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DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat; OPPTS 870.7485; OECD 417.

PC CODE: 107103 **DP BARCODE:** 341456

TEST MATERIAL (RADIOCHEMICAL PURITY): [^{14}C]-5-chloro-2-methyl-3(2H) isothiazolone (99%). This chemical is component of RH-886.

SYNONYMS: [4,5-^{14}C]-RH-651

CITATION: Kim-Kang, H. and D. Wu (2005). Metabolism of ^{14}C-RH-651 in the rat. XenoBiotic Laboratories, Inc., Plainsboro, NJ. XBL Report No. RPT01224, August 4, 2005. MRID 47154007. Unpublished.

SPONSOR: Rohm and Haas Company, Spring House, PA.

EXECUTIVE SUMMARY: In a 96-hour metabolism study (MRID 47154007), ^{14}C-RH-651 (Lot #1018.0013, 99.95% radiochemical purity) dissolved in water was administered by gavage at a dose of 3.75 or 22.5 mg/kg to male rats and at a dose of 3.75 or 11.25 mg/kg to female rats (4 animals/sex/dose). The dosing solutions also contained Kathon 886F (14.11% a.i.) composed of RH-573 (51.4% a.i.) and RH-651 in an approximate 3:1 ratio.

In the low-dose group, 85.73%-87.16% of the radioactive dose was recovered by 96 hours post-dosing, with 29.75%-30.75% found in the urine, 47.27%-48.77% in the feces, 0.93%-1.44% in selected tissues, and 6.29%-7.71% in cage rinse. In the high-dose group, 94.56%-95.60% of the radioactive dose was recovered, with 38.57%-43.03% found in the urine, 43.87%-45.90% in the feces, 3.94%-4.72% in selected tissues, and 4.76%-5.38% in cage rinse. Whole blood (not including plasma) had the highest percent radioactivity in tissues (0.67%-1.09% for the low-dose group, 3.41%-4.11% for the high-dose group). Each of the other tissues contained less than 1% of the dose. Most of the radioactive dose was eliminated within 24 hours post-dosing (77.20%-86.68%).

A total of approximately 29 metabolites of RH-651 were detected in urine and feces samples collected 0-48 hours post-dosing. The main metabolite in the urine, M1A, contained 15.35%-18.19% of the radioactive dose. The main component (approximately 94.38%) of M1A, M1A-1, was identified as N-methylmalonamic acid. The main metabolite in the feces, designated as M15, contained 26.38%-32.54% of the radioactive dose. Metabolite M1B, found in the feces, contained 5.19%-9.62% of the dose. Each of the other metabolites comprised <5% of the dose. Structures were determined for M1A (component M1A-1), M1B (components M1B-1 and M1B-3), M2, M7, M13, M15, M20, and M24 (component M24-A). A metabolic pathway was

proposed for RH-651 in the rat.

This metabolism study in the rat is classified **ACCEPTABLE - NONGUIDELINE** and does not satisfy the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in the rat. The study is upgradable if justification is provided for not collecting expired air samples, or urine samples at 6 and 12 hours post-dosing and/or if these requirements are waived. **The absence of these data does not significantly affect the results of the study.**

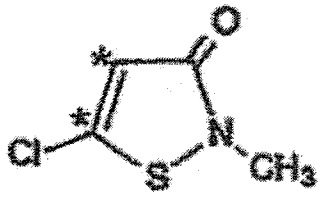
COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

1. **Test compound:**

Radiolabelled test material:	4,5-^{14}C-RH-651
Radiochemical purity:	99.9% (HPLC)
Specific activity:	51.65 mCi/g
Lot/batch #:	1018.0013 in Study Protocol (p. 146) and the Certificate of Analysis (p. 167) the lot number is listed as 1018.0013; however, 1018.0012 is in the text as the lot number.
Non-Radiolabelled test material:	Kathon 886-F (contains 10.45% RH-651, 3.66% RH-573)
Description:	Amber liquid
Lot/batch #:	Kathon 886F: Lot #0000371525 RH-573: Lot #800IJ123 (TD#01-119)
Purity:	Kathon 886F: 14.11% RH-573: 51.4%
Contaminants:	None reported
CAS # of TGAI:	Not provided

Structure:



* ^{14}C position

Taken from page 17 of the study report.

2. **Vehicle and/or positive control:** The dosing solution was prepared by dissolving an appropriate amount of ^{14}C-RH-651, Kathon 886F, and RH-573 in NANOPure™ water.

3. **Test animals:**

Species:	Rat	
Strain:	Sprague Dawley	
Age/weight at study initiation:	Adult (8-10 weeks); ~232-265 grams	
Source:	Hilltop Lab Animals, Inc. (Scottdale, PA)	
Housing:	Individual stainless steel metabolism cages	
Diet:	Certified Purina Rodent Chow® #5002 in powder form, *ad libitum*, except overnight prior to dosing and 4 hr post-dosing; feed analyzed by manufacturer for nutritional contents and contamination	
Water:	Tap water, *ad libitum*; water samples analyzed by International Hydronics Corp. (Rocky Hill, NJ) for selected organophosphates and chlorinated hydrocarbons	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	50±20%
	Air changes:	not provided
	Photoperiod:	~12 hrs dark/12 hrs light

Acclimation period:	5 days in polycarbonate cages (<5/cage); fed Certified Purina Rodent Chow® #5002 in pellet form, *ad libitum* ~24 hr acclimation in study cages; fasted overnight prior to dosing; fresh water available *ad libitum* during acclimation through a bottle dispenser attached to cage

4. **Preparation of dosing solutions:** The low-dose solution (target concentration of 0.5 mg/gram for both sexes) and high-dose solution (target concentration of 3 mg/gram for males, 1.5 mg/gram for females) were prepared by weighing an appropriate amount of Kathon 886F, RH-573, and ^{14}C-RH-651 into the dose bottle, adding a sufficient amount of NANOPure™ water, and mixing until dissolved. Each dosing solution contained ^{14}C-RH-651 and RH-573 in ~3:1 ratio.

   Pre- and post-dose concentrations were assayed by weighing duplicate aliquots of 100 µL of the dosing solution into 10 mL volumetric flasks and diluting to 10 mL with water. After mixing thoroughly, duplicate aliquots (100 µL) were analyzed with a liquid scintillation spectrometer (LSS). The mean concentration of ^{14}C-RH-651 in the low-dose solution was 0.51 mg/gram (containing 0.18 mg/gram RH-573); the mean concentration of ^{14}C-RH-651 in the high-dose solution was 3.03 mg/gram for males (containing 1.05 mg/gram RH-573) and 1.53 mg/gram for females (containing 0.53 mg/gram RH-573). The stability of the test substance in the dose formulations was confirmed using high-performance liquid chromatography (HPLC).

B. STUDY DESIGN AND METHODS:

1. **Group arrangements:** Animals were identified upon receipt by a unique identification number on an ear tag and marked on the tail with indelible ink. Animals were assigned to the test groups noted in Table 1 (method of assignment not specified). In the original protocol, the dose for females in Group B (high dose) was 22.5 mg/kg. According to the protocol amendment, animals in this group were dying several hours after dosing. The dose for this group was subsequently reduced. The data from the original Group B females were not used. Animals in Groups A and B were sacrificed at 96 hours; animals in Group C were sacrificed 24 hours after the start of the experiment.

TABLE 1: Dosing groups for pharmacokinetic studies for ^{14}C-RH-651[a]

Test group	Dose of labeled material (mg/kg)	Dose volume (mL/kg)	No. of rats/sex	Sample
A (low dose)[b]	3.75 males 3.75 females	~7.5	4	Urine, feces, tissues
B (high dose)[c]	22.5 males 11.25 females	~7.5	4	Urine, feces, tissues
C (control)	0	0	1	Urine, feces, tissues

[a] Data obtained from page 20 of the study report

[b] Nominal 3.75 mg/kg ^{14}C-RH-651 and 1.25 mg/kg ^{12}C-RH-573

[c] Nominal male concentrations 22.5 mg/kg ^{14}C-RH-651 and 7.5 mg/kg ^{12}C-RH-573; female concentrations 11.25 mg/kg ^{14}C-RH-651 and 3.75 mg/kg ^{12}C-RH-573

2. **Dosing and sample collection:** Each animal received a single dose by oral gavage based on its body weight prior to dosing. The actual administered dose was determined by weighing the syringe when loaded and after delivery of the dose.

   a. **Pharmacokinetic studies:**

   Sample Collection: Urine and feces were collected from Groups A and B pre-dose and at post-dose intervals of 0-24, 24-48, 48-72, and 72-96 hours. Urine samples were collected into tared cups and freeze-trapped using dry ice. Feces samples were collected at room temperature from the cage screen, then weighed and stored frozen. Cages were rinsed with NANOPure® water at 24, 48, and 72 hours post-dose. At the end of the study, cages were washed with IPA/water (1:1). Cage rinse samples were collected in tared containers. Urine, feces, and cage rinse were collected from Group C at the time of sacrifice.

   At sacrifice, animals were anesthetized with carbon dioxide and blood (~3-5 mL) collected by cardiac puncture. The following tissues were collected from Groups A, B, and C and rinsed with 0.9% saline, as needed, to remove excess blood: liver, fat, kidneys, bone marrow (femur), heart, lungs, brain, testes, ovaries, muscle (hind leg), spleen, adrenals, thyroids, and remaining carcass. Tissues were stored at ~ -20°C until analysis.

   Sample Preparation: Blood was mixed manually by inverting the collection tubes, a 1 mL subsample removed, and ~ 0.1 mL duplicate aliquots weighed out for combustion. The remaining blood was centrifuged (2500 rpm, 10 min, 4°C) to obtain plasma. The plasma was mixed by vortex. Duplicate subsamples were prepared and weighed (~0.05-0.1 gram, as allowable).

   Urine and cage rinse samples were mixed thoroughly. Duplicate aliquots (~0.1 gram for urine, ~1 gram for cage rinse) were weighed into scintillation vials and mixed with scintillation cocktail. Feces were homogenized with ~3-5x (w/v) NANOPure® water (except 24-48 hr feces, with 6x water), and triplicate aliquots of homogenate (equivalent to ~100 mg fresh feces weight) were combusted in a Harvey Biological Sample Oxidizer. Smaller samples were analyzed if expected to contain very high radioactivity.

   Tissues were homogenized with 2x (w/v) NANOPure® water. Duplicate aliquots (equivalent to ~100 mg fresh tissue weight, as allowable) were combusted in a Harvey Biological Sample Oxidizer.

   Sample (Radioactivity) Analysis: Radioactivity in plasma, urine, cage rinse, and feces extract samples was counted directly by a liquid scintillation counter (LSC). Total radioactive residue (TRR) levels in tissues, feces, or post-extraction solid (PES) samples were determined by combusting aliquots of homogenized samples in a Harvey OX-500 or OX-300 Biological Sample Oxidizer; the evolved $^{14}CO_2$ was counted in 15 mL of Harvey Scintillation Carbon-14 Cocktail. Samples analyzed for excretion data were counted in duplicate (direct counting) or triplicate (combustion analysis). Samples for metabolite profiling (See following subsection) were usually limited to a single or duplicate analysis. Samples were analyzed with a Beckman LS 6000IC, LS 6000LL, or LS 6000TA. Samples

were counted for 10 min or until the 2-sigma error was less than or equal to 2%. The counting time for HPLC fractions was 2 min. Quench correction was performed using an external standard method. Oxidizer efficiencies were validated by combusting a known amount of ^{14}C-mannitol.

For TRR (total radioactivity residue) levels, the scintillation spectrometer was set to zero background. Control and treated samples were counted for each matrix, and counts per minute (cpm) automatically converted to disintegrations per minute (dpm). The dpm from the control sample was subtracted from the treated sample and the net dpm per aliquot used for subsequent calculations.

b. **Metabolite characterization studies:** Samples of urine and feces collected at 0-24 hr and 24-48 hr post-dose intervals were analyzed for metabolic profiling. Urine and feces samples were pooled proportionally according to gender, time interval, and treatment. Reverse-phase HPLC (RP-HPLC) was used to obtain radioprofiles of pooled samples. Eight pooled urine samples and eight pooled feces samples were analyzed. Metabolites were designated according to their retention times. Further characterization and identification of some metabolites was obtained by LC/MS and/or LC/MS/MS analysis.

Prior to HPLC analysis, the pooled feces samples were extracted and concentrated. The feces samples were extracted using CH_3OH (~5x v/w); following centrifugation the precipitate was mixed with water and CH_3OH (1:9, 5x v/w), sonicated, and centrifuged. The resulting supernatant was combined with the first supernatant, the volume adjusted to 25 mL with methanol, and aliquots taken for LSC. The entire samples of dried PES (triplicate aliquots) were combusted and radioassayed using LSC. The same procedure was followed with a control feces sample (Group C) fortified with ^{14}C-RH-651 to evaluate the extractability and extraction stability of the parent compound.

HPLC was conducted under one of four conditions (Condition 2, 3, 4, or 5). The initial metabolite radioprofiles for urine and feces extracts were determined using a Shimadzu HPLC System coupled with a StopFlow ARC/RAM (Condition 2). Normal-phase (NP)-HPLC (Condition 4) was used for the analysis of urine and feces metabolite isolates. Condition 3 and Condition 5 were also used for urine metabolite isolation. Appropriate reference standards were analyzed using HPLC.

Because of a large amount of co-eluting matrices, direct LC/MS analyses of the urine samples and feces extracts could not provide definitive molecular ions for most of the metabolites. Two urine samples and one feces extract were subjected to preparative HPLC (Condition 3 or Condition 5) and the eluates collected every 15 sec into 2-mL, 96-well fraction collection plates. Multiple collections were made for each sample to obtain a sufficient quantity of metabolites for subsequent HPLC or LC/MS and/or LC/MS/MS analyses. LC/MS was conducted under one of seven conditions (Condition 1, 2, 3, 4, 5, 6, or 7).

3. **Statistics:** Calculations were performed using an electronic calculator or an IBM-compatible PC with Microsoft® Excel software.

The percent dose recovered in each matrix at each interval was calculated as the total dpm in matrix/dpm dosed x 100. The percentages of doses recovered in blood, fat, and muscle were extrapolated using the following factors: 20.4 grams blood, 10 grams fat, and 125 grams muscle per 250 grams body weight.

For metabolite profiling, radioactivity in pooled urine and feces samples was expressed as percent of administered dose. The percent region of interest (ROI) in each HPLC chromatogram was calculated as the dpm in a peak region/total dpm in interested peak regions x 100. The percent dose, recovered as RH-651 or metabolite in pooled urine was calculated as the (%ROI x % dose in the sample)/100. The percent dose in pooled feces extracts was calculated as the % fractional distribution x % dose in the pooled feces sample)/100. The percent dose recovered as RH-651/metabolite in feces was calculated as (%ROI x % dose in the extracts)/100.

II. RESULTS:

A. PHARMACOKINETIC STUDIES:

1. **Preliminary experiment:** According to the study protocol, the dose levels used in the study were chosen based on previous toxicological studies using the test compound. No further information regarding these studies or their results was provided.

2. **Absorption:** Absorption was not directly addressed in the report. The report references a previous metabolism study with RH-651 in biliary cannulated rats. The study results indicated that biliary excretion is not a major excretory route.[1] Therefore, the majority of the radioactive dose found in the feces in the current study was considered to be unabsorbed dose. The percent radioactive dose found in the feces ranged from 43.87%-48.77%; no signs of sex difference in excretion were observed (see Section IIA4).

3. **Tissue distribution:** The percent of the radioactive dose measured in selected tissues is summarized in Table 2. The mean total radioactivity in selected tissues was 0.93%-1.44% in the low-dose group (Group A) and 3.44%-4.11% in the high-dose group (Group B). Whole blood (not including plasma) had the highest percent radioactivity (mean of 0.67%-1.09% in the low-dose group and 3.41-4.11% in the high-dose group). The next highest percent radioactivity for the low- and high-dose groups was found in the muscle (mean of 0.13%-0.15% and 0.21%-0.25%, respectively) and the liver (mean of 0.06%-0.12% and 0.13%-0.25%, respectively). The percent radioactivity in each of the other analyzed tissues was ≤0.06% of the administered dose.

[1]MRID 47154008. Kim-Kang, H. and D. Wu (2005) Metabolism of ^{14}C-RH-651 in the biliary cannulated rat. XBL Study No. 04042, Report No. RPT01229.

TABLE 2: Distribution of radioactivity in rat tissues/organs 96 hours after oral administration of ^{14}C-RH-651[a]

Tissue/organ	Percent of radioactive dose administered (mean±SD)			
	Low dose (Group A)		High dose (Group B)	
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Adrenal	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Bone marrow	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Blood	1.09±0.25	0.67±0.22	4.11±0.26	3.41±0.53
Brain	0.00±0.00	0.00±0.00	0.01±0.01	0.01±0.01
Fat	0.02±0.01	0.01±0.00	0.03±0.02	0.03±0.02
Heart	0.00±0.00	0.00±0.00	0.01±0.00	0.01±0.01
Kidneys	0.03±0.01	0.03±0.01	0.06±0.01	0.05±0.01
Liver	0.12±0.03	0.06±0.01	0.25±0.04	0.13±0.02
Lungs	0.02±0.01	0.01±0.00	0.03±0.01	0.03±0.00
Muscle	0.15±0.04	0.13±0.02	0.21±0.02	0.25±0.00
Spleen	0.01±0.01	0.01±0.01	0.02±0.00	0.00±0.00
Testes or Ovaries	0.00±0.00	0.00±0.00	0.00±0.01	0.02±0.01
Thyroid	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Plasma[b]	0.01±0.01	0.01±0.00	0.01±0.00	0.01±0.00
Total	**1.44±0.28**	**0.93±0.23**	**4.72±0.22**	**3.94±0.52**

[a] Data were obtained from pages 54 and 55 (Table V and Table VI) of the study report.

[b] The percent dose of plasma is not included in the total because it is included in the value for blood.

4. **Excretion:** The excreted dose was considered to be the sum of the radioactivity in the urine, feces, and cage rinse. The recovery of radioactivity in excreta and cage rinse is summarized in Table 3. By 96 hours post-dose, a mean of 84.81%-85.72% of the radioactive dose was excreted by the low-dose group; 89.84%-91.66% was excreted by the high-dose group. In the low-dose group, fecal excretion (47.27%-48.77%) was noticeably greater than urinary excretion (29.75%-30.75%); in the high-dose group, urinary excretion was only slightly higher than fecal excretion (38.57%-43.03% in urine, 43.87%-45.90% in feces). Most of the radioactive dose was excreted within the first 24 hours (77.20%-77.56% in the low-dose group; 82.93%-86.68% in the high-dose group).

TABLE 3: Recovery of radioactivity in excreta and cage rinse of rats after oral administration of ^{14}C-RH-651[a]				
Matrix/interval	Percent of radioactive dose administered (mean±SD)			
	Low dose (Group A)		High dose (Group B)	
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Urine				
0-24 hr	28.93±3.89	28.07±4.40	37.05±3.23	41.54±3.76
24-48 hr	1.26±0.26	1.22±0.41	0.91±0.20	0.89±0.19
48-72 hr	0.39±0.09	0.35±0.15	0.40±0.05	0.40±0.10
72-96 hr	0.16±0.04	0.11±0.01	0.22±0.03	0.20±0.04
Subtotal	**30.75±3.61**	**29.75±4.82**	**38.57±3.44**	**43.03±3.78**
Cage Rinse				
0-24 hr	4.59±2.80	4.34±2.50	4.39±1.37	3.93±2.83
24-48 hr	0.79±0.34	0.68±0.37	0.46±0.28	0.14±0.02
48-72 hr	0.96±0.55	0.28±0.13	0.15±0.03	0.22±0.16
72-96 hr	1.37±0.89	0.99±1.00	0.38±0.15	0.47±0.21
Subtotal	**7.71±3.50**	**6.29±2.31**	**5.38±1.63**	**4.76±2.74**
Feces				
0-24 hr	43.68±2.25	45.15±4.30	41.49±4.08	41.21±3.11
24-48 hr	2.92±0.38	3.11±2.25	3.61±2.35	2.04±1.06
48-72 hr	0.53±0.10	0.44±0.19	0.55±0.31	0.47±0.15
72-96 hr	0.15±0.04	0.07±0.02	0.25±0.05	0.17±0.09
Subtotal	**47.27±2.02**	**48.77±3.20**	**45.90±2.48**	**43.87±2.41**
Total	**85.72±2.41**	**84.81±1.70**	**89.84±0.86**	**91.66±1.12**

[a] Data were obtained from page 52 (Table III) of the study report.

Table 4 summarizes the percent dose recovery from excreta (urine, feces), cage rinse, and selected tissues 96 hours post-administration. The mean total percent recovery of the radioactive dose in these matrices was 85.73%-87.16% for the low-dose group, and 94.56%-95.60% for the high-dose group.

TABLE 4: Summary of percent dose recovery from excreta, tissues, and cage rinse 96 hours after oral administration of ^{14}C-RH-651[a]				
Matrix	Percent of radioactive dose administered (mean±SD)			
	Low dose (Group A)		High dose (Group B)	
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Urine	30.75±3.61	29.75±4.82	38.57±3.44	43.03±3.78
Feces	47.27±2.02	48.77±3.20	45.90±2.48	43.87±2.41
Cage rinse	7.71±3.50	6.29±2.31	5.38±1.63	4.76±2.74
Tissues	1.44±0.28	0.93±0.23	4.72±0.22	3.94±0.52
Total	**87.16±2.46**	**85.73±1.61**	**94.56±0.85**	**95.60±0.85**

[a] Data were obtained from pages 56 and 57 (Table VII and Table VIII) of the study report.

B. METABOLITE CHARACTERIZATION STUDIES: Approximately 29 metabolites were detected in urine and feces samples during the initial HPLC radioprofiling.

1. **Urine:** The mean percent distribution of metabolites detected in urine is summarized for Group A (low dose) and Group B (high dose) in Tables 5 and 6, respectively. The metabolites are identified by retention time. No parent compound was identified in urine samples during

radioprofiling and its absence was further confirmed by LC/MS. Metabolite M1A accounted for 15.35%-18.19% of the radioactive dose. Each of the other metabolites accounted for ≤ 4% of the dose.

TABLE 5: Profile of metabolites detected in urine of rats in Group A (low dose) dosed with ^{14}C-RH-651[a]

Metabolite ID	Retention Time (min)	Percent of radioactive dose administered					
		Male (n=4)			Female (n=4)		
		0-24 hr	24-48 hr	Total 0-48 hr	0-24 hr	24-48 hr	Total 0-48 hr
M1A[b]	3.41-3.72	15.00	0.73	15.72	14.69	0.66	15.35
M2[c]	4.59-5.19	0.93	ND	0.93	2.36[d]	ND	2.36
M3	5.46-5.78	0.82	ND	0.82	-[d]	0.10	0.10
M4	6.05-6.66	1.66[e]	0.10	1.76	-[d]	0.05	0.05
M5	6.93-6.95	-[e]	-[e]	-[e]	1.36	ND	1.36
M6	7.53-7.54	1.13	ND	1.13	0.89	ND	0.89
M7[c]	8.41-8.74	0.49	ND	0.49	1.14	0.04	1.18
M8	9.01-9.61	0.28	ND	0.28	0.60	ND	0.60
M10	13.13-13.14	0.50	ND	0.50	ND	ND	ND
M13[c]	16.66-16.98	0.22	ND	0.22	ND	ND	ND
M15[c]	17.55-17.88	0.64	ND	0.64	0.83	ND	0.83
M16	18.43-19.02	0.47	ND	0.47	0.40	0.14	0.54
M19	24.35-24.61	ND	0.13	0.13	ND	ND	ND
M20[c]	25.23-25.50	0.54	ND	0.54	ND	ND	ND
M21	26.38-26.45	0.54	ND	0.54	ND	ND	ND
M22	26.72-27.27	0.51	ND	0.51	0.77	ND	0.77
M24[f]	28.73-29.39	1.94	0.31	2.25	2.09	0.17	2.26
M26	30.22-30.55	1.06	ND	1.06	1.21	ND	1.21
M29	34.69-35.01	0.74	ND	0.74	0.49	ND	0.49
M31	36.42	0.20	ND	0.20	ND	ND	ND
M32	37.31-37.67	0.44	ND	0.44	ND	ND	ND
M33	38.49-38.72	0.42	ND	0.42	ND	0.06	0.06
M34	39.08-39.12	0.39	ND	0.39	1.02	ND	1.02
M37	43.85	ND	ND	ND	0.23	ND	0.23
Total		**28.92**	**1.26**	**30.18**	**28.08**	**1.22**	**29.30**

ND – Not detected

[a] Data were obtained from page 63 (Table XIV) and pages 71-72 (Table XXII) of the study report.

[b] The major component of the M1A isolate (M1A-1) was identified as N-methylmalonamic acid.

[c] A structure has been proposed for the metabolite.

[d] M2, M3, and M4 were integrated together.

[e] M4 and M5 were integrated together.

[f] A structure has been proposed for one component (M24-A) of the M24 isolate.

TABLE 6: Profile of metabolites detected in urine of rats in Group B (high dose) dosed with ^{14}C-RH-651[a]							
Metabolite ID	Retention Time (min)	Percent of radioactive dose administered					
		Male (n=4)			Female (n=4)		
		0-24 hr	24-48 hr	Total 0-48 hr	0-24 hr	24-48 hr	Total 0-48 hr
M1A[b]	3.41-3.72	17.60	0.60	18.19	16.95	0.42	17.37
M2[c]	4.59-5.19	3.31	ND	3.31	3.34	0.05	3.39
M3	5.46-5.78	0.99	ND	0.99	3.87	0.05	3.91
M4	6.05-6.66	3.86[d]	ND	3.86	3.97[d]	0.04	4.00
M5	6.93-6.95	-[d]	ND	ND	-[d]	0.04	0.04
M6	7.53-7.54	-[d]	ND	ND	-[d]	ND	ND
M7[c]	8.41-8.74	1.64	ND	1.64	3.64	ND	3.64
M8	9.01-9.61	ND	ND	ND	0.66	ND	0.66
M9	11.96-12.24	0.27	ND	0.27	ND	0.05	0.05
M10	13.13-13.14	0.44	ND	0.44	ND	ND	ND
M15[c]	17.55-17.88	2.30	0.07	2.37	1.70	ND	1.70
M16	18.43-19.02	ND	ND	ND	ND	0.04	0.04
M17	21.43	ND	ND	ND	0.56	ND	0.56
M19	24.35-24.61	0.68	ND	0.68	ND	ND	ND
M20[c]	25.23-25.50	0.73	0.06	0.79	ND	ND	ND
M21	26.38-26.45	0.45	ND	0.45	0.91	ND	0.91
M22	26.72-27.27	0.34	ND	0.34	ND	ND	ND
M23	27.92	ND	ND	ND	0.80	ND	0.80
M24[e]	28.73-29.39	1.78	0.18	1.96	2.33	0.21	2.54
M25	29.95	0.44	ND	0.44	ND	ND	ND
M26	30.22-30.55	0.47	ND	0.47	ND	ND	ND
M28	33.23	ND	ND	ND	0.49	ND	0.49
M29	34.69-35.01	0.58	ND	0.58	0.88	ND	0.88
M30	35.86	0.63	ND	0.63	ND	ND	ND
M32	37.31-37.67	ND	ND	ND	0.81	ND	0.81
M34	39.08-39.12	0.28	ND	0.28	ND	ND	ND
M36	40.88-41.20	0.28	ND	0.28	0.63	ND	0.63
Total		**37.05**	**0.91**	**37.96**	**41.54**	**0.89**	**42.43**

ND – Not detected

[a] Data were obtained from page 64 (Table XV) and pages 71-72 (Table XXII) of the study report.

[b] The major component of the M1A isolate (M1A-1)was identified as N-methylmalonamic acid.

[c] A structure has been proposed for the metabolite.

[d] M4, M5, and M6 were integrated together.

[e] A structure has been determined for one component (M24-A) of the M24 isolate.

Metabolites M1A, M2, M3, M4, M7, M13, M15, M20, and M24 in urine were further analyzed for characterization. A component of M1A (M1A-1) was identified as N-methylmalonamic acid. Based on the analytical results, a structure was also proposed for M2, M7, M13, M15, M20, and M24-A (a component of M-24).

The radioactive peak designated as M1A, when analyzed by NP-HPLC (Condition 4) was found to consist of a major component (94.38% of the ROI) and a minor component (5.62% of the ROI). When M1A was analyzed by NP-HPLC (Condition 5), M1A-1 was identified as

N-methylmalonamic acid, based on its molecular weight and HPLC retention time, which were consistent with the reference standard for that compound. LC-ESI(+)/MS/MS analysis of M1A-1 also yielded product ions similar to those of N-methylmalonamic acid. M1A-2 could not be identified because of its low level.

Based on retention times in RP-HPLC and NP-HPLC (Condition 4), M2 was considered to be the same compound identified during a rat metabolism study of RH-573[2].

M7 was further analyzed by NP-HPLC (Condition 4), LC/RAM/ESI(+)/MS (LC/MS Condition 1), and MS/MS in the positive ion mode. Based on the data, M7 was considered to be derived from a glutathione conjugate of the oxidized form of RH-651, which was further degraded to form a thiol, which then underwent S-methylation and oxidation. The conjugation position and stereo confirmation could not be assigned based on the available data. Based on reported findings that glutathione conjugation favors the β-position of an α, β-unsaturated carbonyl group, the conjugation was proposed to occur at the C_5-position.

M13 was further analyzed by LC/RAM/ESI(+)/MS (LC/MS Condition 1) and MS/MS in the positive ion mode. M13 could also have been derived from RH-573, another component of the dosing solution. M13 corresponds to the metabolite designated as M9 in the rat metabolism study of RH-573 (see footnote 2).

M15, a minor metabolite in urine, was analyzed by LC/RAM/ESI(+)/MS (LC/MS Condition 1), LC/RAM/ESI(+)-/MS/MS, LC/RAM/ESI(+)/MS/MS/MS, LC/RAM/ESI(-)/MS (LC/MS Condition 2), and LC/ESI(-)-MS/MS. To further confirm the proposed structure, the M15 urine isolate was subjected to methylation and H/D exchange; the methylation and H/D exchange product was subjected to LC/MS (LC/MS Condition 1 and Condition 7, respectively). M15 was characterized as a 3-mercapturic acid conjugate of 3-sulfinyl-N-methyl propanamide.

M20 was further analyzed by direct LC/RAM/ESI(+)/MS (LC/MS Condition 1), LC/ESI(+)-MS/MS, and MS/MS/MS analyses. The results were similar to those for M12, a major metabolite identified in the rat metabolism study of RH-573 (see footnote 2); the structure of M12 was thoroughly investigated in that study.

LC/RAM/ESI(+)/MS (LC/MS Condition 1) analysis of M24 showed two distinctive peaks, designated as M24-A and M24-B. The structure of M24-A was based on LC/ES(+)-MS/MS, LC/RAM/ESI(-)/MS (LC/MS Condition 2), and LC/ESI(-)MS/MS analyses. A definitive structure of M24-B could not be determined.

The urinary metabolites that were identified by name and/or structure comprised approximately 67% to 75% of the urinary metabolites. The identified metabolites comprised approximately 21 to 29% of the administered dose (*Note: these estimates include the entire M1A and M24 fractions*).

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[2] MRID47154009. Kim-Kang, H., Cai, L. and D. Wu (2005) Metabolism and pharmacokinetics of ^{14}C-RH-573 in the rat. XBL Study No. 03172, Report No. RPT01957.

2. Feces: The mean percent distribution of metabolites detected in extracted feces samples is summarized for Group A (low dose) and Group B (high dose) in Tables 7 and 8, respectively. The metabolites are identified by retention time. No parent compound was identified in the feces. Metabolite M15 accounted for 27.06%-32.54% of the radioactive dose. M1B accounted for 5.19%-9.62% of the dose; the major component in M1B is the same compound as M15. Each of the other metabolites accounted for less than 1% of the dose.

TABLE 7: Profile of metabolites detected in feces of rats in Group A (low dose) dosed with ^{14}C-RH-651[a]

Metabolite ID	Retention Time (min)	Percent of radioactive dose administered					
		Male (n=4)			Female (n=4)		
		0-24 hr	24-48 hr	0-48 hr Total	0-24 hr	24-48 hr	0-48 hr Total
M1B[b]	3.40-3.71	9.03	0.59	9.62	8.02	0.68	8.70
M15[c]	17.55-17.88	25.66	1.40	27.06	25.81	1.40	27.21
Total		**34.69**	**1.99**	**36.68**	**33.83**	**2.08**	**35.91**

[a] Data were obtained from page 69 (Table XX) and pages 71-72 (Table XXII) of the study report.
[b] The major component of the M1B fraction (M1B-3) was identified as the same compound as M15.
[c] A structure has been proposed for the metabolite.

TABLE 8: Profile of metabolites detected in feces of rats in Group B (high dose) dosed with ^{14}C-RH-651[a]

Metabolite ID	Retention Time (min)	Percent of radioactive dose administered					
		Male (n=4)			Female (n=4)		
		0-24 hr	24-48 hr	0-48 hr Total	0-24 hr	24-48 hr	0-48 hr Total
M1B[b]	3.40-3.71	4.45	0.74	5.19	5.20	0.18	5.38
M2[c]	4.59-5.19	ND	ND	ND	0.90	ND	0.90
M7[c]	8.41-8.74	ND	ND	ND	0.67	ND	0.67
M11	14.59	ND	ND	ND	ND	0.06	0.06
M15[c]	17.55-17.88	30.53	2.01	32.54	25.18	1.20	26.38
M32	37.37	ND	ND	ND	0.84	ND	0.84
Total		**34.98**	**2.75**	**37.73**	**32.79**	**1.44**	**34.23**

ND – Not detected
[a] Data were obtained from page 70 (Table XXI) and pages 71-72 (Table XXII) of the study report.
[b] The major component of the M1B fraction (M1B-3)was identified as the same compound as M15.
[c] A structure has been proposed for the metabolite.

Metabolites M1B and M15 in feces samples were further analyzed for characterization.

M1B was isolated from a feces extract using a preparative HPLC (Condition 3). When analyzed under an acidic condition using NP-HPLC (Condition 4), M1B showed two minor components and one major component. These were designated as M1B-1, M1B-2, and M1B-3, respectively. LC/MS and MS/MS analysis of M1B-3 (LC/MS Condition 4) indicated that the compound might be the same as M15. This was further supported by analysis of M1B by LC/MS under RP-HPLC conditions (LC/MS Condition 2).

Based on its retention time in NP-HPLC (Condition 4), M1B-1 may be the same as one of the

compounds identified in the rat metabolism study of RH-573 (see footnote 2). In that study, M1B-1 was found to contain two compounds, designated as M2-A and M2-B. M1B-1 was proposed to contain one or both of the compounds, designated for the current study as M1B-1A and M1B-1B, respectively.

Because of its low level, a structure could not be determined for M1B-2. Based on its retention time in NP-HPLC (Condition 4), the peak for M1B-2 may contain N-methylmalonamic acid.

M15 in feces extract was further analyzed by LC/MS (LC/MS Condition 4). LC/(+)ESI/LC/MS and MS/MS analyses yielded results similar to those from urine samples, confirming that the compound in feces is the same as that detected in urine. Because the majority of M15 was detected in the feces, and bile is not the primary excretion route (see reference, footnote 1), M15 could have been formed by intestinal flora.

Although metabolites M2 and M7 were not characterized based on feces extract samples, they were characterized for urine samples.

The fecal metabolites that were identified by name and/or structure comprised 92% or greater of the fecal metabolites. The identified metabolites comprised approximately 34.23%-36.68% of the administered dose (*Note: these estimates include the entire M1B fraction*).

3. **Metabolic Pathways:** The main metabolic pathways of RH-651 in the rat were proposed as follows:

   - Phase I metabolites derived from oxidative cleavage of the isothiazolinone moiety with the loss of the chlorine and sulfur atoms and the C_4, C_5 double bond reduction of the moiety (M1A).
   - Phase II metabolites derived from:
     - reductive cleavage of the S-N bond, followed by methylation and glutathione conjugation, with further metabolism of the glutathione moiety to yield a cysteine conjugate, which then formed a mercapturic acid moiety (M20)
     - reductive cleavage of the S-N bond, followed by glutathione conjugation, with further metabolism of the glutathione moiety to yield a cysteinylglycine conjugate, which then underwent N-acetylation (M24-A)
     - reductive cleavage of the S-N bond, followed by the formation of a mercapturic acid conjugate (M13)
     - reductive cleavage of the S-N bond, followed by the oxidation and the formation of a mercapturic acid conjugate (M15)

III. DISCUSSION AND CONCLUSIONS:

A. **CONCLUSIONS:** RH-651 was rapidly excreted from the rat; greater than 77% of the administered dose was eliminated within 24 hours post-dosing. Urinary and fecal elimination were equally important. Fecal and urinary elimination of the administered dose was similar for the high-dose group (43.87%-45.90% in feces, 38.57%-43.03% in

urine). For the low-dose group, feces contained a notably higher percentage of the administered dose (47.27%-48.77% in feces, 29.75%-30.75% in urine).

The results of a previous metabolism study using RH-651 in biliary-cannulated rats indicated that biliary excretion is not a major route. Therefore, the majority of the percent dose in the feces represents unabsorbed dose. The parent compound (RH-651) was not found in the urine or feces. The unabsorbed dose may be glutathione conjugates but no data were presented on conjugated metabolites in this test.

RH-651 was extensively metabolized and excreted mainly into the urine following a single dose. A large amount of the administered dose was found in the feces.

The initial HPLC radiochromatography indicated the presence of at least 29 components derived from RH-651. All metabolites accounting for >5% of the administered dose were identified and/or characterized by LC/MS and LC/MS/MS. Some of the minor metabolites were also identified and/or characterized by these methods.

The metabolites of RH-651 are comprised of a variety of Phase I metabolites consisting of reductive and oxidative cleavage products of RH-651 and Phase II metabolites derived from glutathione conjugates of Phase I metabolites.

N-methylmalonamic acid (M1A) was the main component in urine samples, accounting for >15% of the dose. Other than M1A, only a 3-mercapturic acid conjugate of 3-sulfinyl-N-methyl propanamide (M15) accounted for >5% of the dose). (Note: M15 was a major metabolite in the feces). All other metabolites accounted for <5% of the dose. However, M1A was composed of two components, M1A-1 (N-methylmalonamic acid) and M1A-2 (unidentified). According to the HPLC radiochromatogram of M1A, (page 85 of the study report, Figure 13), M1A-1 and M1A-2 comprised 94.38% and 5.62% of the region of interest..

No significant gender or dose group differences in metabolites were noted Because of toxicity in the female high dose group, the dose to females in this group was reduced to less than that of the males. Therefore, the results for the male and females in the high dose group are not directly comparable.

In the low-dose group, recovery of the dose was slightly less than 90% for both males and females. No explanation is offered for the fate of the remaining radioactivity in this group. Expired air was not collected. Although the residual carcass was reported to have been retained, it was not analyzed for radioactivity.

B. REVIEWER COMMENTS: The investigators' conclusions are generally correct, with the following exceptions:

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- M1A did account for >15% of the dose.
- The authors stated "the majority of the percent dose in the feces represents unabsorbed

dose.

C. STUDY DEFICIENCIES: The following deficiencies are based on the requirements for a Tier I study under OPPTS 870.7485.

Major Deficiencies:

- Radioactivity in urine was not determined at 6 and 12 hours on day 1 of collection.
- Expired air was not collected. No explanation was provided for not measuring radioactivity in expired air.
- For the low-dose group, the study was terminated before seven days or before 90% of the administered dose was recovered.

Minor Deficiencies:

- The rationale for the selection of the doses was not fully explained.
- The method of assignment of animals to test groups was not explained.

D. STUDY CLASSIFICATION: This metabolism study in the rat is classified **ACCEPTABLE – NONGUIDELINE** and does not satisfy the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in the rat. The study is upgradable if justification is provided for not collecting expired air samples, or urine samples at 6 and 12 hours post-dosing. **The absence of these data does not have a significant effect on the study results.**

Appendix A:
Proposed Metabolism Pathway