accounted for 2.38 – 5.91% and for 10.88% in bile cannulated rats 48 hours after administration (Table 5.1.1- 5).

After 168 hours, the highest dose normalised concentrations were detected in the organs and tissues responsible for metabolism and excretion: in liver (0.077 – 0.246) and in kidney (0.071 – 0.218) but also in the adrenal gland (0.016 – 0.185). In all other cases the radioactivity level was low (≤ 0.6; Table 5.1.1- 6).

Table 5.1.1- 4: Pharmacokinetic parameters of [phenyl-UL-^{14}C]fluopyram after oral administration to male and female rats, derived from plasma curve analysis

Test no.	**Test 2**	**Test 3**	**Test 4**	**Test 5**	**Test 6**
Dose, route	**5 mg/kg bw, p.o.**	**5 mg/kg bw, p.o.**	**250 mg/kg bw, p.o.**	**250 mg/kg bw, p.o.**	**5 mg/kg bw, p.o.**
Experiment	**single low dose, EPA**	**single low dose**	**single high dose**	**single high dose**	**multiple low dose***
Duration, sex	**168 h, male**	**168 h, female**	**168 h, male**	**168 h, female**	**168 h, male**
$AUC_{(0-\infty)}$ [µg/g*h]	107	148	5680	7060	80
$t_{1/2\ abs}$ [h]	0.1	0.4	0.5	0.5	0.5
$t_{1/2\ elim(1)}$ [h]	3.9	16.2	4.8	4.8	4.6
$t_{1/2\ elim(2)}$ [h]	30.9	53.0	23.6	29.0	36.8
C_{max} (calc.) [µg/mL]	1.54	2.16	60.9	62.2	1.54
t_{max} (calc.) [h]	15.0	11.2	34.5	41.9	0.8
C_{max} (exp.) [µg/g]	1.45	2.11	59.94	63.09	1.44
t_{max} (exp.) [h]	24.0	8.0	48.0	48.0	0.7
MRT [h]	51.8	65.2	68.8	83.6	53.3
MRT_{abs} [h]	11.7	8.6	34.8	41.8	0.3
MRT_{disp} [h]	40.0	56.7	34.0	41.9	53.1

* one radiolabelled dose after a daily non-labelled dose at 5 mg/kg bw for 14 consecutive days

Table 5.1.1- 5: Radioactive residues in organs and tissues at sacrifice expressed as % dose administered

Test no.	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Dose, route	5 mg/kg bw, p.o.	5 mg/kg bw, p.o.	5 mg/kg bw, p.o.	250 mg/kg bw, p.o.	250 mg/kg bw, p.o.	5 mg/kg bw, p.o.
Experiment	Bile duct cannulation	single low dose, EPA	single low dose	single high dose	single high dose	multiple low dose*
Duration, sex	48 h, male	168 h, male	168 h, female	168 h, male	168 h, female	168 h, male
Erythrocytes**	0.0902	0.0456	0.0554	0.0566	0.0535	0.0448
Plasma**	0.0732	0.0303	0.0434	0.0432	0.0491	0.0244
GIT	3.1660	0.2365	0.4152	0.3166	0.3216	0.1807
Spleen	n.a.	0.0097	0.0126	0.0075	0.0126	0.0066
Liver	n.a.	0.7432	1.0310	0.3720	0.4288	0.5280
Kidney	n.a.	0.1262	0.1555	0.0575	0.0524	0.0919
Perirenal fat**	n.a.	0.0009	0.0071	0.0035	0.0045	0.0033
Adrenal gland	n.a.	0.0003	0.0060	0.0011	0.0022	0.0014
Testis	n.a.	0.0375	n.a.	0.0345	n.a.	0.0302
Ovary	n.a.	n.a.	0.0090	n.a.	0.0032	n.a.
Uterus	n.a.	n.a.	0.0091	n.a.	0.0097	n.a.
Skeleton muscle**	n.a.	0.0212	0.0445	0.0238	0.0362	0.0148
Bone femur**	n.a.	0.0036	0.0065	0.0053	0.0062	0.0028
Heart	n.a.	0.0161	0.0241	0.0109	0.0162	0.0077
Lung	n.a.	0.0163	0.0281	0.0211	0.0268	0.0124
Brain	n.a.	0.0189	0.0337	0.0197	0.0287	0.0138
Thyroid gland	n.a.	0.0001	0.0003	0.0003	0.0003	0.0002
Skin	1.3400	0.4665	0.7640	0.5453	0.6834	0.2978
Carcass	6.2120	1.7830	3.2640	1.3410	1.9800	1.1230

p.o. = per os, oral

n.a. = not applicable

* one radiolabelled a dose after daily non-labelled dose at 5 mg/kg bw for 14 consecutive days

** organ aliquot (of these organs/tissues only a part was sampled at sacrifice, % of the dose administered is relating to the part of organ/tissue sampled and analysed, the contribution of the part not sampled is included in the value of the carcass)

Table 5.1.1- 6: Radioactive residues in organs and tissues at sacrifice expressed as dose normalised concentration

Test no.	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Dose, route	5 mg/kg bw, p.o.	5 mg/kg bw, p.o.	5 mg/kg bw, p.o.	250 mg/kg bw, p.o.	250 mg/kg bw, p.o.	5 mg/kg bw, p.o.
Experiment	Bile duct cannulation	single low dose, EPA	single low dose	single high dose	single high dose	multiple low dose*
Duration, sex	48 h, male	168 h, male	168 h, female	168 h, male	168 h, female	168 h, male
Erythrocytes	0.077	0.038	0.049	0.050	0.046	0.037
Plasma	0.090	0.022	0.038	0.031	0.043	0.019
GIT	0.321	0.022	0.040	0.022	0.036	0.022
Spleen	n.a.	0.037	0.056	0.035	0.043	0.033
Liver	n.a.	0.164	0.246	0.077	0.094	0.137
Kidney	n.a.	0.164	0.218	0.076	0.071	0.125
Perirenal fat	n.a.	0.002	0.019	0.013	0.017	0.008
Adrenal gland	n.a.	0.046	0.185	0.050	0.061	0.079
Testis	n.a.	0.031	n.a.	0.031	n.a.	0.024
Ovary	n.a.	n.a.	0.135	n.a.	0.051	n.a.
Uterus	n.a.	n.a.	0.022	n.a.	0.034	n.a.
Skeleton muscle	n.a.	0.029	0.051	0.027	0.036	0.016
Bone femur	n.a.	0.012	0.020	0.018	0.018	0.009
Heart	n.a.	0.042	0.066	0.033	0.041	0.023
Lung	n.a.	0.030	0.048	0.034	0.042	0.024
Brain	n.a.	0.025	0.044	0.030	0.038	0.020
Thyroid gland	n.a.	0.011	0.060	0.036	0.045	0.029
Skin	0.059	0.021	0.035	0.025	0.032	0.014
Carcass	0.101	0.034	0.060	0.026	0.036	0.020

p.o. = per os, oral

n.a. = not applicable

* one radiolabelled a dose after daily non-labelled dose at 5 mg/kg bw for 14 consecutive days

D. Excretion

No significant difference was observed in the excretion behaviour among the dose groups. Renal excretion was slightly slower in high dose tests compared to low dose tests. The excretion was mostly completed 168 hours post administration (48 hours for the bile-duct cannulation experiment). In total, amounts of 89.5 – 99.3% of the administered radioactivity were excreted with urine, bile and faeces. The remaining radioactivity in the body ranged from 2 – 6% of the administered dose. The main excretion route was biliary with 78.5% of the administered radioactivity measured in the bile 48 hours after dosing in the test with the bile-duct cannulated rats. In the tests with the not bile canulated rats 46.6 to 63.6% of the administered radioactivity was excreted via faeces, while 35.1 to 45.3% of the administered radioactivity was excreted with the urine (Table 5.1.1- 7).

Slight sex difference was observed in the ratio of renal to faecal excretion in low dose tests, where females excreted slightly more of the radioactivity via urine than via faeces. The ratio of the renal and faecal excretion in the others test were comparable (approximately 40% *via* urine and approximately 60% *via* faeces, respectively).

Amounts of 2.9 to 5.9% of the administered radioactivity were found in the total bodies at sacrifice 168 hours after administration, and 10.9% in the bodies of the bile-duct cannulated rats 48 hours after administration (Table 5.1.1- 1). These results show that the excretion was mostly complete at sacrifice.

Table 5.1.1- 7: Cumulative excretion of radioactivity at time intervals expressed as % dose administered

Test no.	**Test 1**	**Test 2**	**Test 3**	**Test 4**	**Test 5**	**Test 6**
Dose, route	**5 mg/kg bw, p.o.**	**5 mg/kg bw, p.o.**	**5 mg/kg bw, p.o.**	**250 mg/kg bw, p.o.**	**250 mg/kg bw, p.o.**	**5 mg/kg bw, p.o.**
Experiment	**Bile duct cannulation**	**single low dose, EPA**	**single low dose**	**single high dose**	**single high dose**	**multiple low dose**
Duration, sex	**48 h, male**	**168 h, male**	**168 h, female**	**168 h, male**	**168 h, female**	**168 h, male**
Urine						
4	---	1.02	1.45	0.40	0.13	0.98
8	1.38	2.69	2.00	0.86	0.35	2.66
12	---	5.15	---	---	---	---
24	5.12	15.67	16.64	4.92	5.21	13.38
32	5.89	---	---	---	---	---
48	7.29	25.27	28.62	14.77	17.35	22.52
72	---	30.53	34.89	33.75	24.76	27.98
96	---	33.87	39.30	29.10	29.60	30.98
120	---	35.87	41.89	32.54	32.32	32.85
144	---	37.26	43.82	34.39	34.22	34.06
168	---	38.25	45.27	35.70	35.53	35.14
Bile						
4	31.54	---	---	---	---	---
8	48.94	---	---	---	---	---
24	61.03	---	---	---	---	---
32	67.80	---	---	---	---	---
48	78.54	---	---	---	---	---
Faeces						

24	3.14	26.12	27.02	13.11	22.01	33.44
48	3.70	46.36	41.20	48.37	49.44	48.78
72	---	50.40	44.07	58.83	53.76	53.04
96	---	51.52	45.15	61.66	55.42	54.36
120	---	52.08	45.75	62.66	56.26	54.99
144	---	52.51	46.12	63.14	56.70	55.21
168	---	53.06	46.58	63.60	57.12	55.45
Sum	89.53	91.32	91.85	99.30	92.65	90.59

* one radiolabelled dose after a daily non-labelled dose at 5 mg/kg bw for 14 consecutive days

E. Metabolism

[Phenyl-UL-^{14}C]fluopyram was extensively metabolised. In total 29 metabolites and the parent compound were identified in urine, faeces, and bile (

Table 5.1.1- 8). The total identification rate in all tests was between approximately 74% and 87% of the administered dose. The biotransformation pathway of male and female rats was qualitatively identical, but revealed sex related differences in the quantity of metabolites.

The proposed biotransformation pathway of fluopyram in the rat is shown in Figure 5.1.1- 1.. The unchanged parent compound was found only in very low amounts (<2% of the dose) in faeces of the low dose tests. In high dose tests, the parent compound amounted to 10.5% of the administered dose in male and to 16.7% in female rats, respectively. The ethyl linking group of the molecule was the main site for metabolisation. The principal metabolic transformation was the molecular cleavage of the ethyl linking group yielding fluopyram-benzamide (M25), which was subsequently hydrolysed to fluopyram-benzoic acid (M33), or hydroxylated and conjugated to form various fluopyram-benzamide and fluopyram-hydroxybenzamide conjugates. fluopyram-benzamide and fluopyram-benzoic acid were major metabolites representing up to 24.49% and 6.65% of the dose administered, respectively.

Prior to the cleavage of the ethyl moiety, was the hydroxylation of the ethyl linking group and the phenyl ring followed by further hydroxylation. Various hydroxylated metabolites conjugated mostly with glucuronic acid and to a lower extent sulphate were identified. Conjugation of the phenyl ring moiety with glutathione followed by further degradation gave rise to fluopyram-7-OH-methyl-sulfone (M16), fluopyram-BA-methyl-sulfoxide (M29) (BA = benzamide) and fluopyram-BA-methyl-sulfone (M30). Further degradation of the phenyl ring was less prominent. Metabolites containing both intact ring moieties of the parent compound accounted in total for up to 72.05% of the given dose and were found in all matrices. Of these, the predominant metabolites were isomer 1 of fluopyram-7-OH-GA (M09) (up to 20.20% of the administered radioactivity), and isomer 2 of fluopyram-enol-GA (M04) (up to 19.95% of the administered radioactivity), both mostly found in bile.

All metabolites present in the total excreta at ≥ 5% of the administered dose and many other metabolites representing < 5% of the administered dose were identified.

Table 5.1.1- 8: Balance of [phenyl-UL-^{14}C]fluopyram and metabolites excreted expressed as % dose administered

Test no.	**Test 1**			**Test 2**			**Test 3**		
Dose, route	**5 mg/kg bw, p.o.**			**5 mg/kg bw, p.o.**			**5 mg/kg bw, p.o.**		
Experiment	**Bile duct cannulation**			**single low dose, EPA**			**single low dose**		
Duration, sex	**48 h, male**			**168 h, male**			**168 h, female**		
Excreta	**Bile**	**Urine**	**Total**	**Urine**	**Faeces**	**Total**	**Urine**	**Faeces**	**Total**
Sampling (h)	**0 – 48**	**0 – 48**	**0 – 48**	**0 – 168**	**0 – 72**	**0 – 72/168**	**0 – 168**	**0 – 72**	**0 – 72/168**
parent compound	**---**	**---**	**---**	**---**	**0.80**	**0.80**	**---**	**1.16**	**1.16**
Common metabolites	67.81	4.23	72.05	10.48	28.08	38.57	8.77	19.30	28.06
fluopyram-methoxy-di-OH-GA (M23)	0.39	---	0.39	0.29	---	0.29	0.18	---	0.18
fluopyram-7-OH-phenol-GA (M14)	2.76	0.17	2.93	4.02	---	4.02	3.33	---	3.33
fluopyram-7-OH-GA (M09), isomer 1	19.09	1.10	20.2	1.15	0.72	1.88	1.22	0.31	1.53
fluopyram-7-OH-GA (M09), isomer 2	2.41	0.20	2.61	0.14	---	0.14	0.32	---	0.32
fluopyram-7-OH-hydroxy-phenol-SA (M17)	---	0.06	0.06	0.35	---	0.25	0.24	---	0.24
fluopyram-di-OH-GA (M21)	1.42	0.12	1.55	0.04	---	0.04	0.24	---	0.24
fluopyram-enol-GA (M04), isomer 1	0.59	---	0.59	---	---	0.00	---	---	0.00
fluopyram-7-OH-phenol-SA (M15)	0.40	0.26	0.66	2.76	---	2.76	0.24	---	0.24
fluopyram-phenol-GA (M07)	1.45	0.10	1.55	0.18	---	0.18	0.26	---	0.26
fluopyram-8-OH-GA (M20), isomer 1	3.71	0.08	3.79	0.04	---	0.04	0.57	---	0.57
fluopyram-8-OH-GA (M20), isomer 2	16.36	0.23	16.59	---	---	0.00	1.33	---	1.33
fluopyram-enol-GA (M04), isomer 2	18.16	1.79	19.95	0.89	---	0.89	0.25	---	0.25
fluopyram-7-OH-phenol (M13)	0.84	0.06	0.90	0.57	10.79	11.36	0.22	3.34	3.55
fluopyram-7-OH-methyl-sulfone (M16)	---	---	---	0.10	0.30	0.40	0.37	0.52	0.89
fluopyram-7-hydroxy (M08)	0.23	0.06	0.28	0.04	10.26	10.30	---	7.46	7.46
fluopyram-8-hydroxy (M18)	---	---	---	0.01	6.01	6.02	---	7.67	7.67
Phenyl-label specific metabolites	2.42	2.81	5.24	26.57	8.08	34.65	33.91	9.50	43.40
fluopyram-benzamide-OH-GA (M27), isomer 1	---	---	---	1.08	---	1.08	1.75	---	1.75

Test no.	Test 1			Test 2			Test 3		
Dose, route	5 mg/kg bw, p.o.			5 mg/kg bw, p.o.			5 mg/kg bw, p.o.		
Experiment	Bile duct cannulation			single low dose, EPA			single low dose		
Duration, sex	48 h, male			168 h, male			168 h, female		
Excreta	**Bile**	**Urine**	**Total**	**Urine**	**Faeces**	**Total**	**Urine**	**Faeces**	**Total**
Sampling (h)	**0 – 48**	**0 – 48**	**0 – 48**	**0 – 168**	**0 – 72**	**0 – 72/168**	**0 – 168**	**0 – 72**	**0 – 72/168**
fluopyram-benzoyl-serine (M24)	---	0.21	0.21	0.39	---	0.39	0.44	---	0.44
fluopyram-benzamide-SA (M31), isomer 1	---	---	---	1.50	---	1.50	1.49	---	1.49
fluopyram-benzamide-N,O-GA (M32)	---	0.60	0.60	1.96	0.08	2.04	1.49	0.47	1.96
fluopyram-benzoic acid (M33)	---	0.74	0.74	4.03	0.36	4.39	5.88	0.52	6.40
fluopyram-benzamide-OH-GA (M27), isomer 2	---	---	---	0.11	---	0.11	0.41	---	0.41
fluopyram-benzamide-OH-GA (M27), isomer 3	---	0.12	0.12	1.81	---	1.81	3.12	---	3.12
fluopyram-hydroxy-benzamide (M26)	---	---	---	0.22	---	0.22	---	---	0.00
fluopyram-benzamide-SA (M31), isomer 2	---	---	---	0.57	---	0.57	0.93	---	0.93
fluopyram-benzamide-cysteine (M28)	---	0.07	0.07	1.65	---	1.65	1.99	---	1.99
fluopyram-BA-methyl-sulfone (M30)	---	---	---	2.65	1.52	4.17	2.17	0.78	2.94
fluopyram-BA-methyl-sulfoxide (M29)	---	---	---	0.54	---	0.54	0.45	---	0.45
fluopyram-benzamide (M25)	2.42	1.07	3.5	10.06	6.12	16.18	13.79	7.73	21.52
Total identified	70.24	7.05	77.28	37.05	36.96	74.02	42.70	29.95	72.65
Sum of unknown (largest unknown)	8.30 (1.44)	0.24 (0.13)	8.55 (1.44)	1.20 (0.19)	9.08 (3.86)	10.27 (3.86)	2.57 (0.59)	9.66 (1.77)	12.23 (1.77)
Total characterised	8.30	0.24	8.55	1.20	9.08	10.27	2.57	9.66	12.23
Total identified and characterised	78.54	7.29	85.83	38.25	46.04	84.29	45.27	39.61	84.88
Solids (non-extractable residue)	---	---	---	---	4.36	4.36	---	4.46	4.46
Faeces not analysed	---	---	---	---	2.66	2.66	---	2.51	2.51
Total radioactivity excreted	78.54	7.29	85.83	38.25	53.06	91.31	45.27	46.58	91.85

Values given in *italics* were recalculated based on values of the report.

Table 5.1.1- 8: Balance of [phenyl-UL-14C]fluopyram and metabolites excreted expressed as % dose administered **(continued)**

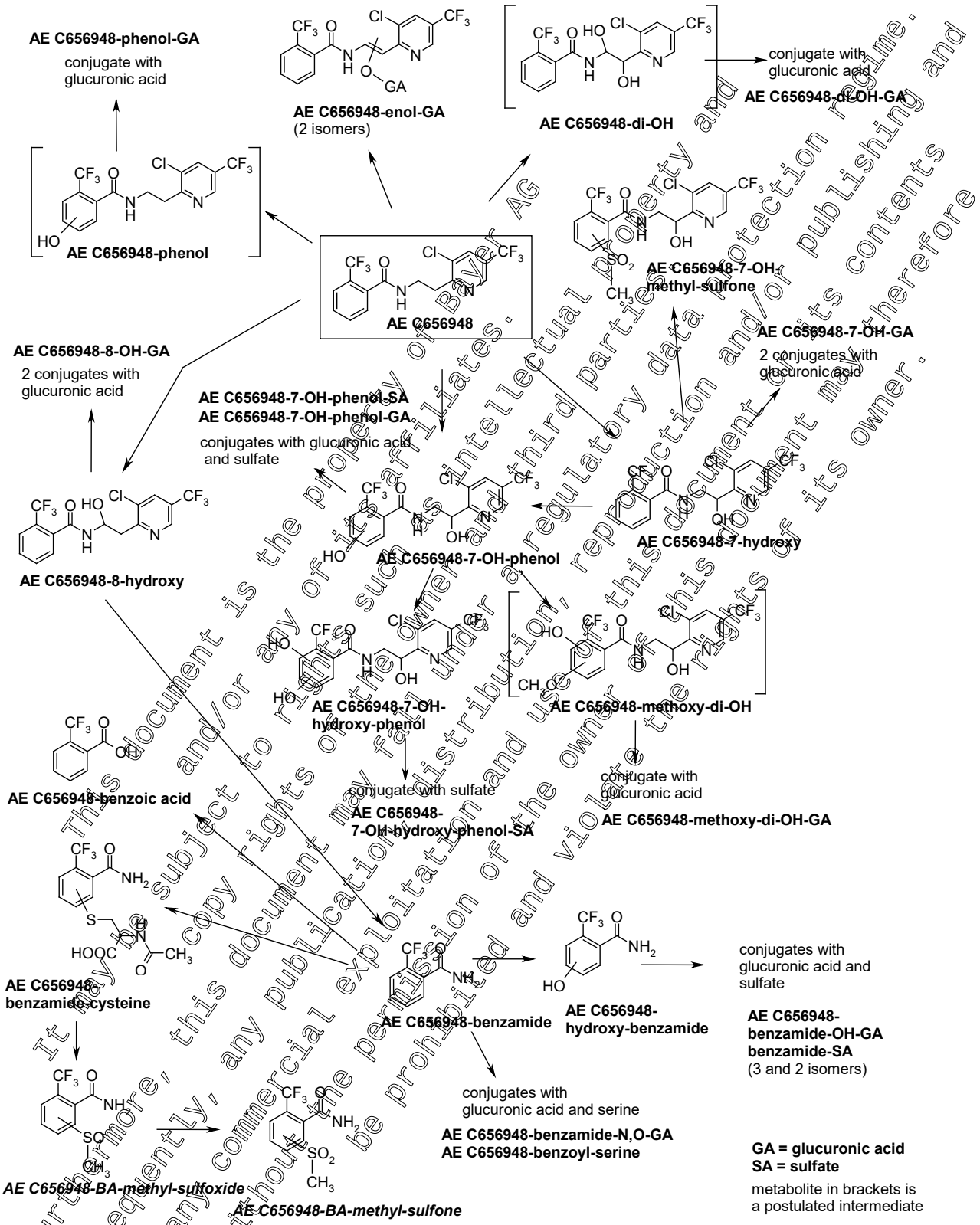
Test no.	**Test 4**			**Test 5**			**Test 6**		
Dose, route	**250 mg/kg bw, p.o.**			**250 mg/kg bw, p.o.**			**5 mg/kg bw, p.o.**		
Experiment	**single high dose**			**single high dose**			**multiple low dose**		
Duration, sex	**168 h, male**			**168 h, female**			**168 h, male**		
Excreta	**Urine**	**Faeces**	**Total**	**Urine**	**Faeces**	**Total**	**Urine**	**Faeces**	**Total**
Sampling (h)	**0 – 168**	**0 – 72**	**0 – 72/168**	**0 – 168**	**0 – 72**	**0 – 72/168**	**0 – 168**	**0 – 72**	**0 – 72/168**
parent compound	**---**	**10.52**	**10.52**	**---**	**16.70**	**16.70**	**---**	**0.41**	**0.41**
Common metabolites	7.35	28.96	36.31	10.14	20.79	30.93	6.89	28.71	35.60
fluopyram-methoxy-di-OH-GA (M23)	0.15	---	0.15	---	---	0.00	---	---	0.00
fluopyram-7-OH-phenol-GA (M14)	0.93	---	0.93	1.33	---	1.33	3.20	---	3.20
fluopyram-7-OH-GA (M09), isomer 1	2.16	---	2.16	2.34	---	2.34	0.79	1.21	2.00
fluopyram-7-OH-GA (M09), isomer 2	0.44	---	0.44	0.31	---	0.31	---	---	0.00
fluopyram-7-OH-hydroxy-phenol-SA (M17)	0.05	---	0.05	0.20	---	0.20	---	---	0.00
fluopyram-di-OH-GA (M21)	0.62	---	0.62	0.80	---	0.80	---	---	0.00
fluopyram-enol-GA (M04), isomer 1	---	---	0.00	---	---	0.00	---	---	0.00
fluopyram-7-OH-phenol-SA (M15)	0.62	---	0.62	0.15	---	0.15	1.27	---	1.27
fluopyram-phenol-GA (M07)	---	---	0.00	---	---	0.00	---	---	0.00
fluopyram-8-OH-GA (M20), isomer 1	0.24	---	0.24	0.84	---	0.84	0.24	---	0.24
fluopyram-8-OH-GA (M20), isomer 2	---	---	0.00	3.37	---	3.37	---	0.71	0.71
fluopyram-enol-GA (M04), isomer 2	1.91	---	1.91	0.59	---	0.59	1.23	---	1.23
fluopyram-7-OH-phenol (M13)	0.13	1.69	1.82	---	0.90	0.90	---	7.84	7.84
fluopyram-7-OH-methyl-sulfone (M16)	0.10	1.08	1.18	0.21	0.57	0.78	0.16	0.56	0.72
fluopyram-7-hydroxy (M08)	---	15.82	15.82	---	8.07	8.07	---	14.33	14.33
fluopyram-8-hydroxy (M18)	---	10.37	10.37	---	11.25	11.25	---	4.06	4.06
Phenyl-label specific metabolites	26.98	12.70	39.68	24.18	13.34	37.53	26.45	14.55	41.01
fluopyram-benzamide-OH-GA (M27), isomer 1	0.32	---	0.32	0.61	---	0.61	1.11	---	1.11

Test no.	Test 4			Test 5			Test 6		
Dose, route	250 mg/kg bw, p.o.			250 mg/kg bw, p.o.			5 mg/kg bw, p.o.		
Experiment	single high dose			single high dose			multiple low dose*		
Duration, sex	168 h, male			168 h, female			168 h, male		
Excreta	**Urine**	**Faeces**	**Total**	**Urine**	**Faeces**	**Total**	**Urine**	**Faeces**	**Total**
Sampling (h)	**0 – 168**	**0 – 72**	**0 – 72/168**	**0 – 168**	**0 – 72**	**0 – 72/168**	**0 – 168**	**0 – 72**	**0 – 72/168**
fluopyram-benzoyl-serine (M24)	0.20	---	0.20	0.06	---	0.06	0.37	---	0.37
fluopyram-benzamide-SA (M31), isomer 1	1.01	---	1.01	0.71	---	0.71	1.51	---	1.51
fluopyram-benzamide-N,O-GA (M32)	3.72	---	3.72	2.78	0.17	2.95	1.74	0.46	2.21
fluopyram-benzoic acid (M33)	5.96	0.69	6.65	4.49	0.60	5.09	4.34	1.09	5.43
fluopyram-benzamide-OH-GA (M27), isomer 2	0.18	---	0.18	0.22	---	0.22	0.27	---	0.27
fluopyram-benzamide-OH-GA (M27), isomer 3	---	---	0.00	0.08	---	0.08	---	---	0.00
fluopyram-hydroxy-benzamide (M26)	---	---	0.00	---	---	0.00	---	---	0.00
fluopyram-benzamide-SA (M31), isomer 2	0.74	---	0.74	0.69	---	0.69	0.69	---	0.69
fluopyram-benzamide-cysteine (M28)	1.28	---	1.28	1.03	---	1.03	1.52	---	1.52
fluopyram-BA-methyl-sulfone (M30)	1.32	0.37	1.69	0.92	0.55	1.47	1.85	1.46	3.31
fluopyram-BA-methyl-sulfoxide (M29)	---	---	0.00	0.13	---	0.13	0.51	---	0.51
fluopyram-benzamide (M25)	12.25	11.64	23.89	12.46	12.02	24.49	12.54	11.54	24.08
Total identified	34.36	52.18	86.53	34.32	50.84	85.17	33.32	43.69	77.00
Sum of unknown (largest unknown)	1.35 (0.34)	4.36 (0.64)	5.71 (0.70)	1.21 (0.59)	1.32 (0.74)	2.52 (0.74)	1.82 (1.09)	6.27 (2.86)	8.10 (2.86)
Total characterised	1.35	4.36	5.71	1.21	1.32	2.52	1.82	6.27	8.10
Total identified and characterised	35.70	56.54	92.24	35.53	52.16	87.69	35.14	49.96	85.10
Solids (non-extractable residue)	---	2.29	2.29	---	1.60	1.60	---	3.08	3.08
Faeces not analysed	---	4.77	4.77	---	3.36	3.36	---	2.41	2.41
Total radioactivity excreted	35.70	63.60	99.30	35.53	57.12	92.65	35.14	55.45	90.59

* one radiolabelled dose after a daily non-labelled dose at 5 mg/kg bw for 14 consecutive days

Values given in *italics* were recalculated based on values of the report.

Figure 5.1.1- 1: **Proposed metabolic pathway of [phenyl-UL-^{14}C]fluopyram in the rat**



III. Conclusions

The kinetic and metabolic behaviour of [phenyl-UL-^{14}C]fluopyram in male and female rats after low and high oral dosage can be characterised by the following observations:

- The administered dose was rapidly and nearly quantitatively absorbed, shown by short half-lives of absorption ($t_{1/2}$ abs ≤ 0.5 h) and the high rate of biliary excretion in the bile-duct cannulation experiment.
- Slight sex difference was observed in the ratio of renal to faecal excretion in low dose tests, where females excreted slightly more of the radioactivity via urine than via faeces. The ratio of the renal and faecal excretion in the others test were comparable (approximately 40% *via* urine and approximately 60% *via* faeces, respectively).
- The oral absorption was at least approximately 94% of the administered dose.
- Plasma peak levels were observed within 0.8 – 41.9 hours after dosing. From peak levels, the radioactivity concentrations in plasma declined continuously. fluopyram and its metabolites were mostly eliminated from the body. The calculated first elimination half-lives ranged from 3.9 – 16.2 hours and the second elimination half-lives ranged from 23.6 – 53.0 hours. Slightly oscillating time course of the plasma radioactivity in female rats of the high dose group indicated that the radioactivity was at least partially subjected to an enterohepatic circulation.
- The excretion was mostly complete at 168 hours post administration. At sacrifice, the radioactivity remaining in the total body accounted only for 2.9% – 5.9% of the administered radioactivity after 168 hours and 10.9% in the bodies of the bile-duct cannulated rats after 48 hours. The highest concentrations were detected in the organs and tissues responsible for metabolism and excretion: in liver, kidney, but also adrenal gland. The main route of excretion was biliary and correspondingly faecal. At 48 hours post administration 78.5% of the dose was excreted via bile. In the tests with the intact rats 46.6% to 63.6% of the administered radioactivity was excreted *via* faeces, while 35.1% to 45.3% of the administered radioactivity was excreted *via* urine.
- [Phenyl-UL-^{14}C]fluopyram was extensively metabolised in rat. In total 29 metabolites and the parent compound were identified in urine, faeces, and bile. All metabolites present in the total excreta at ≥ 5% of the administered dose were identified as well as many other metabolites representing < 5% of the administered dose. The total identification rate in all tests was between *ca.* 74 and 87% of the administered dose.
- The unchanged parent compound was found at *ca.* 0.4 – 16.7% of the administered radioactivity.
- The principal metabolic route involved the molecular cleavage of the ethyl linking group yielding fluopyram-benzamide, which was subsequently hydrolysed, hydroxylated and conjugated to fluopyram-benzoic acid and various fluopyram-benzamide and fluopyram-hydroxybenzamide conjugates.
- Hydroxylation of the ethyl linking group and the phenyl ring followed by further hydroxylation and conjugation preceded the cleavage of the ethyl linking group. Various hydroxylated metabolites conjugated mostly with glucuronic acid and to a lower extent sulphate were identified.
- Major metabolites were isomer 1 of fluopyram-7-OH-GA (M09), isomer 2 of fluopyram-enol-GA (M04), fluopyram-benzamide (M25) and fluopyram-benzoic acid (M33).

Assessment and conclusion by applicant:

The study is valid and acceptable.

Data Point:	KCA 5.1.1/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	[Pyridyl-2,6-14C]AE C656948: Absorption, distribution, excretion and metabolism in the rat
Report No:	MEF-07/486
Document No:	M-298924-01-1
Guideline(s) followed in study:	US EPA OPPTS 870.7485; EU 91/414/EEC amended by 94/79/EC; Canadian PMRA Ref.: DACO 4.5.9; OECD 417; Japanese MAFF, 12 Nousan 8147
Deviations from current test guideline:	None
Previous evaluation:	Yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The toxicokinetic behaviour (absorption, distribution, excretion) and metabolism of fluopyram (AE C656948) were investigated in male and female Wistar rats. The test item was labelled with ^{14}C in position 2 and 6 of the pyridyl ring of the molecule.

[Pyridyl-2,6-^{14}C]fluopyram was administered orally to three groups of four male (test 1), six male (test 2; bile fistulation experiment) and four female rats (test 3), respectively, at a dose rate of 5 mg/kg.

Animals were sacrificed seven days (168 h) after dosing in test 1 and test 3, and two days(48 h) after dosing in the bile fistulation experiment (test 2). The total radioactivity that included the test item and metabolites was determined in bile, urine, faeces as well as in plasma samples, organs and tissues at sacrifice. The metabolism was investigated by radio-HPLC and spectroscopic methods in selected bile and urine samples, and faeces extracts.

Between 98.8% and 100.3% of the administered dose were recovered from measurement of the total radioactivity in plasma samples, bile, urine, faeces as well as in organs and tissues at sacrifice. fluopyram was very rapidly absorbed from the gastrointestinal tract of male and female rats in all test groups. The absorption commenced immediately after oral dosing as shown by the plasma curves and the values calculated for the absorption half-lives (0.3–0.4 hours).

The absorption rate of [pyridyl-2,6-^{14}C]fluopyram was calculated from bile, urine and body excluding GIT and accounted for 97.7% of the recovered dose for males rats (test 2) and led to the conclusion that the administered dose was absorbed completely and systemically bioavailable.

The maximum of the plasma concentration of radioactivity was reached approx. 0.7 h after dosage in male rats (t_{max}). The absorption time in female rats was higher and the plasma maximum reached at approx. 3.3 hours. The maximum equivalent concentrations (C_{max}) were in a similar range for males and females and amounted to approx. 1.8 and 1.5 µg/g.

The plasma concentration in males and females declined to approx. 1% of the maximum concentration within 120 hours post administration, indicating that no retention of the compound related residues in the body of the animals took place.

The level of the plasma curve of females was slightly higher than for males. The plasma curves of both genders revealed slight oscillation in the declining part of the curve during the elimination phase indicating enterohepatic circulation between small intestine and liver.

The values for the initial elimination phase ($t_{1/2\ elim\ 1}$) were about 11 hours for males and 10 hours for females, followed by a slower terminal elimination phase ($t_{1/2\ elim\ 2}$) of about 56 and 73 hours for males and females, respectively. The area under the curves ($AUC_{(0-\infty)}$) indicated a slightly higher systemic exposure for females (37 µg/g x h) than for males (22 µg/g x h).

The mean residence time (MRT_{tot}) of fluopyram-related radioactivity was short for both genders, ranging from about 29 to 33 hours.

Excretion was almost completed 72 h after administration. At this time, males and females had excreted more than 98% of the administered dose *via* urine and faeces. Significant sex differences were observed in the ratio of renal to faecal excretion.

Excretion in males was slightly higher by the faecal than by the renal route of excretion (approx. 53% faecal and approx. 45% renal of the administered dose). In females, renal excretion was higher than the faecal one (approx. 39% faecal and approx. 60% renal excretion of the administered dose). Renal excretion in females was faster and the values in the individual sampling intervals higher than in males. On the other hand, faecal excretion and the values in the individual sampling intervals were higher in males than in females. The total excretion via urine and faeces was comparable in the individual sampling intervals of both genders.

Bile duct-cannulated male rats showed a total excretion of 99.5% of the administered dose. The main radioactivity, 86.8% of the administered dose, was eliminated via bile and further 10.4% via urine. Already about 86% of the administered dose were excreted via the bile within the first 24 hours post administration.

The high biliary and low renal excretion in the bile fistulation experiment (test 2) compared to the high renal excretion in the low dose experiment (test 1) was a further indication for enterohepatic circulation.

The residues in most of the organs and tissues of male and females rats were low (<0.1% of the dose).

A slightly sex related difference was observed for the residues in perirenal fat, where females showed about 3 times higher residues than males (0.01 µg/g and 0.03 µg/g).

Fluopyram was extensively metabolised and 23 metabolites identified. The identification rates in all tests were high and accounted for about 80–83% of the administered dose. The unchanged parent compound was of minor occurence and represented only 1.4–1.9% of the administered dose in faeces of male and female rats.

Molecular cleavage in the range of about 42–48% of the administered dose was observed in both genders represented by numerous label specific metabolites (tests 1 and 3). The amount of label specific metabolites in the bile fistulation experiment (test 2) was distinctively lower and amounted to 12% of the administered dose.

It is assumed that the three main metabolites in bile (fluopyram-7-OH-GA (M09), fluopyram-8-OH-GA (M20) and fluopyram-enol-GA (M04)) were formed during the first pass of the parent compound in the liver. Following enterohepatic circulation, the conjugates were cleaved in the small intestine and

their aglyca partly excreted *via* faeces. The aglyca were also partly re-absorbed and further metabolised by cleavage of the molecule.

These metabolites were mainly excreted via urine.

A sex difference in the ratio of label specific metabolites was observed since the amount of fluopyram-pyridyl-acetic acid (M40) was about three times higher in female than in male rats, and the amounts of fluopyram-7-hydroxy (M08) and fluopyram-7-OH-phenol (M13) two times lower in females than in males. Fluopyram-ethyl-diol metabolite and its GA conjugate (M38 and M39) were about four times higher in males than in females.

It is assumed that the proportion of the various common hydroxylated and conjugated metabolites formed during the first pass effect of the parent compound in the liver was different in males and females. Following enterohepatic circulation, these metabolites were further metabolised and different label specific metabolites obtained. The degree and the type of the label specific metabolites in turn were responsible for the differences in the excretion behaviour of males and females.

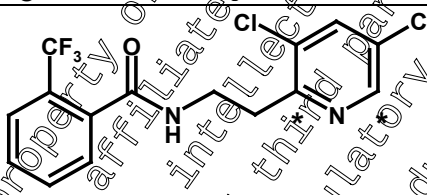
The ethyl linking group of the molecule was the preferred site for metabolism. The metabolic transformations detected were:

- Hydroxylation of the ethyl linking group of the parent compound forming fluopyram-7-hydroxy (M08) and 8-hydroxy (M18) metabolites.
- Further oxidation of fluopyram-7-hydroxy (M08) and 8-hydroxy (M18) metabolites leading to the fluopyram-enol metabolite (M04).
- Hydroxylation of the phenyl ring giving fluopyram-7-OH-phenol (M13).
- Conjugation of the hydroxylated metabolites mainly with glucuronic acid and to a lower extend with sulfate.
- Hydrolytic cleavage and subsequent oxidation to mainly fluopyram-pyridyl-acetic acid (M40), fluopyram-pyridyl-carboxylic acid (M43) and fluopyram-ethyl-diol (M38) and conjugates with GA (M39).
- Further metabolic transformation of the phenyl ring was less prominent (reaction with methoxy and methylsulfone).

I. Materials and methods

A. Materials

1. Test Material

IUPAC name	N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)- (9CI)
Code name	AE C656948
Common name	Fluopyram
Empirical formula	$C_{16}H_{11}ClF_6N_2O$
CAS no.:	658066-35-4 (non-labelled AE C656948)
Molar mass	396.72 g/mol
Chemical structure:	 * position of radiolabel
Radiolabelled test material	[Pyridyl-2,6-^{14}C]AE C656948
Batch no.:	SEL/1775
Specific radioactivity:	3.85 MBq/mg (104.15 µCi/mg)
Radiochemical purity:	> 99% (HPLC) > 99% (TLC)
Chemical purity:	99.8% and 99.4% identity determined by LC/MS, GC/MS & 1-HNMR
Stability of test compound:	Prior to and following structure elucidation, aliquots of all isolated metabolites were analysed with profiling method AE948-33 to prove their stability during preparation and storage.

2. Vehicle:

0.5% aqueous Tragacanth

3. Test animals

Species:	Rat, male and female rats derived from *Rattus norvegicus domesticus*
Strain:	Wistar Hsd/Cpb: WU
Age:	7–8 weeks (male rats) and 8–10 weeks (female rats) at the time of delivery
Weight at dosing:	Approximately 200 g at study initiation
Source:	[redacted]
Acclimation period:	The animals were acclimated to the laboratory conditions in Makrolon cages on wood shavings in the test facility for about 7 days prior to the administration.
Identification:	The animals were identified by cage cards listing the study number, test item name and individual animal number. They were additionally labelled with water-insoluble spots on the tail.
Diet:	The rats were fed *ad libitum* with rat/mice maintenance long life diet (no. 3883.0.15), supplied by Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland. The animals received approximately 16 g per animal and day. Prior to the start of the radioactive administration, the rats were fed about 16 hrs before dosing and again approx. 6 hours after dosing.
Water:	Tap water from the local mains supply was given *ad libitum* (water specification in accordance to the local drinking water regulations). In case of the bile duct cannulation experiment, the animals received after surgery and during the test tap water which was supplied with 10% glucose, 0.5% NaCl and 0.05% KCl.
Housing:	The animals were kept under conventional hygienic conditions in air-conditioned rooms.
Environmental conditions	
Temperature:	20–24 °C
Humidity:	40–68%
Air changes:	10–15 fold air change per hour
Photoperiod:	12 hours light/dark-cycle

4. Preparation of dosing solutions

The stock solution was prepared by dissolving the test item in acetonitrile. For the preparation of each administration suspension an adequate portion of the stock solution was pipetted and evaporated to near dryness. The nearly dry residue was suspended in a 0.5% aqueous Tragacanth solutionin an ultrasonic bath and the suspension stirred overnight on a magnetic stirrer.

B. Study design and methods

All tests were performed according to the current EPA, EU, PMRA, OECD and Japanese MAFF test guidelines for supporting the registration of chemical pesticides.

1. Dose regimen and design of tests

Test no.	Administered single dose of ^{14}C-fluopyram, route (experiment)	Number of rats and sex	Collection of samples during the test and at sacrifice	Duration
1	5 mg/kg bw, oral (single low dose)	4 male	Microplasma, urine and faeces, blood, organs and tissues, GIT, skin and carcass	168 hours
2	5 mg/kg bw, oral (bile duct cannulation)	6 male	Bile, urine and faeces, blood, GIT, skin and carcass	48 hours
3	5 mg/kg bw, oral (single low dose)	4 female	Microplasma, urine and faeces, blood, organs and tissues, GIT, skin and carcass	168 hours

The rats of all test groups received a single dose of fluopyram radiolabelled in the pyridyl ring (pyridyl-2,6-^{14}C).

[Pyridyl-2,6-^{14}C]fluopyram was administered orally to two groups of four male or female rats at a dose of 5 mg/kg bw by oral gavage (test 1 and 3) and to one group of six bile-cannulated male rats by gavage (test 2).

Microplasma, urine and faeces were collected at various times after administration, blood, organs and tissues, GIT, skin and carcass at sacrifice, 168 h after administration for group 1 and 3. Bile, urine and faeces were collected at various times after administration, blood, GIT, skin and carcass at sacrifice, 48 h after administration for group 2.

2. Cholangiostomy

The animals of test 2 were anaesthetised for the cholangiostomy surgery. After shaving the stomach area below the rib cage, a small incision was made. The portion of the small intestine with the pancreatic tissue containing the bile duct was pulled out carefully. Surgical thread was pulled through prior to puncturing the bile duct. Afterwards, the indwelling biliary duodenal catheter was implanted in the bile duct for collection of the bile. The operated zone was relocated into the body by lifting the skin. A small incision was made on the back at the basis of the tail of the animal to pull the bile catheter through. The muscle layer was closed by careful sewing and the use of surgical clamps. The incisions were sealed with allpurpose glue. The rats were placed onto warming pads while regaining consciousness. After coming out of the anaesthesia, they were kept individually in the metabolism cages and the cannula of each animal led through the roof of the cage to permit bile collection into an appropriate weighed container which could be removed and replaced without disturbing the animal. The animals received a diluted analgesic during and after surgery by intramuscular injection.

3. Dosing

Two mL of the administration solution were dosed to each animal of test 1 and test 3, and 1 mL in case of test 2. Oral dosing was performed by oral gavage using a syringe attached to an animal-feeding knob cannula. The concentration of each administration suspension was calculated to reach an administered amount of about the nominal value of the parent compound of 5 mg per kg body weight (bw). Due to different animal weights at administration, the actual dose per kg varied slightly with the body weight.

The administration suspensions were prepared one day before dosing. The purity was checked by radio-HPLC. The amount of radioactivity in each administration suspension was determined by LSC. From these results the radioactivity (dpm, MBq) and the actual dosing rate (mg/kg) applied to each animal in the different tests were calculated.

4. Collection of excreta

After administration of the radiolabelled test item, the rats were kept individually in Makrolon metabolism cages, which allowed for separate and quantitative collection of urine and faeces.

Urine was collected 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 h post administration for test groups 1 and 3. For test group 2, urine was collected 8, 24, 32 and 48 h post administration.

Faeces were collected 24, 48, 72, 96, 120, 144 and 168 h post administration for test group 1 and 3. For test group 2 faeces were collected 24 and 48 h post administration.

For test group 2, bile was collected 4, 8, 24, 32, 48 h post administration, urine was collected 8, 24, 32 and 48 h post administration and faeces were collected 24 and 48 h post administration separately for each animal. The test tubes were cooled by crashed ice. The radioactivity was determined by LSC.

5. Collection of plasma

Blood samples were collected separately for each animal by pressing a capillary coated with heparin in a small cut in the tail vein. For test groups 1 and 3, microplasma was collected 0.17, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 24, 28, 32, 48, 52, 56, 72, 96, 120, 144, 152 and 168 h post administration. The capillaries were centrifuged using a hematocrit centrifuge to separate the plasma from the formed blood constituents (mainly erythrocytes). The radioactivity in the plasma samples was measured by LSC (liquid scintillation counting).

6. Sacrifice

The rats were sacrificed in Pentobarbital-Na anaesthesia by transection of the cervical vessels and exsanguinated.

7. Blood, tissues and organs at sacrifice

After transection of the cervical blood vessels, the oozed out blood was collected into test tubes coated with heparin that was separated afterwards into plasma and erythrocytes by centrifugation. For test

groups 1 and 3, blood, organs and tissues, GIT, skin, carcass were collected at sacrifice, 168 h post administration and 24 and 48 h post administration for test group 2.

The organs and tissues prepared at the end the experiments were weighed immediately after the dissection and again following lyophilisation. Finally, they were homogenised before aliquots were taken for the determination of radioactivity by combustion/LSC. The radioactivity remaining in the GIT, skin, and residual carcass was determined by combustion/LSC in order to establish the radioactivity balance. For the small organs and tissues (e.g. renal fat, uterus, adrenal glands, thyroid gland and ovaries), only the wet weight was determined before they were solubilised and radioassayed by LSC.

8. Sample handling and storage

Freeze dried samples such as organs were stored in plastic vials at room temperature or at approx. +4 °C in a refrigerator. All liquid samples such as urine, bile or with water diluted faeces were kept frozen at ≤ -18°C at all times except during aliquotation for analysis. During the analytical work, the samples were stored either at approx. +4 °C in a refrigerator or at ≤ -18 °C in a freezer.

9. Measurement of radioactivity

The measurement of the radioactivity in liquid samples was carried out by LSC.

Small organs or tissues (adrenal glands, thyroid, ovaries, perirenal fat, and uterus) were solubilised by means of a tissue solubiliser. Portions of these solutions were filled into scintillation vials together with a suitable scintillation cocktail. The radioactivity was then measured by LSC.

All solid samples with the exception of those solubilized were weighed and combusted in an oxygen atmosphere. The released $^{14}CO_2$ was trapped in an alkaline and radio-assayed by LSC.

For all samples, the limit of detection (LOD) was established at approximately 20 dpm measured per aliquot after correction for the background radioactivity. The limit of quantitation (LOQ) for each individual measurement was established as 2 times of the background radioactivity (dpm) of each instrument/method.

Samples with individually measured values below two times of the background radioactivity and for which the average counting efficiency was lower than 50% and the percentage error greater than 10% were not quantified.

10. Toxicokinetic analysis

In this study, the software TOPFIT (version 2.0) was used to calculate the pharmacokinetic parameters by plasma concentration-time curve analysis. The standard 3-compartment model (test 1 and 3) was applied for curve fitting computations. The calculations are based on the average values of plasma concentrations sampled from the rats of the test group.

11. Preparation of urine, faeces and bile for analysis

Pools of urine samples were prepared to represent the following excretion intervals:

0–4, 4–8, 8–12, 12–24, 24–48 and 48–72 for test 1 (72–168 h not analysed)

additionally 0–72 h for test 1

0–24 h for test 2 (24–48 h not analysed)

0–72 h for test 3 (72–168 h not analysed)

Aliquots of these pools were analysed by HPLC without sample preparation.

Bile samples from 0–48 h of test 2 were combined and aliquots analysed by without sample preparation.

Pools of faeces samples were prepared to represent the following excretion intervals:

0–24 and 24–48 for test 1 (48–168 h not analysed)

0–48 hours for test 3 (48–168 h not analysed).

Aliquots of the respective faeces pool were successively extracted six times using 1x acetonitrile and 1x acetonitrile/water (8/2; v/v) (=neutral extracts), 2x acetonitrile/water/formic acid (80/18/2; v/v/v) (= acidic extracts) and 2 x acetonitrile/water/ammonia solution (2/1/1; v/v/v) (= alkaline extracts).

For each extraction step, the sample was mixed with the respective solvent and 50 µL defoamer, and extracted by maceration with a Polytron or Ultraturrax homogeniser. The sample was then centrifuged, the supernatant decanted and the residue extracted again. The volume of each extract was determined and aliquots radioassayed by LSC.

The post extraction solids were air-dried and aliquots radioassayed by LSC following combustion. Acidic and alkaline extracts were each evaporated to the aqueous remainder and adjusted to pH 7. All extracts were adjusted to 400 mL with acetonitrile. 200 mL of each extract were taken and combined. The combined extract was evaporated to the aqueous remainder. The volume of the concentrate was measured and aliquots radioassayed by LSC. The distillate was trapped, its volume measured and aliquots radioassayed by LSC. The distillate was discarded because it contained only negligible radioactivity. The concentrates were subjected to HPLC analysis with the profiling method AE948-33.

12. Analytical methodology

Urine, faeces and bile were analysed for parent compound and metabolites by HPLC with radioactivity detection, with MS and with LC-NMR-MS methods for structure elucidation.

13. High permormance liquid chromatography

The metabolic profiles in bile, urine and faeces extracts, and the purity of the stock solution and the administration suspensions were analysed by reversed phase radio-HPLC with a neutral acetonitrile/methanol/water gradient.

This method was also used for the identification of metabolites by co-chromatography, and for the isolation and purification of metabolites. For HPLC co-chromatography, the sample was mixed with the radiolabelled or non-radiolabelled reference item before injection. Radiolabelled reference items were detected by radiodetection and the non-radiolabelled ones with UV-detection at 270 nm.

Chromatographic matching was assessed by comparison of the ^{14}C- or the ^{14}C- and the UV-chromatograms of the sample with and without the reference items.

14. Mass spectrometry

The electro-spray ionisation MS spectra (ESI) were obtained either with a TSQ 7000 instrument or with a LTQ mass spectrometer. The FT-MS spectra were obtained with an APEX III 70e Fourier Transform Mass Spectrometer. The HPLC instrument used for chromatography was an Agilent HP1100. The flow from the HPLC column was split between UV-detector followed by a radioactivity detector and MS spectrometer.

15. LC-NMR-MS spectroscopy

The 600 MHz NMR-spectra were recorded on a BRUKER AV 600 instrument. The HPLC instrument used for chromatography was an Agilent HP1100. After the HPLC column the flow was split between the UV-detector followed by an SPE unit and an Esquire 3000 plus mass spectrometer.

16. Identification/characterisation and quantification

Urine and bile pools and extracts of faeces pools were analysed by HPLC and compared among each other and among the different matrices, and metabolites assigned. Metabolites from bile and faeces extracts were identified by HPLC co-chromatography with isolated and identified metabolites from urine and with reference items.

Metabolites in urine were isolated, purified and identified spectroscopically. Additionally, metabolites were identified by HPLC co-chromatography with isolated and identified metabolites and with reference items.

For isolation of metabolites in urine, an aliquot of urine pool 0–72 h of test 1 was freeze-dried and the dry residue mixed with acetonitrile. The sample was sonificated, mixed with methanol and sonificated again. The sample was filtered, mixed with water and evaporated to the aqueous remainder. An aliquot was analysed by HPLC and the metabolic profile compared to the respective native urine. The concentrated urine sample was separated by HPLC. The metabolites were collected and fractions containing the same metabolite combined and concentrated. The metabolites fractions were purified by re-injection and separation with the acidic reversed phase method. The metabolites were collected again, concentrated and subjected to structure elucidation.

Two metabolites (BN178103 and BN178010) were collected from urine fractionation, but not purified with the acidic reversed phase method AE948-45. These metabolites were purified and subjected to structure elucidation.

Prior to and following structure elucidation, aliquots of all isolated metabolites were analysed to assign them in the urine profile and to prove their stability during preparation and storage.

II. Results and discussion

A. Recovery

The radioactivity balances of all tests were between 98.8% and 100.3% of the administered dose were recovered from measurement of the total radioactivity in urine, bile and faeces as well as in organs and tissues at sacrifice (Table 5.1.1-9 and Table 5.1.1-10).

Table 5.1.1-9: Tests 1 to 3: Balance of radioactivity in excreta and tissues of rats after oral administration of [pyridyl-2,6-^{14}C]AE C656948 expressed as % of dose administered

Test n°	Test 1	Test 2	Test 3
Dose	5 mg/kg bw	5 mg/kg bw	5 mg/kg bw
Experiment	Single low dose	Bile fistulation experiment	Single low dose
Duration (h)	168	48	168
Gender	Male	Male	Female
Radioactivity in % of dose administered (mean values)			
Urine	45.44	10.40	60.44
Bile	-	86.81	-
Faeces	53.04	2.30	39.49
Total excreted	98.48	99.51	99.93
Skin	0.049	0.061	0.043
Sum organs	0.298	0.393	0.263
Body excluding GIT	0.342	0.454	0.306
GIT	0.020	0.047	0.026
Total in body	0.361	0.501	0.332
Balance	98.84	100.0	100.3

Table 5.1.1-10: Tests 1 to 3: Balance of radioactivity in excreta and tissues of rats after oral administration of [pyridyl-2,6-^{14}C]AE C656948, expressed as % of total radioactivity recovered

n°	Test 1	Test 2	Test 3
Dose	5 mg/kg bw	5 mg/kg bw	5 mg/kg bw
Experiment	Single low dose	Bile fistulation experiment	Single low dose
Duration (h)	168	48	168
Gender	Male	Male	Female
Radioactivity in % of total radioactivity recovered (mean values)			
Urine	45.96	10.37	60.27
Bile		86.86	-
Faeces	53.67	2.28	39.40
Total excreted	99.63	99.51	99.67
Skin	0.050	0.061	0.043
Sum organs	0.296	0.393	0.262
Body excluding GIT	0.346	0.453	0.306
GIT	0.020	0.047	0.026
Total in body	0.366	0.501	0.331
Norm.-factor	1.012	1.001	0.998
Absorption rate (sum of urine, bile	-	97.68	-

and body excluding GIT)			

B. Absorption

fluopyram was very rapidly absorbed from the gastrointestinal tract of male and female rats in all test groups. The absorption commenced immediately after oral dosing. The values calculated for the absorption half-lives (0.3–0.4 hours, see Table 5.1.1-12).

The absorption of the compound related radioactivity from the gastrointestinal tract and distribution into the different organs and tissues was investigated by measuring the radioactivity concentrations in the plasma. The mean equivalent concentrations (C) and dose-normalised concentrations (CN) of all tests are shown in Table 5.1.1-11.

The absorption rate of [pyridyl-2,6-^{14}C]fluopyram was calculated from bile, urine and body excluding GIT and accounted for 97.7 % of the recovered dose for males rats (test 2, see Table 5.1.1-10) and led to the conclusion that the administered dose was absorbed completely and systemically bioavailable.

Table 5.1.1-11: Tests 1 and 3: Time course of ^{14}C-concentration in the plasma of rats after oral administration of [pyridyl-2,6-^{14}C]fluopyram

Equivalent concentration C [µg/g]			Dose normalized Concentrations CN		
Test n°	Test 1	Test 3		Test 1	Test 3
Dose	5 mg/kg bw	5 mg/kg bw		5 mg/kg bw	5 mg/kg bw
Experiment	Single low dose	Single low dose		Single low dose	Single low dose
Duration (h)	168	168		168	168
Gender	Male	Female		Male	Female
Time [h post admin.]			Time [h post admin.]		
0.16	0.441	0.193	0.16	0.088	0.041
0.33	1.195	0.518	0.33	0.240	0.109
0.66	1.781	0.894	0.66	0.358	0.188
1	1.759	1.147	1	0.354	0.242
1.5	1.446	1.277	1.5	0.291	0.269
2	1.075	1.420	2	0.216	0.299
3	0.772	1.499	3	0.155	0.316
4	0.796	1.385	4	0.16	0.292
6	0.775	1.332	6	0.156	0.281
8	0.770	1.421	8	0.155	0.300
24	0.255	0.538	24	0.051	0.113
28	0.210	0.458	28	0.042	0.096
32	0.206	0.413	32	0.041	0.087
48	0.086	0.139	48	0.017	0.029
52	0.072	0.111	52	0.015	0.023
56	0.067	0.098	56	0.014	0.021
72	0.042	0.043	72	0.009	0.009
96	0.025	0.023	96	0.005	0.005
120	0.017	0.015	120	0.004	0.003
144	0.012	0.012	144	0.002	0.003
152	0.012	0.012	152	0.002	0.003
168	0.009	0.009	168	0.002	0.002

C. Distribution

After a single oral administration of 5 mg/kg bw [pyridyl-2,6-^{14}C]fluopyram to male and to female rats (low dose), the maximum of the plasma concentration of radioactivity female rats was higher and the plasma maximum reached at approximately 3.3 h.

The maximum equivalent concentrations (C_{max}) were in a similar range for males and females and amounted to approx. 1.8 and 1.5 µg/g.

The plasma concentration in males and females declined to approx. 1% of the maximum concentration within 120 hours post administration, indicating that no retention of the compound related residues in the body of the animals took place. Raw data are presented in Table 5.1.1-11. Values calculated are presented in Table 5.1.1-12.

The plasma curves of both genders showed a very fast elimination phase at the beginning of the test followed by a slower terminal elimination phase. The level of the plasma curve of females was slightly higher than for males. The plasma curves of both genders revealed slight oscillation of the declining part of the curve during the elimination phase indicating enterohepatic circulation between small intestine and liver.

The values for the initial elimination phase ($t_{1/2\ elim\ 1}$) were about 11 hours for males and 10 hours for females, followed by a slower terminal elimination phase ($t_{1/2\ elim\ 2}$) of about 56 and 73 hours for males and females, respectively. The area under the curves ($AUC_{(0-\infty)}$) indicated a slightly higher systemic exposure for females (37 µg/g x h) than for males (22 µg/g x h).

The mean residence time (MRT_{tot}) of fluopyram-related radioactivity was short for both genders, ranging from about 29 to 33 hours.

Table 5.1.1-12: Tests 1 and 3: Pharmacokinetic parameters following oral administration of [pyridyl-2,6-^{14}C]fluopyram derived from three-compartment plasma curve

Test n°	**Test 1**	**Test 3**
Dose	**5 mg/kg bw**	**5 mg/kg bw**
Experiment	**Single low dose**	**Single low dose**
Duration (h)	**168**	**168**
Gender	**Male**	**Female**
t_{max} [h] measured	0.7	3.0
t_{max} [h] calculated	0.7	3.3
C_{max} [µg/mL] measured	1.78	1.50
C_{max} [µg/mL] calculated	1.79	1.43
$t_{1/2\ abs}$ [h]	0.3	0.4
$t_{1/2\ elim\ 1}$ [h]	11.2	9.8
$t_{1/2\ elim\ 2}$ [h]	55.9	72.9
$AUC_{0-\infty}$ [mg/L*h]	22	37
MRT_{tot} [h]	32.9	29.1
MRT_{abs} [h]	0.6	7.3
MRT_{disp} [h]	32.3	21.8

Exponential analysis was performed using the average values of plasma concentrations of four animals for the time range of 0 to 168 hours post administration.

D. Excretion

Excretion was almost completed 72 h after administration. At this time, males and females had excreted more than 98% of the administered dose via urine and faeces.

Significant sex differences were observed in the ratio of renal to faecal excretion. Excretion in males was slightly higher by the faecal than by the renal route of excretion (53% faecal and 45% renal of the

administered dose). In females, renal excretion was higher than the faecal one (39% faecal and 60% renal of the administered dose). Renal excretion in females was faster and the values in the individual sampling intervals higher than in males. On the other hand, faecal excretion and the values in the individual sampling intervals were higher in males than in females. The total excretion via urine and faeces was comparable in the individual sampling intervals of both genders.

Bile duct-cannulated male rats showed a total excretion of 99.5% of the administered dose. The main radioactivity, 86.8% of the administered dose, was eliminated via bile and further 10.4% via urine. Already 86% of the administered dose were excreted via the bile within the first 24 hours.

Details of the cumulative excretion of radioactivity are given in Table 5.1.1-13 (% of dose administered) and in Table 5 (% of dose recovered).

The residues of most of the organs and tissues of male and females rats were low (< 0.1% of the dose). Negligible amounts of radioactivity were found in the skin (0.04 – 0.05%) of the administered dose. The radioactivity in liver and GIT was slightly higher (about 0.1% of the administered dose).

A slightly sex related difference was observed for the residues in perirenal fat, where females showed about 3 times higher residues than males (0.01 µg/g and 0.03 µg/g). The highest residues were detected in erythrocytes (about 0.1 µg/g), as well as in liver (about 0.11 µg/g) and in kidney (about 0.05 µg/g)), the responsible organs for degradation and excretion. The concentrations in the other organs and tissues were low and ranged from 0.004 µg/g to 0.02 µg/g.

Table 5.1.1-13: Tests 1 to 3: Cumulative excretion of radioactivity in % of dose administered

Test n°	**Test 1**	**Test 2**	**Test 3**
Dose	**5 mg/kg bw**	**5 mg/kg bw**	**5 mg/kg bw**
Experiment	**Single low dose**	**Bile fistulation experiment**	**Single low dose**
Duration (h)	**168**	**48**	**168**
Gender	**Male**	**Male**	**Female**
Urine			
4	5.46	---	6.25
8	12.16	6.47	16.68
12	17.96	---	---
24	35.44	10.15	46.73
32	---	10.29	---
48	43.77	10.4	57.34
72	44.91	---	59.28
96	45.22	---	59.82
120	45.33	---	60.1
144	45.39	---	60.31
168	45.44	---	60.44
Bile			
4	---	39.04	---
8	---	73.21	---
24	---	86.36	---
32	---	86.68	---
48	---	86.81	---
Faeces			
24	40.59	2.11	25.12
48	50.96	2.30	37.12
72	52.32	---	38.86
96	52.73	---	39.23
120	52.85	---	39.36
144	52.95	---	39.44
168	53.04	---	39.49
Sum excreted	98.48	99.51	99.94

Table 5.1.1-14: Tests 1 to 3: Cumulative excretion of radioactivity in % of total radioactivity recovered

Test n°	Test 1	Test 2	Test 3
Dose	5 mg/kg bw	5 mg/kg bw	5 mg/kg bw
Experiment	Single low dose	Bile fistulation experiment	Single low dose
Duration (h)	168	48	168
Gender	Male	Male	Female
Urine			
4	5.49	---	6.25
8	12.29	6.43	16.68
12	18.16	---	---
24	35.83	10.11	46.73
32	---	10.25	---
48	44.27	10.37	57.36
72	45.43	---	59.31
96	45.75	---	59.85
120	45.88	---	60.12
144	45.91	---	60.34
168	45.96	---	60.47
Bile			
4	---	39.15	---
8	---	73.25	---
24	---	86.4	---
32	---	86.73	---
48	---	86.86	---
Faeces			
24	41.05	2.09	25.13
48	51.57	2.28	37.14
72	52.94	---	38.9
96	53.35	---	39.27
120	53.48	---	39.4
144	53.58	---	39.48
168	53.67	---	39.53
Norm. - factor	1.012	1.001	1.001

E. Metabolism

The identification rates in the three tests were high and accounted for 80.3–83.1% of the administered dose. The parent compound was of minor importance and represented only 1.4–1.9% of the administered dose in the faeces of test 1 and test 3.

The metabolite profiles of urine, bile and faeces were different and 23 metabolites identified in total. The main and prominent identified metabolites were sorted and summarised in Table 5.1.1-15.

Table 5.1.1-15: Main and prominent identified metabolites (sum of urine and faeces)

Test n°	**Test 1**	**Test 2**	**Test 3**
Dose	**5 mg/kg bw**	**5 mg/kg bw**	**5 mg/kg bw**
Experiment	**Single low dose**	**Bile fistulation experiment**	**Single low dose**
Duration (h)	**168**	**48**	**168**
Gender	**Male**	**Male**	**Female**
	% of dose administered		
Common metabolites:	**38.9**	**70.8**	**30.8**
fluopyram-7-hydroxy (M08)	15.7	0.2	7.5
fluopyram-7-OH-GA, 2 isomers (M09)	1.6	27.2	2.1
fluopyram-8-hydroxy (M18)	5.7		8.1
fluopyram-8-OH-GA, 2 isomers (M20)	0.2	16.8	1.5
fluopyram-enol-GA, 2 isomers (M04)	2.1	16.7	0.5
fluopyram-7-OH-phenol (M13)	9.3	1.3	3.9
fluopyram-7-OH-phenol-conjugates (M14 and M15)	2.7	5.2	4.0
fluopyram-7-OH-methyl-sulfone (M16)	0.8	0.1	0.9
Various hydroxy/methoxy-phenol conjugates (M07+M17+M21+M23)	0.8	3.1	2.3
Pyridyl-label specific metabolites:	**42.0**	**12.3**	**47.6**
fluopyram pyridyl-acetic acid (M40)	12.3	5.0	37.8
fluopyram ethyl-diol (M38) conjugates with glucuronic acid (3 isomers M39)	15.8	4.9	4.5
fluopyram-pyridyl-carboxylic acid (M43)	5.0	0.7	0.8
fluopyram hydroxy-PAA (M41)	5.2	1.3	1.6
fluopyram -hHydroxyethyl-GA (M37)	3.7	0.4	2.9

Fluopyram was extensively metabolised and 23 metabolites identified. Molecular cleavage in the range of 42–48% of the administered dose was observed in both genders represented by numerous label specific metabolites. The amount of label specific metabolites in the bile fistulation test was distinctively lower and amounted to 12% of the administered dose.

Three metabolite groups (each two isomers of fluopyram-7-OH-GA (M09), 8-OH-GA (M20) and enol-GA (M04)) were the major metabolites in bile. Following enterohepatic circulation, the conjugates were cleaved and their aglyca partly excreted via faeces and further metabolised by cleavage of the molecules. These metabolites were mainly excreted via urine.

A sex difference in the ratio of label specific metabolites was observed since the amount of fluopyram-pyridyl-acetic acid (M40) was about three times higher in female than in male rats, and the amounts of fluopyram-7-hydroxy (M08) and fluopyram-7-OH-phenol (M13) two times lower in females than in

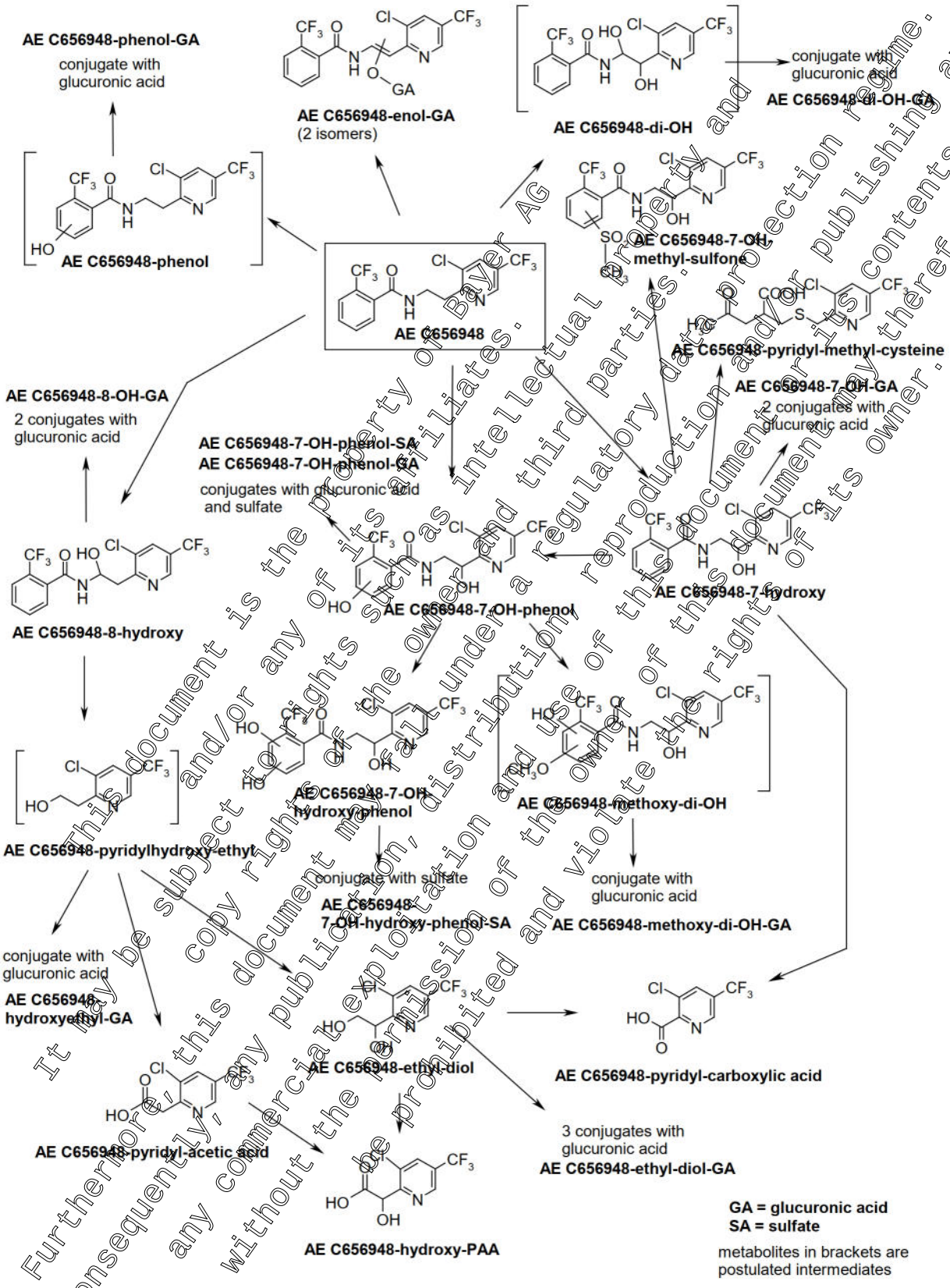
males. fluopyram-ethyl-diol metabolites (M38 and M39) were about four times higher in males than in females.

It is assumed that the proportion of the three metabolite groups (fluopyram-7-OH-GA (M09), 8-OH-GA (M20) and enol-GA (M04) formed during the first pass effect of the parent compound in the liver was different in males and females. Following enterohepatic circulation, these metabolites were degraded and different label specific metabolites obtained. The degree and the type of the label specific metabolites in turn were responsible for the differences in the excretion behaviour of males and females.

The proposed biotransformation pathway of [pyridyl-2,6-^{14}C]fluopyram is presented in Figure 5.1.1-2. The ethyl linking group of the molecule was the preferred site for degradation. The metabolic transformations detected were:

- Hydroxylation of the ethyl linking group of the parent compound forming fluopyram-7-hydroxy (M08) and 8-hydroxy metabolites (M18 and M20).
- Further oxidation of fluopyram-7-hydroxy (M08) and 8-hydroxy metabolites (M18 and M20) leading to the fluopyram-enol metabolite (M04).
- Hydroxylation of the phenyl ring giving fluopyram-7-OH-phenol (M13).
- Conjugation of the hydroxylated metabolites mainly with glucuronic acid and to a lower extend with sulfate.
- Hydrolytic cleavage and subsequent oxidation to mainly fluopyram-pyridyl-acetic acid (M40), fluopyram-pyridyl-carboxylic acid (M43) and fluopyram-ethyl-diol (M38) and conjugates with GA (M39).
- Further degradation of the phenyl ring was less prominent (reaction with methoxy and methylsulfone).

Figure 5.1.1- 2 **Proposed metabolic pathway of [pyridyl-2,6-^{14}C]AE C656948 (fluopyram) in the rat**



III. Conclusions

The toxicokinetic and metabolic behaviour of the test item can be characterised by the following facts:

- Fluopyram was rapidly absorbed and quickly and efficiently eliminated from the body in all tests.
- Excretion of total radioactivity (more than 98% of the administered dose) was renally and faecally and almost complete 72 h after administration. The excretion behaviour of male and female rats revealed a sex related difference: females excreted more and faster renally and males more and faster faecally. The values for the total excretion were comparable.
- Based on the bile fistulation test, the systemic bioavailability was about 98% of the recovered dose. The predominant route of excretion was biliary and about 87% of the administered dose excreted via bile – and already about 86% of the administered dose within the first 24 hours.
- Considerable enterohepatic circulation was indicated by high biliary and low renal exretion in the bile fistulation test compared to the high renal excretion in the low dose tests, and by the different metabolite pattern in bile and in urine.
- It was assumed that the three main metabolite groups in bile (fluopyram-7-OH-GA (M09), 8-OH-GA (M20) and enol-GA (M04)) were formed during the first pass of the parent compound in the liver. Following enterohepatic circulation, the conjugates were cleaved in the small intestine and their aglyca partly excreted via faeces. The aglyca were also partly re-absorbed and further metabolised by cleavage of the molecule. These metabolites were mainly excreted via urine.
- No significant residues remained in the bodies of the rats at sacrifice, 168 h after administration (max. 0.1% of the administered dose).
- The identification rates in all tests were high and accounted for about 80–83% of the administered dose. The parent compound was of minor importance and represented only 1.4–1.9% of the administered dose in faeces of male and female rats.

The metabolic transformations detected were:

- Hydroxylation of the ethyl linking group of the parent compound forming fluopyram-7-hydroxy (M08) and 8-hydroxy metabolites (M18) and (M20).
- Further oxidation of fluopyram-7-hydroxy (M08) and 8-hydroxy metabolites (M18 and M20) leading to the fluopyram-enol metabolite (M04).
- Hydroxylation of the phenyl ring giving fluopyram-7-OH-phenol (M13).
- Conjugation of the hydroxylated metabolites mainly with glucuronic acid and to a lower extend with sulfate.
- Hydrolytic cleavage and subsequent oxidation to mainly fluopyram-pyridyl-acetic acid (M40), fluopyram-pyridyl-carboxylic acid (M43) and fluopyram-ethyl-diol (M38) and conjugates with GA (M39).
- Further degradation of the phenyl ring was less prominent (reaction with methoxy and methylsulfone).
-

Assessment and conclusion by applicant:

The study is valid and acceptable.

Data Point:	KCA 5.1.1/03
Report Author:	[redacted]
Report Year:	2010
Report Title:	[Phenyl-UL-14C]AE C656948: Distribution of the total radioactivity in male and female rats determined by quantitative whole body autoradiography (QWBA), determination of the exhaled 14CO2 and metabolic profiling in excreta
Report No:	MEF-07/456
Document No:	M-296623-02-1
Guideline(s) followed in study:	US EPA OPPTS 870.7485; EU 91/414/EEC amended by 94/97/EC; Canadian PMRA Ref.: DACO 4.5.9; OECD 417; Japanese MAFF, 12 Nousan 8147
Deviations from current test guideline:	None
Previous evaluation:	Yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This study was already evaluated in the previous Annex I inclusion but an amendment was produced subsequently (*Amendment No.1- : On page 34, the animal number "786" in Table 1, Part C was wrong (mistyping error) and must be replaced by "278"*)

Executive Summary

The kinetic behaviour of Fluopyram (AE C656948), uniformly labelled with ^{14}C in the phenyl ring of the molecule, was investigated in male and female Wistar rats for seven days following single oral administration of about 3 mg a.s./kg bodyweight (bw). The distribution of total radioactivity to and elimination from organs and tissues were analysed by quantitative whole-body autoradiography (QWBA) using the radioluminography (RLG) technique. One rat each was taken for sectioning at 1, 4, 8, 24, 48, 72, 120, and 168 hours after administration. Additional to that, the excretion of radioactivity via urine and faeces as well as expiration and via expired air were investigated.

Fluopyram was readily absorbed from the gastrointestinal tract, distributed among almost all organs and tissues investigated, and excreted constantly over the whole experimental period. The rats showed higher faecal (65% for males and 53% for females) and lower renal excretion (32% for males and 41% for females). Less than 0.1% of the administered was expired as $^{14}CO_2$ or other volatiles during a sampling period of 48 hours. This demonstrated the stability of the phenyl-UL-^{14}C label with regard to formation of volatile products.

In male rats, the maximum equivalent concentrations (= CEQ_{max}: expressed as µg a.s.-equiv./g) were reached for nearly all organs and tissues during the first day after administration. Within this period, the respective values remained on a comparable high level. For most organs of the central compartment (e.g. liver, kidney), and the peripheral tissues fat, muscle, some glands (e.g. adrenal, thyroid, Harderian) and nasal mucosa, they were higher than in blood at the times of t_{max} and t_{168h}, suggesting a rapid clearance from blood and distribution to organs and tissues of the animals. Due to the relative high values at the terminal time a delayed depletion of test compound related radioactivity from the organs and tissues is assumed.

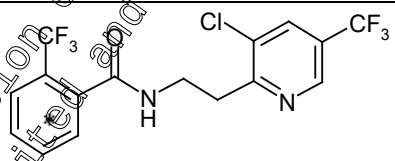
In female rats, the maximum equivalent concentrations were reached for most organs and tissues during the first day post dosing as well. The respective values remained within this period on a comparable high level. Only for the nasal mucosa and glandula preputialis, the maximum concentrations peaked after 48 hours. For most organs of the central compartment (e.g. liver, kidney) and the peripheral tissues, fat, muscle, some glands (e.g. adrenal, thyroid, preputialis, Harderian), ovary and nasal mucosa, they were higher than in blood at the times of t_{max} and for most of them also at t_{168h}. Similarly to the males, the absorbed radioactivity is rapidly cleared from blood and distributed to organs and tissues of the animals. The higher values for the organs at t_{168h} indicate a still ongoing degradation (liver) and excretion (kidney) on a high level and additionally a delayed depletion of test compound related radioactivity from the other organs.

From peak values, a continuous but slow decline of radioactivity concentrations was observed for all organs and tissues during the whole testing period. Seven days after administration, quantifiable radioactive residues were still measured in all of them except the pineal body, the only one that had reached the LOQ. This indicated that distribution, metabolism and elimination of radioactivity from all these organs and tissues as well as excretion via urine and faeces were still ongoing processes. Therefore, a retention of fluopyram related radioactivity in any of the organs and tissues investigated can be excluded.

I. Materials and methods

A. Materials

1. Test Material

IUPAC name	N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)- (9CI)
Code name	AE C656948
Common name	Fluopyram
Empirical formula	$C_{16}H_{11}ClF_6N_2O$
CAS no.:	658066-35-4 (non-labelled AE C656948)
Molar mass	396.72 g/mol
Chemical structure:	 * position of radiolabel
Radiolabelled test material:	[phenyl-UL-^{14}C]AE C656948
Batch no.:	FC633-04 (non-labelled AE C656948)
Specific radioactivity:	3.85 MBq/mg = 2.31 x 105 dpm/µg = 104.05 µCi/mg = 41.28 Ci/mol
Radiochemical purity:	> 98% (HPLC)
Chemical purity:	> 99% (HPLC)
Stability of test compound:	Not determined

2. Vehicle: 0.5% aqueous Tragacanth

3. Test animals

Species:	Rat, male and female rats (*Rattus norvegicus domesticus*)
Strain:	Wistar
Age:	7 weeks (male rats) and 11–12 weeks (female rats) at the time of delivery
Weight at dosing:	Approximately 190 g (male), 199 g (female) at study initiation
Source:	[redacted]
Acclimation period:	The animals were acclimated to laboratory conditions in Makrolon® cages on wood shavings in the test facility for 8 days prior to the administration.
Identification:	The animals were identified by cage cards on which the study number, test compound name and individual animal number were displayed; additional labelleling with water-insoluble spots on the tail.
Diet:	Rat/mice maintenance long life diet (no. 3883.0.15) *ad libitum*, supplied by Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland (*ca.* 16 g per animal and day); last feeding *ca.* 16 hours prior to administration, next feeding *ca.* 6 hours after administration.
Water:	Tap water from the local mains supply (*ad libitum*)
Housing:	The animals were kept under conventional hygienic conditions in air-conditioned rooms.
Environmental conditions	
Temperature:	21–23 °C
Humidity:	45–55%
Air changes:	10–15 fold air change per hour
Photoperiod:	12 hours light/dark-cycle

4. Preparation of dosing solutions

Upon delivery, the compounds were dissolved in acetonitrile. For the preparation of the administration suspensions of test 1 and test 2, an aliquot of the respective radioactive stock solution was concentrated nearly to dryness and suspended in 0.5% aqueous Tragacanth-solution by treatment with an ultrasonic bath. During the individual administrations, the suspensions were stirred at room temperature.

For the preparation of the administration suspensions of the non-radiolabelled test item used for the control animals, the compound was suspended in 0.5% aqueous Tragacanth-solution by treatment with an ultrasonic bath. During the individual administrations, the suspensions were stirred at room temperature. The preparation of all administration suspensions was started on the day before administration.

B. Study design and methods

All tests were performed according to the current EPA, EU, PMRA, OECD and Japanese MAFF test guidelines for supporting the registration of chemical pesticides.

1. Dosing

Nine rats were taken for each experiment. The suspensions were administered to the rats by oral gavage. Each rat received 2 mL of the respective administration suspension of [phenyl-UL-^{14}C]fluopyram and each control animal 2 mL of the administration suspension of non-radiolabelled fluopyram. The concentration of the administration suspensions was calculated to reach a target dose

of 3 mg/kg body weight (bw). Due to different animal weights at administration (mean test 1, males: 190 g; mean test 2, females: 199 g), the mean actual doses per kilogram in the radioactive tests varied slightly with the body weight. The amounts of the test item administered in both experiments are shown in the table below:

2. Amount of test item administered in both experiments

Test No. and sex	[^{14}C] Administration suspensions							
	Total prepared		Administered per rat					
	Amount [mg]	Volume [mL]	Volume/ Amount	Target dose mg/kg bw	Radioactivity [dpm]	Amount [mg]	Actual dose [mg/kg bw]	Mean rat weight [g]
1, Males	7.8	26	2.0 mL / 0.6 mg	3	131788356	0.57	3.00	190
2, Females	7.2	24	2.0 mL / 0.6 mg	3	125449240	0.54	2.73	199

After administration of the radiolabelled test item, the rats were kept individually in Makrolon® metabolism cages, which allowed for separate and quantitative collection of urine, faeces, and the expired air.

3. Collection of excreta

Urine was collected separately for each animal in a cryogenic trap cooled with dry ice. The funnels for urine collection were rinsed with demineralised water at the end of each sampling period. The rinsing solutions were drained into the same vial as the corresponding urine fraction. The radioactivity was determined by liquid scintillation counting (LSC).

The faeces samples were collected every 24 h separately for each animal before they were lyophilised (freeze-dried), weighed, and homogenised. An aliquot was taken for determination of the radioactivity by combustion/LSC.

4. Trapping of expired air

Carbon dioxide and other volatiles from expired air were collected from four male and four female animals. The respective metabolism cages were attached to a high velocity air pump and ventilated with *ca.* 2 L of air per minute and cage. The out-coming air was passed through a trapping system of two gaswashing bottles each containing a 1:1-mixture of ethanolamine/ethanol. At sampling, the exact volume was determined and aliquots radioassayed by LSC.

5. Sacrifice

The animals which received the radiolabelled test were sacrificed using carbon dioxide at 1, 4, 8, 24, 48, 72, 120 and 168 h post administration. The control animals which were used for detection of possible chemo-graphic effects were sacrificed at 4 h after administration.

6. Sample handling and storage

All collected urine samples were kept frozen at all times except during aliquotation for radioactivity analysis. Freeze-dried faeces samples were stored at room temperature.

7. Measurement of radioactivity

The measurement of the radioactivity in the liquid samples was carried out by LSC. All solid samples and blood standards were combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

8. Quantitative whole body autoradiography

The distribution of total radioactivity in organs and tissues was determined at various time points by means of quantitative whole-body autoradiography (radioluminography = RLG). For this purpose, the sacrificed animals were deep frozen at *ca.* -70 °C and embedded in carboxymethylcellulose (CMC). Sagittal whole-body sections of 50 µm thickness were cut at *ca.* -25 °C using a cryomicrotome and freeze-dried for at least 24 hours. The radioactivity distribution in the dry whole-body section was detected employing a phosphor-imaging system. The quantification of equivalent concentrations in the organ and tissues was performed using ^{14}C-spiked blood standards for calibration.

9. Analytical methods

Samples were analysed for parent compound and metabolites by HPLC with radioactivity detection, with MS and with LC-MS-NMR methods for structure elucidation.

10. High performance liquid chromatography (HPLC)

All analytical samples in this study were investigated by radio-HPLC. The HPLC-methods were based on the use of reversed phase columns and an acidic or neutral acetonitrile/methanol/buffer (pH 7) gradient. Detection was performed by UV (270 nm) and a radioisotope detector equipped with a with glass scintillator measuring cell.

11. Mass spectrometry (MS)

The electro-spray ionisation MS spectra (ESI) were obtained either with a TSQ 7000 instrument, with a LTQ or with a TSQ Quantum Ultra AM mass spectrometer. The FT-MS spectra were obtained with an APEX III 70e Fourier Transform Mass Spectrometer. The HPLC instrument used for chromatography was an Agilent HP1100. The flow from the HPLC column was split between UV-detector followed by a radioactivity detector and MS spectrometer.

12. LC-NMR-MS-Spectrometer

The 600 MHz NMR-spectra were recorded on a BRUKER AV 600 instrument. The HPLC instrument used for chromatography was an Agilent HP1100. After the HPLC column the flow was split between the UV-detector followed by an SPE unit and an Esquire 3000 plus mass spectrometer.The compounds were trapped in an SPE unit on a polymer triggered by their quasi molecular ion detected by the Esquire mass spectrometer. After trapping the HPLC peak, the SPE cartridge was dried. The sample was then transferred into the NMR probe head using the solvent specified in the spectrum header.

13. Identification, characterization and quantification

Individual urine samples were combined and adjusted with formic acid to approximately pH 2. The sample was afterwards partitioned into an unpolar and polar phase using ethyl acetate as the first followed by methanol as the second eluent. Both phases were concentrated to the respective aqueous remainders, filled up with ammonium acetate buffer (pH 7) or water to a final volume of about 10 mL and adjusted to a pH-value of about 7.0. All further purification steps of the unipolar and polar

fractions were performed by semipreparative HPLC using the above mentioned neutral or acidic HPLC-gradients. The purified metabolites were identified by spectroscopic methods (MS and NMR).

II. Results and discussion

A. Distribution of radioactivity to and elimination from organs and tissues

In male rats, for nearly all organs and tissues, maximum equivalent concentrations were reached during the first day after administration (Table 5.1.1-16).

Within this period, the respective values remained on a comparable high level. For most organs of the central compartment (e.g. liver, kidney), the peripheral tissues fat, muscle, some glands (e.g. adrenal, thyroid, Harderian) and nasal mucosa they were higher than in blood at the times of t_{max} and $t_{168\ h}$ (Table 5.1.1-18), suggesting a rapid clearance from blood and distribution to organs and tissues of the animals.

Due to the relative high values at the terminal time, a delayed depletion of test compound related radioactivity from the organs and tissues is assumed. The tissue/blood-concentration ratios at t_{max} were highest for liver (factor 4.6), followed by the nasal mucosa (3.5), kidney (3.3), brown fat (2.7), and adrenal gland (2.5). The relatively lowest uptake was found for skeleton muscle (0.6), thymus (0.6) and vitreal body (0.3). No relevant differences of the equivalent concentrations at one and four hours after administration were detected for the perirenal and brown fatty tissues. After this period higher values were found in the brown fat.

The tissue/blood concentration ratios at day seven were highest for the nasal mucosa (24.3), kidney (6.3), liver (3.9), and the Harderian (3.9) and adrenal (3.4) glands. Lowest values were detected in thymus (0.5), skin (0.6) and vitreal body (0.6).

Similar to the results of male rats, in female rats the maximum equivalent concentrations were reached for most organs and tissues in the course of the first day after administration (Table 5.1.1-17).

Within this period, the respective values remained on a comparable high level. Only for the nasal mucosa and glandula preputialis, the maximum concentrations peaked after 48 hours.

For most organs of the central compartment (e.g. liver, kidney), the peripheral tissues fat, muscle, some glands (e.g. adrenal, thyroid, preputialis, Harderian), ovary and nasal mucosa, they were higher than in blood at the times of t_{max} and for most of them also at $t_{168\ h}$ (Table 5.1.1-19), suggesting a rapid clearance of absorbed radioactivity from blood and distribution to organs and tissues of the animals.

The higher values for the organs at $t_{168\ h}$ indicate a still ongoing degradation (liver) and excretion (kidney) on a high level and additionally a delayed depletion of test compound related radioactivity from the other organs.

The tissue/blood-concentration ratios at t_{max} were first of all highest for the glandula preputialis (factor 80.2), nasal mucosa (5.0) and brown fat (4.9), followed by liver (3.6), glandula infraorbitalis (3.6), adrenal gland (3.4), Harderian gland (3.0) and kidney (2.6).

The relatively lowest uptake was found for skin (1.0), spinal cord (1.0), uterus (0.9), lung (0.9), brain (0.8) vitreal body (0.8), thymus (0.7), and skeleton muscle (0.6). No relevant differences of the equivalent concentrations until 24 hours after administration were detected for the perirenal and brown fatty tissues. After this period higher values were found in the brown fat.

The tissue/blood concentration ratio at day seven was very high for the glandula preputialis (factor 219). Significant lower values were calculated for the nasal mucosa (31.6), liver (6.2), kidney (4.8), as well as the Harderian (3.6) and adrenal glands (3.2). Lowest values were detected in thymus (0.5), vitreal body (0.6) and skin (0.5).

Table 5.1.1-16: Test 1: Individual equivalent concentrations of radioactivity in blood, organs and tissues of male rats

Organ or tissue	Equivalent concentration CEQ [µg a.s.-equiv./g]							
	Animal no.							
	129	130	131	132	133	134	135	136
	Time of sacrifice [hours post administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	***0.837***	0.404	0.695	0.628	0.333	0.247	0.116	0.063
Liver	***3.873***	2.433	2.797	1.617	1.196	0.983	0.523	0.246
Renal cortex	1.561	2.037	***3.740***	2.949	3.366	1.931	0.869	0.546
Renal medulla	1.338	1.049	***1.726***	1.706	1.089	0.889	0.477	0.243
Kidney total	1.450	1.543	***2.733***	2.328	2.228	1.410	0.670	0.394
Brown fat	***2.263***	0.523	0.697	0.461	0.25	0.166	0.115	0.073
Perirenal fat	***1.914***	0.596	0.408	0.157	0.086	0.088	0.042	0.028
Skeleton muscle	0.497	0.283	0.440	***0.506***	0.277	0.202	0.115	0.070
Myocardium	***0.985***	0.445	0.754	0.660	0.388	0.308	0.175	0.125
Lung	***0.408***	0.351	0.298	0.314	0.242	0.211	0.091	0.043
Spleen	0.614	0.41	0.664	***0.684***	0.394	0.269	0.143	0.059
Pancreas	***0.972***	0.42	0.658	0.645	0.354	0.254	0.150	0.077
Bone marrow	***0.484***	0.281	0.440	0.470	0.22	0.146	0.063	0.028
Testes	0.560	0.356	***0.644***	0.610	0.365	0.244	0.127	0.059
Brain	***0.558***	0.294	0.493	0.523	0.290	0.184	0.092	0.047
Spinal cord	***0.680***	0.337	0.563	0.573	0.332	0.221	0.114	0.059
Pituitary gland	***0.716***	0.333	0.577	0.596	0.328	0.22	0.108	0.064
Pineal body	0.703	0.293	***1.420***	0.443	0.244	0.170	0.084	-
Adrenal gland	***2.051***	1.009	1.419	1.489	0.903	0.877	0.446	0.213
Thymus	***0.510***	0.265	0.465	0.469	0.236	0.157	0.066	0.03
Thyroid gland	***0.929***	0.549	0.865	0.871	0.501	0.309	0.155	0.084
Salivary gland	***1.032***	0.489	0.771	0.759	0.439	0.311	0.146	0.087
Nasal mucosa	1.323	1.355	2.194	***2.927***	2.249	2.053	2.072	1.524
Skin	***0.649***	0.336	0.532	0.534	0.266	0.181	0.088	0.037
Vitreal body	0.240	***0.258***	0.408	0.613	0.317	0.224	0.100	0.038
Harderian gland	***1.882***	1.017	0.814	2.118	1.187	0.782	0.418	0.242
Glandula infraorbitalis	***1.926***	0.600	0.795	0.684	-	0.352	0.192	0.133

- Organ or tissue is usually visible in the rat sections but not discernible in the radioluminograms.

Values shown in italics and bold style: Maximum CEQ-values (= CEQ_{max})

Table 5.1.1-17: Test 2: Individual equivalent concentrations of radioactivity in blood, organs and tissues of female rats

Organ or tissue	Equivalent concentration CEQ [µg a.s.-equiv./g]							
	Animal no.							
	271	272	273	274	275	276	277	278
	Time of sacrifice [hours post administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	0.649	0.708	0.666	0.841	0.523	0.296	0.143	0.074
Liver	2.796	2.844	3.033	2.732	2.092	1.319	0.839	0.462
Renal cortex	1.106	1.621	1.705	2.090	1.804	1.175	0.750	0.415
Renal medulla	1.053	1.387	2.064	2.298	1.753	1.484	0.723	0.390
Kidney total	1.080	1.504	1.885	2.194	1.779	1.330	0.737	0.353
Brown fat	4.101	2.218	1.473	0.775	0.524	0.317	0.148	0.071
Perirenal fat	2.464	2.896	1.405	0.520	0.125	0.065	0.037	0.017
Skeleton muscle	0.511	0.457	0.480	0.542	0.436	0.275	0.176	0.099
Myocardium	1.051	0.939	0.862	0.872	0.599	0.415	0.239	0.149
Lung	0.727	0.754	0.664	0.334	0.304	0.163	0.084	0.047
Spleen	0.610	0.713	0.743	0.927	0.679	0.403	0.212	0.080
Pancreas	1.004	0.966	0.843	0.850	0.606	0.354	0.171	0.085
Bone marrow	1.209	0.748	0.503	0.583	0.379	0.192	0.089	0.038
Ovary	1.113	1.406	2.123	2.265	1.607	1.015	0.465	0.209
Uterus	0.596	0.653	0.663	0.789	0.499	0.292	0.127	0.047
Brain	0.660	0.603	0.599	0.592	0.430	0.278	0.124	0.052
Spinal cord	0.827	0.715	0.644	0.734	0.504	0.318	0.153	0.067
Pituitary gland	0.787	0.849	0.661	0.773	0.484	0.320	0.159	0.069
Pineal body	0.980	0.791	0.677	-	-	-	-	-
Adrenal gland	2.828	2.325	2.958	2.008	1.618	0.993	0.478	0.239
Thymus	0.515	0.528	0.542	0.590	0.376	0.226	0.096	0.036
Thyroid gland	1.104	1.078	1.023	1.061	0.757	0.473	0.215	0.102
Salivary gland	1.069	1.026	0.954	0.970	0.691	0.470	0.240	0.116
Nasal mucosa	1.442	2.330	2.855	3.410	4.226	3.587	2.256	2.338
Skin	0.700	0.796	0.613	0.618	0.393	0.256	0.109	0.038
Vitreal body	0.217	0.281	0.447	0.652	0.466	0.262	0.127	0.045
Harderian gland	2.508	1.601	1.621	2.116	0.946	1.078	0.314	0.265
Glandula infraorbitalis	2.993	1.882	1.365	1.051	0.778	0.504	0.309	0.159
Glandula parotidea	0.985	1.227	1.789	2.018	1.782	1.068	0.491	0.281
Glandula preputialis	5.020	13.832	22.657	47.156	67.447	35.328	14.843	16.203

- Organ or tissue is usually visible in the rat sections but not discernible in the radioluminograms.

Values shown in italics and bold style: Maximum CEQ-values (= CEQ_{max})

Table 5.1.1-18: Test 1: Pharmacokinetic parameters derived from individual concentration vs. time data of radioactivity in blood, organs and tissues of male rats

Organ or tissue	CEQ_{max}[µg a.s.-equiv./g]	CEQ_{max} ratio organ/blood	t_{max} [h]	$CEQ_{168\ h}$ [µg a.s.-equiv. /g]	$CEQ_{168\ h}$ ratio organ/blood
Blood	0.837	1.00	1	0.063	1.00
Liver	3.873	**4.63**	1	0.246	**3.92**
Renal cortex	3.740	**4.47**	8	0.546	**8.69**
Renal medulla	1.726	**2.06**	8	0.243	**3.80**
Kidney total	2.733	**3.27**	8	0.394	**6.27**
Brown fat	2.263	**2.71**	1	0.073	**1.16**
Perirenal fat	1.914	**2.29**	1	0.028	0.44
Skeleton muscle	0.506	0.60	24	0.070	**1.12**
Myocardium	0.985	**1.18**	1	0.125	**1.99**
Lung	0.408	0.49	1	0.043	0.69
Spleen	0.684	0.82	24	0.059	0.93
Pancreas	0.972	1.16	1	0.077	**1.22**
Bone marrow	0.484	0.58	1	0.028	0.45
Testes	0.644	0.77	8	0.059	0.94
Brain	0.558	0.67	1	0.047	0.75
Spinal cord	0.680	0.81	1	0.059	0.94
Pituitary gland	0.716	0.86	1	0.064	**1.02**
Pineal body	1.420	**1.70**	8	< LOQ	n.c.
Adrenal gland	2.051	**2.45**	1	0.213	**3.39**
Thymus	0.510	0.61	1	0.030	0.48
Thyroid gland	0.929	**1.11**	1	0.084	**1.33**
Salivary gland	1.032	**1.23**	1	0.087	**1.38**
Nasal mucosa	2.927	**3.50**	24	1.524	**24.26**
Skin	0.649	0.78	1	0.037	0.60
Vitreal body	0.258	0.31	4	0.038	0.60
Harderian gland	1.882	**2.25**	1	0.242	**3.85**
Glandula infraorbitalis	1.926	**2.30**	1	0.133	**2.12**

CEQ Equivalent concentration [µg a.s.-equiv. /g].

n.c. Not calculated

Bold style Values > 1.0

Table 5.1.1-19: Test 2: Pharmacokinetic parameters derived from individual concentration vs. time data of radioactivity in blood, organs and tissues of female rats

Organ or tissue	CEQ_{max}[µg a.s.-equiv./g]	CEQ_{max} ratio organ/blood	t_{max} [h]	$CEQ_{168\ h}$ [µg a.s.-equiv./g]	$CEQ_{168\ h}$ ratio organ/blood
Blood	0.841	1.00	24	0.074	1.00
Liver	3.033	**3.61**	8	0.462	**6.24**
Renal cortex	2.09	**2.48**	24	0.315	**4.25**
Renal medulla	2.298	**2.73**	24	0.390	**5.27**
Kidney total	2.194	**2.61**	24	0.353	**4.76**
Brown fat	4.101	**4.87**	1	0.071	0.96
Perirenal fat	2.896	**3.44**	4	0.017	0.23
Skeleton muscle	0.542	0.64	24	0.099	**1.34**
Myocardium	1.051	**1.25**	1	0.149	**2.01**
Lung	0.754	0.9	4	0.047	0.63
Spleen	0.927	**1.10**	24	0.080	**1.08**
Pancreas	1.004	**1.19**	1	0.085	**1.15**
Bone marrow	1.200	**1.43**	1	0.038	0.51
Ovary	2.265	**2.69**	24	0.209	**2.82**
Uterus	0.789	0.94	24	0.047	0.64
Brain	0.660	0.78	1	0.052	0.70
Spinal cord	0.827	0.98	1	0.067	0.91
Pituitary gland	0.849	**1.01**	4	0.069	0.93
Pineal body	0.980	**1.16**	1	---	n.c.
Adrenal gland	2.828	**3.36**	1	0.239	**3.23**
Thymus	0.590	0.7	24	0.036	0.49
Thyroid gland	1.104	**1.31**	1	0.102	**1.38**
Salivary gland	1.069	**1.27**	1	0.116	**1.57**
Nasal mucosa	4.226	**5.02**	48	2.338	**31.59**
Skin	0.796	0.95	4	0.038	0.51
Vitreal body	0.652	0.78	24	0.045	0.61
Harderian gland	2.508	**2.98**	1	0.265	**3.59**
Glandula intraorbitalis	2.993	**3.56**	1	0.159	**2.15**
Glandula parotidea	2.018	**2.40**	24	0.281	**3.80**
Glandula preputialis	67.447	**80.17**	48	16.203	**218.96**

CEQ Equivalent concentration [µg a.s.-equiv. /g].

n.c. Not calculated

Bold style Values > 1.0

B. Residual concentrations in organs and tissues

For males, until 168 hours post administration, the equivalent concentrations in blood, organs and tissues declined down to 7.5% of the maximum concentration in blood, down to 0–6.9% in liver, fat, bone, skin and some glands, down to 7.5–10% in further samples (e.g. spleen, pancreas, testes, brain, spinal cord and some glands) and down to 10–14% in kidneys, muscle, myocardium, lung, vitreal body, adrenal and Harderian glands. For more details please refer to Table 5.1.1-20.

The highest value was detected in the nasal mucosa (52.1%) and the lowest in the pineal body the only one that had reached the LOQ. It should be mentioned that a reduction relative to CEQ_{max} was obvious for all organs up to the test end.

For females, until 168 hours post administration, the equivalent concentrations in blood, organs and tissues declined down to 8.8% of the maximum concentration in blood down, down from 0.6–8.6% in the fatty tissues, bone marrow, skin, glandula infraorbitalis, vitreal body, uterus, thymus, brain and spinal cord, pituitary and adrenal glands, down to 9.2% each in the ovary and thyroid gland and down to 10–18% in the Harderian, parotidea and salivary glands, myocardium and skeleton muscle, liver and kidney.

The highest values were detected in the glandula preputialis (24%) and in the nasal mucosa (55%) and the lowest in the pineal body the only one that had reached the LOQ. It should be mentioned that a reduction relative to CEQ_{max} was obvious for all organs and tissues up to the test end. For more details please refer to Table 5.1.1-21.

Table 5.1.1-20: Test 1: Percentage ratio of the individual equivalent concentrations in the blood, organs and tissues over the maximum concentrations (CEQ_{max}) of male rats

Organ or tissue	Ratio [%] CEQindiv.time / CEQ_{max}							
	Animal no.							
	129	130	131	132	133	134	135	136
	Time of sacrifice [hours post administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	100.00	48.25	83.07	75.03	39.80	29.47	13.84	7.51
Liver	100.00	62.82	72.22	41.73	30.87	25.44	13.49	6.35
Renal cortex	41.74	54.46	100.00	78.85	89.99	51.64	23.08	14.59
Renal medulla	77.52	60.74	100.00	98.86	63.11	51.48	27.63	14.06
Kidney total	53.04	56.45	100.00	85.17	81.51	51.59	24.52	14.42
Brown fat	100.00	23.10	30.81	20.37	11.06	7.33	5.09	3.22
Perirenal fat	100.00	31.16	21.33	8.21	4.52	4.62	2.22	1.45
Skeleton muscle	98.30	55.97	86.86	100.00	54.73	39.87	22.63	13.87
Myocardium	100.00	45.12	76.48	66.96	39.34	31.23	17.76	12.69
Lung	100.00	86.21	73.11	76.95	59.46	51.64	22.30	10.66
Spleen	89.79	59.87	97.00	100.00	57.63	39.32	20.87	8.56
Pancreas	100.00	43.15	67.71	66.34	36.45	26.16	15.38	7.87
Bone marrow	100.00	58.07	90.96	97.06	45.38	30.17	12.95	5.78
Testes	86.99	55.25	100.00	94.66	56.70	37.84	19.74	9.18

Organ or tissue	Ratio [%] CEQindiv.time / CEQ_{max}							
	Animal no.							
	129	130	131	132	133	134	135	136
	Time of sacrifice [hours post administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Brain	100.00	52.63	88.31	93.78	52.04	33.05	16.48	8.48
Spinal cord	100.00	49.57	82.88	84.31	48.81	32.44	16.77	8.69
Pituitary gland	100.00	46.56	80.58	83.17	43.75	30.66	15.03	8.97
Pineal body	49.52	20.63	100.00	31.19	17.17	11.94	5.88	n.c.
Adrenal gland	100.00	49.20	69.19	72.61	44.05	42.73	21.75	10.37
Thymus	100.00	51.95	91.26	92.04	46.31	30.73	13.01	5.89
Thyroid gland	100.00	59.13	99.18	93.77	53.95	33.25	16.65	9.01
Salivary gland	100.00	47.40	74.68	73.54	42.59	30.19	14.20	8.40
Nasal mucosa	45.20	46.30	74.93	100.00	76.83	50.13	70.78	52.06
Skin	100.00	51.68	82.01	82.21	40.92	27.83	13.57	5.76
Vitreal body	92.83	100.00	158.12	237.49	122.91	86.83	38.92	14.55
Harderian gland	100.00	54.03	43.24	112.54	63.99	41.53	22.19	12.86
Glandula infraorbitalis	100.00	31.12	41.28	35.52	n.c.	18.30	9.97	6.90

CEQ Equivalent concentration [µg a.s.-equiv. /g]

n.c. Not calculated

Table 5.1.1-21: Test 2: Percentage ratio of the individual equivalent concentrations in the blood, organs and tissues over the maximum concentrations (CEQ_{max}) of female rats

Organ or tissue	Ratio [%] CEQindiv.time / CEQ_{max}							
	Animal no.							
	271	272	273	274	275	276	277	278
	Time of sacrifice [hours post administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	77.12	84.15	79.11	100.00	62.13	35.23	17.05	8.80
Liver	92.19	93.77	100.00	90.07	68.96	43.48	27.66	15.23
Renal cortex	52.95	77.59	81.61	100.00	86.32	56.25	35.91	15.07
Renal medulla	45.83	60.33	89.82	100.00	76.29	64.59	31.46	16.98
Kidney total	49.22	68.55	85.91	100.00	81.06	60.62	33.58	16.07
Brown fat	100.00	54.08	35.93	18.89	12.78	7.73	3.62	1.73
Perirenal fat	85.08	100.00	48.51	17.97	4.32	2.25	1.28	0.58
Skeleton muscle	94.20	84.35	88.43	100.00	80.31	50.76	32.42	18.32
Myocardium	100.00	89.31	82.04	82.91	56.98	39.49	22.78	14.15
Lung	96.37	100.00	88.09	44.30	40.26	21.57	11.08	6.20
Spleen	65.84	76.97	80.20	100.00	73.22	43.48	22.84	8.61
Pancreas	100.00	96.29	83.98	84.73	60.36	35.31	17.03	8.46
Bone marrow	100.00	62.33	41.89	48.56	31.60	16.02	7.44	3.17
Ovary	49.14	62.07	93.72	100.00	70.96	44.82	20.51	9.21
Uterus	75.58	82.82	84.00	100.00	63.26	37.03	16.04	5.99
Brain	100.00	91.36	90.63	89.62	65.08	42.05	18.82	7.80
Spinal cord	100.00	86.45	77.87	88.80	61.02	38.42	18.55	8.15
Pituitary gland	92.64	100.00	77.85	91.05	57.02	37.68	18.76	8.13
Pineal body	100.00	80.74	69.15	n.c.	n.c.	n.c.	n.c.	n.c.
Adrenal gland	100.00	82.22	90.48	71.00	57.23	35.10	16.91	8.45
Thymus	87.20	89.38	91.83	100.00	63.73	38.27	16.28	6.09
Thyroid gland	100.00	97.05	92.68	96.15	68.55	42.85	19.48	9.22
Salivary gland	100.00	95.99	89.24	90.78	64.63	43.96	22.48	10.85
Nasal mucosa	34.13	55.12	67.56	80.69	100.00	84.87	53.38	55.32
Skin	87.96	100.00	77.06	77.60	49.44	32.12	13.73	4.75
Vitreal body	33.30	43.06	68.58	100.00	71.46	40.12	19.46	6.91
Harderian gland	100.00	63.82	64.61	84.36	37.71	42.98	12.54	10.58
Glandula infraorbitalis	100.00	62.90	45.61	35.13	25.98	16.84	10.31	5.31
Glandula parotidea	48.81	60.78	88.62	100.00	88.29	52.90	24.32	13.92
Glandula preputialis	7.44	20.51	33.59	69.92	100.00	52.38	22.01	24.02

CEQ Equivalent concentration [µg a.s.-equiv./g].

n.c. Not calculated

C. Excretion and expiration

In male rats, the major part of the dosed radioactivity (max. 65%) was excreted with faeces and the minor one (max. 32%) with urine (Table 5.1.1-22).

Around three days after dosage, the faecal excretion was nearly completed. Only a very minor part of the dose was excreted in the time range between 72 and 168 hours after administration. The urinary excretion showed a slightly different behaviour because a clear plateau level was not reached within the whole sampling period of seven days.

The expiration of ^{14}C-carbon dioxide and other ^{14}C-labelled volatiles was tested with animals for a testing period of 48 hours (No's 133–136; Table 5.1.1-22). Less than 0.07% of the total administered dose was expired during this sampling period: about two third until 24 hours and one third from 24 to 48 hours. This low amount demonstrates the stability of the phenyl-UL-^{14}C label with regard to possible formation of volatile products.

In female rats, the major part of the dosed radioactivity (max. 53%) was excreted with faeces and the minor one (max. 41%) with urine (Table 5.1.1-23).

Three days after dosage, the faecal excretion was nearly completed. An only very minor part of the dose was excreted in the time range between 72 and 168 hours after administration. The urinary excretion showed a slightly different behaviour because a clear plateau level was not reached within the whole sampling period of seven days.

The expiration of ^{14}C-carbon dioxide and other ^{14}C-labelled volatiles was tested with animals for a testing period of 48 hours (No's 275–278; Table 5.1.1-23). Less than 0.09 % of the administered dose was expired during this sampling period: about two third until 24 hours and one third from 24 to 48 hours. This low amount demonstrates the stability of the phenyl-UL-^{14}C label with regard to possible formation of volatile products.

Table 5.1.1-22: Test 1: Cumulative excretion of radioactivity in urine, faeces and expired air of male rats

	Percentage of total dose administered							
	Animal no.							
Sample	**129**	**130**	**131**	**132**	**133**	**134**	**135**	**136**
	Time of sacrifice [hours post administration]							
	1 h	**4 h**	**8 h**	**24 h**	**48 h**	**72 h**	**120 h**	**168 h**
Expired air								
24h					0.046	0.049	0.049	0.044
48h					0.064	0.07	0.069	0.065
Urine								
1h	0.45							
4h		1.35	1.71	0.38	2.07	1.47	1.86	1.49
8h			3.62	5.08	4.07	2.41	3.48	3.12
24h				16.19	14.84	15.76	14.8	12.39
48h					23.16	23.98	22.75	19.17
72h						28.39	27.05	23.34
96h							29.66	26.02
120h							31.68	27.92
144h								29.25
168h								30.13
Faeces								
24h	*	*	*	43.06	42.22	49.4	46.57	49.88
48h	*	*	*	*	527	62.38	58.03	57.4
72h	*	*	*	*	*	64.63	60.48	60.93
96h	*	*	*	*	*	*	61.41	61.74
120h	*	*	*	*	*	*	62.02	62.25
144h	*	*	*	*	*	*	*	62.54
168h	*	*	*	*	*	*	*	63.39
Total excreted	**0.45**	**1.35**	**3.62**	**59.25**	**75.92**	**93.09**	**93.76**	**93.58**

* Faeces not collected

Table 5.1.1-23: Test 2: Cumulative excretion of radioactivity in urine, faeces and expired air of female rats

	Percentage of total dose administered							
	Animal no.							
Sample	**271**	**272**	**273**	**274**	**275**	**276**	**277**	**278**
	Time of sacrifice [hours post administration]							
	1 h	**4 h**	**8 h**	**24 h**	**48 h**	**72 h**	**120 h**	**168 h**
Expired air								
24h					0.059	0.053	0.059	0.068
48h					0.08	0.068	0.081	0.087
Urine								
1h	0.21							
4h		0.64	0.62	0.44	1.83	0.07	0.65	0.36
8h			3.89	3.25	3.25	3.85	3.94	2.54
24h				13.29	17.46	19.47	16.82	19.27
48h					27.69	29.66	27.65	28.53
72h						35.59	33.98	33.50
96h							37.82	36.39
120h							40.21	38.19
144h								39.67
168h								40.51
Faeces								
24h	*	*	*	0.68	29.09	28.36	26.62	36.69
48h	*	*	*	*	39.40	42.27	41.78	49.50
72h	*	*	*	*	*	44.13	45.57	51.59
96h	*	*	*	*	*	*	46.67	52.23
120h	*	*	*	*	*	*	47.22	52.64
144h	*	*	*	*	*	*	*	52.95
168h	*	*	*	*	*	*	*	53.20
Total excreted	0.21	0.64	3.89	32.97	67.17	79.78	87.51	93.80

* Faeces not collected

III. Conclusions

Fluopyram, labelled with ^{14}C in the phenyl ring of the molecule, was readily absorbed from the gastrointestinal tract of male and female Wistar rats after single oral administration. Test item related radioactivity was well distributed to all organs and tissues.

The highest exposure during the whole observation period was found for the gastrointestinal tract (overexposed regions), glandula preputialis (females only) and nasal mucosa for both sexes. For the

GI-tract, this may indicate on the one hand a possible incomplete absorption of the administered compound but on the other hand also a pronounced enterohepatic cycle between the small intestine and liver. For the glandula preputialis of the female rats a temporary accumulation and delayed depletion of test item related radioactivity is assumed. The high values detected in the nasal mucosa(s) of both genders resulted possibly from a retention of test item related radioactivity in the nasal mucus. High equivalent concentrations until the terminal time point at day seven were also found for liver and kidney, likely due to their metabolising and biliary, as well as urinary excretion functions.

From peak values, a continuous but slow decline of radioactivity concentrations was observed for all organs and tissues during the whole testing period. Seven days after administration, quantifiable radioactive residues were still measured in all of them except the pineal body which only had reached the LOQ. This indicated that distribution, metabolism and elimination of radioactivity from all these organs and tissues as well as excretion via urine and faeces were still ongoing processes. Therefore, a retention of fluopyram related radioactivity in any of the organs and tissues investigated can be excluded.

Assessment and conclusion by applicant:

The study is valid and acceptable.

Data Point:	KCA 5.1.1/04
Report Author:	[redacted]
Report Year:	2008
Report Title:	[Pyridyl-2,6-14C]AE C656948: Distribution of the total radioactivity in male and female rats determined by quantitative whole body autoradiography (QWBA), determination of the exhaled 14CO2
Report No:	MEF-07/457
Document No:	M-296485-01-1
Guideline(s) followed in study:	US EPA OPPTS 870.7485; EU 91/414/EEC amended by 94/79/EC; Canadian PMRA Ref.: DACO 4.5.9; OECD 417; Japanese MAFF, 12 Nousan 8147
Deviations from current test guideline:	None
Previous evaluation	Yes, evaluated and accepted
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The kinetic behaviour of fluopyram (AE C656948), labelled with ^{14}C in the 2,6 position of the pyridyl ring of the molecule, was investigated in male and female Wistar rats for seven days following single oral administration of about 3 mg (males) and 4.5 mg (females) a.s./kg bodeweight (bw). The distribution of total radioactivity to and elimination from organs and tissues were analysed by quantitative whole-body autoradiography using the radioluminography (RLG) technique. One rat each was taken for sectioning at 1, 4, 8, 24, 48, 72, 120, and 168 hours after administration. Additional to that, the excretion of radioactivity via urine and faeces as well as expiration via expired air were investigated.

AE C656948 was readily absorbed from the gastrointestinal tract, distributed among almost all organs and tissues investigated. The excretion via urine and faeces was nearly completed after 72 hours. The male rats showed slightly higher faecal (54%) and lower urinary (51%) excretion. For female rats, the urinary excretion was slightly higher (50%) as the faecal excretion (43%). Less than 1% of the administered was expired as $^{14}CO_2$ or other volatiles during a sampling period of 48 hours. This demonstrated the stability of the pyridyl-2,6-^{14}C label with regard to formation of volatile products.

In male rats, the maximum equivalent concentrations (= CEQ_{max}: expressed as µg a.s.-equiv./g) were reached for all organs and tissues at one hour after administration. For most organs of the central compartment (e.g. liver, kidney), and the peripheral tissues fat, some glands (e.g. adrenal, thyroid, Harderian) and nasal mucosa, they were higher than in blood at t_{max} and for liver also at $t_{168\ h}$, suggesting a rapid clearance from blood and distribution to organs and tissues of the animals. For the high value in liver at $t_{168\ h}$ a still ongoing degradation on a high level is assumed.

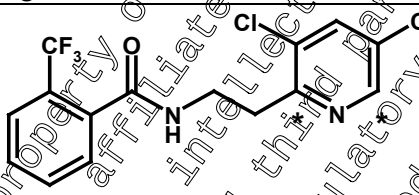
In female rats, the maximum equivalent concentrations were reached for most organs and tissues during the first hour post dosing as well except for the kidney and perirenal fat which peaked after four hours. For most organs of the central compartment (e.g. liver, kidney), the peripheral tissues, fat, brain, some glands (e.g. adrenal, thyroid, preputialis, Harderian), ovary, and nasal mucosa, they were higher than in blood at the times of tmax and for liver, nasal mucosa, Harderian gland and glandula preputialis also at $t_{168\ h}$. Similarly to the males, the absorbed radioactivity is rapidly cleared from blood and distributed to organs and tissues of the animals. The higher values for the before mentioned organs at $t_{168\ h}$ indicate a still ongoing degradation on a high level in the liver and additionally a delayed depletion of test compound related radioactivity from the other organs.

From peak values of both genders, a continuous but slow decline of radioactivity concentrations was observed for all organs and tissues during the whole testing period. Seven days after administration, quantifiable radioactive residues were still measured in most of them. This indicated that distribution, metabolism and elimination of radioactivity from all these organs and tissues as well as excretion via urine and faeces were still ongoing processes. Therefore, a retention of fluopyram related radioactivity in any of the organs and tissues investigated can be excluded.

I. Materials and methods

A. Materials

1.Test Material

IUPAC name	N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)- (9CI)
Code name	AE C656948
Common name	Fluopyram
Empirical formula	$C_{16}H_{11}ClF_6N_2O$
CAS no.:	658066-35-4 (non-labelled AE C656948)
Molar mass	396.72 g/mol
Chemical structure:	 * position of radiolabel
Radiolabelled test material	[Pyridyl-2,6-^{14}C]AE C656948
Batch no.:	RFC633-04 (non-labelled AE C656948)
Specific radioactivity:	5.48 MBq/mg = 3.29 x 105 dpm/µg = 148.11 µCi/mg = 58.75 Ci/mol (Test 1) 3.85 MBq/mg = 2.31 x 105 dpm/µg = 104.05 µCi/mg = 41.28 Ci/mol (Test 2)
Radiochemical purity:	> 99% (HPLC), Test 1 and > 98% (HPLC), Test 2
Chemical purity:	> 99% (HPLC), Test 1 and Test 2
Stability of test compound:	Not determined

2. Vehicle: 0.5% aqueous Tragacanth

3. Test animals

Species:	Rat, male and female rats (*Rattus norvegicus domesticus*)
Strain:	Wistar
Age:	7 weeks (male rats) and 11–12 weeks (female rats) at the time of delivery
Weight at dosing:	Approximately 192–207 g (male), 194–202 g (female) at study initiation
Source:	[redacted]
Acclimation period:	The animals were acclimated to laboratory conditions in Makrolon® cages on wood shavings in the test facility for 8 days prior to the administration.
Identification:	The animals were identified by cage cards on which the study number, test compound name and individual animal number were displayed; additional labelleling with water-insoluble spots on the tail.
Diet:	Rat/mice maintenance long life diet (no. 3883.0.15) *ad libitum*, supplied by Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland (*ca.* 16 g per animal and day), last feeding *ca.* 16 hours prior to administration, next feeding *ca.* 6 hours after administration.

Water:	Tap water from the local mains supply (*ad libitum*)
Housing:	The animals were kept under conventional hygienic conditions in air-conditioned rooms.
Environmental conditions	
Temperature:	18–25 °C
Humidity:	45–71%
Air changes:	10–15 fold air change per hour
Photoperiod:	12 hours light/dark-cycle

4. Preparation of dosing solutions

Upon delivery, the compounds were dissolved each in. For the preparation of the administration suspensions of test 1 and test 2, an aliquot of the respective radioactive stock solution was concentrated to near dryness and suspended in 0.5% aqueous Tragacanth solution by treatment with an ultrasonic bath.

For the preparation of the administration suspensions of the non-radiolabelled test item used for the control animals, the compound was suspended in 0.5% aqueous Tragacanth-solution by treatment with an ultrasonic bath. During the individual administrations, the suspensions were stirred at room temperature. The preparation of all administration suspensions was started on the day before administration.

B. Study design and methods

All tests were performed according to the current EPA, EU, PMRA, OECD and Japanese MAFF test guidelines for supporting the registration of chemical pesticides.

1. Dosing

Nine rats were taken for each experiment. The suspensions were administered to the rats by oral gavage. Each rat received 2 mL of the respective administration suspension of [pyridyl-2,6-^{14}C]fluopyram and each control animal 2 mL of the administration suspension of non-radiolabelled fluopyram. The concentration of the administration suspensions was calculated to reach a target dose of 3 mg/kg bw. Due to different animal weights at administration (mean test 1, males: 190 g; mean test 2, females: 199 g), the mean actual doses per kilogram in the radioactive tests varied slightly with the body weight. The amounts of the test item administered in both experiments are shown in the table below:

2. Amount of test item administered in both experiments

Test No. and sex	[^{14}C] Administration suspensions							
	Total prepared		Administered per rat					
	Amount [mg]	Volume [mL]	Volume/ Amount	Target dose mg/kg bw	Radioactivity [dpm]	Amount [mg]	Actual dose [mg/kg bw]	Mean rat weight [g]
1, Males	8.4	28	2.0 mL / 0.6 mg	3	190829412	0.58	2.93	198
2, Females	10.32	24	2.0 mL / 0.86 mg	4.3	203644977	0.88	4.48	197

After administration of the radiolabelled test item, the rats were kept individually in Makrolon® metabolism cages, which allowed for separate and quantitative collection of urine, faeces, and the expired air.

3. Collection of excreta

Urine was collected separately for each animal in a cryogenic trap cooled with dry ice. The funnels for urine collection were rinsed with demineralised water at the end of each sampling period. The rinsing solutions were drained into the same vial as the corresponding urine fraction. The radioactivity was determined by LSC.

The faeces samples were collected every 24 h separately for each animal before they were lyophilised (freeze-dried), weighed, and homogenised. An aliquot was taken for determination of the radioactivity by combustion/LSC.

4. Trapping of expired air

Carbon dioxide and other volatiles from expired air were collected from four animals each (No's.: 946–949, males and 230–233, females). The respective metabolism cages were attached to a high velocity air pump and ventilated with ca. 2 L of air per minute and cage. The out coming air was passed through a trapping system of two gaswashing bottles each containing about 150–200 mL of a 1:1-mixture of ethanolamine/ethanol. At sampling, the exact volume was determined, from which an aliquot was taken for the determination of radioactivity.

5. Sacrifice

The animals which received the radiolabelled test item (942–949, males and 226–233, females) were sacrificed using carbon dioxide at 1, 4, 8, 24, 48, 72, 120 and 168 h post administration. The control animals (950, male and 234, female) which were used for detection of possible chemo-graphic effects were sacrificed at 4 h after administration.

6. Sample handling and storage

All collected urine samples were kept frozen at all times except during aliquotation for radioactivity analysis. Freeze-dried faeces samples were stored at room temperature.

7. Measurement of radioactivity

The measurement of the radioactivity in the liquid samples was carried out by LSC. All solid samples and blood standards were combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

8. Quantitative whole body autoradiography

The distribution of total radioactivity i.e. without differentiation of unchanged test item and labelled biotransformation products in organs and tissues was determined at various time points by means of quantitative whole-body autoradiography (radioluminography = RLG). For this purpose, the sacrificed animals were deep frozen at ca. -70 °C and embedded in carboxymethylcellulose (CMC). Sagittal whole-body sections of 50 µm thickness were cut at ca. -25 °C using a cryomicrotome and freeze-dried for at least 24 hours. The radioactivity distribution in the dry whole-body section was detected employing a phosphor-imaging system. The quantification of equivalent concentrations in the organ and tissues was performed using ^{14}C-spiked blood standards for calibration.

II. Results and discussion

A. Distribution of radioactivity to and elimination from organs and tissues

In male rats, the maximum equivalent concentrations were reached for all organs and tissues at one hour after administration (Table 5.1.1-24).

For most organs of the central compartment (e.g. liver, kidney), and the peripheral tissues fat, some glands (e.g. adrenal, thyroid, Harderian) and nasal mucosa, they were higher than in blood at t_{max} and for liver also at at $t_{168\ h}$ (Table 5.1.1-24), suggesting a rapid clearance of test item related radioactivity from blood and distribution to these organs and tissues. For the high liver value at at $t_{168\ h}$ a still ongoing degradation on a high level is assumed.

The tissue/blood-concentration ratios at t_{max} were highest for liver (factor 6.6), followed by the perirenal fat (4.4), brown fat (4.0), glandula infraorbitalis (3.1), adrenal gland (2.9), kidney (2.0) and some other glands, as well as nasal mucosa (around 1.4). The relatively lowest uptake was found for skeleton muscle (0.7), thymus (0.7) and vitreal body (0.3). No relevant differences were seen for the perirenal and brown fatty tissues at one hour after administration. The tissue/blood concentration ratios at day seven were highest for the liver (1.5), nasal mucosa (0.9) and kidney (0.7). Lowest values were detected in thymus (0.14) and testes (0.08).

Similar to the male rats, the maximum equivalent concentrations in femalre rats were reached for most organs and tissues during the first hour post dosing as well (Table 5.1.1-25) except for kidney and perirenal fat which peaked after 4 hours.

For most organs of the central compartment (e.g. liver, kidney), the peripheral tissues fat, brain, some glands (e.g. adrenal, thyroid, preputialis, Harderian) and nasal mucosa, they were higher than in blood at the times of t_{max} and for liver, nasal mucosa, Harderian gland and glandula preputialis also at $t_{168\ h}$ (Table 5.1.1-27).

Similarly to the males, the absorbed radioactivity is rapidly cleared from blood and distributed to organs and tissues of the animals. The higher values for the before mentioned organs at t168 h indicate a still ongoing degradation on a high level in the liver and additionally a delayed depletion of test compound related radioactivity from the other organs.

The tissue/blood-concentration ratios at CEQ_{max} were highest for the brown (factor 7.3) and renal fat (6.0) followed by liver (4.6), glandula infraorbitalis (4.5), adrenal gland (4.1), glandula preputialis (factor 4.0), Harderian gland (3.8), nasal mucosa (3.8) and kidney (2.5). The relatively lowest uptake was found for the skin (1.1), brain (1.0), thymus, spleen and lung (all 0.8), and skeleton muscle (0.7). No relevant differences were seen for the perirenal and brown fatty tissues at one hour after administration.

The tissue/blood concentration ratios at day seven were highest for the nasal mucosa (4.5) and liver (1.7), Harderian gland (1.3), glandula parotidea (1.2) and kidney (0.8). Lowest value was detected in the skeleton muscle (0.1).

Table 5.1.1-24: Test 1: Individual equivalent concentrations of radioactivity in blood, organs and tissues of male rats

Organ or tissue	Equivalent concentration CEQ [µg a.s.-equiv./g]							
	Animal no.							
	942	943	944	945	946	947	948	949
	Time of sacrifice [hours post administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	**0.504**	0.241	0.231	0.124	0.052	0.049	0.048	0.046
Liver	**3.330**	2.152	1.512	0.680	0.257	0.176	0.149	0.070
Renal cortex	**0.972**	0.481	0.348	0.175	0.078	0.050	0.040	0.034
Renal medulla	**1.010**	0.588	0.352	0.173	0.069	0.051	0.043	0.033
Kidney total	**0.991**	0.534	0.350	0.174	0.074	0.051	0.041	0.033
Brown fat	**2.022**	0.526	0.322	0.090	0.028	0.018	0.015	0.010
Perirenal fat	**2.236**	1.144	0.401	0.071	0.022	0.012	0.006	< LOQ
Skeleton muscle	**0.364**	0.136	0.099	0.029	0.009	0.007	0.006	0.006
Myocardium	**0.681**	0.255	0.190	0.071	0.020	0.019	0.016	0.010
Lung	**0.425**	0.225	0.168	0.088	0.027	0.015	0.025	0.024
Spleen	**0.372**	0.154	0.123	0.050	0.022	0.020	0.017	0.015
Pancreas	**0.673**	0.261	0.205	0.061	0.024	0.014	0.010	0.009
Bone marrow	**0.343**	0.160	0.138	0.057	0.026	0.019	0.012	0.007
Testes	**0.364**	0.140	0.095	0.033	0.009	0.007	0.006	0.004
Brain	**0.395**	0.122	0.086	0.019	0.005	0.004	< LOQ	< LOQ
Spinal cord	**0.481**	0.150	0.100	0.025	0.007	0.006	0.005	< LOQ
Pituitary gland	**0.506**	0.259	0.124	0.051	0.018	0.014	0.013	0.007
Pineal body	**0.345**	0.210	0.143	0.056	0.018	0.016	0.009	0.008
Adrenal gland	**1.436**	0.471	0.352	0.119	0.037	0.027	0.027	0.020
Thymus	**0.345**	0.140	0.102	0.040	0.017	0.015	0.011	0.007
Thyroid gland	**0.738**	0.252	0.183	0.076	0.028	0.021	0.017	0.013
Salivary gland	**0.717**	0.293	0.202	0.066	0.020	0.014	0.011	0.007
Nasal mucosa	**0.710**	0.503	0.454	0.271	0.198	0.085	0.072	0.043
Skin	**0.567**	0.216	0.144	0.055	0.021	0.015	0.012	-
Vitreal body	**0.150**	0.076	0.052	0.019	0.007	< LOQ	< LOQ	< LOQ
Harderian gland	**0.708**	0.573	0.342	0.134	0.027	0.020	0.013	-
Glandula infraorbitalis	**1.568**	0.581	0.415	0.147	0.036	0.026	0.016	-

- Organ or tissue is usually visible in the rat sections but not discernible in the radioluminograms.

Values shown in italics and bold style: Maximum CEQ-values (= CEQ_{max})

Table 5.1.1-25: Test 2: Individual equivalent concentrations of radioactivity in blood, organs and tissues of female rats

Organ or tissue	Equivalent concentration CEQ [µg a.s.-equiv./g]							
	Animal no.							
	226	227	228	229	230	231	232	233
	Time of sacrifice [hours post administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	***1.027***	0.731	0.685	0.235	0.082	0.070	0.044	0.042
Liver	***4.709***	3.922	3.468	1.049	0.345	0.232	0.106	0.072
Renal cortex	***1.659***	1.614	1.500	0.386	0.100	0.082	0.042	0.031
Renal medulla	2.087	***3.422***	2.169	0.425	0.106	0.095	0.044	0.038
Kidney total	1.873	***2.518***	1.834	0.406	0.103	0.089	0.043	0.034
Brown fat	***7.492***	4.904	3.048	0.490	0.069	0.051	0.019	---
Perirenal fat	4.880	6.193	4.619	0.587	0.060	0.045	0.019	0.010
Skeleton muscle	***0.722***	0.488	0.474	0.115	0.018	0.013	0.006	0.005
Myocardium	***1.677***	1.263	1.056	0.270	0.044	0.035	0.015	0.012
Lung	***0.808***	0.714	0.723	0.154	0.055	0.040	0.025	0.018
Spleen	***0.866***	0.664	0.605	0.162	0.044	0.032	0.018	0.015
Pancreas	***1.447***	1.238	1.013	0.231	0.039	0.028	0.013	0.009
Bone marrow	***1.214***	0.623	0.598	0.167	0.047	0.027	-	-
Ovary	***1.541***	1.307	1.203	0.240	0.054	0.027	0.015	0.012
Uterus	***0.857***	0.767	0.572	0.205	0.048	0.026	0.020	0.009
Brain	***1.054***	0.759	0.537	0.070	0.013	0.007	< LOQ	< LOQ
Spinal cord	***1.351***	0.957	0.614	0.084	0.014	0.008	< LOQ	---
Pituitary gland	***1.153***	0.797	0.750	0.167	0.034	0.027	0.011	0.011
Pineal body	***1.334***	1.103	0.938	0.216	0.037	0.031	0.014	0.011
Adrenal gland	***4.171***	3.518	2.508	0.546	0.085	0.063	0.028	0.019
Thymus	***0.794***	0.625	0.547	0.140	0.039	0.026	0.014	0.010
Thyroid gland	***1.652***	1.221	1.182	0.284	0.059	0.044	0.020	0.013
Salivary gland	***1.675***	1.348	1.067	0.241	0.045	0.031	0.013	0.009
Nasal mucosa	***3.870***	2.661	2.312	1.248	0.354	0.398	0.246	0.191
Skin	***1.092***	0.921	0.561	0.194	0.046	0.028	-	-
Vitreal body	***0.244***	0.230	0.194	0.058	0.014	0.009	< LOD	< LOQ
Harderian gland	***3.917***	2.758	2.308	0.486	0.153	0.163	0.077	0.053
Glandula infraorbitalis	***4.591***	3.356	2.269	0.448	0.108	0.067	-	-
Glandula parotidea	***2.174***	1.000	1.365	0.290	0.168	0.139	0.090	0.051
Glandula preputialis	***4.077***	5.274	3.831	0.525	0.080	0.052	-	-

- Organ or tissue is usually visible in the rat sections but not discernible in the radioluminograms.

Values shown in italics and bold style: Maximum CEQ-values (= CEQ_{max})

Table 5.1.1-26: Test 1: Pharmacokinetic parameters derived from individual concentration vs. time data of radioactivity in blood, organs and tissues of male rats

Organ or tissue	CEQ_{max}[µg a.s.-equiv./g]	CEQ_{max} ratio organ/blood	t_{max} [h]	$CEQ_{168\ h}$ [µg a.s.-equiv. /g]	$CEQ_{168\ h}$ ratio organ/blood
Blood	0.504	1.00	1	0.046	1.00
Liver	3.330	6.61	1	0.070	1.52
Renal cortex	0.972	1.93	1	0.034	0.73
Renal medulla	1.010	2.01	1	0.033	0.73
Kidney total	0.991	1.97	1	0.033	0.73
Brown fat	2.022	4.02	1	0.010	0.22
Perirenal fat	2.236	4.44	1	< LOQ	n.c.
Skeleton muscle	0.364	0.72	1	0.006	0.14
Myocardium	0.681	1.35	1	0.010	0.22
Lung	0.425	0.84	1	0.024	0.51
Spleen	0.372	0.74	1	0.015	0.32
Pancreas	0.673	1.34	1	0.009	0.19
Bone marrow	0.343	0.68	1	0.007	0.16
Testes	0.364	0.72	1	0.004	0.08
Brain	0.395	0.78	1	< LOQ	n.c.
Spinal cord	0.484	0.96	1	< LOQ	n.c.
Pituitary gland	0.506	1.00	1	0.007	0.16
Pineal body	0.345	0.69	1	0.008	n.c.
Adrenal gland	1.436	2.85	1	0.020	0.44
Thymus	0.345	0.69	1	0.007	0.14
Thyroid gland	0.738	1.47	1	0.013	0.28
Salivary gland	0.717	1.42	1	0.007	0.16
Nasal mucosa	0.710	1.41	1	0.043	0.94
Skin	0.567	1.13	1	-	-
Vitreal body	0.150	0.3	1	< LOD	n.c.
Harderian gland	0.708	1.41	1	-	-
Glandula infraorbitalis	1.568	3.11	1	-	-

CEQ Equivalent concentration [µg a.s.-equiv. /g].

n.c. Not calculated

Bold style Values > 1.0

Table 5.1.1-27: Test 2: Pharmacokinetic parameters derived from individual concentration vs. time data of radioactivity in blood, organs and tissues of female rats

Organ or tissue	CEQ_{max}[µg a.s.-equiv./g]	CEQ_{max} ratio organ/blood	t_{max} [h]	$CEQ_{168\ h}$ [µg a.s.-equiv./g]	$CEQ_{168\ h}$ ratio organ/blood
Blood	1.027	1.00	1	0.042	
Liver	4.709	**4.59**	1	0.072	1.71
Renal cortex	1.659	**1.62**	1	0.031	0.74
Renal medulla	3.422	**3.33**	4	0.038	0.89
Kidney total	2.518	**2.45**	4	0.034	0.81
Brown fat	7.492	**7.30**	1	---	n.c.
Perirenal fat	6.193	**6.03**	4	0.010	0.23
Skeleton muscle	0.722	0.70	1	0.005	0.12
Myocardium	1.677	**1.63**	1	0.012	0.28
Lung	0.808	0.79	1	0.018	0.42
Spleen	0.866	0.84	1	0.015	0.35
Pancreas	1.447	**1.41**	1	0.009	0.22
Bone marrow	1.214	**1.18**	1	---	n.c.
Ovary	1.541	**1.50**	1	0.012	0.28
Uterus	0.857	0.83	1	0.009	0.22
Brain	1.054	**1.03**	1	< LOQ	n.c.
Spinal cord	1.351	**1.32**	1	---	---
Pituitary gland	1.153	**1.12**	1	0.011	0.27
Pineal body	1.334	**1.30**	1	0.011	0.26
Adrenal gland	4.171	**4.06**	1	0.019	0.46
Thymus	0.794	0.77	1	0.010	0.23
Thyroid gland	1.652	**1.61**	1	0.013	0.32
Salivary gland	1.675	**1.63**	1	0.009	0.22
Nasal mucosa	3.870	**3.77**	1	0.191	**4.53**
Skin	1.092	**1.06**	1	---	---
Vitreal body	0.244	0.24	1	< LOQ	n.c.
Harderian gland	3.917	**3.82**	1	0.053	**1.25**
Glandula infraorbitalis	4.591	**4.47**	1	---	---
Glandula parotidea	2.174	**2.12**	1	0.051	**1.22**
Glandula preputialis	4.077	**3.97**	1	---	---

CEQ Equivalent concentration [µg a.s.-equiv. /g].

n.c. Not calculated

Bold style Values > 1.0

B. Residual concentrations in organs and tissues

In male rats, until 168 hours post administration, the equivalent concentrations in blood, organs and tissues declined down to 9.1% of the maximum concentration in blood, down to 0–2.3% in the glands (e.g. adrenal, thyroid, Harderian), down to 2.1–3.4% in liver and kidney and down to 0–6.1% in further samples (e.g. fatty tissues, lung, spleen, nasal mucosa). For more details please refer to Table 5.1.1-28.

From all the organs and tissues, the highest value was detected in the nasal mucosa (6.1%). It should be mentioned that a reduction relative to CEQ_{max} was obvious for all organs up to the test end.

In female rats, until 168 hours post administration, the equivalent concentrations in blood, organs and tissues declined down to 4.1% of the maximum concentration in blood, down to 0–2.4% in the glands (e.g. adrenal, thyroid, Harderian, preputialis), down to 1.4–1.5% in liver and kidney and down to 0–4.9% in further samples (e.g. fatty tissues, lung, spleen, nasal mucosa). From all these organs and tissues, the highest value was detected in the nasal mucosa (4.9%). It should be mentioned that up to the test end a reduction relative to CEQ_{max} was obvious for all organs and tissues. For more details please refer to Table 5.1.1-29.

Table 5.1.1-28: Test 1: Ratio [%] of the individual equivalent concentrations in the blood, organs and tissues over the maximum concentrations (CEQ_{max}) of male rats

Organ or tissue	Ratio [%] CEQindiv.time / CEQ_{max}							
	Animal no.							
	942	943	944	945	946	947	948	949
	Time of sacrifice [hours post administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	100.00	47.81	45.80	24.53	10.36	9.82	9.61	9.14
Liver	100.00	64.65	45.40	20.44	7.72	5.28	4.48	2.10
Renal cortex	100.00	49.44	35.84	17.97	8.04	5.17	4.07	3.45
Renal medulla	100.00	58.23	34.82	17.14	6.87	5.05	4.30	3.31
Kidney total	100.00	53.92	35.32	17.55	7.44	5.11	4.18	3.38
Brown fat	100.00	26.01	15.94	4.45	1.39	0.89	0.72	0.51
Perirenal fat	100.00	51.18	17.95	3.17	0.99	0.54	0.27	n.c.
Skeleton muscle	100.00	37.25	27.32	8.08	2.52	1.92	1.65	1.75
Myocardium	100.00	37.40	27.91	10.43	3.00	2.80	2.39	1.49
Lung	100.00	52.86	39.58	20.78	6.36	3.43	5.77	5.54
Spleen	100.00	41.44	33.00	13.38	6.05	5.32	4.64	3.92
Pancreas	100.00	38.80	30.47	9.06	3.50	2.06	1.51	1.32
Bone marrow	100.00	46.70	40.06	16.69	7.44	5.47	3.37	2.13
Testes	100.00	38.50	25.96	9.19	2.60	2.05	1.66	1.04
Brain	100.00	30.84	21.90	4.91	1.34	1.08	n.c.	n.c.
Spinal cord	100.00	31.16	20.82	5.10	1.41	1.24	1.06	n.c.
Pituitary gland	100.00	51.19	24.46	10.14	3.47	2.75	2.51	1.45
Pineal body	100.00	60.75	41.44	16.15	5.26	4.75	2.64	2.28
Adrenal gland	100.00	32.80	24.52	8.29	2.56	1.86	1.89	1.41
Thymus	100.00	40.66	29.53	11.66	4.99	4.28	3.28	1.92
Thyroid gland	100.00	34.10	24.85	10.27	3.83	2.90	2.37	1.75
Salivary gland	100.00	40.84	28.16	9.24	2.79	1.93	1.52	1.04
Nasal mucosa	100.00	70.90	63.94	38.23	27.96	11.94	10.19	6.10
Skin	100.00	38.07	25.40	9.72	3.72	2.69	2.09	n.c.
Vitreal body	100.00	51.07	34.74	12.69	4.83	n.c.	n.c.	n.c.
Harderian gland	100.00	80.96	48.27	18.93	3.80	2.86	1.85	n.c.
Glandula infraorbitalis	100.00	37.07	26.44	9.37	2.27	1.66	1.01	n.c.

CEQ Equivalent concentration [µg a.s.-equiv. /g].
n.c. Not calculated

Table 5.1.1-29: Test 2: Ratio [%] of the individual equivalent concentrations in the blood, organs and tissues over the maximum concentrations (CEQ_{max}) of female rats

Organ or tissue	Ratio [%] CEQindiv.time / CEQ_{max}							
	Animal no.							
	226	227	228	229	230	231	232	233
	Time of sacrifice [hours post administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Blood	100.00	71.16	66.72	22.90	7.98	6.86	4.29	1.11
Liver	100.00	83.28	73.64	22.27	7.32	4.93	2.25	1.53
Renal cortex	100.00	97.26	90.41	23.27	6.02	4.93	2.54	1.88
Renal medulla	60.99	100.00	63.38	12.43	3.09	2.78	1.28	1.10
Kidney total	74.39	100.00	72.86	16.11	4.08	3.52	1.71	1.36
Brown fat	100.00	65.46	40.68	6.53	0.92	0.68	0.26	n.c.
Perirenal fat	78.80	100.00	74.59	9.48	0.96	0.73	0.31	0.16
Skeleton muscle	100.00	67.63	62.66	15.91	2.49	1.87	0.78	0.68
Myocardium	100.00	75.34	62.97	16.12	2.61	2.10	0.90	0.69
Lung	100.00	88.42	89.52	19.04	6.85	4.95	3.09	2.17
Spleen	100.00	76.62	69.84	18.72	5.10	3.73	2.09	1.71
Pancreas	100.00	85.55	69.98	15.94	2.69	1.95	0.88	0.65
Bone marrow	100.00	51.32	49.23	13.72	3.86	2.20	n.c.	n.c.
Ovary	100.00	84.82	78.06	15.56	3.49	1.78	0.98	0.76
Uterus	100.00	89.46	66.71	23.91	5.54	2.98	2.28	1.11
Brain	100.00	72.02	50.98	6.62	1.19	0.67	n.c.	n.c.
Spinal cord	100.00	70.85	45.45	6.23	1.01	0.56	n.c.	n.c.
Pituitary gland	100.00	69.11	65.05	14.51	2.98	2.35	0.97	0.98
Pineal body	100.00	82.66	70.33	16.18	2.79	2.31	1.09	0.81
Adrenal gland	100.00	81.96	60.14	13.09	2.04	1.52	0.67	0.47
Thymus	100.00	78.70	68.91	17.59	4.92	3.28	1.81	1.22
Thyroid gland	100.00	73.91	71.57	17.18	3.60	2.67	1.18	0.81
Salivary gland	100.00	80.48	63.71	14.38	2.66	1.85	0.76	0.57
Nasal mucosa	100.00	68.77	57.18	32.25	9.14	10.27	6.35	4.93
Skin	100.00	84.37	51.33	17.77	4.17	2.60	n.c.	n.c.
Vitreal body	100.00	94.36	79.59	23.57	5.65	3.66	n.c.	n.c.
Harderian gland	100.00	70.41	58.92	12.40	3.90	4.15	1.96	1.35
Glandula infraorbitalis	100.00	73.11	49.42	9.75	2.35	1.46	n.c.	n.c.
Glandula parotidea	100.00	46.00	62.81	13.35	7.74	6.38	4.16	2.36
Glandula preputialis	100.00	129.38	93.99	12.88	1.97	1.27	n.c.	n.c.

CEQ Equivalent concentration [µg a.s.-equiv./g].

n.c. Not calculated

C. Excretion and expiration

In male rats, the excretion of radioactivity (Table 5.1.1-22) amounted to max. 51% in urine and max. 54% in faeces. About three days after dosage, the urinary and faecal excretion were nearly completed. An only very minor part of the dose was excreted in the time range between 72 and 168 hours after administration. The expiration of ^{14}C-carbon dioxide and other ^{14}C-labelled volatiles was tested with animals for a testing period of 48 hours (No's 946–949; Table 5.1.1-22). Slightly less than 1% of the administered dose was expired during this sampling period which demonstrated the stability of the pyridyl-2,6-^{14}C label with regard to formation of volatile products.

In female rats, the major part of the dosed radioactivity (max. 54%) was excreted with urine and the minor one (max. 43%) with urine (Table 5.1.1-31).

About three days after dosage, the urinary and faecal excretion were nearly completed. An only very minor part of the dose was excreted in the time range between 72 and 168 hours after administration. The expiration of ^{14}C-carbon dioxide and other ^{14}C-labelled volatiles was tested with animals for a testing period of 48 hours (No's 275–278; Table 5.1.1-31). Less than 1% of the administered dose was expired during this sampling period which demonstrated the stability of the pyridyl-2,6-^{14}C label with regard to formation of volatile products.

Table 5.1.1-30: Test 1: Cumulative excretion of radioactivity in urine, faeces and expired air of male rats

	Percentage of total dose administered							
	Animal no.							
	942	943	944	945	946	947	948	949
Sample	Time of sacrifice [hours post administration]							
	1 h	4 h	8 h	24 h	48 h	72 h	120 h	168 h
Expired air								
24h					0.046	0.063	0.061	0.061
48h					0.693	0.864	0.889	0.783
Urine								
1h	1.84							
4h		4.61	4.02	2.69	0.72	9.12	1.87	4.20
8h			12.33	14.84	14.28	17.03	12.88	19.22
24h				36.97	33.54	37.45	34.41	43.43
48h					41.52	42.89	41.78	49.70
72h						43.70	42.91	50.57
96h							43.15	50.82
120h							43.28	50.94
144h								51.01
168h								51.05
Faeces								
24h	*	*	*	40.51	39.80	43.18	41.11	30.61
48h	*	*	*	*	50.72	50.64	52.00	45.91
72h	*	*	*	*	*	51.65	53.64	47.06
96h	*	*	*	*	*	*	54.06	47.39
120h	*	*	*	*	*	*	54.19	47.50
144h	*	*	*	*	*	*	*	47.58
168h	*	*	*	*	*	*	*	47.65
Total excreted	1.84	4.61	12.33	77.48	92.93	96.21	98.36	99.48

* Faeces not collected

Table 5.1.1-31: Test 2: Cumulative excretion of radioactivity in urine, faeces and expired air of female rats

	Percentage of total dose administered							
	Animal no.							
Sample	**226**	**227**	**228**	**229**	**230**	**231**	**232**	**233**
	Time of sacrifice [hours post administration]							
	1 h	**4 h**	**8 h**	**24 h**	**48 h**	**72 h**	**120 h**	**168 h**
Expired air								
24h					0.963	0.642	0.755	0.603
48h					1.076	0.763	0.855	0.681
Urine								
1h	0.91							
4h		1.71	2.04	2.84	2.56	2.1	1.87	2.33
8h			12.15	12.15	12.09	13.96	10.05	9.23
24h				39.58	37.85	38.09	33.29	38.83
48h					50.58	51.1	44.77	47.88
72h						54.17	46.8	49.43
96h							47.2	49.98
120h							47.35	50.26
144h								50.36
168h								50.42
Faeces								
24h	*	*	*	29.5	29.41	23.84	25.44	26.21
48h	*	*	*	*	40.55	37.29	39.62	40.74
72h	*	*	*	*	*	40.55	42.24	42.49
96h	*	*	*	*	*	*	42.63	42.97
120h	*	*	*	*	*	*	42.81	43.15
144h	*	*	*	*	*	*	*	43.23
168h	*	*	*	*	*	*	*	43.27
Total excreted	0.91	1.71	12.15	69.08	92.2	95.49	91.02	94.37

* Faeces not collected

III. Conclusions

Fluopyram, labelled with ^{14}C in the 2 and 6 position of the pyridyl ring of the molecule, was readily absorbed from the gastrointestinal tract of male and female Wistar rats after single oral administration. Test item related radioactivity was distributed to all organs and tissues quite well. The highest exposure during the whole observation period was found for the gastrointestinal tract (overexposed regions), glandula parotidea and Harderian gland (females only) and nasal mucosa for both sexes. For the GI-tract, this may indicate on the one hand a possible incomplete absorption of the administered compound but on the other hand also a pronounced enterohepatic cycle between the small intestine and liver. For the glandula parotidea and Harderian gland of the female rats, a delayed depletion of test

item related radioactivity is assumed. The high values detected in the nasal mucosa(s) of both genders resulted possibly from a retention of test item related radioactivity in the nasal mucus. High equivalent concentrations until the terminal time point at day seven were also found for liver and kidney, likely due to their metabolising and biliary, as well as urinary excretion functions.

From peak values, a continuous but slow decline of radioactivity concentrations was observed for all organs and tissues during the whole testing period. Seven days after administration, quantifiable but rather low radioactive residues were still measured in some of them. This indicated that distribution, metabolism and elimination of radioactivity from all these organs and tissues as well as excretion via urine and faeces were still ongoing processes. Therefore, a retention of fluopyram related radioactivity in any of the organs and tissues investigated can be excluded.

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Assessment and conclusion by applicant:

The study is valid and acceptable.

Data Point:	KCA 5.1.1/03
Report Author:	[redacted]
Report Year:	2008
Report Title:	[Pyridyl-2,6-^{14}C]AE C656948 - Metabolism in organs and tissues of male and female rats (three timepoints)
Report No:	MEF-08/15
Document No:	M-298834-01-1
Guideline(s) followed in study:	US EPA OPPTS 870.7485; EU 91/414/EC amended by 94/79/EC; Canadian PMRA Ref. DACO 4.5.8; OECD 417; Japanese MAFF 12 Nohsan 8147
Deviations from current test guideline:	None
Previous evaluation:	Yes, evaluated and accepted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The depletion of radioactive residues from organs and tissues, the excretion with urine and metabolism of radiolabelled [Pyridyl-2,6-^{14}C]fluopyram were investigated in male and female Wistar rats.

Three groups of 4 male and 4 female rats each were orally administered by oral gavage with a single dose of [pyridyl-2,6-^{14}C]fluopyram at a nominal dose level of 5 mg/kg bw, respectively.

The animals were sacrificed 1 h, 4 h and 24 h after dosing. The total radioactivity that included the unchanged test item and metabolites was determined in the excreted urine samples over the sampling times (0–1 h, 0–4 h and 0–24 h) as well as in plasma, liver, kidney and perirenal fat at sacrifice. The metabolism was investigated in urine and plasma samples and in extracts from liver, kidney and fat using HPLC and TLC with radiodetection.

The overall recovery accounted for *ca.* 97.2% and *ca.* 98.8% of the administered dose in male rats (tests 1–3) and *ca.* 97.8% and *ca.* 99.7% in female rats (tests 4–6).

The urinary excretion started immediately after administration and increased to *ca.* 28.7% and *ca.* 43.1% of the administered dose after 24 hours. The amount excreted by female rats was about 1.5-times higher than by male rats.

The highest TRR-values were detected in the organs and tissues as well as in the combined GIT (gastrointestinal tract) plus faeces at one hour after administration. The distribution of the radioactivity within the central compartments of the body was fast and showed a distinctive preference towards the liver and kidney. All values decreased until the test end to significantly lower values. It is expected that residual amounts in these samples are subject to further elimination from the body and retention of the test item can therefore be excluded. In contrast to the male rats, the TRR-values of organs and tissues from female rats were higher at nearly all points in time which was particularly noticeable for the perirenal fat values (factor 4.3 at 24 h).

After purification (degreasing by C18 SPE) and concentration, the resulting extracts represented between *ca.* 55% and *ca.* 100% of the TRR. The extraction procedure was clearly sufficient for kidney and perirenal fat samples (>87%). For the liver samples of males, the extractability decreased from *ca.* 93% (1 h) to *ca.* 55% (24 h) and of females from *ca.* 97% (1 h) to *ca.* 65% (24 h).

Fluopyram was extensively metabolised and more than 20 metabolites were identified. Molecular cleavage occurred at least in a range of 23–34% of the administered dose in both sexes represented by numerous label specific metabolites. In the various samples, sex specific differences were observed in the ratio of pyridyl label specific and common metabolites which contained still the intact molecular structure. The metabolic transformation of the parent compound was generally more pronounced in male rats.

Following radioactive components were identified in order of their elution in the HPLC-profiles: fluopyram-pyridyl-carboxylic acid (M43), fluopyram-hydroxy-PAA (M41), fluopyram-ethyl-diol-GA (M39, 3 isomers), fluopyram-pyridyl-acetic acid (M40), fluopyram-hydroxyethyl-GA (M37), fluopyram-ethyl-diol (M38), fluopyram-methoxy-di-OH-GA (M23), fluopyram-pyridyl-methyl-cysteine (M44), fluopyram-7-OH-phenol-GA (M14), fluopyram-7-OH-GA (M09, 2 isomers), fluopyram-7-OH-hydroxy-phenol-SA (M17), fluopyram-di-OH-GA (M21), fluopyram-enol-GA (M04, isomer 1), fluopyram-7-OH-phenol-SA (M15), fluopyram-phenol-GA (M07), fluopyram-8-OH-GA (M20, 2 isomers), fluopyram-enol-GA (M04, isomer 2), fluopyram-7-OH-phenol (M13), fluopyram-7-OH-methyl-sulfone (M16), fluopyram-7-hydroxy (M08), fluopyram-8-hydroxy (M18), parent compound, fluopyram-*E*-olefin (M02) and fluopyram-*Z*-olefin (M03).

Identification rates ranged from *ca.* 92% to *ca.* 98% of the TRR in urine, *ca.* 61% to 100% of the TRR in plasma, *ca.* 45% to *ca.* 92% of the TRR in liver, *ca.* 74% to *ca.* 97% of the TRR in kidney and *ca.* 86% to *ca.* 99% of the TRR in the perirenal fat. Further small peaks or peak groups additionally detected in the metabolic profiles (<1% of administered dose) were characterized by their behaviour during extraction and clean up and the retention times in the HPLC-chromatograms.

The metabolism of [pyridyl-2,6-^{14}C]fluopyram in male and female rats was principally oxidative and took place mainly at the ethylene bridge of the molecule. The major metabolites were fluopyram-7-hydroxy (M08) and fluopyram-pyridyl-acetic acid (M40).

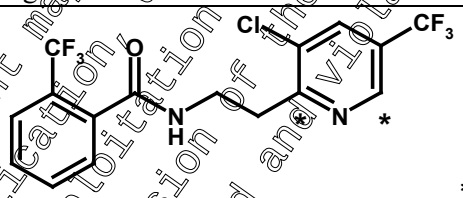
The metabolic transformation reactions in detail were:

- hydroxylation of the ethylene bridge of the molecule resulting in fluopyram-7-hydroxy, fluopyram-8-hydroxy, and a dihydroxylated compound;
- hydroxylation of the phenyl ring leading to fluopyram-phenol and fluopyram-7-OH-phenol;
- molecular cleavage and conjugation to fluopyram-pyridyl-hydroxyethyl (conjugates);
- molecular cleavage and oxidation to fluopyram-pyridyl-carboxylic acid;
- oxidation of fluopyram-pyridyl-hydroxyethyl to mainly fluopyram-pyridyl-acetic acid and to a lower extent to fluopyram-ethyl-diol, fluopyram-hydroxy-PAA and fluopyram-pyridyl-carboxylic acid;
- elimination of water from compounds hydroxylated in the ethylene bridge leading to fluopyram-*Z*-olefin and *E*-olefin (*E*- and *Z*-olefin can isomerise into each other); and
- conjugation of several hydroxylated metabolites with glucuronic acid and to a lower extent with sulfate.

I. Materials and methods

A. Materials

1. Test Material

IUPAC name	*N*-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name	Benzamide, N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)- (9CI)
Code name	AE C656948
Common name	Fluopyram
Empirical formula	$C_{16}H_{11}ClF_6N_2O$
CAS no.:	658066-35-4 (non-labelled AE C656948)
Molar mass	396.72 g/mol
Chemical structure:	 * position of radiolabel
Radiolabelled test material	[phenyl-UL-^{14}C]AE C656948
Batch no.:	SEL/1775
Specific radioactivity:	3.85 MBq/mg
Radiochemical purity:	> 99% (HPLC) > 99% (TLC)
Chemical purity:	> 99% (HPLC)
Stability of test compound:	Following preparation of the administration suspension used for tests 1 to 6, the purity of the test item was again checked by HPLC. The radiochemical purity of the test item used in all experiments was >99% which showed a sufficient stability in 0.5% aqueous Tragacanth.

2. Vehicle: 0.5% aqueous Tragacanth solution

3. Test animals

Species:	Rat, male and female rats derived from *Rattus norvegicus*
Strain:	Wistar Hsd/Cpb: WU
Age:	7 weeks (males), 11–12 weeks (females)
Weight at dosing:	Approximately 207–260 g
Source:	[redacted]
Acclimation period:	7 days
Identification:	Cage cards on which the study number, test compound name and individual animal number were displayed; additional labelleing with water-insoluble spots on the tail.
Diet:	Rat/mice maintenance long life diet (no. 3883.0.15), Fa. Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland, *ad libitum*. The animals were fed with approximately 16 g per day and animal. The animals were fed the last time *ca.* 16 h prior to administration of the dose and again 6 h after dosing.
Water:	Tap water, *ad libitum*
Housing:	After administration of the radiolabelled test item, the rats were kept individually in Makrolon metabolism cages.
Environmental conditions	
Temperature:	21–24 °C
Humidity:	54–66%
Air changes:	10 – 15 fold air change per hour
Photoperiod:	12 hours light/dark-cycle

4. Preparation of dosing solutions

The whole amount was dissolved in 25 mL acetonitrile and the radioactivity in this stock solution was determined by LSC. The radioactivity concentration was 8.33 MBq/mL corresponding to 2.16 mg/mL. The sample was stored in a freezer (≤ -18 °C) until preparation of the administration suspension. The purity of the test item in the stock solution was checked by HPLC and the identity by spectroscopic methods. One administration suspension was prepared for all six tests. At the day before starting the first test, a definite volume of the stock solution was pipetted into a glass flask and concentrated under a gentle stream of nitrogen. The nearly dry residue was suspended in 75 mL 0.5% aqueous Tragacanth® with the aid of magnetic stirrer, glass balls and an ultrasonic bath for 5 minutes. The suspension was stirred continuously on a magnetic stirrer all the time in a cooling chamber at approx +4 °C. Only during the individual administrations, the suspension was stirred at room temperature.

The purity of the test item was checked by HPLC and the concentration was determined by LSC. The total amount of radioactivity administered to each animal served as reference (100%) for the percentage calculation of the total radioactivity in the biological samples.

B. Study design and methods

All tests were performed according to the current EPA, PMRA, OECD and Japanese MAFF test guidelines for supporting the registration of chemical pesticides.

1. Dose regimen and design of tests

Test no.	Test description	Number of rats and sex	Sample collection schedule	Duration
1	5 mg/kg bw, single oral	4 male	Urine, faeces, blood (plasma and blood cells), liver, kidney, perirenal fat, GIT (including faeces), skin and carcass	1 hours
2	5 mg/kg bw, single oral	4 male	Urine, faeces, blood (plasma and blood cells), liver, kidney, perirenal fat, GIT (including faeces), skin and carcass	4 hours
3	5 mg/kg bw, single oral	4 male	Urine, faeces, blood (plasma and blood cells), liver, kidney, perirenal fat, GIT (including faeces), skin and carcass	24 hours
4	5 mg/kg bw, single oral	4 female	Urine, faeces, blood (plasma and blood cells), liver, kidney, perirenal fat, GIT (including faeces), skin and carcass	1 hours
5	5 mg/kg bw, single oral	4 female	Urine, faeces, blood (plasma and blood cells), liver, kidney, perirenal fat, GIT (including faeces), skin and carcass	4 hours
6	5 mg/kg bw, single oral	4 female	Urine, faeces, blood (plasma and blood cells), liver, kidney, perirenal fat, GIT (including faeces), skin and carcass	24 hours

The rats of all test groups received a single dose of Fluopyram (AE C656948) radiolabelled in the pyridyl ring (pyridyl-2,6-^{14}C).

[Pyridyl-2,6-^{14}C]fluopyram was administered orally to six groups of four male or female rats at a low dose of 5 mg/kg bw. Urine was collected from administration until sacrifice, and blood, organs and tissues were collected at sacrifice at 1, 4 or 24 hours.

2. Dosing

The animals received the calculated volume by oral gavage using a syringe attached to an animal-feeding knob cannula. The volume was based on the nominal average animal weight of 250 g. The concentration of each administration solution was calculated to reach an administered amount of about the nominal value of the active substance per kg body weight (bw). Due to different animal weights at administration, the actual dose per kg varied slightly with the body weight. Two millilitres of the administration suspension were dosed to each animal.

The purity of the test item was checked by HPLC and the concentration was determined by LSC. The total amount of radioactivity administered to each animal served as reference (100%) for the percentage calculation of the total radioactivity in the biological samples.

3. Collection of excreta

After the administration of the radiolabelled test substance, the rats were kept individually in Makrolon® metabolism cages, which allowed for separate and quantitative collection of urine and faeces.

Urine was collected at various times separately for each animal in a cryogenic trap cooled with dry ice. The funnels for urine collection were rinsed with demineralized water at the end of each sampling period. The rinsing solutions were drained into the same vial as the corresponding urine fraction. The radioactivity was determined by LSC.

Faeces were collected separately for each animal in a cryogenic trap cooled with dry ice for the whole time of the tests. All individual samples were added to the respective gastrointestinal tract (GIT) of the corresponding rat at sacrifice.

4. Sacrifice

At the end of each test period (1 h, 4 h or 24 h after administration), the animals were anaesthetized with Pentobarbital-Na. They were sacrificed and exsanguinated by transection of the cervical blood vessels.

5. Blood, tissues and organs at sacrifice

After transection of the cervical blood vessels the oozed out blood was collected into test tubes coated with heparin and afterwards plasma and blood cells were separated by centrifugation. After weighing of the whole plasma sample, an aliquot was taken for determination of the radioactivity by LSC.

The dissected tissue sample (GIT including faeces, skin, and carcass including sedimented blood cells) were transferred into tared plastic vessels for straight recording of their individual fresh weights. The combined GIT faeces sample and an aliquot sample of depilated skin were lyophilised by freeze-drying. After weighing they were homogenised before aliquots were taken for determination of the radioactivity by combustion/LSC.

The original whole carcass/blood cells-sample was passed up to five times through a mincing machine in half-frozen state. The equipment of the mincing machine was carefully cleaned prior to a new sample. From this tissue pulp an aliquot was lyophilised, homogenised and weighed, before aliquots were taken for determination of the radioactivity by combustion/LSC.

Liver, renal fat and kidneys of the individual rats were weighed and pooled for the extraction of radioactive residues. No separate determination of radioactivity in these organs was performed in order to get enough sample material for extraction and metabolic profiling. For the calculations, the mean values of the total radioactive residues based on the sum of extracts and solids were used for the individual animals.

6. Sample handling and storage

Freeze dried samples like GIT (together with faeces) or organs were stored in plastic vials at room temperature or at approx. +4 °C in a refrigerator. Liquid samples like urine were kept frozen at ≤ -18 °C at all times except during aliquotation for analysis. During the analytical work, the samples were stored either at approx. +4 °C in a refrigerator or at ≤ -18 °C in a freezer.

7. Measurement of radioactivity

The measurement of the radioactivity in liquid samples was carried out by liquid scintillation counting (LSC). All solid samples were combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.

The amounts of radioactivity found in the excreta, organs and tissues of the rat at sacrifice were calculated from the radioactivity concentrations determined by radioassay and were related to the administered radioactivity. The percentage amounts in the organs were obtained from the multiplication of the respective dose normalised concentrations (C_{norm}) with the corresponding gamma-values. The gamma value of an organ is equivalent to its percentage weight contribution to the total body weight of the animal.

The radioactive residues for liver, renal fat, and kidney were calculated by summation of the residues in the extracts and solids after solvent extraction. No determination of radioactivity was performed for these organs and tissues of individual animals to get enough sample material for extraction and profiling.

8. Preparation of urine, plasma, organs and tissues for analysis

Generally, a combined samples from all animals were prepared per test group and selected time periods

The urine and plasma samples were analysed by HPLC without further purification.

For combined liver and kidney, two solvent extractions were performed by macerating the sample with acetonitrile/water (8/2, v/v). At each step, the respective sample was separated into the extract and solids by filtration. The total volume of each extract was measured and the radioactivity of an aliquot was determined by LSC. The final solids were dissolved in Beckman tissue solubilizer from which an aliquot was taken for radioactivity measurement by LSC. Both extracts were combined and applied to a C18 SPE cartridge to remove the lipid fraction of the matrix. The SPE percolate and column wash with acetonitrile/water were combined and concentrated to the aqueous remainder for HPLC analysis.

For combined fat, two solvent extractions were performed by macerating the sample with ACN/water (8/2, v/v). At each step, the respective sample was separated into the extract and solids by centrifugation. The total volume of each extract was measured and the radioactivity of an aliquot was determined by LSC. The liquid phase remaining after extraction was dissolved in tetrahydrofurane/methanol (1/1, v/v) and the remaining solids in Beckman tissue solubilizer. An aliquot each was taken for radioactivity measurement by LSC. Both extracts were combined and applied to a C18 SPE cartridge to remove the lipid fraction of the matrix. The SPE percolate and column wash with acetonitrile/water were combined and concentrated to the aqueous remainder for HPLC analysis.

9. High performance liquid chromatography (HPLC)

For metabolic profiling, the individual samples of this study were analysed by HPLC. These investigations were based on the use of a reversed phase column and a neutral acetonitrile/methanol/water gradient. Detection was performed by UV (270 nm) and a radioisotope detector with glass scintillator.

10. Thin layer chromatography (TLC)

For detection and identification of fluopyram-*E*- and *Z*-olefins in liver and fat samples, pre-layered, glass-backed HPTLC-plates were used for one-dimensional TLC. The absorbents were silica gel 60 F_{254} (normal phase) and LiChrospher RP-18 WF_{254} (reversed phase). The plates were developed using dichloromethane (normal phase) and acetonitrile / water (7/3, v/v; reversed phase).

For co-chromatography, a solution of the reference items was applied to the plate overlapping with the sample solution. After development of the TLC plate, co-chromatographic correspondence was assessed by analysis of the individual radioluminogram.

11. LC-NMR-MS Spectroscopy

The 600 MHz NMR-spectrum was recorded on a BRUKER AV 600 instrument.

12. Identification, characterisation and quantification

The liver extract from test group 1 was further purified twice by reversed phase SPE and finally by semipreparative HPLC for the isolation of the fluopyram-*E* and *Z*-olefins. The major substance peak which eluted at 99.3 min was collected, concentrated to near dryness and dissolved in acetonitrile. An aliquot each was taken for determination of radio-activity by LSC and for TLC-analysis using the normal phase and reversed phase mode.

The fat extracts from test groups 3 and test 6 were further purified by semipreparative HPLC for the isolation of the fluopyram-*E*- and *Z*-olefins. The major substance peaks which eluted at about 100 min were collected, concentrated to near dryness and dissolved in acetonitrile. An aliquot each was taken for determination of radioactivity by LSC and for TLC-analysis using the normal phase and reversed phase mode.

The assignment of the unchanged parent Fluopyram (AE C656948) and metabolites in all samples of the respective test was achieved by comparison of the HPLC profiles among each other and respective retention times with those of rat and lactating goat metabolism studies in which they were unambiguously identified by chromatographic and spectroscopic methods.

Further proof of identity was obtained by comparison of the elution patterns of two selected urine samples with two representative urine samples of male and female rats from the rat ADME study and of the perirenal fat extracts from all tests with the fat extract from the lactating goat metabolism study.

In order to show the commonality of metabolic pathways with other metabolism studies, the metabolites fluopyram-*E*-olefin and fluopyram-*Z*-olefin were identified following isolation and purification in the liver extract of test 1 (males, 1 h), fat extract of test 3 (males, 24 h), and fat extract of test 6 (females, 24 h) by TLC-cochromatography with the authentic nonradiolabelled reference items in two independent chromatographic systems (normal phase and reversed phase TLC).

Further small peaks or peak groups additionally detected in the metabolic profiles were characterized by their behaviour during extraction and clean up and the retention times in the HPLC-chromatograms.

All identified and unknown metabolites were determined quantitatively in composite samples of urine, plasma, and extracts from organs and tissues. For quantification, the ^{14}C-signals in the HPLC-chromatograms were integrated.

II. Results and discussion

A. Recovery

The overall recovery accounted for 97.2% to 98.8% of the administered dose in male rats (tests 1–3) and 97.8% to 99.7% in female rats (tests 4–6). The entire balances for the total radioactivity detected in urine, the combined GIT plus faeces sample, skin, and organs and tissues at sacrifice are shown in Table 5.1.1- 1.

Table 5.1.1-32: Balance of radioactivity in urine and in organs and tissues of male and female rats sacrificed 1 h, 4 h, and 24 h after a single oral administration of [pyridyl-2,6-^{14}C]fluopyram

Test no.	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Dose, route	**5 mg/kg bw**	**5 mg/kg bw**	**5 mg/kg bw**	**5 mg/kg bw**	**5 mg/kg bw**	**5 mg/kg bw**
Sex	**male**	**male**	**male**	**female**	**female**	**female**
Duration	**1 h**	**4 h**	**24 h**	**1 h**	**4 h**	**24 h**
[% dose administered]						
Excretion						
Urine	1.438	4.806	28.690	1.902	6.744	43.060
Samples						
Plasma	0.317	0.214	0.094	0.403	0.259	0.110
Carcass	13.820	6.016	2.713	29.160	23.090	6.118
Kidneys	0.404	0.289	0.116	0.573	0.424	0.120
Liver	4.570	3.079	1.711	5.518	4.004	1.225
GIT + faeces	71.230	82.610	64.200	42.760	47.520	47.360
Skin	4.723	1.511	0.763	17.650	10.290	1.437
Perirenal fat	0.663	0.288	0.062	1.093	1.496	0.309
Balance	**97.160**	**98.810**	**98.350**	**99.060**	**97.820**	**99.740**
Factor	1.029	1.012	1.017	1.011	1.022	1.003
Virtual matrix groups						
Body excluding GIT	24.500	11.400	5.461	54.400	43.560	9.320
GIT + faeces	71.230	82.610	64.200	42.760	47.520	47.360
Body	95.720	94.010	69.660	97.160	91.080	56.680

Table 5.1.1-33: Total radioactive residues in organs and tissues of male and female rats sacrificed 1 h, 4 h, and 24 h after a single oral administration of [pyridyl-2,6-^{14}C]fluopyram

Test no.	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Dose, route	5 mg/kg bw	5 mg/kg bw	5 mg/kg bw	5 mg/kg bw	5 mg/kg bw	5 mg/kg bw
Sex	male	male	male	female	female	female
Duration	1 h	4 h	24 h	1 h	4 h	24 h
[mg equiv./kg]*						
Plasma	1.273	0.690	0.343	1.702	1.260	0.407
Carcass	1.180	0.498	0.221	2.976	2.660	0.525
Kidneys	2.966	1.863	0.812	5.056	3.982	0.960
Liver	7.216	4.977	1.827	8.666	6.005	1.537
GIT + faeces	47.530	58.810	20.820	34.260	37.130	18.290
Skin	0.997	0.292	0.150	4.601	2.562	0.334
Perirenal fat	7.261	2.740	0.573	11.490	13.150	2.505
Body excluding GIT	1.397	0.619	0.291	3.710	2.842	0.539
GIT + faeces	47.530	58.810	20.820	34.260	37.130	18.290
Body	5.026	4.743	3.186	6.100	5.479	2.849

*Mean values

B. Distribution

The total radioactive residues (= TRR, expressed as percentage of administered dose and as equivalent concentration) detected in the organs and tissues at sacrifice are given in Table 5.1.1- 1 and Table 5.1.1-33.

In male rats, sacrificed one hour post dosing, *ca.* 24.5% of the dose were detected in the organs and tissues and *ca.* 71.2% in the GIT plus faeces sample. After 24 hours, the percentage of the dose in the organs declined to *ca.* 5.5%. This value indicated a fast distribution of the absorbed test item related radioactivity within the body followed by a quick elimination from the organs and tissues that led finally to a significant increase of the urinary excretion from *ca.*1.4% to *ca.* 28.7%.

The highest TRR-values were detected in the organs and tissues at one hour after administration. As shown in Table 5.1.1-34, all values decreased until the test end to significantly lower values. It is therefore expected that the residual amounts in these samples are subject to further elimination from the body.

In female rats, sacrificed one hour post dosing, *ca.* 54.4% of the dose was detected in the organs and tissues and *ca.* 42.8% in the GIT plus faeces sample. After 24 hours, the percentage of the dose in the organs declined to *ca.* 9.3%. These value indicated a very fast distribution of the absorbed test item related radioactivity within the body followed by a quick elimination from the organs and tissues that led finally to a significant increase of the urinary excretion from *ca.* 1.9% to *ca.* 43.1%.

Similar to male rats, the highest TRR-values were detected in the organs and tissues at one hour after administration. As shown in Table 5.1.1-35, all values decreased until the test end to significantly lower values and it is again expected that the residual amounts in these samples are subject to further elimination from the body. In contrast to the male rats, the TRR-values in nearly all organs and tissues

were higher at all points in time which was particularly noticeable for the perirenal fat values higher (factor 4.3 at 24 h).

Table 5.1.1-34: Decrease of radioactive residues residues in organs and tissues of male rats after a single oral administration of [Pyridyl-2,6-^{14}C]fluopyram

Test number	1	3	
Test period [h]	1	24	Decline by [%]
Samples	TRR [mg eq./kg]		
Plasma	1.273	0.343	73
Liver	7.216	1.827	75
Kidneys	2.966	0.812	73
Carcass	1.180	0.221	81
Perirenal fat	7.261	0.573	92
Skin	0.997	0.150	85
Body excluding GIT	1.397	0.291	79

Table 5.1.1-35: Decrease of radioactive residues residues in organs and tissues of female rats after a single oral administration of [Pyridyl-2,6-^{14}C]fluopyram

Test number	4	6	
Test period [h]	1	24	Decline by [%]
Samples	TRR [mg eq./kg]		
Plasma	1.702	0.407	76
Liver	8.666	1.537	82
Kidneys	5.056	0.960	81
Carcass	2.976	0.525	82
Perirenal fat	11.490	2.505	78
Skin	4.601	0.334	93
Body excluding GIT	3.710	0.539	85

D. Excretion

The urinary excretion started immediately after administration and increased to *ca*. 28.7% and *ca.* 43.1% of the administered dose after 24 hours. The amount excreted by female rats was about 1.5-times higher than by male rats (Table 5.1.1-32).

E. Metabolism

In urine of male rats, the major metabolite was fluopyram-pyridyl-acetic acid followed by fluopyram-ethyl-diol-GA (sum of 3 isomers) and fluopyram-hydroxy-PAA which accounted for *ca*. 7.9%, *ca*. 6.9% and *ca*. 4.4% of the dose, respectively, at sacrifice. Between 1% and 2% were calculated for fluopyram-hydroxy-ethyl-GA, fluopyram-enol-GA (sum of 2 isomers) and fluopyram-pyridyl carboxylic acid. The values for all other metabolites were less than 1% and the amount of the

Fluopyram (AE C656948) did not exceed 0.02% of the dose. The identification rate was higher than 90% of the TRR.

In urine of female rats, the major metabolite was fluopyram-pyridyl-acetic acid which increased to *ca.* 29.3% of the dose at 24 hours after administration. Between 1% and 2% were calculated for fluopyram-hydroxy-ethyl-GA, fluopyram-7-OH-phenol-GA, fluopyram-8-OH-GA (sum of 2 isomers) and fluopyram-ethyl-diol-GA (sum of 3 isomers) with maximum values of *ca.* 1.9%, *ca.* 1.6%, *ca.* 1.5% and *ca.* 1.3%, respectively. The values for all other metabolites accounted for less than 1% and the amount of parent fluopyram did not exceed 0.03% of the dose.

The identification rate was higher than 91% of the TRR. The quantitative results of the metabolic profiles of the urine samples from all tests are summarised in Table 5.1.1-36.

In plasma of male rats, the major components of the one hour sample were fluopyram-7-hydroxy (*ca.* 0.2%; 0.807 mg/kg), parent fluopyram (*ca.* 0.06%; 0.234 mg/kg), fluopyram-pyridyl-acetic acid (*ca.* 0.03%; 0.104 mg/kg) and fluopyram-7-OH-GA, isomer 1 (0.014%; 0.055 mg/kg). The identification rate was >99% of the TRR.

After four hours of the administration, fluopyram-7-hydroxy and fluopyram-pyridyl-acetic acid were again the prominent metabolites which accounted for *ca.* 0.09% (0.293 mg/kg) and *ca.* 0.03% (0.096 mg/kg), respectively. Until 24 hours, a further decline was observed and only two prominent metabolites (fluopyram-ethyl-diol-GA, isomer 3 and fluopyram-7-hydroxy) with values of more than 0.02% were detected.

In plasma of female rats, the major components of the one hour sample were the parent fluopyram (*ca.* 0.28%; 1.187 mg/kg), fluopyram-7-hydroxy (*ca.* 0.07%; 0.292 mg/kg) and fluopyram-pyridyl-acetic acid (*ca.* 0.04%; 0.153 mg/kg). The values for other metabolites were lower. The identification rate was >98% of the TRR.

No significant change in the metabolic pattern was observed in the four hour sample. At 24 hours, the percentage value for each component was less than 0.05% of the dose.

The quantitative results of the metabolic profiles of the plasma samples from all tests are shown in Table 5.1.1-37.

In liver of male rats, fluopyram-7-hydroxy was the only major component which accounted for more than 1% of the dose in the one hour sample. This amount decreased to *ca.* 0.13% until the test end at 24 hours. The unchanged parent Fluopyram was detected in the one and four hour sample with *ca.* 0.82% and *ca.* 0.08%, respectively. The percentage values for all other metabolites were less than 1% of the dose and the prominent components within this group were fluopyram-ethyl-diol-GA (sum of 3 isomers), fluopyram-7-OH-phenol, fluopyram-7-OH-GA (sum of 2 isomers), fluopyram-enol-GA (sum of 2 isomers), fluopyram-8-OH-GA (sum of 2 isomers) and fluopyram-8-hydroxy. fluopyram-*E*-olefin and fluopyram-*Z*-olefin were very low and amounted to less than 0.01% of the dose (≤ 0.01 mg/kg).

In consequence of the decreasing extractability, the identification rates in liver of male rats declined from *ca.* 79% of the TRR at one hour to *ca.* 48% of the TRR at 24 hours after administration. The TRR-value in the extract was high for the first sampling period (6.697 mg/kg) which declined to 4.008 mg/kg until four and 0.994 mg/kg until 24 hours after administration. At one hour after administration, higher values than 1 mg/kg were calculated for the parent fluopyram (1.287 mg/kg) and fluopyram-7-hydroxy (1.663 mg/kg). Up to the test end, only fluopyram-7-hydroxy and fluopyram-ethyl-diol-GA (sum of 3 isomers) reached values of more than 0.1 mg/kg.

In liver of female rats, the unchanged parent Fluopyram was the only major component which accounted for more than 1% of the dose in the one hour sample. This amount decreased to *ca*. 0.08% until the test end at 24 hours. The percentage values for all other metabolites were less than 1% and the prominent components within this group were fluopyram-7-hydroxy, fluopyram-8-OH-GA (sum of 2 isomers) and fluopyram-pyridyl-acetic acid. The values for fluopyram-*E*-olefin and fluopyram-*Z*-olefin were very low and did not exceed 0.01% of the dose (sum of both).

In consequence of the decreasing extractability, the identification rate in liver of female rats declined from *ca*. 92% of the TRR at one hour to *ca*. 45% of the TRR at 24 hours after administration. The TRR-value of the extract was high for the first sampling period (8.42 mg/kg) which declined to 5.593 mg/kg until four and 0.996 mg/kg until 24 hours after administration. At one hour after administration, higher values than 1 mg/kg were calculated only for the parent Fluopyram (5.317 mg/kg). At the test end, only fluopyram-7-hydroxy reached a value of more than 0.1 mg/kg.

The quantitative results of the metabolic profiles in the liver extracts of all tests are shown in Table 5.1.1-38.

In kidney of male rats, fluopyram-pyridyl-acetic acid and fluopyram-7-hydroxy were the major metabolites, which accounted for more than 0.1% of the dose each in the one hour sample. This amount decreased to less than 0.03% until the test end at 24 hours. The unchanged parent Fluopyram was detected in the one and four hour sample with *ca*. 0.06% and *ca*. 0.01%, respectively. The percentage values for all other metabolites were less than 0.1% of the dose and the prominent components within this group were fluopyram-8-hydroxy and fluopyram-hydroxy-PAA.

The identification rate in kidney of male rats declined only slightly from *ca*. 94% at one hour to *ca*. 85% of the TRR at 24 hours after administration. The TRR-value of the extract was high for the first sampling period (2.915 mg/kg) which declined to 1.774 mg/kg until four and 0.703 mg/kg until 24 hours after administration. At one hour after administration, higher values than 0.05 mg/kg were calculated for fluopyram-pyridyl-acetic acid (0.89 mg/kg), fluopyram-7-hydroxy (0.854 mg/kg), fluopyram-8-hydroxy (0.184 mg/kg), fluopyram-hydroxy-PAA (0.11 mg/kg) and the parent Fluopyram (0.456 mg/kg). Up to the test end, only fluopyram-pyridyl-acetic acid, fluopyram-ethyl-diol-GA (sum of 3 isomers), fluopyram-hydroxy-PAA and fluopyram-7-hydroxy reached values of more than 0.05 mg/kg.

In kidney of female rats, the parent Fluopyram and fluopyram-pyridyl-acetic acid were the major components which accounted for more than 0.1% of the dose in the one hour sample. This amount decreased to less than 0.05% until the test end (24 h). For all other metabolites, lower values than 0.05% were measured and with in this group fluopyram-8-hydroxy was prominent.

The identification rate in kidney of female rats declined from *ca*. 97% of the TRR at one hour to *ca*. 74% of the TRR at 24 hours after administration. The TRR-value of the extract was high for the first sampling period (5.024 mg/kg) which declined to 3.93 mg/kg until four and 0.865 mg/kg until 24 hours after administration. At one hour after administration, higher values than 0.05 mg/kg were calculated for the parent Fluopyram (2.775 mg/kg), fluopyram-pyridyl-acetic acid (1.406 mg/kg), fluopyram-7-hydroxy (0.423 mg/kg) and fluopyram-8-hydroxy (0.29 mg/kg). Up to the test end, only fluopyram-pyridyl-acetic acid and fluopyram-7-hydroxy reached values of more than 0.06 mg/kg.

The quantitative results of the metabolic profiles in the kidney extracts of all tests are shown in Table 5.1.1-39.

In fat of male rats, the parent Fluopyram and fluopyram-7-hydroxy were the prominent components which accounted for more than 0.2% of the dose in the one hour sample. This amount decreased to

less than 0.03% until the test end at 24 hours. The percentage values for all other metabolites were lower than 0.03%. Similarly to the one hour liver extract, the values for fluopyram-*E*-olefin and fluopyram-*Z*-olefin were very low and amounted to less than 0.01% of the dose each.

The identification rate in fat of male rats declined only slightly from *ca.* 96% of the TRR after one hour to *ca*. 90% of the TRR after 24 hours. The TRR-value of the extract was high for the first sampling period (7.228 mg/kg) which declined to 2.699 mg/kg until four and 0.544 mg/kg until 24 hours after administration. At one hour after administration, higher values than 0.05 mg/kg were calculated for the parent Fluopyram (3.972 mg/kg), fluopyram-7-hydroxy (2.604 mg/kg), fluopyram-8-hydroxy (0.279 mg/kg) and fluopyram-pyridyl-carboxylic acid (0.056). Up to the test end, only fluopyram-7-hydroxy, fluopyram-ethyl-diol-GA (sum of 3 isomers) and fluopyram-*Z*-olefin reached values of more than 0.05 mg/kg.

In fat of female rats, the parent Fluopyram was the only prominent component which accounted for more than 0.9% of the dose in the one hour sample. This amount decreased to *ca.* 0.2% until the test end at 24 hours after administration. The only significant metabolites were fluopyram-7-hydroxy and fluopyram-8-hydroxy with maximum values of *ca*. 0.11% and *ca*. 0.03%, respectively. fluopyram-*E*-olefin and fluopyram-*Z*-olefin were identified at a low amount of less than 0.01% of the dose each.

The identification rate in fat from female rats declined only slightly from *ca.* 95% of the TRR at one hour to *ca*. 86% of the TRR at 24 hours after administration. The TRR-value of the extract was high for the first sampling period (11.078 mg/kg) which increased to 13.111 mg/kg until four and decreased to 2.476 mg/kg until 24 hours after administration. At one hour after administration, higher values than 0.05 mg/kg were calculated for the parent Fluopyram (9.922 mg/kg), fluopyram-7-hydroxy (0.731 mg/kg) and fluopyram-8-hydroxy (0.294 mg/kg). Up to the test end, parent Fluopyram, fluopyram-7-hydroxy and fluopyram-*Z*-olefin reached values of more than 0.05 mg/kg. The quantitative results of the metabolic profiles in the fat extracts of all tests are shown in Table 5.1.1-40.

Table 5.1.1-36: Quantitative evaluation of Fluopyram (AE C656948) and metabolites in urine of male and female rats sacrificed 1 h, 4 h, and 24 h after a single oral administration

Test no.		1	2	3	4	5	6
Sex		male	male	male	female	female	female
Duration		1 h	4 h	24 h	1 h	4 h	24 h
		[% dose administered]					
Total radioactive residue in urine pool sample		1.438	4.806	28.690	1.902	6.744	43.060
Peak ID	Compound						
80	fluopyram (AE C656948), a.s.	0.024	---	---	0.026	0.019	0.027
6	fluopyram-pyridyl-carboxylic acid (M43)	0.089	0.243	1.160	0.058	0.084	0.341
7	fluopyram-hydroxy-PAA (M41)	0.165	0.590	4.393	0.028	0.085	0.793
9	fluopyram-ethyl-diol-GA (M39), isomer 1	---	0.067	0.905	---	0.023	0.386
11	fluopyram-ethyl-diol-GA (M39), isomer 2	---	0.040	0.568	---	---	0.132
12	fluopyram-ethyl-diol-GA (M39), isomer 3	0.032	0.283	5.464	---	0.038	0.804
16	fluopyram-pyridyl-acetic acid (M40)	0.828	2.402	7.893	1.545	5.343	29.265
26	fluopyram-hydroxy-ethyl-GA (37)	---	0.088	1.812	0.019	0.076	1.896
29	fluopyram-ethyl-diol (M38)	---	0.019	0.460	---	---	0.096
33	fluopyram-methoxy-di-OH-GA (M23)	---	0.016	0.100	---	0.016	---
34	fluopyram-pyridyl-methyl-cysteine (M44)	---	---	0.050	---	---	---
35	fluopyram-7-OH-phenol-GA (M14)	---	0.037	0.912	---	0.027	1.579
40	fluopyram-7-OH-GA (M09), isomer 1	0.020	0.103	0.361	0.025	0.075	0.697
42	fluopyram-7-OH-GA (M09), isomer 2	---	---	0.020	---	0.026	0.248
43	fluopyram-7-OH-hydroxy-phenol-SA (M17)	---	---	0.065	---	0.008	0.085
47	fluopyram-di-OH-GA (M21)	---	0.016	0.121	---	0.017	0.383
48	fluopyram-enol-GA (M04), isomer 1	0.057	0.218	0.770	0.036	0.015	0.141
49	fluopyram-7-OH-phenol-SA (M15)	---	---	---	---	---	0.038
50	fluopyram-phenol-GA (M07)	---	---	0.275	---	0.065	0.997
51	fluopyram-8-OH-GA (M20), isomer 1	---	---	0.058	---	0.034	0.189
57	fluopyram-8-OH-GA (M20), isomer 2	---	---	---	---	0.197	1.327
60	fluopyram-enol-GA (M04), isomer 2	0.087	0.242	1.232	0.093	0.013	0.145
64	fluopyram-7-OH-phenol (M13)	---	---	0.151	---	0.002	0.102
68	fluopyram-7-OH-methyl-sulfone (M16)	0.020	0.035	0.070	0.018	0.062	0.370
73	fluopyram-7-hydroxy (M08)	0.026	0.019	0.026	0.010	0.014	0.042
75	fluopyram-8-hydroxy (M18)	---	---	---	---	---	0.018
Sum identified		1.349	4.388	26.867	1.859	6.240	40.100
Sum characterized *		0.089	0.418	1.823	0.043	0.504	2.960
Sum total		1.438	4.806	28.690	1.902	6.744	43.060

* Sum of unknown peaks

Table 5.1.1-37: Quantitative evaluation of Fluopyram (AE C656948) and metabolites in plasma of male and female rats sacrificed 1 h, 4 h, and 24 h after a single oral administration

Test no.		1	2	3	4	5	6
Sex		male	male	male	female	female	female
Duration		1 h	4 h	24 h	1 h	4 h	24 h
		[% dose administered]					
Total radioactive residue in plasma pool sample		0.317	0.214	0.094	0.403	0.259	0.110
Peak ID	Compound						
80	fluopyram (fluopyram), a.s.	0.058	0.008	---	0.281	0.148	0.012
7	fluopyram-hydroxy-PAA (M41)	---	0.007	0.003	---	---	---
12	fluopyram-ethyl-diol-GA (M39), isomer 3	---	0.006	0.019	---	---	---
16	fluopyram-pyridyl-acetic acid (M40)	0.026	0.030	0.004	0.036	0.026	---
26	fluopyram-hydroxyethyl-GA (M37)	---	---	0.002	---	---	---
29	fluopyram-ethyl-diol (M38)	---	0.005	0.002	---	---	---
35	fluopyram-7-OH-phenol-GA (M14)	---	0.006	0.007	---	---	---
40	fluopyram-7-OH-GA (M09), isomer 1	0.014	0.012	0.002	0.007	0.003	0.005
43	fluopyram-7-OH-hydroxy-phenol-SA (M17)	---	0.005	---	---	---	---
48	fluopyram-enol-GA (M04), isomer 1	0.011	0.012	0.002	---	---	---
68	fluopyram-7-OH-methyl-sulfone (M16)	0.007	0.002	---	0.003	0.005	---
73	fluopyram-7-hydroxy (M08)	0.201	0.091	0.016	0.069	0.062	0.040
75	fluopyram-8-hydroxy (M18)	---	---	---	---	---	0.010
Sum identified		0.317	0.182	0.056	0.397	0.244	0.067
Sum characterized *		---	0.031	0.039	0.006	0.015	0.043
Sum total		0.317	0.214	0.094	0.403	0.259	0.110

* Sum of unknown peaks

Table 5.1.1-38: Quantitative evaluation of fluopyram (AE C656948) and metabolites in liver of male and female rats sacrificed 1 h, 4 h, and 24 h after a single oral administration

Test no.		1	2	3	4	5	6
Sex		male	male	male	female	female	female
Duration		1 h	4 h	24 h	1 h	4 h	24 h
		[% dose administered]					
Total radioactive residue in liver pool sample		4.570	3.079	1.711	5.518	4.004	1.225
Peak ID	Compound						
80	fluopyram (AE C656948), a.s.	0.815	0.075	---	3.385	1.885	0.078
6	fluopyram-pyridyl-carboxylic acid (M43)	0.028	0.020	0.012	---	---	---
7	fluopyram-hydroxy-PAA (M41)	0.015	0.017	0.017	---	---	---
9	fluopyram-ethyl-diol-GA (M39), isomer 1	---	---	0.022	---	---	---
11	fluopyram-ethyl-diol-GA (M39), isomer 2	---	---	0.014	---	---	---
12	fluopyram-ethyl-diol-GA (M39), isomer 3	0.035	0.053	0.243	0.004	0.010	0.021
16	fluopyram-pyridyl-acetic acid (M40)	0.069	0.073	0.023	0.108	0.092	0.026
26	fluopyram-hydroxy-ethyl-GA (M37)	0.009	0.013	0.020	0.006	0.007	0.004
29	fluopyram-ethyl-diol (M38)	0.035	0.020	0.029	0.006	0.008	0.011
33	fluopyram-methoxy-di-OH-GA (M23)	---	0.010	0.005	---	---	---
34	fluopyram-pyridyl-methyl-cysteine (M44)	---	0.012	---	---	---	---
35	fluopyram-7-OH-phenol-GA (M14)	0.052	0.079	0.062	0.017	0.029	0.021
40	fluopyram-7-OH-GA (M09), isomer 1	0.183	0.142	0.081	0.081	0.067	0.027
42	fluopyram-7-OH-GA (M09), isomer 2	0.029	0.029	0.005	0.017	0.019	0.013
43	fluopyram-7-OH-hydroxy-phenol-SA (M17)	0.030	0.016	0.006	0.023	0.023	0.003
47	fluopyram-di-OH-GA (M21)	---	---	0.011	0.010	0.025	---
48	fluopyram-enol-GA (M04), isomer 1	0.333	0.283	0.069	0.036	0.015	---
49	fluopyram-7-OH-phenol-SA (M15)	0.041	---	---	0.017	0.007	0.005
50	fluopyram-phenol-GA (M07)	---	---	0.010	0.058	0.040	0.007
51	fluopyram-8-OH-GA (M20), isomer 1	---	---	---	0.060	0.048	0.009
57	fluopyram-8-OH-GA (M20), isomer 2	0.134	0.091	0.023	0.252	0.173	0.041
60	fluopyram-enol-GA (M04), isomer 2	0.012	0.054	0.017	0.015	0.023	0.003
64	fluopyram-7-OH-phenol (M13)	0.487	0.141	0.029	0.038	0.061	0.021
68	fluopyram-7-OH-methyl-sulfone (M46)	0.027	0.109	0.010	0.166	0.145	0.022
73	fluopyram-7-hydroxy (M08)	1.053	0.509	0.125	0.460	0.499	0.164
75	fluopyram-8-hydroxy (M18)	0.236	0.150	0.037	0.288	0.242	0.070
84	fluopyram-*E*-olefin (M02)	0.003	---	---	---	---	0.002
87	fluopyram-*Z*-olefin (M03)	0.005	0.006	0.003	0.004	0.005	0.007
Subtotal extracts		4.241	2.480	0.931	5.362	3.730	0.794
Sum identified		3.609	1.902	0.823	5.051	3.423	0.552
Sum characterized *		0.632	0.578	0.108	0.311	0.307	0.242
Not analysed fraction		0.023	0.032	0.012	0.010	0.012	0.007
Solids (post extraction)		0.305	0.567	0.768	0.146	0.263	0.424
Sum total		4.570	3.079	1.711	5.518	4.004	1.225

* Sum of unknown peaks

Table 5.1.1-39: Quantitative evaluation of fluopyram (AE C656948) and metabolites in kidney of male and female rats sacrificed 1 h, 4 h, and 24 h after a single oral administration

Test no.		1	2	3	4	5	6
Sex		male	male	male	female	female	female
Duration		1 h	4 h	24 h	1 h	4 h	24 h
		[% dose administered]					
Total radioactive residue in kidney pool sample		0.404	0.289	0.116	0.573	0.424	0.120
Peak ID	Compound						
80	fluopyram (AE C656948), a.s.	0.062	0.009	---	0.304	0.170	0.007
6	fluopyram-pyridyl-carboxylic acid (M43)	0.004	0.005	0.003	---	---	---
7	fluopyram-hydroxy-PAA (M41)	0.015	0.024	0.011	---	---	0.003
9	fluopyram-ethyl-diol-GA (M39), isomer 1	---	---	0.005	---	---	---
11	fluopyram-ethyl-diol-GA (M39), isomer 2	---	---	0.002	---	---	---
12	fluopyram-ethyl-diol-GA (M39), isomer 3	0.004	0.008	0.022	---	---	0.002
16	fluopyram-pyridyl-acetic acid (M40)	0.121	0.129	0.023	0.159	0.156	0.040
26	fluopyram-hydroxy-ethyl-GA (M37)	---	---	0.006	---	---	0.002
29	fluopyram-ethyl-diol (M38)	0.002	0.003	0.004	---	---	---
35	fluopyram-7-OH-phenol-GA (M14)	0.002	0.004	0.003	---	---	0.003
40	fluopyram-7-OH-GA (M09), isomer 1	0.003	0.004	0.001	---	---	0.001
42	fluopyram-7-OH-GA (M09), isomer 2	0.002	0.003	0.001	---	---	---
43	fluopyram-7-OH-hydroxy-phenol-SA (M17)	0.002	---	---	---	---	---
48	fluopyram-enol-GA (M04), isomer 1	0.007	0.004	0.002	---	---	---
49	fluopyram-7-OH-phenol-SA (M15)	---	---	---	---	---	0.001
50	fluopyram-phenol-GA (M07)	---	---	---	---	---	0.001
57	fluopyram-8-OH-GA (M20), isomer 2	---	---	---	---	---	0.001
60	fluopyram-enol-GA (M04), isomer 2	0.004	0.004	---	---	---	0.001
64	fluopyram-7-OH-phenol (M13)	0.008	0.004	0.002	---	0.002	---
68	fluopyram-7-OH-methyl-sulfone (M16)	0.002	---	0.001	---	---	0.004
73	fluopyram-7-hydroxy (M08)	0.116	0.050	0.010	0.048	0.046	0.017
75	fluopyram-8-hydroxy (M18)	0.025	0.012	0.003	0.033	0.022	0.007
84	fluopyram-*E*-olefin (M02)	---	---	0.001	---	---	---
Subtotal extracts		0.397	0.275	0.101	0.569	0.418	0.108
Sum identified		0.379	0.263	0.099	0.554	0.395	0.089
Sum characterized *		0.018	0.012	0.002	0.015	0.023	0.019
Not analysed fraction		0.001	0.002	0.001	0.001	0.001	0.002
Solids (post extraction)		0.006	0.012	0.015	0.003	0.004	0.010
Sum total		0.404	0.289	0.116	0.573	0.424	0.120

* Sum of unknown peaks

Table 5.1.1-40: Quantitative evaluation of fluopyram (AE C656948) and metabolites in perirenal fat of male and female rats sacrificed 1 h, 4 h, and 24 h after a single oral administration

Test no.		1	2	3	4	5	6
Sex		male	male	male	female	female	female
Duration		1 h	4 h	24 h	1 h	4 h	24 h
		[% dose administered]					
Total radioactive residue in fat pool sample		0.663	0.288	0.062	1.093	1.496	0.309
Peak ID	Compound						
80	fluopyram (AE C656948), a.s.	0.363	0.139	0.001	0.944	1.336	0.203
6	fluopyram-pyridyl-carboxylic acid (M43)	0.005	0.002	0.002	---	---	---
9	fluopyram-ethyldiol-GA (M39), isomer 1	---	---	0.002	---	---	---
11	fluopyram-ethyl-diol-GA (M39), isomer 2	---	---	0.001	---	---	---
12	fluopyram-ethyl-diol-GA (M39), isomer 3	---	0.001	0.010	---	---	---
16	fluopyram-pyridyl-acetic acid (M40)	---	0.001	0.001	---	---	---
26	fluopyram-hydroxy-ethyl-GA (M37)	---	---	0.002	---	---	---
29	fluopyram-ethyl-diol (M38)	0.001	0.001	0.003	---	---	---
35	fluopyram-7-OH-phenol-GA (M14)	---	<0.001	0.001	---	---	---
40	fluopyram-7-OH-GA (M09), isomer 1	0.002	0.002	<0.001	---	---	---
48	fluopyram-enol-GA (M04), isomer 1	0.001	0.002	<0.001	---	---	---
50	fluopyram-phenol-GA (M07)	---	0.003	0.001	---	---	---
51	fluopyram-8-OH-GA (M20), isomer 1	---	---	0.001	---	---	---
57	fluopyram-8-OH-GA (M20), isomer 2	---	0.001	---	---	---	---
60	fluopyram-enol-GA (M04), isomer 2	---	---	0.001	---	---	---
64	fluopyram-7-OH-phenol (M13)	0.002	0.001	---	---	---	---
68	fluopyram-7-OH-methyl-sulfone (M16)	---	---	---	---	0.002	---
73	fluopyram-7-hydroxy (M08)	0.238	0.107	0.020	0.070	0.110	0.042
75	fluopyram-8-hydroxy (M18)	0.026	0.010	0.002	0.028	0.027	0.010
84	fluopyram-*E*-olefin (M02)	---	---	0.001	---	---	0.004
87	fluopyram-Z-olefin (M03)	0.001	0.002	0.007	0.001	---	0.007
Subtotal extracts		0.660	0.283	0.059	1.054	1.492	0.305
Sum identified		0.638	0.274	0.056	1.042	1.476	0.266
Sum characterized *		0.022	0.009	0.003	0.012	0.015	0.039
Not analysed fraction		0.001	0.001	0.001	0.001	0.001	0.001
Solids (post extraction)		0.002	0.003	0.002	0.038	0.003	0.003
Sum total		0.663	0.288	0.062	1.093	1.496	0.309

* Sum of unknown peaks

III. Conclusions

Fluopyram was extensively metabolised in male and female Winstar rats and more than 20 metabolites were identified. Molecular cleavage occurred at least in a range of 23–34% of the administered dose in both sexes represented by numerous label specific metabolites. In the various samples, sex specific differences in the ratio of pyridyl label specific and common metabolites which contained still the intact molecular structure were observed. The metabolic transformation of fluopyram was generally more pronounced in male rats.

The metabolic transformation reactions of [pyridyl-2,6-^{14}C]fluopyram in male and female rats in detail were:

- hydroxylation of the ethylene bridge of the molecule resulting in fluopyram-7-hydroxy, fluopyram-8-hydroxy, and a dihydroxylated compound
- hydroxylation of the phenyl ring leading to fluopyram-phenol and fluopyram-7-OH-phenol
- molecular cleavage and conjugation to fluopyram-pyridyl-hydroxyethyl conjugates
- molecular cleavage and oxidation to fluopyram-pyridyl-carboxylic acid
- oxidation of fluopyram-pyridyl-hydroxyethyl to mainly fluopyram-pyridyl-acetic acid and to a lower extent to fluopyram-ethyl-diol, fluopyram-hydroxy-PAA and fluopyram-pyridyl-carboxylic acid
- elimination of water from compounds hydroxylated in the ethylene bridge leading to fluopyram-*Z*-olefin and *E*-olefin (*E*- and *Z*-olefin can isomerise into each other)
- conjugation of several hydroxylated metabolites with glucuronic acid and to a lower extent with sulfate

On the basis of the nature and amount of metabolites found in rat tissues, the metabolic pathway of [pyridyl-2,6-^{14}C]fluopyram is proposed in Figure 5.1.1-3.

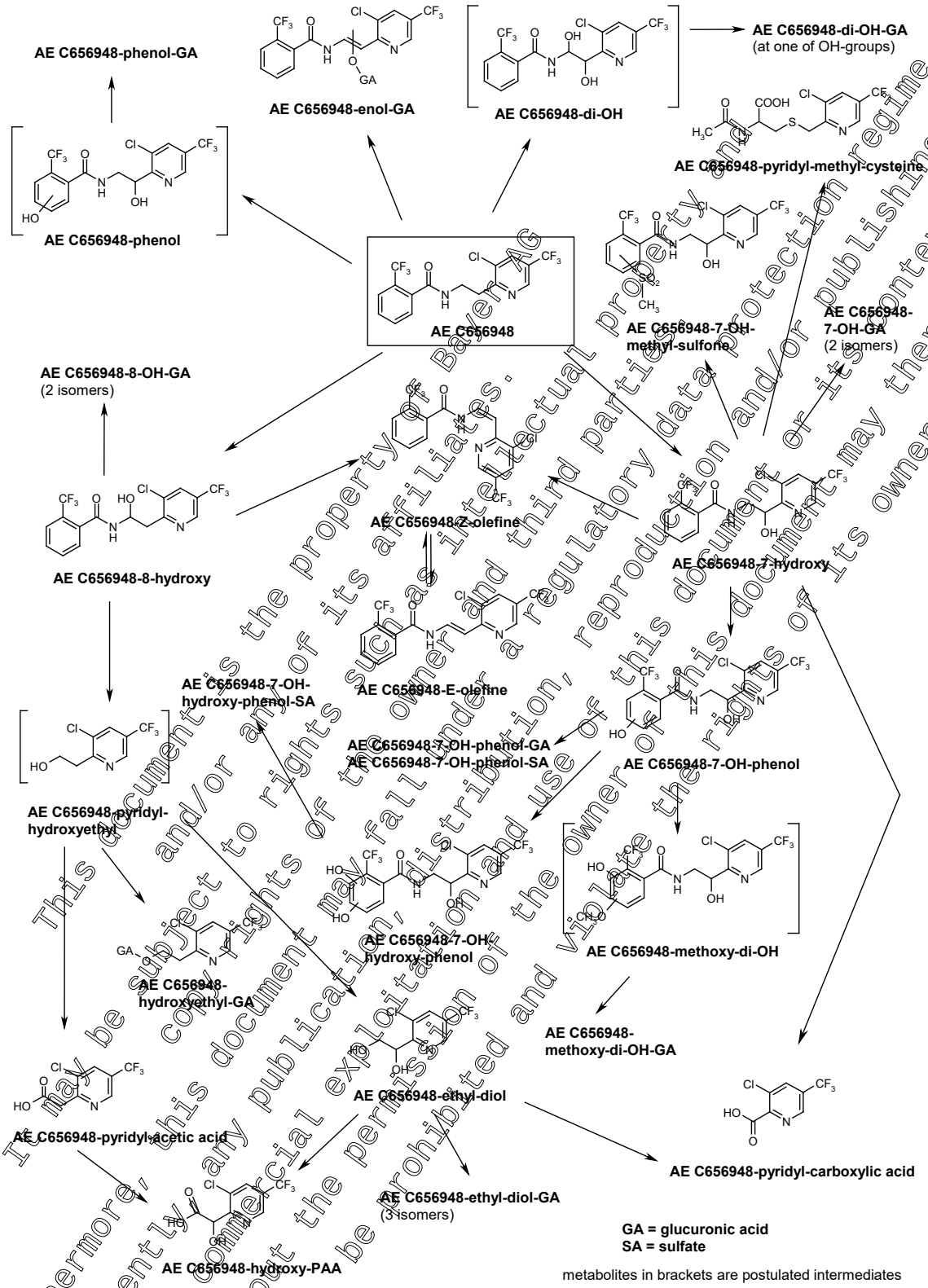


Figure 5.1.1-3: Proposed metabolic pathway of [pyridyl-2,6-^{14}C]AE C656948 (fluopyram) in the rat

III. Conclusions

The kinetic and metabolic behaviour of [pyridyl-2,6-^{14}C]fluopyram in male and female Wistar rats can be characterised by the following observations:

- Sex-related differences were observed for the amount of radioactivity excreted with urine.
- The distribution of the radioactivity within the central compartments (i.e. blood, liver, kidney) of the body was fast and showed a distinctive preference towards the liver as the main organ responsible for metabolism and – to a lower extent – to the kidney.
- As anticipated, the highest TRR-values were measured in plasma, liver and kidney at one hour after administration. They decreased significantly within the test period of 24 hours. A retention of test item related radioactivity in any of the organs and tissues investigated can therefore be excluded.
- The extraction efficiency using conventional methods was sufficient for kidney, perirenal fat and the one hour liver samples (>87%). At later times, the extractability of radioactive residues from liver samples of both sexes decreased significantly.
- Fluopyram (AE C656948), all major and several minor metabolites were identified in all matrices. Identification rates ranged from *ca.* 93% to *ca.* 98% in urine, *ca.* 61% to 100% in plasma, *ca.* 45% to *ca.* 92% in liver, *ca.* 74% to *ca.* 97% in kidney and *ca.* 86% to *ca.* 99% in the perirenal fat.
- The metabolic transformation of fluopyram was principally oxidative and took place mainly at the ethylene bridge of the molecule. The major metabolites were fluopyram-7-hydroxy and fluopyram-pyridylacetic acid.
- Sex specific differences in relation to the extent of metabolic transformation of the parent Fluopyram (AE C656948) were observed. This resulted in significantly higher levels of the unchanged test item in nearly all samples of female rats.
- The metabolism results are in good accordance with those obtained from the ADME-study which was conducted with the pyridyl radiolabelled test item as well.

Based on the results obtained the depletion of radioactive residues from the organs and tissues of male and female rats and metabolism of [pyridyl-2,6-^{14}C]fluopyram are assumed as adequately understood.

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Assessment and conclusion by applicant:

The study is valid and acceptable.

Data Point:	KCA 5.1.1/06
Report Author:	[redacted]
Report Year:	2009
Report Title:	Fluopyram - Toxicokinetics in ADME studies - Differences between the phenyl- and pyridyl-radiolabel
Report No:	M-345016-01-1
Document No:	M-345016-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	None
Previous evaluation:	No, submitted but not evaluated
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

This position paper was provided upon request of BfR in order to explain the differences in the toxicokinetic data depending on the part of the molecule that had been radiolabeled. Please refer to page 3 of revised DAR Vol 3 B6 from 30 August 2012.

From the findings of the ADME studies, it can be concluded that the early phase of biokinetics is similar for both radiolabels and mainly reflect the behaviour of the primary metabolites which still contain the whole molecular moiety. Later on the cleavage products are dominating the biokinetic behaviour of the radioactive residues. After the cleavage, absorption and distribution in the body of the rat, the pyridyl label specific metabolites are obviously faster excreted compared to the phenyl-label specific metabolites, i.e. mainly AE C656948-benzamide. This different behaviour can be explained by the more polar nature of the metabolites from the pyridyl part of the molecule.
Consequently, the application of phenyl-labelled fluopyram resulted in higher t_{max} and AUC values for the radioactive residues compared to the application of the pyridyl label.

Data Point:	KCA 5.1.1/07
Report Author:	[redacted]
Report Year:	2009
Report Title:	Fluopyram - Evaluation of OECD joint review dossier - Questions addressed from German BfR - Metabolism, toxicology, dated February 23, 2009
Report No:	M-345006-01-1
Document No:	M-345006-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	None
Previous evaluation:	No, submitted but not evaluated
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

This position paper was provided upon request of BfR. It contains responses to multiple questions. The response relevant for this section is presented here. Question: Remarkable inter-species differences became apparent when the amounts of certain metabolites in rats on the one hand and goats/cows/hen on the other hand were compared. Metabolites of potential toxicological concern (fluopyram-benzamide or the fluopyram olefins) were affected by these differences. Is there an explanation for these differences?

The metabolism of fluopyram in rats and livestock is principally the same. Due to the fact that in the ADME studies the excreta are analysed for metabolites in contrast to the organs and tissues in the livestock metabolism studies, the quantitative equivalence is more difficult to show. Nevertheless, it is possible to set up a simple comparison for the metabolites in question. Due to the hydrophobic nature of the fluopyram-olefins they were predominantly found in the fatty tissues of the rat (organ depletion study, [redacted]; 2008; M-298834-01) and in livestock. In rat the highest residue (sum of both isomers at 24 h after dosing) was found in the perirenal fat at a maximum amount of 0.014% of the dose which is equivalent to 0.086 mg parent compound equivalents/kg. In the laying hen the highest amount of the fluopyram-olefins in fat was 0.462 mg/kg ([redacted]; 2008; M-297093-01). The rat received a dose of 1x 5 mg/kg bw. and the hen 14x 2 mg/kg bw. If this is taken into account, then the fluopyram-olefine residues are on a comparable level. The same holds true for the goat. The highest amount of fluopyram- olefins in fat was 0.133 mg/kg ([redacted] 2008; M-297849-01).

The rat received a dose of 1x 5 mg/kg bw. and the goat 5x 2 mg/kg bw. If these different dose regimes are taken into account, then the olefine residues are again on a comparable level.

The results are consistent with the findings from the feeding studies, where fluopyram- olefins were always < LOD or LOQ at the 0.1X and 1X feeding levels.

For the fluopyram-benzamide the comparison can be done via the excreta, which contain by far the major amount of radioactive residues: In the ADME study with phenyl-labeled fluopyram 3.5% of the dose were excreted as fluopyram-benzamide after 48 h (bile duct cannulation test) and 16-24% of the dose were excreted as fluopyram-benzamide after 168 h (see Table 1 of this document). In the laying hen study with phenyl-labeled fluopyram ([redacted]; 2008; M-297093-01) the amount of fluopyram-benzamide in the excreta after 14 days of dosing was 45% of the dose. This percentage is derived from the amount of the metabolite in the excreta given as 54.6% of the TRR and a TRR given in the report as 82.67% of the dose which is excreted after 14 days (cumulative). These 45% are roughly comparable with the 16-24% of the dose found after 168 h taking the different dose rates into account.

In the lactating goat study with phenyl-labeled fluopyram ([redacted]; 2008; M-299111-01) the amount of fluopyram-benzamide in the excreta after 5 days of dosing was 4.52% of the dose which has to be compared to the findings in the rat of 3.5% after 48 h.

The higher fluopyram-benzamide residues in livestock tissues measured in the feeding studies are also in agreement with the results of the quantitative whole-body autoradiography study with the phenyl-labeled fluopyram. As explained before, the delayed distribution, delayed and ongoing excretion of the fluopyram-benzamide related radioactivity showed that quantifiable residues in livestock are possible. It can be concluded that there are no inter-species differences in the metabolism of fluopyram in rat and livestock.

CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

Data Point:	KCA 5.1.2/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Amendment to study report no.1 - Metabolic stability and profiling of [phenyl-UL-14C]fluopyram in liver microsomal fractions from human, rat, dog, rabbit and mouse for inter-species comparison
Report No:	S18-07838
Document No:	M-667764-02-1
Guideline(s) followed in study:	US EPA OCSPP Not Applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 5.1.2/03
Report Author:	[redacted]
Report Year:	2021
Report Title:	Metabolic stability and profiling of [pyridyl-2,6-14C]fluopyram in liver microsomal fractions from human, rat, dog, rabbit and mouse for inter-species comparison and m/z-value determination of metabolite Unkown4 derived from [phenyl-UL-14C]fluopyram in liver microsomal fractions from human
Report No:	S19-23624
Document No:	M-762838-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 amended by the Commission Regulation (EU) No. 283/2013 (Europe) EU Regulation 1107/2009 (SANCO/11802/2010 Rev. 7 US EPA OCSPP Not Applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Metabolic profiles of fluopyram were compared between different species using an *in-vitro* system based on liver microsomal fraction from humans (mixed gender), rat (mixed gender), dog (male), mouse (mixed gender) and rabbit (female). 1 and 10 µM [phenyl-UL-^{14}C]-fluopyram were incubated for 0, 60 and 120 min with incubation buffer and the NADPH generating biochemical system consisting of NADP, glucose-6-phosphate and the enzyme glucose-6-phosphate-dehydrogenase. The metabolic capability of the tested liver microsomal fractions was proven with [4-^{14}C]-testosterone.

In general, without the NADP-generating biochemical system the test item was proven stable for at

least 120 minutes in liver microsomal incubation solutions from all species and sexes.

Recoveries of radioactivity after incubation were determined by LSC after sample processing and ranged from 95.5% to 106.7% with three exceptions at less than 93% or more than 111% after 60 minutes and from 94.0% to 110.6% after 120 minutes. Radioactivity after incubation of 10 µM [4-^{14}C]-testosterone was recovered at levels ranging from 104.0% to 107.5% after 120 minutes.

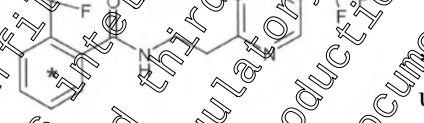
[Phenyl-UL-^{14}C]-fluopyram was biotransformed at a moderate rate after incubation with human and rat liver microsomes (up to 53.42% and 38.53% transformation rate, respectively), at a moderate to high rate after incubation with dog and mouse liver microsomes (up to 73.69% and 80.86% transformation rate, respectively) and at a high rate after incubation with rabbit liver microsomes (up to 92.88% transformation rate).

In total up to six (human, rat, dog), eight (mouse) and seven (rabbit) metabolites were detected after incubation.

All metabolites >5% AR detected in human mixed liver microsomes were detected in other species than human, too. As one metabolite was only identified in human and rabbit liver microsomes identification was necessary and was subsequently carried out in study S19-23624 (M-762838-01-1). The metabolite was identified as fluopyram benzamide (M25), a metabolite that was also identified during a rat metabolism study (M-298614-02-1). Metabolites were only characterised based on their chromatographic behaviour and no metabolic pathway was derived.

A. Materials and methods

1. Test Material:

IUPAC Name	*N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridyl]ethyl]-α,α,α-trifluoro-ortho-toluamide
Chemical Name	*N*-(2-(3-chloro-5-(trifluoromethyl)-2-pyridinyl)ethyl)-2-(trifluoromethyl)benzamide
Code names	BCS-AR83685, M-00020621
Common name	Fluopyram
Empirical formula	$C_{16}H_{11}ClF_6N_2O$
CAS Number	658066-35-4
Molar mass	396.7 g/mol
Chemical structure	 * position of the uniformly-labelled phenyl ring
Radiolabelled test material	[Phenyl-UL-^{14}C]Fluopyram
Batch number	KML 10676
Original specific radioactivity	5.15 MBq/mg
Radiochemical purity	>98% by radio-HPLC
Chemical purity	>98% by HPLC, UV-detector, 210 nm
Radiolabelled control compound	[4-^{14}C]-testosterone with a specific radioactivity of 1880 MBq/mmol, >97% radiochemical purity

2. Test system:

Test system: Pooled microsomal liver fractions from humans (mixed), rat (mixed), dog (male), mouse (mixed) and rabbit (female) from Corning (Woburn, MA 01801, USA), BD Bioscience (Woburn, MA 01801, USA) and XenoTech (Lenexa, KS, USA), respectively

Strain, sex: Human: male Caucasian, Afro American and female Caucasian, 35 male and 24 female donors, age 20-77 years (male) and 35-77 (female), batches 6123001 and 7331001

Rat: Sprague Dawley, male and female, 223 male and 20 female donors, age 8-10 weeks, batches 7031003 and 7313001 (female) and 8169001 and 7152001 (male)

Dog: Beagle, male, batch 7184001, 5 dogs age ≥12 months

Mouse: male and female CD-1 strain, 20 male donors, 150 female donors, age 11 weeks, batches 2215859 and 4338001

Rabbit: female New Zealand rabbit, 4 donors, age sexually mature

3. Preparation of dosing solutions

The test substance was dissolved in acetonitrile (ACN) to form a stock solution. The identity and purity of the test item in the stock solution was confirmed by radio-HPLC analysis. The identity was additionally confirmed by NP-TLC (using two different TLC methods). From this stock solutions working solutions were prepared in acetonitrile/water (1/1, v/v) to prepare incubations at 1 or 10 µM.

For the positive control experiments, [4-^{14}C]-testosterone was dissolved in acetonitrile. The identity of [4-^{14}C]-testosterone in the stock solution was confirmed by HPLC analysis. Working solutions were prepared in incubation buffer (K_2HPO_4/ KH_2PO_4, approximately 4/1, v/v, pH 7.4). The purity of the test item in the working solutions was analysed by HPLC.

4. Study design and methods

[Phenyl-UL-^{14}C]-fluopyram was incubated separately with liver microsomal fractions of mixed human, mixed rat, male dog, mixed mouse and female rabbit at 37±2°C using a water bath with gentle shaking at concentrations of 1 µM or 10 µM. The incubation times were 0, 60 and 120 minutes for all species, both sexes and all concentrations. The incubation system includes NADPH-generating biochemical system (~26 mM Na_2NADP, ~66 mM glucose-6-phosphate, ~66 mM magnesium chloride and 40 U/mL glucose-6-phosphate dehydrogenase).
To show the stability of the test item in the incubation system control incubation at 1 µM and 10 µM [phenyl-UL-^{14}C]-fluopyram was performed without NADPH-generating biochemical system in all species and sexes at incubation times of 0 and 120 minutes.
To evaluate the ability of the liver microsomal fractions a positive control incubation system with testosterone was performed. 10 µM [4-^{14}C]-testosterone was incubated with liver microsomal fraction from each species at 37±2°C for 120 minutes.
Microsomal incubates were stopped by addition of acetonitrile.

Concentration of test item in incubation system [µM]	Species, sex of liver microsomal fractions	Incubation Times [minutes]	Remark
1, 10	Human, mixed Rat, mixed Dog, male Mouse, mixed Rabbit, female	0, 60, 120	Test item [Phenyl-UL-^{14}C]-fluopyram With NADPH-generating biochemical system
1, 10	Human, mixed Rat, mixed Dog, male Mouse, mixed Rabbit, female	0, 120	Test item [Phenyl-UL-^{14}C]-fluopyram Stability control – without NADPH-generating biochemical system
10	Human, mixed Rat, mixed Dog, male Mouse, mixed Rabbit, female	0, 120	Test item: [4-^{14}C]-testosterone Positive control – with NADPH-generating biochemical system

5. Analytical methods

The liver microsomal incubations were centrifuged, the supernatant was removed and concentrated under a nitrogen stream. The pellet was washed with 1 mL acetonitrile and homogenised before centrifugation. The supernatant was added to the concentrated supernatant and concentrated further under a nitrogen stream. The volume was set to 1 and 2 mL with water, respectively, and the radioactivity was determined by LSC and investigated qualitatively and quantitatively by HPLC.

6. High Performance Liquid Chromatography (HPLC)

HPLC was used to confirm the identity and purity of [phenyl-UL-^{14}C]-fluopyram in acetonitrile and to analyse its metabolites. HPLC analysis was also used to determine the purity of the control [4-^{14}C]-testosterone in acetonitrile; a reverse phase column with a water and acetonitrile mobile phase was used.

7. Thin Layer Chromatography (TLC)

The identity and purity of [phenyl-UL-^{14}C]-fluopyram was additionally confirmed by NP-TLC using two different mobile phases of either dichloromethane/ethyl acetate (80/20, v/v) or dichloromethane/ethyl acetate (50/50, v/v).

8. Recoveries and metabolic transformation rate

Recovery of radioactivity from the HPLC column was determined to ensure quantitative elution of injected radioactivity from the HPLC column by measuring radioactivity before and after injection.

Recovery of radioactivity was determined as relative percentage of the applied radioactivity recovered in the supernatant of the specific incubates as compared with the application controls.

B. Results and discussion

1. Recovery of radioactivity

HPLC recoveries after 120 min incubation at 10 µM [phenyl-UL-^{14}C]-fluopyram ranged from 97.5% to 106.3% of applied radioactivity for all test systems (Table 5.1.2- 1).

Table 5.1.2- 1: HPLC-Recovery of radioactivity after incubation of 10 µM [phenyl-UL-^{14}C]-fluopyram with liver microsomal fractions from human, rat (Sprague Dawley), dog (Beagle), mouse (CD-1) and rabbit (New Zealand)

Test system Liver microsomal fraction from:	HPLC recovery of applied RA after 120 min incubation [%] [phenyl-UL-^{14}C]-fluopyram [10 µM]
Human, mixed	99.9
Rat, mixed	102.8
Dog, male	97.5
Mouse, mixed	103.1
Rabbit, female	106.3

Recoveries of radioactivity after incubation of 1 µM and 10 µM [phenyl-UL-^{14}C]-fluopyram with liver microsomal fractions were determined by LSC after sample processing and ranged from 95.5% to 106.7% with two exceptions at less than 93% or more than 111% after 60 minutes and from 94.0% to 110.6% after 120 minutes (Table 5.1.2- 2). Radioactivity after incubation of 10 µM [4-^{14}C]-testosterone was recovered at levels ranging from 104.0% to 107.5% after 120 minutes (Table 5.1.2-3).

Table 5.1.2- 2: Recovery of radioactivity in supernatants after incubation of 1 µM and 10 µM [phenyl-UL-^{14}C]-fluopyram with liver microsomal fractions from human, rat (Sprague Dawley), dog (Beagle), mouse (CD-1) and rabbit (New Zealand) after 0 min, 60 min and 120 min incubation

Test system Liver microsomal fraction from:	Incubation time [min]	Recovery [%] of applied RA [phenyl-UL-^{14}C]-fluopyram	
		1 µM	10 µM
Human, mixed	0	106.6	110.1
		104.1	109.5
Rat, mixed		106.9	>111[2]
		103.3	109.8
Dog, male		99.9	108.8
		105.4	108.8
Mouse, mixed		99.9	101.7
		104.2	96.0
Rabbit, female		97.1	104.2
		95.4	104.1
Human, mixed	60	104.9	105.6
		102.3	105.5
Rat, mixed		105.4	104.0
		105.5	106.7
Dog, male		<93[1]	98.6
		98.5	>111[2]
Mouse, mixed		99.5	100.4
		102.3	103.3
Rabbit, female		95.5	104.5
		99.5	98.9
Human, mixed	120	102.8	102.1
		103.8	101.4
Rat, mixed		100.7	110.6
		103.4	109.2
Dog, male		98.7	101.2
		98.0	106.4
Mouse, mixed		100.8	99.4
		104.9	96.5
Rabbit, female		94.0	100.7
		95.6	102.1

[1] Recovery less than 93%, the sample was excluded from evaluation

[2] Recovery higher than 111%, the sample was excluded from evaluation

Table 5.1.2- 3: Recovery of radioactivity in supernatants after incubation of 10 µM [4-^{14}C]-testosterone with liver microsomal fractions from human, rat (Sprague Dawley), dog (Beagle), mouse (CD-1) and rabbit (New Zealand) after 120 min incubation

Test system Liver microsomal fraction from:	Incubation Time (min)	Recovery [%] of applied RA - 10 µM [4-^{14}C]-testosterone
Human, mixed	120	105.8
		104.0
Rat, mixed		105.2
		107.1
Dog, male		106.0
		106.7
Mouse, mixed		107.5
		105.8
Rabbit, female		107.4
		104.5

2. Biotransformation of [phenyl-UL-^{14}C]-fluopyram

In general, without the NADP-generating biochemical system the test item was proven stable for at least 120 minutes in liver microsomal incubation solutions from all species (Table 5.1.2- 4 to Table 5.1.2- 8).

Human liver microsomes

Biotransformation of [phenyl-UL-^{14}C]-fluopyram in human (mixed gender) liver microsomes was moderate at all concentrations ranging from 26.11 to 53.42% after incubation times from 60 to 120 minutes (Table 5.1.2- 4). In general, biotransformation rates increase with increasing incubation times. The highest biotransformation rate was achieved after 120 minutes at a test item concentration of 1 µM and amounted to 53.42%. After incubation at a test item concentration of 10 µM, the metabolic transformation rate amounted to 43.00% after 120 minutes.

Table 5.1.2- 4: Metabolic transformation rate of 1 µM and 10 µM [phenyl-UL-^{14}C]-fluopyram after incubation with liver microsomal fractions from human (mixed)

Concentration of [phenyl-UL-^{14}C]-fluopyram [µM]	Incubation time [min] with human microsomes	Area of [phenyl-UL-^{14}C]-fluopyram [%]	Metabolic transformation rate [%]
1	0	100.0	0.00
	60	70.81	29.19
	120	46.58[1]	53.42[1]
10	0	100.00	0.00
	60	73.89	26.11
	120	57.00	43.00
Controls without NADPH generating system			
1	0	100.00	---
	120	100.00	0.00
10	0	100.00	---
	120	100.00	0.00

[1] Mean value of duplicates was used because of high deviation.

Rat liver microsomes

Biotransformation of [phenyl-UL-^{14}C]-fluopyram in rat (mixed gender) liver microsomes was moderate at all concentrations ranging from 23.76 to 38.53% after 60 to 120 minutes incubation times (Table 5.1.2- 5). In general, biotransformation rates increase with increasing incubation times. The highest biotransformation rate was achieved after 120 minutes at a test item concentration of 1 µM and amounted to 38.53%. After 120 minutes, incubation at 10 µM test item concentration the metabolic transformation rate amounted to 28.29%.

Table 5.1.2- 5: Metabolic transformation rate of 1 µM and 10 µM [phenyl-UL-^{14}C]-fluopyram after incubation with liver microsomal fractions from rat (mixed)

Concentration of [phenyl-UL-^{14}C]-fluopyram [µM]	Incubation time [min] with rat microsomes	Area of [phenyl-UL-^{14}C]-fluopyram [%]	Metabolic transformation rate [%]
1	0	100.00	0.00
	60	70.53	29.47
	120	61.47	38.53
10	0	99.62	0.38
	60	76.24	23.76
	120	71.71	28.29
Controls without NADPH generating system			
1	0	100.00	---
	120	100.00	0.00
10	0	100.00	---
	120	100.00	0.00

Dog liver microsomes

Biotransformation of [phenyl-UL-^{14}]-fluopyram in dog (male) liver microsomes was moderate to high at both concentrations ranging from 32.50 to 73.69% after 60 to 120 minutes incubation time (Table 5.1.2- 6). In general, biotransformation rates increase with increasing incubation times. The highest biotransformation rate was achieved after 120 minutes at a test item concentration of 1 µM and amounted to 73.69%. After 120 minutes incubation at 10 µM test item concentration the metabolic transformation rate amounted to 48.67%.

Table 5.1.2- 6: Metabolic transformation rate of 1 µM and 10 µM [phenyl-UL-^{14}C]-fluopyram after incubation with liver microsomal fractions from dog (male)

Concentration of [phenyl-UL-^{14}C]-fluopyram [µM]	Incubation time [min] with dog microsomes	Area of [phenyl-UL-^{14}C]-fluopyram [%]	Metabolic transformation rate [%]
1	0	100.00	0.00
	60	43.63	56.37
	120	26.31	73.69
10	0	100.00	0.00
	60	67.50	32.50
	120	51.33	48.67
Controls without NADPH generating system			
1	0	100.00	---
	120	100.00	0.00
10	0	100.00	---
	120	100.00	0.00

Mouse liver microsomes:

Biotransformation of [phenyl-UL-^{14}C]-fluopyram in mouse (mixed) liver microsomes was high at both concentrations ranging from 45.53 to 80.86% after 60 to 120 minutes incubation time (Table 5.1.2- 7). In general, biotransformation rates increase with increasing incubation times. The highest biotransformation rate was achieved after 120 minutes at a test item concentration of 1 µM and amounted to 80.86%. After 120 minutes incubation at 10 µM test item concentration the metabolic transformation rate amounted to 50.13%.

Table 5.1.2- 7: Metabolic transformation rate of 1 µM and 10 µM [phenyl-UL-^{14}C]-fluopyram after incubation with liver microsomal fractions from mouse (mixed)

Concentration of [phenyl-UL-^{14}C]-fluopyram [µM]	Incubation time [min] with mouse microsomes	Area of [phenyl-UL-^{14}C]-fluopyram [%]	Metabolic transformation rate [%]
1	0	100.0	0.00
	60	26.63	73.37
	120	19.14	80.86
10	0	100.00	0.00
	60	54.47	45.53
	120	49.87	50.13
Controls without NADPH generating system			
1	0	100.00	---
	120	100.00	0.00
10	0	100.00	---
	120	100.00	0.00

Rabbit liver microsomes

Biotransformation of [phenyl-UL-^{14}C]-fluopyram in rabbit (female) liver microsomes was high at both concentrations ranging from 71.28% to 92.88% after 60 to 120 minutes incubation time (Table 5.1.2-8). In general, biotransformation rates increase with increasing incubation times. The highest biotransformation rate was achieved after 120 minutes at a test item concentration of 1 µM and amounted to 92.88%. After 120 minutes incubation at 10 µM test item concentration the metabolic transformation rate amounted to 81.93%.

Table 5.1.2- 8: Metabolic transformation rate of 1 µM and 10 µM [phenyl-UL-^{14}C]-fluopyram after incubation with liver microsomal fractions from rabbit (female)

Concentration of [phenyl-UL-^{14}C]-fluopyram [µM]	Incubation time [min] with rabbit microsomes	Area of [phenyl-UL-^{14}C]-fluopyram [%]	Metabolic transformation rate [%]
1	0	100.00	0.00
	60	17.39	82.16
	120	7.12	92.88
10	0	100.00	0.00
	60	28.72	71.28
	120	18.07	81.93
Controls without NADPH generating system			
1	0	100.00	---
	120	100.00	0.00
10	0	100.00	---
	120	100.00	0.00

3. Biotransformation of [4-^{14}C]-testosterone

The metabolic capability of the liver microsomal fraction was demonstrated with control incubation of [4-^{14}C]-testosterone at 10 µM concentrations (**Table 5.1.2- 9**). After an incubation time of 120 minutes testosterone amount decreases and the amount of testosterone metabolites increases. In total up to 13 metabolites were formed.

6β-Hydroxytestosterone was found after incubation with [4-^{14}C]-testosterone for 120 minutes with liver microsomes in all species and was selected as a biological transformation marker compound. The highest metabolic activities were measured for mouse mixed liver microsomes and amounted to 71.1% for 6β-hydroxytestosterone after 120 minutes. Human mixed liver microsomes had a maximum metabolic activity of 38.3% for 6β-hydroxytestosterone after 120 minutes incubation.

Table 5.1.2- 9: Biotransformation of [4-^{14}C]-testosterone

Species	Concentration of [4-^{14}C]-testosterone [µM]	Incubation time [min]	Area of [4-^{14}C]-testosterone [%]	Area of 6β-hydroxytestosterone [%]
Human, mixed	10	120	18.36	38.32
Rat, mixed	10	120	n.d.	11.67
Dog, male	10	120	13.97	40.43
Mouse, mixed	10	120	3.34	71.10
Rabbit, female	10	120	n.d.	28.15

n.d. = not detected

4. Metabolites after biotransformation of [phenyl-UL-^{14}C]-fluopyram

All detected metabolites were only characterised based on their chromatographic behaviour (see figures below). No metabolic pathway was derived.

Human liver microsomes

In total up to six metabolites were detected after incubation of 1-10 µM [phenyl-UL-^{14}C]-fluopyram with mixed gender human liver microsomes. "Unknown 12" showed the highest abundance and accounted for up to 22.9% of the radioactivity. All other detected metabolites ranged from 0.6-12.7% radioactivity. Parent fluopyram accounted from 46.6% to 72.5% radioactivity after 60-120 minutes incubation.

Rat liver microsomes

In total up to six metabolites were detected after incubation of 1-10 µM [phenyl-UL-^{14}C]-fluopyram with mixed gender rat liver microsomes. "Unknown 12" showed the highest abundance and accounted for up to 30.6% of the radioactivity. All other detected metabolites ranged from 0.3-10.7% radioactivity. Parent fluopyram accounted from 56.7% to 75.7% radioactivity after 60-120 minutes incubation.

Dog liver microsomes

In total up to six metabolites were detected after incubation of 1-10 µM [phenyl-UL-^{14}C]-fluopyram with male dog liver microsomes. "Unknown 5" showed the highest abundance and accounted for up to 37.9% of the radioactivity. All other detected metabolites ranged from 0.6-13.6% radioactivity. Parent fluopyram accounted for 26.1% to 68.2% radioactivity after 60-120 minutes incubation.

Mouse liver microsomes

In total up to eight metabolites were detected after incubation of 1-10 µM [phenyl-UL-^{14}C]-fluopyram with mixed gender mouse liver microsomes. "Unknown 12" showed the highest abundance and accounted for up to 37.8% of the radioactivity. All other detected metabolites ranged from 0.4-15.2% radioactivity. Parent fluopyram accounted from 19.1% to 54.1% radioactivity after 60-120 minutes incubation.

Rabbit liver microsomes

In total up to seven metabolites were detected after incubation of 1-10 µM [phenyl-UL-^{14}C]-fluopyram with female rabbit liver microsomes. “Unknown 13” showed the highest abundance and accounted for up to 39.0% of the radioactivity. All other detected metabolites ranged from 0.7-32.7% radioactivity. Parent fluopyram accounted from 6.6% to 29.7% radioactivity after 60-120 minutes incubation.

Incubations of 1 µM [phenyl-UL-^{14}C]-fluopyram with human mixed, rat mixed, dog male, mouse mixed and rabbit female liver microsomes revealed the parent substance and additional 15 metabolites during the incubation period. “Unknown 13” detected in rabbit female microsomes after an incubation time of 120 min showed the highest abundance of 39.0%. All metabolites >5% AR detected in human mixed liver microsomes were detected in other species than human, too.

Unknown4 (retention time approx. 23.0-24.0 min) was detected after incubation of a concentration of 1 µM [phenyl-UL-14C]Fluopyram with human microsomes at a higher level (4.3-7.5 % AR) and in rabbit (5.4 % AR). Therefore, identification was necessary and was subsequently carried out in study S19-23624 (M-762838-01-1) and found to be fluopyram benzamide (M25, Figure 5.1.2- 1)

Figure 5.1.2- 1: Structure of fluopyram benzamide as elucidated in study S19-23624

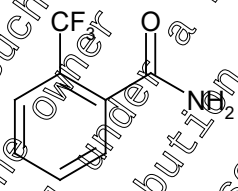


Figure 5.1.2-2: HPLC profiles of [phenyl-UL-^{14}C]-fluopyram application solution and incubation of 1 µM [phenyl-UL-^{14}C]-fluopyram with human, rat, dog, mouse and rabbit liver microsomal fraction after 120 min incubation

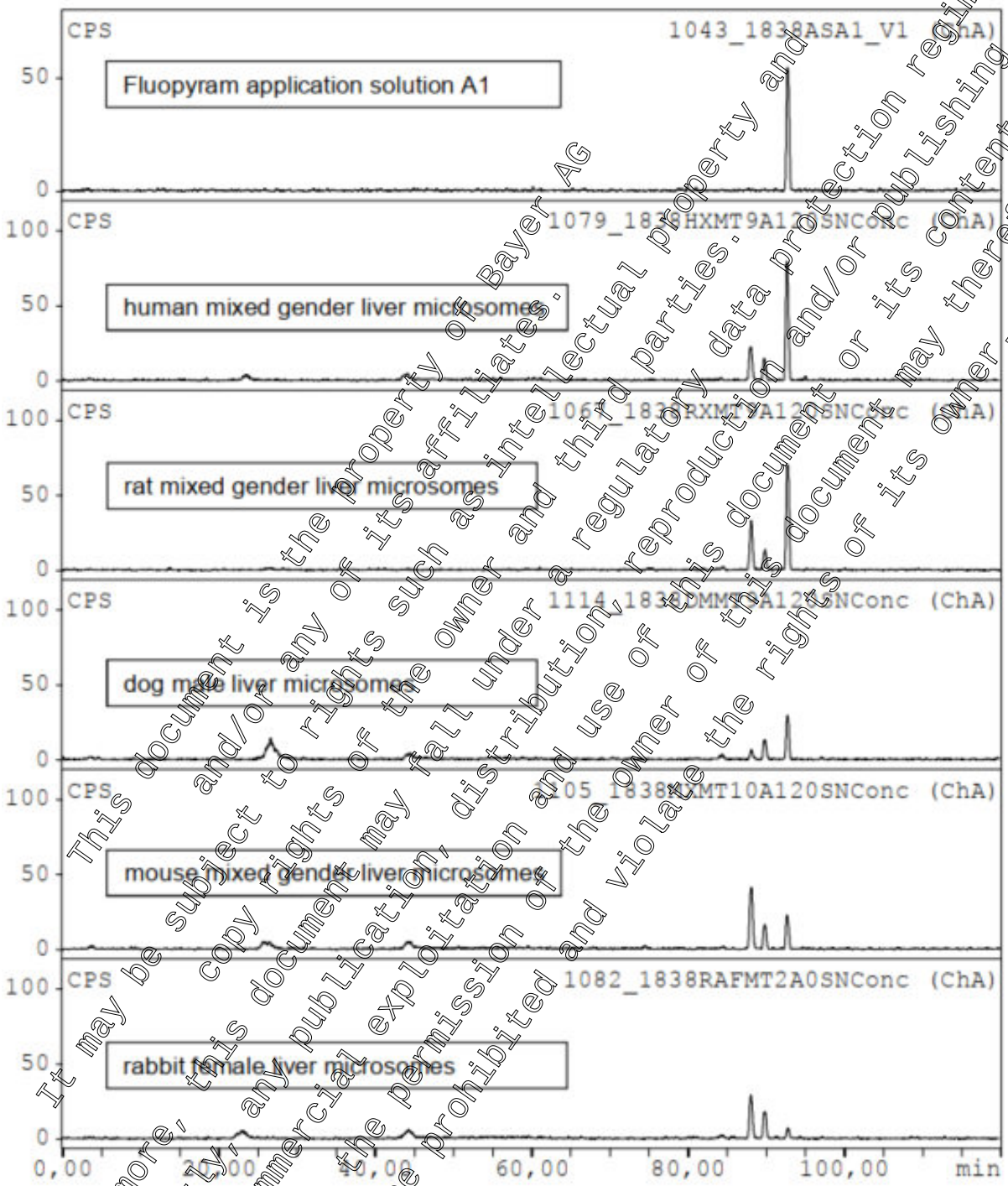
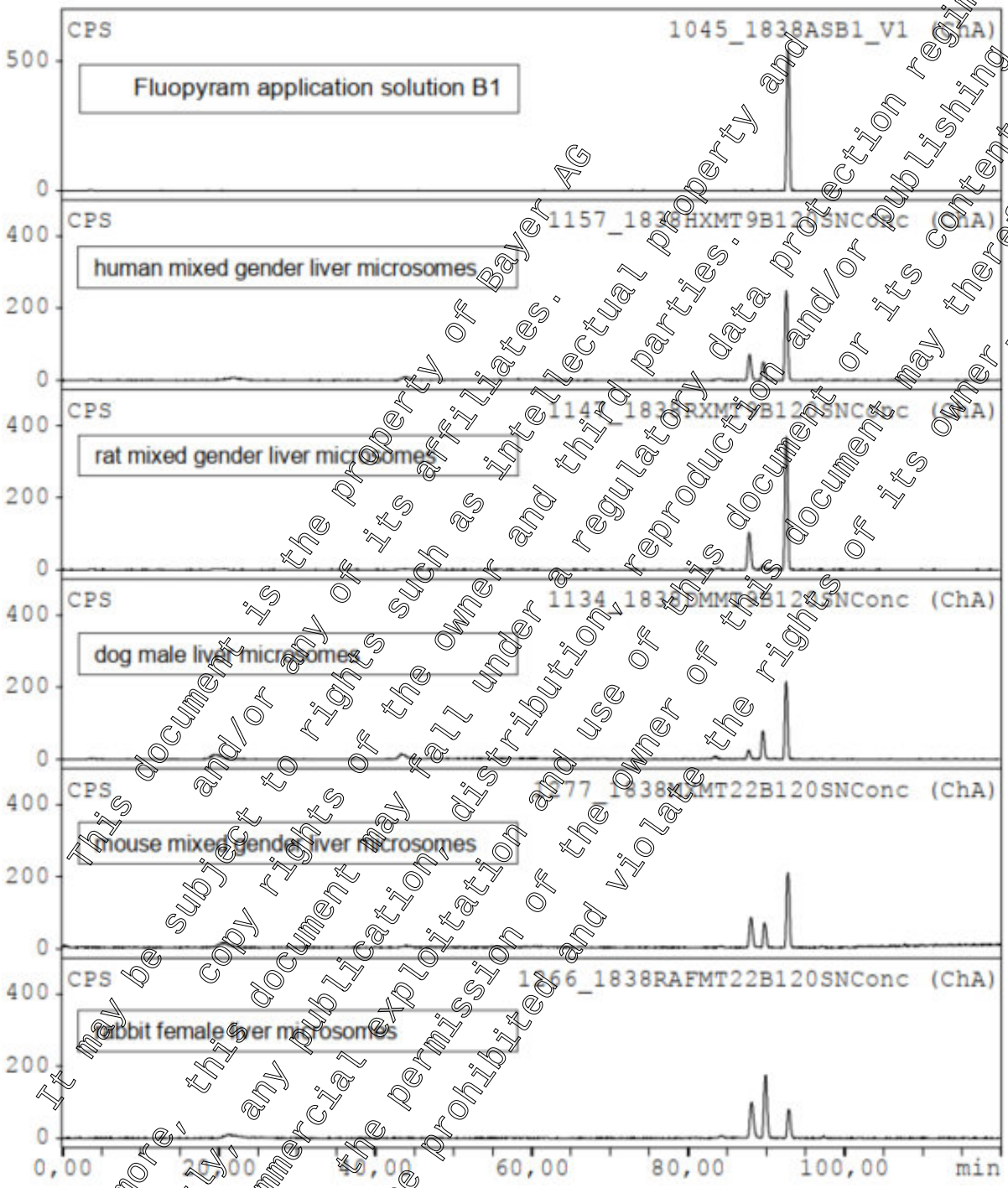


Figure 5.1.2-3: HPLC profiles of [phenyl-UL-^{14}C]-fluopyram application solution and incubation of 10 µM [phenyl-UL-^{14}C]-fluopyram with human, rat, dog, mouse and rabbit liver microsomal fraction after 120 min incubation



C. Conclusion

Metabolic profiles of fluopyram were compared between different species using an *in-vitro* system based on liver microsomal fraction from human (mixed gender), rat (mixed gender), dog (male), mouse (mixed gender), and rabbit (female). 1 and 10 µM [phenyl-UL-^{14}C]-fluopyram were incubated for 0, 60 and 120 min. The metabolic capability of the tested liver microsomal fractions was proven with [4-^{14}C]-testosterone.

[Phenyl-UL-^{14}C]-fluopyram was biotransformed at a moderate rate after incubation with human and rat liver microsomes, at a moderate to high rate after incubation with dog and mouse liver microsomes and at a high rate after incubation with rabbit liver microsomes.

No unique human metabolites radioactivity were detected. Metabolites were only characterised based on their chromatographic behaviour and no metabolic pathway was derived. Unknown4 (retention time approx. 23.0-24.0 min) was detected after incubation of a concentration of 1 µM [phenyl-UL-14C]Fluopyram with human microsomes at a higher level (4.3-7.5 % AR) and in rabbit (5.4 % AR). Therefore, identification was necessary and was subsequently carried out in study S19-23624 (M-762838-01-1) and found to be fluopyram benzamide (M25, Figure 5.1.2- 1), a well know *in vivo* rat metabolite.

Assessment and conclusion by applicant: The study is considered valid, scientifically acceptable and appropriate for the assessment of the toxicology of fluopyram.

Data Point:	KCA 5.1.2/03
Report Author:	[redacted]
Report Year:	2021
Report Title:	Metabolic stability and profiling of [pyridyl-2,6-14C]fluopyram in liver microsomal fractions from human, rat, dog, rabbit and mouse for inter-species comparison and m/z-value determination of metabolite Unkown4 derived from [phenyl-UL-14C]fluopyram in liver microsomal fractions from human
Report No:	S19-23624
Document No:	M-762838-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 amended by the Commission Regulation (EU) No. 283/2013 (Europe) EU Regulation 1107/2009 (SANCO/11802/2010 Rev. 7 US EPA OCSPP Not Applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously evaluated
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCA 5.1.2/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Amendment to study report no.1 - Metabolic stability and profiling of [pyridyl-2,6-14C]fluopyram in liver microsomal fractions from human, rat, dog, rabbit and mouse for inter-species comparison
Report No:	S18-07840
Document No:	M-667765-02-1
Guideline(s) followed in study:	US EPA OCSPP Not Applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Metabolic profiles of fluopyram were compared between different species using an *in-vitro* system based on liver microsomal fraction from humans (mixed gender), rat (mixed gender), dog (male), mouse (mixed gender) and rabbit (female). 1 and 10 µM [pyridyl-2,6-^{14}C]-fluopyram were incubated for 0, 60 and 120 min with incubation buffer and the NADPH generating biochemical system consisting of NADP, glucose-6-phosphate and the enzyme glucose-6-phosphate-dehydrogenase. The metabolic capability of the tested liver microsomal fractions was proven with [4-^{14}C]-testosterone.

Due to technical issues during the HPLC measurements for the 10 µM incubation experiments, these had to be repeated in the separate study S19-23624. The results for both studies are presented in this summary. Results for the 10 µM incubations from study M-667765-02-1 are disregarded.

In general, without the NADP-generating biochemical system the test item was proven stable for at least 120 minutes in liver microsomal incubation solutions from all species and sexes.

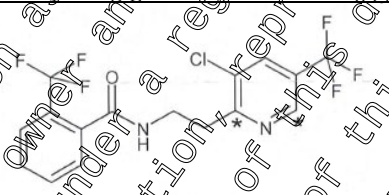
Recoveries of radioactivity after incubation were determined by LSC after sample processing and ranged from 95.3% to 109.6%. Radioactivity after incubation of 10 µM [4-^{14}C]-testosterone was recovered at levels ranging from 99.2% to 109.8% after 120 minutes.

[Pyridyl-2,6-^{14}C]-fluopyram was biotransformed at a moderate to high rate after incubation with human, rat and dog liver microsomes (up to 43.21%, 49.01% and 67.47% transformation rate, respectively), and at a high rate after incubation with mouse and rabbit liver microsomes (up to 78.48% and 92.70% transformation rate, respectively).

In total up to two (human, rat), three (dog), four (mouse) and ten (rabbit) metabolites were detected after incubation. All metabolites >5% AR detected in human mixed liver microsomes were detected in other species than human, too. Metabolites were only characterised based on their chromatographic behaviour and no metabolic pathway was derived.

A. Materials and methods

1. Test Material:

IUPAC Name	*N*-[2-[3-chloro-5-(trifluoromethyl)-2-pyridyl]ethyl]-α,α,α-trifluoro-ortho-toluamide
Chemical Name	*N*-(2-(3-chloro-5-(trifluoromethyl)-2-pyridinyl)ethyl)-2-(trifluoromethyl)benzamide
Code names	BCS-AR83685, M-00020622
Common name	Fluopyram
Empirical formula	$C_{16}H_{11}ClF_6N_2O$
CAS Number	658066-35-4
Molar mass	396.7 g/mol
Chemical structure	 * labelled position
Radiolabelled test material	[Pyridyl-2,6-^{13}C]-fluopyram
Batch number	KML 10677
Original specific radioactivity	3.85 MBq/mg
Radiochemical purity	>98% by radio-HPLC
Chemical Purity	>98% by HPLC, UV-detector, 210 nm
Radiolabelled control compound	[4-^{14}C]-testosterone with a specific radioactivity of 1880 MBq/mmol, >97% radiochemical purity

2. Test system:

Test system: Pooled microsomal liver fractions from humans (mixed), rat (mixed), dog (male), mouse (mixed) and rabbit (female) from Corning (Woburn, MA 01801, USA), BD Bioscience (Woburn, MA 01801, USA) and XenoTech (Lenexa, KS, USA), respectively

Strain, sex: M-667765-02-1:

Human: male Caucasian, African American and female Caucasian, 53 male and 40 female donors, age 20-77 years (male) and 35-77 years (female), batches 5168001, 6123001 and 7331001

Rat: Sprague Dawley, male and female, 91 male and 11 female donors, age 8-10 weeks, batches 7313001 (female) and 8169001 (male)

Dog: Beagle, male, 35 dogs age ≥12 months, batches 5216003, 7184001 and 8176003

Mouse: CD-1 strain, male and female, about 20 male donors, 211 female donors, age 11 weeks, batches 4338001, 8323002 (female) and 2215859

(male)
Rabbit: New Zealand, female, 4 donors, age sexually mature, batch 1010273
S19-23624:
Human: Caucasian, 28 male and 7 female donors, age 21-69 years (male) and 40-61 (female), batch 9050002
Rat: Sprague Dawley, male and female, 126 male and 7 female donors, age 8-10 weeks, batches 9070003 (female) and 8005001 (male)
Dog: Beagle, male, batch 7184001, 5 dogs age ≥12 months
Mouse: CD-1 strain, about 210 male donors, 800 female donors, age 11 weeks, batches 1310224 (female) and 7352001 (male)
Rabbit: New Zealand rabbit, female, 4 donors, age sexually mature, batch 1010273

3. Preparation of dosing solutions

The test substance was dissolved in acetonitrile (ACN) to form a stock solution. The identity and purity of the test item in the stock solution was confirmed by radio-HPLC analysis. The identity was additionally confirmed by NP-TLC. These stock solutions were diluted with acetonitrile/water (1/1, v/v), to obtain working solutions for preparation of incubations at 1 or 10 µM.

For the positive control experiments, [4-^{14}C]-testosterone was dissolved in acetonitrile (ethanol for study S19-23624). The purity of [4-^{14}C]-testosterone in the stock solution was confirmed by radio-HPLC analysis. Working solutions were prepared in incubation buffer (K_2HPO_4/ KH_2PO_4, approximately 4/1, v/v, pH 7.4). The purity of the test item in the working solutions was analysed by HPLC-UV.

4. Study design and methods

[Pyridyl-2,6-^{14}C]-fluopyram was incubated separately with liver microsomal fractions of mixed human, mixed rat, male dog, mixed mouse and female rabbit at 37±2°C using a water bath with gentle shaking at concentrations of 1 µM or 10 µM. The incubation times were 0, 60 and 120 minutes for all species, both sexes and concentrations. The incubation system includes NADPH-generating biochemical system (~26 mM Na_2NADP, ~66 mM glucose-6-phosphate, ~66 mM magnesium chloride and 100 µL 40 U/mL glucose-6-phosphate dehydrogenase).

To show the stability of the test item in the incubation system control incubation at 1 µM and 10 µM [pyridyl-2,6-^{14}C]-fluopyram was performed without NADPH-generating biochemical system in all species and sexes at incubation times of 0 and 120 minutes.

To evaluate the ability of the liver microsomal fractions a positive control incubation system with testosterone was performed. 10 µM [4-^{14}C]-testosterone was incubated with liver microsomal fraction from each species at 37±2°C for 120 minutes.

Microsomal incubates were stopped by addition of acetonitrile.

Concentration of test item in incubation system [µM]	Species, sex of liver microsomal fractions	Incubation Times [minutes]	Remark
1, 10	Human, mixed	0, 60, 120	Test item [Pyridyl-2,6-

	Rat, mixed Dog, male Mouse, mixed Rabbit, female		^{14}C]-fluopyram With NADPH-generating biochemical system
1, 10	Human, mixed Rat, mixed Dog, male Mouse, mixed Rabbit, female	0, 120	Test item [Pyridyl-2,6-^{14}C]-fluopyram Stability control without NADPH-generating biochemical system
10	Human, mixed Rat, mixed Dog, male Mouse, mixed Rabbit, female	0, 120	Test item: [4-^{14}C]-testosterone Positive control – with NADPH-generating biochemical system

5. Analytical methods

The liver microsomal incubations were centrifuged, the supernatant was removed and concentrated under a nitrogen stream. The pellet was washed with 1 mL acetonitrile and homogenised before centrifugation. The supernatant was added to the concentrated supernatant and concentrated further under a nitrogen stream. The volume was set to 1 and 2 mL with water, respectively, and the radioactivity was determined by LSC and investigated qualitatively and quantitatively by HPLC.

6. High Performance Liquid Chromatography (HPLC)

HPLC was used to confirm the identity and purity of [pyridyl-2,6-^{14}C]-fluopyram in acetonitrile and to analyse its metabolites. HPLC analysis was also used to determine the purity of the control [4-^{14}C]-testosterone in acetonitrile; a reverse phase column with a water and acetonitrile mobile phase for testosterone and water/ammonia (25%)/formic acid (99%) (1000/1.54/0.8, v/v/v) and acetonitrile/methanol (1/1, v/v) gradient for fluopyram was used.

7. Thin Layer Chromatography (TLC)

The identity and purity of [pyridyl-2,6-^{14}C]-fluopyram was additionally confirmed by NP-TLC using dichloromethane/ethyl acetate (50/50, v/v) as a mobile phase.

8. Recoveries and metabolic transformation rate

Recovery of radioactivity from the HPLC column was determined to ensure quantitative elution of injected radioactivity from the HPLC column by measuring radioactivity before and after injection.

Recovery of radioactivity was determined as relative percentage of the applied radioactivity recovered in the supernatant of the specific incubates as compared with the application controls.

Metabolic transformation rate of [pyridyl-2,6-^{14}]-fluopyram and [4-^{14}C]-testosterone was determined based on the decrease of the parent radioactivity in the test sample in comparison to the radioactivity of the parent in the control incubation at 0 min.

B. Results and discussion

5. Recovery of radioactivity

HPLC recoveries after 120 min incubation at 10 µM [pyridyl-2,6-^{14}C]-fluopyram ranged from 91.3% to 97.2% of applied radioactivity for all test systems (Table 5.1.2- 10).

Table 5.1.2- 10: HPLC-Recovery of radioactivity after incubation of 10 µM [pyridyl-2,6-^{14}C]-fluopyram with liver microsomal fractions from human, rat (Sprague Dawley), dog (Beagle), mouse (CD-1) and rabbit (New Zealand)

Test system Liver microsomal fraction from:	HPLC recovery of applied RA after 120 min incubation [%] [pyridyl-2,6-^{14}C]-fluopyram [10 µM] S19-23624 (M-762838-01-1)
Human, mixed	91.3
Rat, mixed	96.7
Dog, male	97.2
Mouse, mixed	93.4
Rabbit, female	94.2

Recoveries of radioactivity after incubation of 1 µM and 10 µM [pyridyl-2,6-^{14}C]-fluopyram with liver microsomal fractions were determined by LSC after sample processing and ranged from 95.3% to 109.6% (**Erreur ! Source du renvoi introuvable.**). Radioactivity after incubation of 10 µM [4-^{14}C]-testosterone was recovered at levels ranging from 99.2% to 109.8% after 120 minutes (Table 5.1.2-12)

Table 5.1.2- 11: Recovery of radioactivity in supernatants after incubation of 1 µM and 10 µM [pyridyl-2,6-^{14}C]-fluopyram with liver microsomal fractions from human, rat (Sprague Dawley), dog (Beagle), mouse (CD-1) and rabbit (New Zealand) after 0 min, 60 min and 120 min incubation

Test system Liver microsomal fraction from:	Incubation time [min]	Recovery [%] of applied RA [pyridyl-2,6-^{14}C]-fluopyram	
		1 µM	10 µM[1]
		S18-07840 (M-667765-02-1)	S19-23624 (M-762838-01-1)
Human, mixed	0	96.4 107.9	101.1
Rat, mixed	0	107.4 98.7	103.2
Dog, male	0	103.8 -[2]	104.4
Mouse, mixed	0	103.2 104.4	109.0
Rabbit, female	0	106.7 102.1	109.6
Human, mixed	60	102.0 104.7	98.2
Rat, mixed	60	104.0 102.0	100.6
Dog, male	60	98.6 100.4	102.2
Mouse, mixed	60	96.4 98.5	108.8
Rabbit, female	60	103.9 101.9	107.3
Human, mixed	120	101.7 100.6	97.0
Rat, mixed	120	105.3 101.7	99.8
Dog, male	120	95.7 99.3	101.4
Mouse, mixed	120	97.5 95.3	107.4
Rabbit, female	120	101.0 101.9	104.5

[1] Mean of two replicates.
[2] Sample spilled during work up, the sample was excluded from evaluation

Table 5.1.2- 12: Recovery of radioactivity in supernatants after incubation of 10 µM [4-^{14}C]-testosterone with liver microsomal fractions from human, rat (Sprague Dawley), dog (Beagle), mouse (CD-1) and rabbit (New Zealand) after 120 min incubation

Test system Liver microsomal fraction from:	Incubation Time (min)	Recovery [%] of applied RA - 10 µM [4-^{14}C]-testosterone	
		S18-07840 (M-667765-02-1)	S19-23624 (M-762838-01-1)[1]
Human, mixed	120	107.9 108.7	103.4
Rat, mixed		101.3 104.1	104.2
Dog, male		105.1 105.9	109.8
Mouse, mixed		99.2 104.8	101.3
Rabbit, female		103.5 103.8	105.0

[1] Mean of two replicates.

6. Biotransformation of [pyridyl-2,6-^{14}C]-fluopyram

In general, without the NADP-generating biochemical system the test item was proven stable for at least 120 minutes in liver microsomal incubation solutions from all species (Table 5.1.2- 13 to Table 5.1.2- 17).

Human liver microsomes

Biotransformation of [pyridyl-2,6-^{14}C]-fluopyram in human (mixed gender) liver microsomes was moderate at both concentrations ranging from 16.87 to 43.21% after incubation times from 60 to 120 minutes (Table 5.1.2- 13). In general, biotransformation rates increase with increasing incubation times. The highest biotransformation rate was achieved after 120 minutes at a test item concentration of 1 µM and amounted to 43.21%. After incubation at a test item concentration of 10 µM, the metabolic transformation rate amounted to 24.44% after 120 minutes.

Table 5.1.2- 13: Metabolic transformation rate of 1 µM and 10 µM [pyridyl-2,6-^{14}C]-fluopyram after incubation with liver microsomal fractions from human (mixed)

Concentration of [pyridyl-2,6-^{14}C]-fluopyram [µM]	Incubation time [min] with human microsomes	Area of [pyridyl-2,6-^{14}C]-fluopyram [%]	Metabolic transformation rate [%]
1 (S18-07840, M-667765-02-1)	0	100.00	0.00
	60	65.40	34.60
	120	45.68	49.21
10 (S19-23624, M-762838-01-1)	0	100.00	0
	60	83.13	16.87
	120	75.57	24.44
Controls without NADPH generating system			
1 (S18-07840, M-667765-02-1)	0	100.00	---
	120	100.00	0.00
10 (S19-23624, M-762838-01-1)	0	100.00	---
	120	100.00	0.00

Rat liver microsomes

Biotransformation of [pyridyl-2,6-^{14}C]-fluopyram in rat (mixed gender) liver microsomes was moderate at all concentrations ranging from 14.87 to 49.01% after 60 to 120 minutes incubation times (Table 5.1.2- 14**Erreur ! Source du renvoi introuvable.**). In general, biotransformation rates increase with increasing incubation times. The highest biotransformation rate was achieved after 120 minutes at a test item concentration of 1 µM and amounted to 49.01%. After 120 minutes incubation at 10 µM test item concentration the metabolic transformation rate amounted to 22.94%.

Table 5.1.2- 14: Metabolic transformation rate of 1 µM and 10 µM [pyridyl-2,6-^{14}C]-fluopyram after incubation with liver microsomal fractions from rat (mixed)

Concentration of [pyridyl-2,6-^{14}C]-fluopyram [µM]	Incubation time [min] with rat microsomes	Area of [pyridyl-2,6-^{14}C]-fluopyram [%]	Metabolic transformation rate [%]
1 (S18-07840, M-667765-02-1)	0	100.00	0.00
	60	65.57	34.43
	120	50.99	49.01
10 (S19-23624, M-762838-01-1)	0	99.66	0
	60	84.84	14.87
	120	76.80	22.94
Controls without NADPH generating system			
1 (S18-07840, M-667765-02-1)	0	100.00	---
	120	100.00	0.00
10 (S19-23624, M-762838-01-1)	0	100.00	---
	120	100.00	0.00

Dog liver microsomes

Biotransformation of [pyridyl-2,6-^{14}C]-fluopyram in dog (male) liver microsomes was moderate to high at both concentrations ranging from 32.50 to 67.47% after 60 to 120 minutes incubation time (**Table 5.1.2- 15**). In general, biotransformation rates increase with increasing incubation times. The highest biotransformation rate was achieved after 120 minutes at a test item concentration of 1 µM and amounted to 67.47%. After 120 minutes incubation at 10 µM test item concentration the metabolic transformation rate amounted to 42.84%.

Table 5.1.2- 15: Metabolic transformation rate of 1 µM and 10 µM [pyridyl-2,6-^{14}C]-fluopyram after incubation with liver microsomal fractions from dog (male)

Concentration of [pyridyl-2,6-^{14}C]-fluopyram [µM]	Incubation time [min] with dog microsomes	Area of [pyridyl-2,6-^{14}C]-fluopyram [%]	Metabolic transformation rate [%]
1 (S18-07840, M-667765-02-1)	0	100.00	0.00
	60	48.26	51.74
	120	32.53	67.47
10 (S19-23624, M-762838-01-1)	0	100.00	0
	60	67.50	32.50
	120	57.17	42.84
Controls without NADPH generating system			
1 (S18-07840, M-667765-02-1)	0	100.00	--
	120	100.00	0.00
10 (S19-23624, M-762838-01-1)	0	100.00	--
	120	100.00	0.00

Mouse liver microsomes

Biotransformation of [pyridyl-2,6-^{14}C]-fluopyram in mouse (mixed) liver microsomes was high at both concentrations ranging from 54.50 to 78.48% after 60 to 120 minutes incubation time (Table 5.1.2- 16). For the 1 µM incubation experiments, the metabolic transformation range was stable with increasing incubation time. For the 10 µM incubation experiments the biotransformation rates increased with increasing incubation time. The highest biotransformation rate was achieved after 60 minutes at a test item concentration of 1 µM and amounted to 78.48%. After 120 minutes incubation at 1 µM and 10 µM test item concentrations the metabolic transformation rates amounted to 78.47% and 56.77%, respectively.

Table 5.1.2- 16: Metabolic transformation rate of 1 µM and 10 µM [pyridyl-2,6-^{14}C]-fluopyram after incubation with liver microsomal fractions from mouse (mixed)

Concentration of [pyridyl-2,6-^{14}C]-fluopyram [µM]	Incubation time [min] with mouse microsomes	Area of [pyridyl-2,6-^{14}C]-fluopyram [%]	Metabolic transformation rate [%]
1 (S18-07840, M-667765-02-1)	0	100.00	0.00
	60	21.52	78.48
	120	21.53	78.47
10 (S19-23624, M-762838-01-1)	0	99.42	0
	60	45.24	54.50
	120	42.98	56.77
Controls without NADPH generating system			
1 (S18-07840, M-667765-02-1)	0	100.00	--
	120	100.00	0.00
10 (S19-23624, M-762838-01-1)	0	100.00	--
	120	100.00	0.00

Rabbit liver microsomes

Biotransformation of [pyridyl-2,6-^{14}C]-fluopyram in rabbit (female) liver microsomes was high at both concentrations ranging from 58.47% to 92.70% after 60 to 120 minutes incubation time (Table 5.1.2-17). In general, biotransformation rates increase with increasing incubation times. The highest biotransformation rate was achieved after 120 minutes at a test item concentration of 1 µM and amounted to 92.70%. After 120 minutes incubation at 10 µM test item concentration the metabolic transformation rate amounted to 70.92%.

Table 5.1.2- 17: Metabolic transformation rate of 1 µM and 10 µM [pyridyl-2,6-^{14}C]-fluopyram after incubation with liver microsomal fractions from rabbit (female)

Concentration of [pyridyl-2,6-^{14}C]-fluopyram [µM]	Incubation time [min] with rabbit microsomes	Area of [pyridyl-2,6-^{14}C]-fluopyram [%]	Metabolic transformation rate [%]
1 (S18-07840, M-667765-02-1)	0	100.00	0.00
	60	15.26	84.74
	120	7.30	92.70
10 (S19-23624, M-762838-01-1)	0	99.53	0
	60	41.34	58.47
	120	28.95	70.92
Controls without NADPH generating system			
1 (S18-07840, M-667765-02-1)	0	100.00	--
	120	100.00	0.00
10 (S19-23624, M-762838-01-1)	0	100.00	--
	120	100.00	0.00

7. Biotransformation of [4-^{14}C]-testosterone

The metabolic capability of the liver microsomal fraction was demonstrated with control incubation of [4-^{14}C]-testosterone at 10 µM concentrations (Table 5.1.2-18). After an incubation time of 120 minutes testosterone amount decreases and the amount of testosterone metabolites increases. In total up to 21 metabolites were formed.

6β-Hydroxytestosterone was found after incubation with [4-^{14}C]-testosterone for 120 minutes with liver microsomes in all species and was selected as a biological transformation marker compound. The highest metabolic activities were measured for dog male liver microsomes and amounted to 39.7% for 6β-hydroxytestosterone after 120 minutes. Human mixed liver microsomes had a maximum metabolic activity of 30.7% for 6β-hydroxytestosterone after 120 minutes incubation.

Table 5.1.2- 18: Biotransformation of [4-^{14}C]-testosterone (concentration 10 µM)

Species	Incubation time [min]	Area of [4-^{14}C]-testosterone [%]		Area of 6β-hydroxytestosterone [%]	
		S18-07840 (M-667765-02-1)	S19-23624 (M-762838-01-1)	S18-07840 (M-667765-02-1)	S19-23624 (M-762838-01-1)
Human, mixed	120	8.12	30.7	40.79	23.8
Rat, mixed	120	n.d.	0.6	13.72	n.d.
Dog, male	120	10.59	39.7	32.54	19.6
Mouse, mixed	120	n.d.	4.6	67.96	n.d.
Rabbit, female	120	n.d.	2.3	32.71	33.3

n.d. = not detected

8. Metabolites after biotransformation of [pyridyl-2,6-^{14}C]-fluopyram

All detected metabolites were only characterised based on their chromatographic behaviour (see figures below). No metabolic pathway was derived.

Human liver microsomes

Two metabolites were detected after incubation of 1 µM [pyridyl-2,6-^{14}C]-fluopyram (S18-07840, M-667765-02-1) with mixed gender human liver microsomes. "Unknown 9" showed the highest abundance and accounted for up to 34.5% of the radioactivity. The other detected metabolite ranged from 12.7-20.2% radioactivity. Parent fluopyram accounted from 45.4% to 65.6% radioactivity after 60-120 minutes incubation.

Two metabolites were detected after incubation of 10 µM [pyridyl-2,6-^{14}C]-fluopyram (S19-23624, M-762838-01-1) with mixed gender human liver microsomes. "Unknown 9" showed the highest abundance and accounted for up to 16.3% of the radioactivity. The other detected metabolite ranged from 5.6-8.2% radioactivity. Parent fluopyram accounted from 75.6% to 83.1% radioactivity after 60-120 minutes incubation.

Rat liver microsomes

Two metabolites were detected after incubation of 1 µM [pyridyl-2,6-^{14}C]-fluopyram (S18-07840, M-667765-02-1) with mixed gender rat liver microsomes. "Unknown 8" showed the highest abundance and accounted for up to 34.0% of the radioactivity. All other detected metabolites ranged from 10.4-14.7% radioactivity. Parent fluopyram accounted from 51.3% to 67.7% radioactivity after 60-120 minutes incubation.

Two metabolites were detected after incubation of 10 µM [pyridyl-2,6-^{14}C]-fluopyram (S19-23624, M-762838-01-1) with mixed gender rat liver microsomes. "Unknown 6" showed the highest abundance and accounted for up to 17.5% of the radioactivity. The other detected metabolite ranged from 3.9-5.7% radioactivity. Parent fluopyram accounted from 76.8% to 84.8% radioactivity after 60-120 minutes incubation.

Dog liver microsomes

In total up to three metabolites were detected after incubation of 1 µM [pyridyl-2,6-^{14}C]-fluopyram (S18-07840, M-667765-02-1) with male dog liver microsomes. “Unknown 9” showed the highest abundance and accounted for up to 56.0% of the radioactivity. All other detected metabolites ranged from 2.5-7.5% radioactivity. Parent fluopyram accounted for 30.4% to 47.5% radioactivity after 60-120 minutes incubation.

Three metabolites were detected after incubation of 10 µM [pyridyl-2,6-^{14}C]-fluopyram (S19-23624, M-762838-01-1) with male dog liver microsomes. “Unknown 9” showed the highest abundance and accounted for up to 38.9% of the radioactivity. All other detected metabolites ranged from 0.4-3.5% radioactivity. Parent fluopyram accounted for 57.2% to 67.5% radioactivity after 60-120 minutes incubation.

Mouse liver microsomes

Two metabolites were detected after incubation of 1 µM [pyridyl-2,6-^{14}C]-fluopyram (S18-07840, M-667765-02-1) with mixed gender mouse liver microsomes. “Unknown 9” showed the highest abundance and accounted for up to 43.0% of the radioactivity. The other detected metabolite ranged from 36.0-38.5% radioactivity. Parent fluopyram accounted from 20.9% to 21.2% radioactivity after 60-120 minutes incubation.

In total up to four metabolites were detected after incubation of 10 µM [pyridyl-2,6-^{14}C]-fluopyram (S19-23624, M-762838-01-1) with mixed gender mouse liver microsomes. “Unknown 9” showed the highest abundance and accounted for up to 35.1% of the radioactivity. All other detected metabolites ranged from 0.3-21.3% radioactivity. Parent fluopyram accounted from 43.0% to 45.2% radioactivity after 60-120 minutes incubation.

Rabbit liver microsomes

In total up to four metabolites were detected after incubation of 1 µM [pyridyl-2,6-^{14}C]-fluopyram (S18-07840, M-667765-02-1) with female rabbit liver microsomes. “Unknown 9” showed the highest abundance and accounted for up to 48.8% of the radioactivity. All other detected metabolites ranged from 2.2-37.1% radioactivity. Parent fluopyram accounted from 6.6% to 14.4% radioactivity after 60-120 minutes incubation.

In total up to ten metabolites were detected after incubation of 10 µM [pyridyl-2,6-^{14}C]-fluopyram (S19-23624, M-762838-01-1) with female rabbit liver microsomes. “Unknown 9” showed the highest abundance and accounted for up to 46.7% of the radioactivity. All other detected metabolites ranged from 0.1-21.1% radioactivity. Parent fluopyram accounted from 28.9% to 41.3% radioactivity after 60-120 minutes incubation.

Incubations of 1 µM [pyridyl-2,6-^{14}C]-fluopyram (S18-07840, M-667765-02-1) with human mixed, rat mixed, dog male, mouse mixed and rabbit female liver microsomes revealed the parent substance and additional 5 metabolites during the incubation period. “Unknown 9” detected in dog male microsomes after an incubation time of 120 min showed the highest abundance of 56.0%. All metabolites ≥5% AR detected in human mixed liver microsomes were detected in other species than human, too.

Incubations of 10 µM [pyridyl-2,6-^{14}C]-fluopyram (S19-23624, M-762838-01-1) with human mixed, rat mixed, dog male, mouse mixed and rabbit female liver microsomes revealed the parent substance and additional 14 metabolites during the incubation period. “Unknown 9” detected in rabbit female

microsomes after an incubation time of 120 min showed the highest abundance of 46.7%. All metabolites >5% AR detected in human mixed liver microsomes were detected in other species than human, too.

Figure 5.1.2-4: HPLC profiles of [pyridyl-2,6-^{14}C]-fluopyram application solution after incubation of 1 µM [pyridyl-2,6-^{14}C]-fluopyram with human, rat, dog, mouse and rabbit liver microsomal fraction after 120 min incubation S18-07840, M-667765-02-1

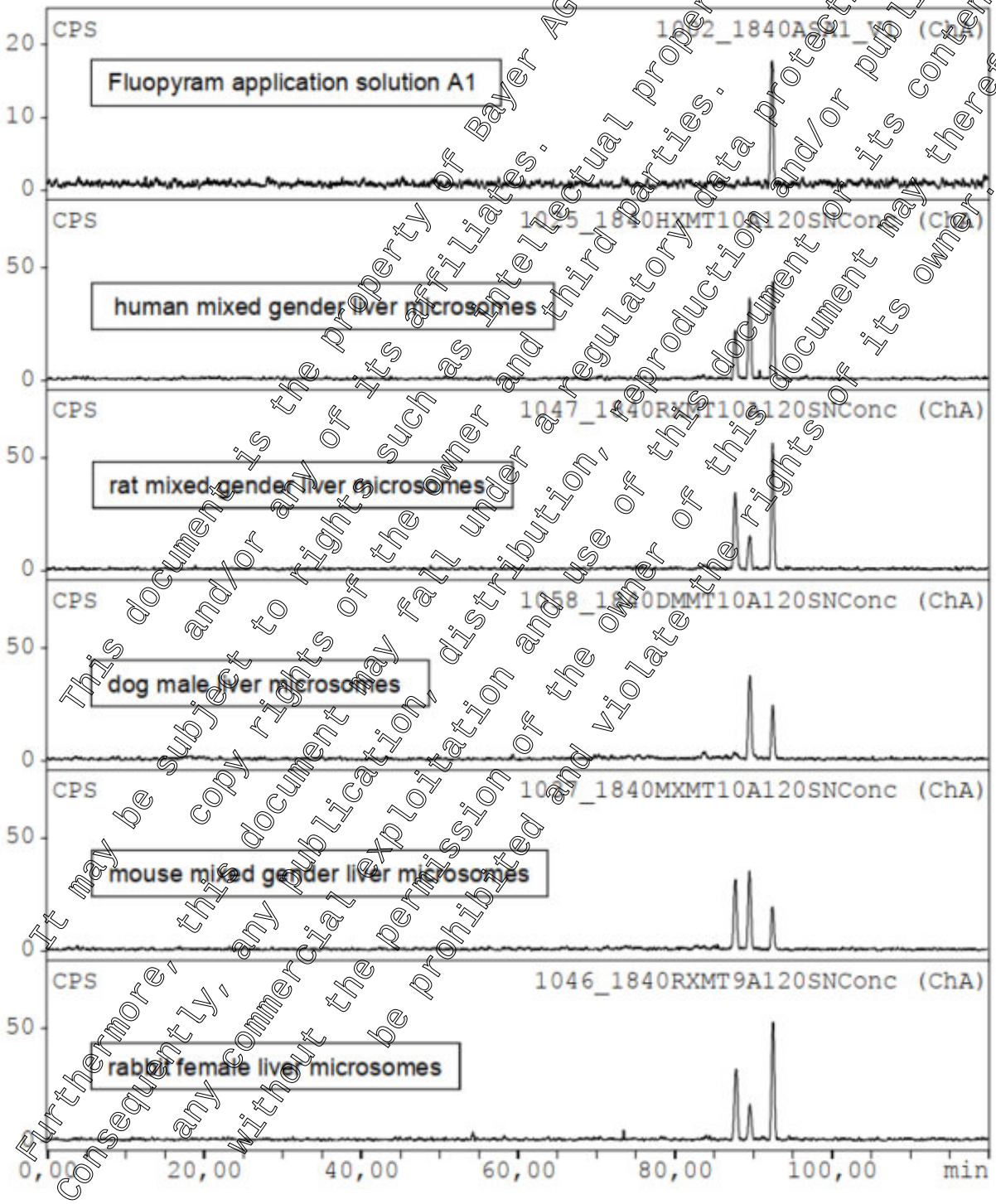
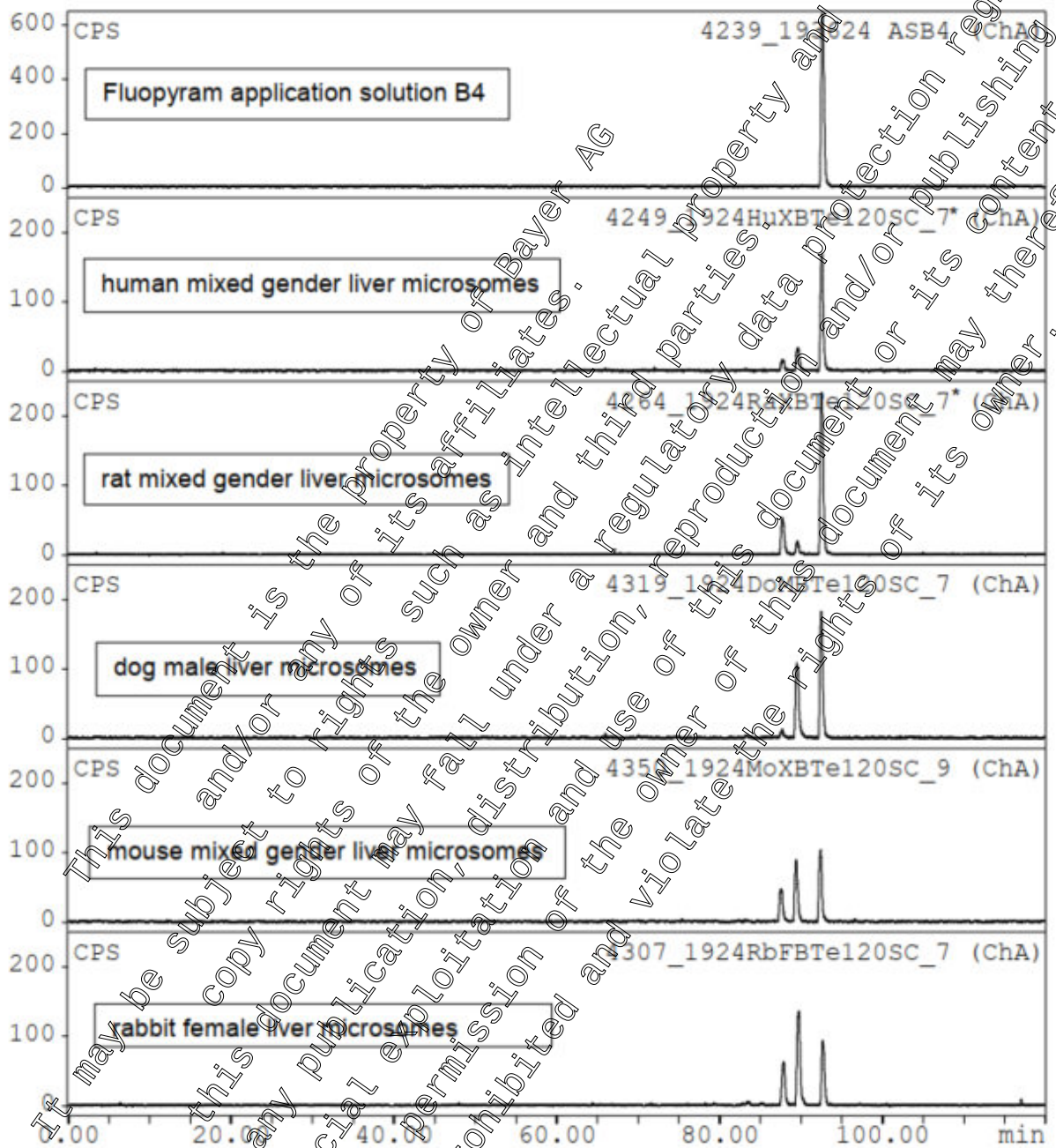


Figure 5.1.2-5: HPLC profiles of [pyridyl-2,6-^{14}C]-fluopyram application solution after incubation of 10 µM [pyridyl-2,6-^{14}C]-fluopyram with human, rat, dog, mouse and rabbit liver microsomal fraction after 120 min incubation (S19-23624, M-762838-01-1)



C. Conclusion

Metabolic profiles of fluopyram were compared between different species using an *in-vitro* system based on liver microsomal fraction from human (mixed gender), rat (mixed gender), dog (male), mouse (mixed gender), and rabbit (female). 1 and 10 µM [pyridyl-2,6-^{14}C]-fluopyram were incubated for 0, 60 and 120 min. The metabolic capability of the tested liver microsomal fractions was proven with [4-^{14}C]-testosterone.

[Pyridyl-2,6-^{14}C]-fluopyram was biotransformed at a moderate to high rate after incubation with human, rat and dog liver microsomes, and at a high rate after incubation with mouse and rabbit liver microsomes.

No unique human metabolites >2% radioactivity were detected. Metabolites were only characterised based on their chromatographic behaviour and no metabolic pathway was derived.

Assessment and conclusion by applicant:

The study is considered valid, scientifically acceptable and appropriate for the assessment of the toxicology of fluopyram.

Data Point:	KCA 5.1.2.04
Report Author:	[redacted]
Report Year:	2018
Report Title:	Prediction of biotransformation products of the fungicide fluopyram by electrochemistry coupled online to liquid chromatography-mass spectrometry and comparison with in vitro microsomal assays
Report No:	M-763230-01-1
Document No:	M-763230-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	None
Previous evaluation:	No, not previously evaluated
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities (literature publication)
Acceptability/Reliability:	Yes

This is an article from the literature. Although it is not relevant for risk assessment, it is summarised here as supportive information.

Executive Summary

Fluopyram is a new succinate dehydrogenase inhibitor fungicide, which has been shown to be extensively metabolized by oxidation, hydroxylation, and N-dealkylation followed by phase II conjugation with glutathione, glucuronic acid, and sulfate or by photodegradation into lactam fluopyram, dechlorinated fluopyram, and hydroxyl substitution of −Cl. Using electrochemistry (EC) coupled online to liquid chromatography (LC) and electrospray mass spectrometry (ESI-MS) and an electrochemical flow-through cell equipped with a boron-doped diamond electrode, oxidative phase I metabolite production was performed. The identification of the transformation products was done with retention time, isotopic patterns, fragmentation, and accurate mass measurements using EC/LC/MS, LC-MS/MS, and/or high-resolution mass spectrometry. The results obtained by EC were used as reference substances and compared with conventional in vitro studies by incubating fluopyram with rat and human liver microsomes (RLM, HLM). As a result, known phase I metabolites of fluopyram like benzamide, benzoic acid, 7-hydroxyl, 8-hydroxyl, 7,8-dihydroxyl fluopyram, lactam fluopyram, pyridyl acetic acid, and Z/E-olefin fluopyram, were confirmed and also new metabolites including an imide, hydroxyl lactam, and 7-hydroxyl pyridyl acetic acid oxidative metabolites were simulated by EC/LC/MS and liver microsomes. As a conclusion, EC/LC/MS is a time- and cost-efficient method for metabolic elucidation which enable matrix-free detection with valuable information about the mechanisms and intermediates of metabolism processes.

I. Material and Methods

Chemicals and reagents

Fluopyram (99.9% purity), NADPH, ammonium formate (NH_4FA), formic acid (FA) and $MgCl_2 \cdot 6H_2O$, KH_2PO_4/K_2HPO_4, HPLC-grade acetonitrile (ACN, 99.90%) and methanol (MeOH, 99.85%) were purchased from commercial suppliers. Ultrapure water was produced by a Seralpur PRO 90 CN system (Ransbach-Baumbach, Germany). Standard solution of fluopyram: 80 mL of 100 µmol/L in ACN/MeOH/H_2O, 40/50/10 (v/v/v) with 0.1% formic acid and 5 mmol/L NH_4FA)

Microsome liver cells

Rat liver microsomes (RLM) from Sprague Dawley male rats and human liver microsomes (HLM) from a female gender pool were obtained from Thermo Fischer ScientificGmbH, both with 20 mg/mL protein concentration.

Electrochemical oxidation of fluopyram and detection of transformation products

Mass voltammogram of fluopyram was recorded using a potentiostat coupled online to an electrospray ionization (ESI) source of a single quadrupole mass spectrometer. The potentiostat was equipped with an electrochemical flow-through cell with a boron-doped diamond working electrode, Pd/H_2 reference electrode, and titanium counter electrode. Two sets of 100 µmol/L fluopyram solution: ACN/H_2O (90/10, v/v) with 0.1% formic acid, 5 mmol/L NH_4FA, and MeOH/ACN (1/1, v/v) with 0.1% FA, were infused. Mass voltammograms in the range of 0–2300 and 1650–2500 mV were recorded separately in order to cover the wide range of products. Blank measurements were performed using the same solvent composition without the analyte. A nominal mass of 50 to 1000 Da was scanned by using ESI-MS in both ESI (+) and ESI (−) polarities. Oxidation products were separated and detected online by coupling an HPLC (Agilent 1200 series) with an eclipse XDB C8 analytical column (Agilent) to the EC outlet. Triplicates of working solution (100 µmol/L) was injected to a flowing mobile phase (50 µL/min) which passes through the EC to the waste. The oxidation was monitored by applying a direct current potential of 2000 to 2700 mV for 30 s. After EC oxidation the products trapped into HPLC were back flashed by the mobile phase. To simulate between EC oxidation and CYP450 metabolites, products from both experiments were analyzed on offline LC/ESI-MS parallel to blanks and a standard.

Microsomal incubations

To identify CYP450-catalyzed metabolites, solution of standard fluopyram was incubated with either HLM or RLM. Potassium phosphate buffer was added to a mixture of microsomal protein and $MgCl_2 \cdot 6H_2O$. After addition of fluopyram in ACN, the mixture was pre-incubated for 5 min at room temperature and then for 90 min at 37 °C after addition of NADPH. To terminate the reactions, ice-cold ACN was added. The final concentrations were 50 µmol/L fluopyram, 0.1 mmol/L $MgCl_2$, 1.6 mg/mL microsomal protein, and 0.5 mg/mL NADPH in 250 µL of final volume for both RLM and HLM. Three negative controls (without either of microsomes, substrate or cofactor) were incubated and analyzed simultaneously. The final supernatant solution was analyzed by LC-MS/MS.

Offline LC-MS/MS

Identification and characterization of products from EC and LMs were performed on an HPLC (Agilent 1200) hyphenated to an AB Sciex 4000 QTRAP® MS/MS. Standard fluopyram was oxidized by boron-doped diamond working electrode at 2300 mV DC potential. An aliquot was collected and analyzed by LC-MS/MS after 0.5-, 1-, 3-, 6-, and 24-h synthesis. From the 24-h synthesized products, the MS/MS spectra of targeted precursor ions were collected by linear ion trap (LIT) with dynamic fill time (DFT) in both ESI polarities. Furthermore, an MRM method was developed for monitoring of selected metabolites/oxidation products. The analytical column and mobile phase were the same as online EC/LC/MS section. Other MS/MS conditions and mass transitions are listed in Table 5.1.2- 19.

Table 5.1.2- 19: Mass transitions (*m/z*) for simultaneous analysis of fluopyram and selected oxidative phase I metabolites

Compounds	Abbreviation	Q1 → Q3	DP (eV)	CE (eV)	CXP (eV)	EP (eV)
Fluopyram	FLP	397 → 208, 173	80	45, 45	13	10
7-/8-Monohydroxylated FLP	M_{4b}/M_{4a}	413 → 395, 145	95	40, 33	14	10
7,8-Dihydroxylated FLP	M_3	429 → 411, 391	95	42, 33	14	10
2-Trifluoromethyl benzamide	M_2 (BZM)	190 → 170, 130	65	37, 32	13	10

Q1, precursor ion; Q3, product ion; DP, declustering potential; CE, collision energy; CXP, cell exit potential; EP, entrance potential

Confirmation by high-resolution mass spectrometry

High-resolution mass spectrometry data were acquired by a hybrid linear quadrupole ion trap (LTQ) high-resolution Fourier transform ion cyclotron resonance mass spectrometer (FTICR-MS) (Thermo Fisher). The FTICR-MS was equipped with an ESI source. Aliquots from 24 h synthesis were diluted and injected into a static nano-ESI emitter and scanned from 100 to 650 m/z with 100,000 nominal mass resolution.

II. Results and Discussion

Electrochemical oxidation of fluopyram

In order to obtain a general overview of the oxidation products of fluopyram, EC/MS experiments were performed without HPLC separation. The oxidation of fluopyram can be identified by the decreasing signal intensity at a potential ca. 1650 mV and increasing signal intensities of transformation products . Electrochemical oxidation attempted by ACN/MeOH, 1/1 (v/v) with either 0.1% formic acid and/or 5 mmol/L NH_4FA gave a very reproducible total ion current and keeps boron-doped diamond surface active. Two experimental setups were used: (i) MeOH/ACN, 1/1 (v/v) with 0.1% formic acid to investigate transformation products via N-dealkylation, lactam formation, and oxidation via dehydrogenation (direct EC) and (ii) ACN/ MeOH/H_2O, 40/50/10 (v/v/v) with 0.1% formic acid and 5 mmol/L NH_4FA to investigate oxidation products by indirect hydroxylation and successive dehydrogenation or N-dealkylation (indirect EC). Several products have additional minor

peaks/shoulders, indicating that a product could be formed at different potentials or from different oxidative pathways. Exact masses of several EC- and HLM-derived products measured by high-resolution mass spectrometry and the corresponding modifications of the parent fluopyram are listed in Table 5.1.2- 20. Some of the transformation products (EC and LMs) were not confirmed by high-resolution mass spectrometry, maybe due to the stability of the products or the differences in the sensitivity of the techniques.

Table 5.1.2- 20: Transformation products of fluopyram with the corresponding modification, exact masses, and deviations measured by high-resolution mass spectrometry after incubation with HLM and oxidation by EC

Transformation products	Molecular formula $[M+H]^+$	Calculated m/z $[M+H]^+$	Measured m/z $[M+H]^+$	δm/m (ppm)	Proposed modification	Formed by
FLP	$C_{16}H_{12}ClF_6N_2O$	397.0535	397.0535	0.0	–	–
M_{1a}/M_{1b}	$C_{16}H_{10}ClF_6N_2O_3$	427.0279	427.0267	−2.8	+2O, −2H	EC, LM
M_2	$C_8H_6F_3O_2$	191.0314	191.0317	1.6	*N*-Dealkylation, +OH	LM
M_3	$C_8H_7F_3NO$	190.0474	190.0475	0.5	*N*-Dealkylation, +H	EC, LM
M_4	$C_{16}H_{13}F_6N_2O_2$	379.0881	379.0880	−0.2	Cl·, +HO·	LM
M_5	$C_{16}H_{12}ClF_6N_2O_3$	429.0435	429.0441	1.4	+2O	EC, LM
M_6	$C_7H_4ClF_3NO_2$	225.9877	225.9874	−1.3	C-Dealkylation, +2O	EC, LM
M_{7a}/M_{7b}	$C_{16}H_{12}ClF_6N_2O_2$	413.0491	413.0489	−0.5	+O	EC, LM
M_{10}	$C_{16}H_{13}F_6N_2O$	363.0932	363.0932	0.0	Cl·, +H·	EC, LM
M_{11} (olefin)	$C_{16}H_{10}ClF_6N_2O$	395.0280	395.0280	0.0	−2H	EC, LM
M_{15}	$C_{16}H_{11}F_6N_2O$	361.0776	361.0786	2.7	−HCl	EC
E_1	$C_8H_6ClF_3N$	208.0135	208.0135	0.0	*N*-Dealkylation, −2H	EC
E_2	$C_8H_6ClF_3NO$	255.9983	255.9975	−3.3	*N*-Dealkylation, +O	EC
E_3	$C_8H_6ClF_3NO_2$	240.0034	240.0032	−0.8	*N*-Dealkylation, +2O	EC
E_5	$C_{16}H_{12}ClF_6N_2O_3$	425.0128	425.0115	−3.0	+2O, −4H	EC
Intermediate	$C_8H_4F_3O$	173.0208	173.0209	0.6	*N*-Dealkylation	EC

M, metabolites detected in LMs; E, products produced by EC but not by LM; LM, liver microsome; δm/m, relative mass deviation error

Identification of oxidative products

Structural elucidation and possible oxidation pathways are postulated based on the accurate mass measurement by high-resolution mass spectrometry, fragmentation pattern of targeted precursor ions, isotopic patterns (Cl-isotope), and/or retention-time mapping (m/z vs. time) and tabulated in Table 5.1.2- 21.

The known olefin metabolite of fluopyram was detected by high-resolution mass spectrometryin both EC and LMs at m/z 395 (δm/m= 0.0 ppm, Table 5.1.2- 20), although the formation on EC was very low. The products at m/z 190 and 208 were proved as M3 and 3- chloro-2-ethenyl-5-trifluoromethylpyridine (E1), respectively (Table 5.1.2- 20). Oxidation product E1 ($C_8H_6ClF_3N$) was not detected in LMs. This leads to a conclusion that M3 could be produced by N-dealkylation of olefin fluopyram in EC and parent fluopyram in CYP450-catalyzed mechanisms. Hence, the formation of olefin and imine is the crucial step for further hydroxylation and N-dealkylation of fluopyram.

Table 5.1.2- 21: Retention time (tR), mass fragments, and proposed mechanisms of miscellaneous transformation products after LM and EC experiments analyzed by LC-MS/MS

Products	ESI(+) (m/z)	t_R (min)	Product ions (m/z)	Proposed mechanisms	Formed by
M_{8a}	257	n.d.	279, 259, 239, 153	+2O, *N*-dealkylation	EC
M_8	223	24.9	225, 209, 196, 187, 167, 91	−2H, *N*-dealkylation	EC, HLM
M_9	237	24.8, 26.1	220, 219, 162	−2H, +2O, *N*-dealkylation	EC, LM
M_{12}	391	26.9	413, 373, 321	+2O, −3H	EC, LM
M_{13}^u	317	27.2	251, 219, 201, 185, 170	−2CO, −HCl	EC, LM
M_{14}^u	371	27.6, 29.1, 31.8	353, 297, 253, 213, 223, 171, 97	−2CO, +2O	EC, LM
M_{16}^u	493	28.55	475, 457, 198, 273, 221	+6O	LM

n.d., not detected; U, unknown

Both mono- (*m/z* 413) and dihydroxylated fluopyram (*m/z* 429) oxidative products were formed around 2000 mV by indirect EC. To have a close insight about hydroxylation mechanisms of fluopyram, different constant potentials were applied and the products were monitored by online EC/LC/MS (Figure 5.1.2-6). At 2000 mV, two monohydroxylated peaks with *m/z* 413 (M7 a and M7 b) and one dihydroxylated peak with *m/z* 429 (M5) were separated and characterized alongside other oxidative products. Hence, M5 could be 7,8-di OH fluopyram and the two peaks (M7a and M7b) are 7- and 8-OH fluopyram isomers. The most stable product ion, *m/z* 395 for the mono- and 341 for the dihydroxylated fluopyram yet leads to a conclusion of aliphatic hydroxylation of fluopyram. Imine (*m/z* 223, M8) and olefin (*m/z* 395, M11) formation supports this idea because hydroxylation of the unsaturated bond occurs easily through an epoxide intermediate that has been detected at *m/z* 411 on EC/MS. As presented in Figure 5.1.2-6, the M5 disappears on ≥ 2300 mV and two new peaks with *m/z* 427 (M1a and M1b) arose at 2700 mV (oxidation of −C–OH to – C=O). In addition, although the hydroxylated products are minor in EC, fluopyram metabolized intensively to monohydroxylated products in LMs. Besides this, too many oxidative products via further N-dealkylation of hydroxylated products were detected in both EC- and CYP450-based experiments.

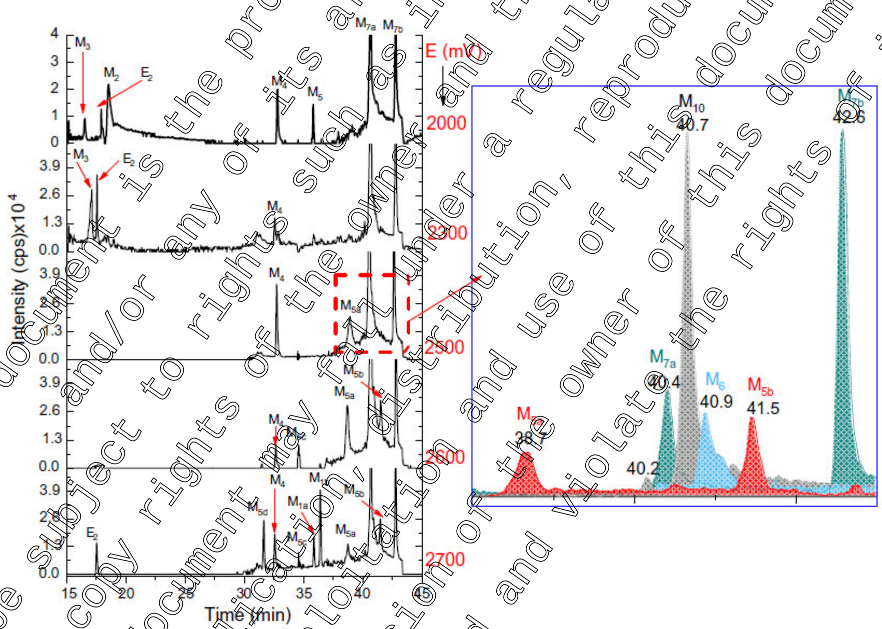


Figure 5.1.2-6: Total ion chromatograms of fluopyram oxidation products at different potentials and ion chromatograms of selected products at 2500 V (right) measured by online EC/LC/MS

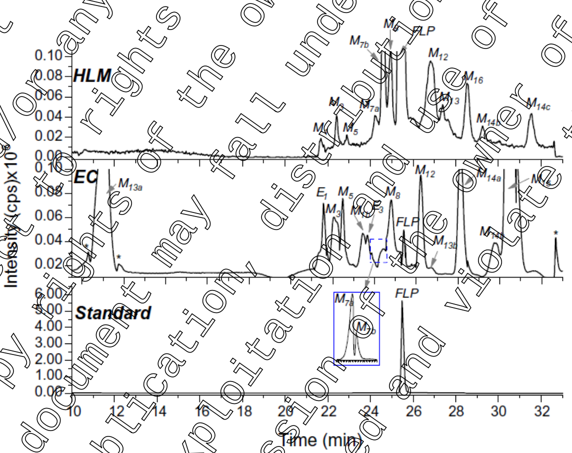
As evidenced by high-resolution mass spectrometry and EC/(LC)/MS measurements (Table 5.1.2- 20), hydroxylated fluopyram further oxidized via N-dealkylation extensively. At 2000 mV, benzoic acid (M2), benzamide (M3), monohydroxyl-[3-chloro-5-trifluoromethylpyridyl]acetic acid (E2), and 3-chloro-5-trifluoromethylpyridyl carboxylic acid (M6) were identified (Figure 5.1.2-6). Both M2 and M3 are known metabolites of fluopyram in fruits and animals. Alongside, oxidation product at *m/z* 223 was determined as 3-chloro-5-trifluoromethylpyridinyl ethanimine (M8). This is another evidence for fluopyram oxidation via imine formation. However, few products from both EC and LMs were not detected by high-resolution mass spectrometry and their possible structures are postulated only by EC/(LC)/MS and LC-MS/MS data (Table 5.1.2- 21). It needs further investigation to elucidate possible competitive products with identical *m/z*. In addition to hydroxylation and N-dealkylation, dichlorination and subsequent hydroxylation products were detected in both LMs and EC. Cyclization

products (between C3 and C16 after −HCl lost) such as lactam fluopyram (*m/z* 361, M15, Table 5.1.2-20) and its 7-hydroxyl imide form (M12, *m/z* 391) were detected in both EC and LMs. However, detection of products via dechlorination in LMs (M4, M10, M12 and M15) was a new result. Unexpectedly, an intense metabolite with *m/z* 493 was detected only in LM experiments by LC-MS/MS analysis. From MS/MS fragmentation and scanning on MRM (+), product ions *m/z* 475 (−H_2O), 457 (−HCl), and 198 (with −^{37}Cl isotope pattern) elute at the same retention time with *m/z* 493 (Table 5.1.2- 21). It could be a multihydroxylated metabolite of fluopyram (at both aliphatic and aromatic positions) or polymers of other metabolites.

Comparison of EC- and microsomal-derived transformation products

All transformation products generated by LM incubation and EC oxidation were analyzed by LC-MS/MS in parallel to a fluopyram standard solution (Figure 5.1.2-7). Although one-to-one comparison is impossible, many identical biotransformation products were detected. Because there were no differences between HLM and RLM metabolites, only the HLM metabolite chromatogram is depicted in Figure 5.1.2-7. The hydroxylation sites can differ between electrochemical and enzymatic reactions which result in the same mass-to-charge ratio but different structures. CYP450 enzymes are highly selective and catalyze specific positions whereas electrochemical oxidation favors hydroxylation of electron-rich groups. Imide species and dechlorinated products are new metabolites, they have not yet been reported. Although selectivity of EC-based oxidation is incomparable with enzymatic catalyzed metabolism, it is a complimentary tool for in silico simulation of biotransformation processes of fluopyram.

Figure 5.1.2-7: Total ion chromatograms of fluopyram metabolites from HLM incubation and electrochemical oxidation products (after 24 h synthesis) with respect to standard fluopyram (asterisk



indicates not known)

III. Conclusions

In this study, biotransformation mechanisms of the fungicide fluopyram were investigated using a non-microsomal method by electrochemistry (EC) coupled online to MS and enzymatic method by incubating liver microsomal assay to predict the oxidative phase I metabolism products of fluopyram and their formation mechanisms. In addition to known metabolites, six new transformation products could be predicted by the outlined work. The main oxidative mechanisms of fluopyram were initiated by proton abstraction/electron transfer from the −NH group which produces imine and olefin. The imine and olefin lead to subsequent hydroxylation and N-dealkylation via epoxide intermediates. On the other hand, this work suggests oxidation by dechlorination as one of fluopyram biotransformation mechanisms. EC/LC/MS gives convincing information regarding metabolism mechanisms and short-

lived intermediates and is a promising technique to simulate the fate of agrochemicals in living organisms.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The purpose of the publication is to describe and discuss the performance of online EC/LC/MS as a technique for fast prediction of oxidative metabolic pathways of fluopyram by elucidating biotransformation products. Specifically, oxidative phase I metabolism of fluopyram was investigated by EC/LC/MS and compared with HLM and RLM metabolites. Besides known phase I metabolites, they predicted new metabolites as imine, olefin with subsequent hydroxylation as well as dichlorination and short live intermediates as benzylium and epoxide. However, GLP comparative *in vitro* as well as *in vivo* studies have already been conducted and are included in the present AIR dossier. From that point of view, it can be concluded that the EC method does not deliver suitable prediction in comparison with the *in vivo* approaches and should be taken with caution since the method leads to unusable or artificial metabolites, not detected with the conventional GLP required methods. As such, the publication is not relevant to risk assessment.

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All following data points of the document MCA 5 Toxicology and Metabolism studies will be found in the second part of the summary.