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RPA 201772

Metabolism Study 85-1

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DATA EVALUATION RECORD

STUDY TYPE: Metabolism - [Rat] (S85-1)

DP BARCODE: D214214

P.C. CODE: 123000

MRID NO.: 435732-24

TEST MATERIAL: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole

SYNONYMS: Isoxaflutole; RPA 201772

STUDY REPORT NUMBER: SA 93026

SPONSOR: Rhone-Poulenc Secteur Agro, Sophia Antipolis, France

TESTING FACILITY: Rhone-Poulenc Secteur Agro, Sophia Antipolis, France

TITLE OF REPORT: RPA 201772: Absorption, Distribution, Metabolism and Excretion in the Rat

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EXECUTIVE SUMMARY:

In a metabolism study (MRID # 435732-24), ¹⁴C-RPA 201772 (98.7%) was administered to groups (5/sex/dose) of male and female Sprague-Dawley (CD) rats by gavage at a single low oral dose (1 mg/kg), repeated low oral dose (1 mg/kg/day as a final dose in a fifteen day repeat dose series), and a single high dose (100 mg/kg). In addition, pharmacokinetics in blood was investigated using two groups of 10 rats (5/sex/dose) that received a single oral dose of 1 or 100 mg/kg of ¹⁴C-RPA 201772. Urine and feces were collected at 0-24, 0-48, 0-96, 0-120, 0-144, and at 0-168 hour intervals, and tissues were collected at 168 hours post-dosing. Metabolite analysis was performed on the urine and feces of all dose groups, and on the liver samples of the two low dose group male and female rats.

¹⁴C-RPA 201772 was rapidly and extensively absorbed and metabolized. RPA 202248, a major metabolite, a diketone nitrile derivative, represented $\geq 70\%$ of the radioactivity excreted in the urine and feces from the two low dose groups. The other minor metabolite, RPA 203328, was more polar. Elimination was rapid and dose-dependent. The mean total recovery ranged from 98.09% to 99.84% (mean 99.21%). Urinary elimination (males: 61.16% to 66.65%, females: 58.80% to 67.41%) was predominant in the two low dose groups while major portion of radiolabel was excreted via the feces (males: 62.99%, females: 55.23%) in the high dose group. The higher fecal elimination possibly resulted from the saturation of absorption resulting in elimination of unchanged parent compound. The majority of the radiolabel was eliminated in the first 24 and 48 hours for the low and the high dose groups, respectively. The extensive systemic clearance of the radiolabel was reflected in the low levels of radioactivity found in tissues at 168 hours post-dosing. For the two low dose groups, liver (0.172 to 0.498 ppm) and kidneys (0.213 to 0.498 ppm) accounted for the major portion of the administered dose found in tissues. In the high dose group, the highest level of radioactivity was found in decreasing order in blood, plasma, liver, and kidney. Sex-related differences were observed in the excretion and distribution pattern among high dose rats. The elimination half-lives were similar among single low and high dose groups, with an estimated mean blood half-life of 60 hours. No sex differences were observed in the metabolism of ¹⁴C-RPA 201772. Based on the identified metabolites, a scheme for metabolic pathways of RPA 201772 was proposed.

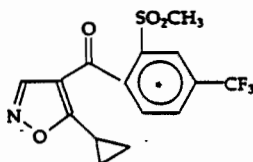
This metabolism study in the rat is classified as *acceptable* and *satisfies* the guideline requirement for a metabolism study (85-1) in rats.

A. MATERIALS:**1. Test Compound:**

RPA 201772 [U-¹⁴C phenyl]
Radiochemical purity: 98.7%
Specific activity: 18.35 mCi/mmole
Lot/Batch: JKS473

Non radioactive Compound RPA 201772,
Purity: 98.3%
Lot/Batch No.: FPI1308
Description: Fine yellow powder, as noted in
MRID #435732-19
CAS No.: 141112-29-0

Structure:



* denotes the position of the radiolabel

2. Vehicle: 0.75% (w/v) Aqueous methyl cellulose

3. Test animals:

Species: Rat
Strain: CD, Sprague-Dawley
Age: Not specified
Weight: Male: 173.99-314.16; Female: 163.45-229.90 at the
initiation of dosing
Source: Iffa Credo, France
Housing: Initially in metal, wire mesh bottomed cages, and
then individually in metabolism cages, except for rats in
the PK group, which were kept in wire mesh bottomed cages
Animals received food: Diet (AOUC, U.A.R.) and water ad
libitum.
Environmental conditions:
Temperature: 20±2°C
Humidity: Not reported
Air changes: Not reported
Photoperiod: 12 hours light/dark
Acclimation period: Not reported

4. Preparation of dosing solutions:

Each dose suspension was prepared fresh on the day of dosing and assayed for active ingredient (by High-Performance-Liquid Chromatography [HPLC]) and for radioactivity (by Liquid Scintillation Counting [LSC]) before, during and after the dosing. The solvent from the ^{14}C -RPA 201772 stock solution was evaporated under a stream of nitrogen to obtain a dry residue which was ground to a fine powder and suspended in a few drops of aqueous methyl cellulose (0.75% w/v). The calculated amount of non-radiolabeled RPA 201772 was added to the radiolabeled RPA 201772 and mixed together by grinding to obtain the required dose level and the specific activity. An appropriate amount of methyl cellulose was added to prepare a homogenous dosing suspension of sufficient quantity and concentration, at the required active ingredient, to dose animals at a rate of 1g dose/200 g rat b.w.

B. STUDY DESIGN AND METHODS:

1. Group Arrangements

Two series of experiments were performed as follows:

- A main study was conducted to provide information concerning the absorption, distribution, metabolism and elimination of the test compound in male and female rats following single and repeated low (at 1 mg/kg/day) and single high oral dosing (at 100 mg/kg/day).
- A second study was conducted to study the pharmacokinetic behavior of the test compound in the blood of male and female rats receiving single oral administrations at 1 or 100 mg/kg/day.

For the above studies, the animals were assigned on a weight-basis to the test groups as shown in table 1.

2. Dosing and sample collection/Preparation

Twenty-four hours before dosing, the animals were placed in individual metabolism cages. The diet was removed approximately 18 hours before administration of the radiolabeled test material, except for rats in the repeated low oral dose group, which received diet *ad libitum* during the non-radiolabelled dosing period.

TABLE 1: Dosing groups for the main and pharmacokinetic studies for RPA 201772

Test Group	Dose of labelled material, mg/kg ^a	Number/sex	Remarks
Main Study:			
Single low dose	1 (M: 1.01; F: 1.00)	5	Sacrificed at 168 hours post-dosing
Single high dose	100 (M: 116.20; F: 117.66)	5	Sacrificed at 168 hours post-dosing
Repeat low dose	1 (M: 1.07; F: 1.07)	5	14 consecutive doses of non-labelled material followed by single dose of labelled material on day 15; sacrificed at 168 hours post-dosing
Pharmacokinetics:			
Single Low dose	1 (M: 0.92; F: 0.93)	5	Sacrificed at 168 hours post-dosing
Single high dose	100 (M: 98.92; F: 98.26)	5	Sacrificed at 168 hours post-dosing

^aThe values in parentheses represent the actual doses received by the animal

The animals were weighed prior to dosing and the dose required for each animal calculated, dispensed and administered by gavage. For the repeat-dose study, non-radiolabeled test material was administered by gavage for 14 consecutive days and a single dose of radiolabeled compound was administered on day 15.

Sample collection

Air was drawn through each metabolism cage (at a rate of 0.3-0.4 l/min) and exhaled carbon dioxide was trapped for the first 48 hours following dosing. After 48 hours, <0.1% of the radioactivity administered was detected in the trap fluid.

The urine and feces were collected over solid carbon dioxide in polystyrene boxes at 24-hour intervals following dosing. These samples were protected from light. The animals were sacrificed after 168 hours. The liver, kidneys, heart, lungs, brain, spleen, muscle, abdominal fat, ovaries, testes, gastrointestinal tract plus contents, bone and marrow, adrenals, uterus, eyes, harderian glands, and the skin and fur were removed at sacrifice; the residual carcass being retained for analysis. Plasma was obtained from cardiac blood samples by centrifugation. The cage washes were performed, at 24-hour intervals (for low dose groups) and at 168 hours (for all groups) post-dosing, with acidified water followed by HPLC-

grade acetonitrile. The aqueous and the solvent washes were combined before radioassay.

Blood samples from the tip of the tail of each rat in the PK group were collected prior to dosing, at 0.5, 1, 2, 3, 4, 6, 8 and 24 hours post-dosing and at 24 hour intervals thereafter until 168 hours post-dosing.

Sample Preparation

Feces were homogenized with methanol (30 to 40 ml) and centrifuged; the solid material was air dried and ground to powder. Samples were combusted in an oxidizer following addition of cellulose powder. The carbon dioxide generated by combustion was absorbed in a trapping agent which was then mixed with 12 ml of Permafluor prior to radioimmunoassay. Minced portions of fat, testes, bone and marrow and uterus, ovaries and adrenals were combusted; blood samples from the single low and repeated oral low dose studies were also combusted after drying weighed aliquots. Other tissues and dry carcasses were homogenized; the portions of homogenates were weighed and combusted in combusto-cones using the sample oxidizer (Packard Model 306 or 387) following addition of cellulose powder (0.05 to 0.1 g). The carbon dioxide generated was absorbed using the same procedure as above.

Blood samples were added to a mixer of Soluene and isopropanol (1:1 v/v; 1 ml) and warmed at 45°C to aid solubilization. After cooling, the samples were decolorised by hydrogen peroxide (500 ul, 30% w/v) before the addition of 10 ml of Hionic Fluor.

All samples were stored in the dark for at least 18 hours before being analyzed by liquid scintillation counting.

2. Metabolite characterization

Urine and Feces: Both urine and fecal samples were analyzed by HPLC to investigate metabolic profiles in individual and pooled samples for both sexes and at each time period. Metabolite analysis was conducted on urine and feces samples from each individual animals of each sex at each time point up to 48 hours post-dosing for the high dose group and only from the time period 0-24 hours for the low and repeat dose groups. The samples were pooled at 0-24 hours, 24-48 hours, and 48-168 hours for the high dose group, and at 0-24 hours and 24-168 hours time periods for the low and repeated dose groups. Fecal samples were extracted in methanol.

β -glucuronidase or sulphatase were added to the pooled urine samples from the high dose group rats and the samples were incubated at 37°C for 18 hours. Positive controls for β -glucuronidase contained pooled samples with enzyme mix and phenolphthalein glucuronide solution. β -glucuronidase inhibitor, D-saccharo-1,4-lactone, was added to samples containing sulphatase enzymes which is known to contain some β -glucuronidase activity. Blank incubations of buffer plus urine and without enzyme activity, incubated at 37°C and non-incubated (maintained at 4°C) were also performed and the results were analyzed by HPLC.

Liver: Liver samples from each individual liver homogenate from the two low dose groups were pooled per sex per group and centrifuged for 10 min. The supernatant were evaporated under a gentle stream of nitrogen to a dry residue before being analyzed by HPLC.

To aid identification of metabolites, non-radiolabeled and radiolabeled reference standards were used. The quantification of the metabolites present in samples was performed using HPLC systems. HPLC was performed on Waters M600E Multisolvant Delivery System. Radioactive bands were detected by Waters Lambda Max Model 481.

Structural identification was performed by corroborating retention times and mass spectrum (ion mass pattern) between chemical standards and RPA 201772 metabolites observed in the analyzed samples. The structural determinations were performed using liquid chromatography and mass spectrometer (LC/MS) coupling allowing the direct injection of chemical standards and representative biological samples collected during single high dose experiments.

3. Statistics:

The percent dose recovery and compound concentrations, including means and standard deviations were calculated using the Microsoft Excel spreadsheet program.

4. Compliance:

Signed and dated GLP, quality assurance and confidentiality statements were provided.

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C. RESULTS:1. Main study:a. Absorption

As there were no intravenous data available for RPA 201772 (due to the lack of an intravenous dose group), the extent of absorption is inferred from available urinary excretion data. Based on urinary elimination, it appears that $\geq 70\%$ of the dose was absorbed in the low dose groups. In the high dose group, the higher fecal elimination indicates low absorption (approx. 25%). These results suggest that significant absorption of the test material occurred at the low dose and as the dose increased there was saturation of absorption resulting in a major portion of the parent compound being excreted unchanged.

b. Tissue distribution

A low level of radioactivity was found in the tissues at 168 hours post-dosing (Refer to Table 2). For the two low dose groups, the mean tissue/plasma ratios were higher for liver (48.11-241.39) and kidneys (47.05-286.92). Also the liver (0.172-0.498 ppm) and kidneys (0.213-0.498) accounted for the major part of the administered dose found in the tissues. These two observations indicate that these organs were primarily involved in the elimination and/or metabolism of the compound. Tissue concentrations were dose-dependent.

1) Single low dose: The percentages of administered dose recovered were close to background level for the majority of tissues, except for the liver and kidneys. The mean recoveries for the kidneys were higher for females (0.498 ppm) than for males (0.223 ppm).

2) Repeated Low dose: Although higher levels of radioactivity were recovered in the liver and kidneys, sex-related differences were noted with mean recoveries for the liver being higher for males (0.427 ppm) than for females (0.172 ppm). There was no change in distribution pattern following repeated dosing.

TABLE 2 Distribution of radioactivity in rat tissues/organs after administration of ^{14}C -RPA 201772^a.

Tissue/ Organ	μg Equivalents of RPA 201772/g of tissue (ppm)					
	Single low dose		Multiple low dose		Single high dose	
	Male	Female	Male	Female	Male	Female
Liver	0.498	0.388	0.427	0.172	4.53	4.59
Kidneys	0.223	0.498	0.213	0.221	2.93	3.78
Heart	0.001	0.001	0.001	n.d.	1.85	3.19
Lungs	0.006	0.001	0.004	0.001	2.46	4.00
Brain	n.d.	n.d.	n.d.	n.d.	0.26	0.38
Spleen	n.d.	0.001	0.001	n.d.	1.52	1.91
Muscle	n.d.	n.d.	n.d.	n.d.	1.18	1.44
fat	0.001	0.001	0.002	0.001	1.71	1.62
Gonads	n.d.	n.d.	n.d.	n.d.	0.80	2.36
GI Tract +Contents	0.004	0.005	0.007	0.004	2.03	1.60
Bone & Marrow	n.d.	n.d.	n.d.	n.d.	0.84	1.09
Adrenal	0.002	0.002	n.d.	n.d.	2.32	2.69
Uterus	----	0.001	----	0.001	----	2.57
Eyes	n.d.	n.d.	n.d.	n.d.	0.65	0.74
Harderian Glands	n.d.	n.d.	n.d.	n.d.	1.06	1.66
Residual Carcass	n.d.	n.d.	n.d.	n.d.	0.72	0.93
Skin & Fur	0.012	0.023	0.015	0.020	0.40	0.56
Blood	0.002	0.004	0.004	0.003	6.28	9.08
Plasma	0.001	0.002	0.005	0.004	5.22	7.28

a = Data extracted from study number SA 93026 and tables 3, 4, 10, 11, 17 and 18; MRID # 435732-24
n.d.= not detected

3) Single high dose: Sex-related differences were noted with females displaying higher levels of radioactivity in the tissues at 168 hours post-dosing than in males. The highest level of radioactivity was detected in decreasing order in whole blood, plasma, liver and kidneys.

c. Excretion

Based on the high mean recoveries shown in Table 3, it appears that in the two low dose groups, ¹⁴C-RPA 201772 was primarily excreted in the urine of rats. In the high dose group, the mean recoveries were mainly in the feces in the form of unchanged parent compound indicating saturation of absorption (Refer to Table 3). The majority of radioactivity was eliminated in the first 24 hours for the two low dose groups and within first 48 hours for the high dose group.

1) Single low dose: As summarized in Table 3, the mean 7-day recovery of radioactivity post-dosing in the urine was slightly higher for the males (61.16%) than for the females (58.80%). The mean 7-day fecal recovery values for males and females were 26.06% and 26.94%, respectively.

2) Repeat Low dose: The mean 7-day recovery of radioactivity post-dosing in the urine was slightly higher for the females (67.41%) than for the males (66.65%). The mean 7-day fecal recovery values for males and females, respectively, were 24.04% and 24.72%, respectively.

3) Single high dose: The mean 7-day recoveries of radioactivity post-dosing in the urine for the males and females were 31.37% and 41.20% respectively. The mean 7-day fecal recovery values for males and females 62.99% and 55.23%, respectively. Sex differences were observed in the elimination of radiolabel with higher urinary elimination in females and fecal elimination in males.

TABLE 3 Recovery of radioactivity in rats after administration of ¹⁴C-RPA 201772^a.

	Percent of radioactive dose recovered					
	Single low dose		Repeated low dose		Single high dose	
	Male	Female	Male	Female	Male	Female
Tissues	4.33	3.36	2.62	1.44	1.47	1.79
Cage wash	7.68	11.34	6.12	6.4	1.48	0.63
Urine	61.16	58.80	66.65	67.41	31.37	41.20
Feces	26.06	26.94	24.04	24.72	62.99	55.23
Total	99.23	100.44	99.43	99.97	97.31	98.85

^a = Data extracted from study number SA 93026 and tables 7, 14, and 21; MRID# 435732-24

3. Metabolite characterization and identification:

i) Urine and feces: The metabolism of ^{14}C -RPA 201772 was qualitatively and quantitatively similar in both sexes of rats and was not influenced by the repeated administration. The compound was rapidly and extensively metabolized as seen by the lack of parent material in the urine of the two low dose group rats; only traces were detected in the urine of the high dose group rats. Unchanged parent compound was detected in the feces of rats from the high dose group indicating saturation of absorption of the compound.

After oral administration, 9 metabolites (UMET/1-9) were detected in the urine and 11 metabolites (FMET/1-11) were detected in the fecal extracts (Table 4). Independent of the time period and the elimination route, the major metabolite detected in the urine (UMET/5) and fecal (FMET/7) extracts for the three dose groups was identified as RPA 202248. It is a diketonitrile derivative of the parent compound, which represented 70% of the radioactivity excreted in the urine and feces. The metabolites UMET/1 and FMET/1 (more polar metabolite) were identified as RPA 203328 (minor metabolite); the metabolites UMET/2 and FMET/3 were not clearly identified but suggested the presence of a strongly acidic proton or the presence of an unstable conjugate. The metabolites UMET/7 and FMET/9 were identified as an amine derivative from RPA 202248. The minor metabolites, UMET/8 and FMET/10, were possibly the Des-SO₂Me derivatives from the parent compound. The unchanged RPA 201772 was detected as UMET/9 and FMET/11.

The urine samples subjected to enzymatic hydrolysis with β -glucuronidase or sulphatase produced metabolites similar to those found in the urine samples left unincubated at (4°C) that displayed metabolites UCMET/3 (RPA 202248) as a major metabolite and UCMET/1 (RPA 203328) and UCMET/4 (unidentified) as two minor metabolites. Sulphatase hydrolysis of the high dose male urine samples revealed minor metabolite UCMET/2 but lacked UCMET/1. Based on these results the study author concluded that neither the ^{14}C -RPA 201772 nor its metabolites were eliminated as glucuronide and/or as sulphate conjugates. This observation suggests that only phase I reactions were involved in the metabolism of ^{14}C -RPA 201772 following single oral administration in the rat. A proposed metabolic pathway is presented in attachment I.

ii) Liver: The liver samples from the two low dose group males and females contained TMET/1 (RPA 202248) as a major metabolite representing 33% to 77.91% of the initial radioactivity measured. TMET/2 (unidentified) was detected as a minor metabolite (0.28 to 2.15% of the initial radioactivity).

4. Pharmacokinetics study:

Pharmacokinetic data indicated that the extent of absorption was less in female rats but the rate of absorption was higher at both the low and high dose levels. In addition, decreased absorption in both sexes was observed at the high dose, as indicated by the presence of greater percentages of radioactivity in feces at the high dose. In this study, the Cmax values obtained for the high and low single dose groups were directly proportional to the dose levels as shown below.

High dose group: The sex-related differences were noted in the mean whole blood Cmax values with 48.10 ± 12.18 μg equivalents/ ^{14}C -RPA 201772 g for males and 25.19 ± 5.85 μg equivalents/g for females; the corresponding mean Tmax values were 0.98 ± 0.40 hours (males) and 0.67 ± 0.05 hours for the females. The elimination half-lives for the high dose group were 59.23 hours for males and 60.04 hours for the females.

Low dose group: The mean whole blood Cmax values were 0.50 ± 0.10 μg equivalents/g (males) and 0.27 ± 0.05 μg equivalents/g (females); the corresponding mean Tmax values were 1.03 ± 0.35 hours (males) and 0.52 ± 0.04 hours for the females. The elimination half-lives for the low dose group were 61.05 hours for males and 59.40 hours for the females.

Based on the decreased Cmax and Tmax values observed for female rats, the pharmacokinetic data suggest that the extent of absorption was less in female versus male rats, but that the rate of absorption was greater in female rats at both the low and high dose levels. Decreased extent of absorption is supported by the lower tissue radioactivity observed in female versus male liver in all dose groups, and by the lower plasma concentrations observed in female rats versus male rats. The observation of consistently higher tissue radioactivity in the kidney of female rats despite lower liver levels of radioactivity is of interest, but is not readily explainable based on the available data.

D. DISCUSSION:

The present study was conducted to examine the excretion, tissue distribution, and biotransformation of RPA 201772 in male and female Sprague-Dawley rats to fulfill the Subdivision F 85-1 guideline. Urinary excretion data indicated that the amount of absorption was similar among low dose groups; but the percentage excreted in the urine was decreased at the high dose. The presence of greater amounts of parent compound in feces at the high dose supports this finding.

Tissue distribution data showed the liver and kidney to be the

major sites for residual radioactivity at 168 hours post-dosing in all dose groups which reflects involvement of these tissues in the metabolism and elimination of the administered dose. The elimination half-lives were approx. 60 hours in single low and high dose groups.

Excretion data showed urine to be the major route of excretion in the two low dose groups (range: 58.80-67.41% of the total dose), with the majority of the remainder in the feces (range: 24.04-26.94% of the total dose). At the high dose, the percentage of urinary excretion was decreased (male: 31.37; female: 41.20 %), while fecal excretion increased (male: 62.99%; female: 55.23); sex differences were observed in the elimination of radiolabel with urinary excretion being higher in females and fecal excretion being higher in males.

The relative importance of urinary elimination reflects the intensive metabolism of the compound. No sex differences were seen. The main metabolite observed in urine and feces from the low dose groups was RPA 202248; the minor metabolite, which was more polar was identified as RPA-203328. The unchanged parent compound was also detected in the urine and feces.

A scheme for metabolism of RPA 201772 was proposed and provided as part of this review.

E. CLASSIFICATION: Acceptable.

TABLE 4. Metabolite profile in excreta of rats dosed with ¹⁴C-RPA 201772^a.

Dose	Percent of administered dose					
	Single low dose		Repeated low dose		Single high dose	
Compound	Male	Female	Male	Female	Male	Female
RPA 201772-Parent (UMET/9, FMET/11)	n.d.	n.d.	n.d.	n.d.	8.25	5.62
RPA 202248 (UMET/5, FMET/7)	79.56	77.56	84.42	85.20	69.98	79.88
RPA 203328 (UMET/1, FMET/1)	2.02	0.58	1.29	2.59	3.61	2.56
Total identified	81.58	78.14	85.71	87.79	81.84	88.06
Unidentified Metabolite (UMET/2, FMET/3)	n.d.	n.d.	2.15	1.49	0.95	0.80
Unidentified Metabolite (UMET/7, FMET/9)	n.d.	n.d.	n.d.	n.d.	2.29	4.35
Unidentified Metabolite (UMET/8, FMET/10)	n.d.	n.d.	n.d.	n.d.	1.97	1.30
Miscellaneous Unidentified metabolites ^b	n.d.	n.d.	0.13	0.07	4.65	5.47
Total unidentified	n.d.	n.d.	2.28	1.56	9.86	11.92
Total accounted for ^c	81.58	78.14	91.03	89.28	91.70	99.98
Lost/unaccounted for ^d	18.42	21.86	8.97	10.72	8.30	0.02
Total	100	100	100	100	100	100

a = Data extracted from study number SA 93026 and Tables 25 and 26

b = Include metabolites: UMET/3, UMET/4, UMET/6, FMET/4, FMET/5, FMET/6, and FMET/8

c = Total accounted for = (Total identified) + (Total unidentified)

d = 100 - (Total accounted for)

n.d. = Not detected

MRID# 435732-24