DATA EVALUATION RECORD

E1-2-05

BAS 670H

Study Type: §85-1a; Metabolism Study in Rats

Work Assignment No. 1-01-11 V (MRIDs 45902305 and 45902306)

Prepared for
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U.S. Environmental Protection Agency
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DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat; OPPTS 870.7485 (§85-1a); OECD 417.

PC CODE: 123009 **DP BARCODE:** D292904

TEST MATERIAL (RADIOCHEMICAL PURITY): BAS 670H (>98%)

SYNONYM: [3-(4,5-Dihydro-isoxazol-3-yl)-4-methanesulfonyl-2-methyl-phenyl]-(5-hydroxy-1-methyl-1H-pyrazol-4-yl)-methanone

CITATION: Leibold, E., and B. van Ravenzwaay (2002) ^{14}C-BAS 670H - Study of biokinetics in rats. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany. Laboratory Project ID.: Project No. 02B0022/996002. BASF Registration Document No. 2002/1006961. May 28, 2002. MRID 45902305. Unpublished

Tilting, N., and H. E. Knoell (2002) Metabolism of ^{14}C-BAS 670H in rats. BASF Aktiengesellschaft, BASF Agricultural Center Limburgerhof, Crop Protection Division, Ecology and Environmental Analytics, Limburgerhof, Germany. Laboratory Project ID.: Study Code 55908. BASF Registration Document No. 2002/1011852. December 6, 2002. MRID 45902306. Unpublished

SPONSOR: BASF Corporation, P.O. Box 13528, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a rat metabolism study (MRIDs 45902305 and 45902306), [^{14}C]-BAS 670H in 0.5% aqueous Tylose or Cremophor EL/CMC was administered to Wistar rats by gavage. In an initial plasma kinetics studies, a single oral dose of [pyrazole-4-^{14}C]-BAS 670H (Batch # 706-1013; radiochemical purity of >= 98%) was administered to 4 Wistar rats/sex/dose at nominal doses of 10, 100, 200, 400, or 500 mg/kg. In the main mass balance/excretion/metabolism studies, 4 rats/sex/dose were given [pyrazole-4-^{14}C]-BAS 670H as a single oral dose of 10 or 300 mg/kg, a repeated oral dose of 300 mg/kg (14 days unlabeled + 1 day radiolabeled), or 300 mg/kg [phenyl-U-^{14}C]-BAS 670H (Batch #714-1026; radiochemical purity of >= 98%). Tissue distribution time course and biliary excretion studies were also performed. Metabolites were identified and quantified in the urine, feces, bile, kidney, and liver

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in the main studies at 10 and 300 mg/kg. In an additional study, metabolite profiles were determined in urine and feces of rats given a single oral dose of 500 mg/kg.
Absorption of [^{14}C]-BAS 670H following a single oral dose was rapid but limited, with the highest plasma concentrations observed at 1 hour (first time point measured). In the 10 mg/kg group, a second smaller peak in plasma concentration occurred at 8 hours post-dose. Plasma concentrations declined bi-phasically at 10 and 100 mg/kg and tri-phasically at >= 200 mg/kg. The change in AUC was proportional to dose in both sexes at <= 200 mg/kg and overproportional with dose at 400 and 500 mg/kg.

In the main mass balance/excretion studies, 94-103% of the dose was recovered after 168 hours, with <= 0.12% dose remaining in the tissues and <0.1% of the dose in exhaled air. The majority of the dose was recovered within 48 hours in the feces (73-91% dose) and urine (8-29% dose). In a separate experiment, bile was collected for up to 48 hours from rats and accounted for 19-32% dose at 10 mg/kg and 7-9% dose at 300 mg/kg. The pattern of excretion was similar between the sexes and dose groups, although urinary excretion was higher in the low dose groups than in the high dose groups. Urinary excretion was also higher in females, while biliary excretion was higher in males.

At 168 hours post-dose, concentrations of radioactivity remaining in the tissues were generally similar between the sexes and across the dose groups. Concentrations were highest in liver and kidneys of all groups, and in the thyroid of rats from the single 300 mg/kg [^{14}C-pyrazole] dose group. Concentrations in the thyroid were also generally above levels in the blood for the other 300 mg/kg dose groups, but not the 10 mg/kg group. For the two groups dosed at 300 mg/kg with different ^{14}C-labels, concentrations in the adrenal glands, ovaries, uterus, bone marrow, and pancreas of the [^{14}C-phenyl] group were higher than in the [^{14}C-pyrazole] group, suggesting some differential distribution of metabolites. Repeated dosing at 300 mg/kg had no effect on the concentration of radioactivity in the tissues.

Similar findings in the relative distribution of radioactivity in tissues were observed in the time course study. Concentrations in the kidneys and liver were higher than in blood in both sexes at all time points in both the 10 and 300 mg/kg groups. Compared to levels in the blood, radioactivity was also higher in the ovaries and uterus beginning at 1 hour in the 10 mg/kg rats and beginning at 8 hours in the 300 mg/kg rats. In the thyroid, increases over blood levels were observed transiently in the low dose at 8 hours and consistently in the high dose beginning at 2 hours.

Radio-HPLC identified and quantified parent and up to four metabolites (M670H01, M670H02, M670H05, and M670H13) in the urine, feces, bile, liver, and kidney, and the identity of each compound was confirmed by LC/MS, LC/MS/MS, and /or NMR analysis. In the main study groups, parent and identified metabolites in excreta accounted for 91.8-104.5% dose, while unidentified compounds accounted for <1% dose.

In all groups, parent was the predominant compound identified in both urine (4.0-21.3% dose) and feces (66.3-91.7% dose). The primary metabolites in urine were M670H02 (1.0-5.3% dose) and M670H01 (0.2-1.2% dose), along with minor amounts (<0.5% dose) of M670H05 and

M670H13. Metabolites identified in feces included M670H02 (1.3-3.1% dose) and M670H01 (0.6-6.7% dose). In the bile, parent was again the predominant compound identified, accounting for 3.4-13.7% of the dose, along with minor amounts of M670H02 (2.2-12.1% dose), M670H01 (0.2-1.3% dose), and M670H13 (<0.5% dose). In liver and kidneys sampled at T_{max} (1 hour) from both 10 and 300 mg/kg dose groups, the major compound identified was parent, accounting for 48.3-82.5% of the total radioactive residues (TRR), along with M670H02 (14.5-34.6% TRR) and M670H01 (0.9-14.0% TRR).

Regardless of sex, dose level, and the position of the ^{14}C-label, the overall metabolism of [^{14}C]BAS 670H in rats was similar. The ^{14}C-dose was excreted primarily as unchanged parent (82.7-98.3% dose), which was recovered primarily in the feces and to a lesser extent in the urine. Biotransformation of ^{14}C-BAS 670H was limited and primarily involved oxygenation of the isoxazole ring to form M670H02 (1.0-5.3% dose) and subsequent ring opening and loss of the acetic acid moiety to yield M670H01 (0.4-7.9% dose). A minor fraction of parent (<1% dose) was also hydrolyzed at methanone bridge to yield M670H13 and M670H05. The proposed pathway for biotransformation of [^{14}C]BAS 670H in rats is presented in Figure 1.

This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OPP 85-1] in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided.

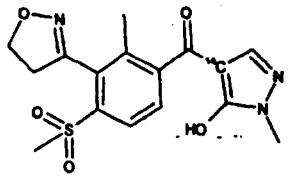
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test compound:

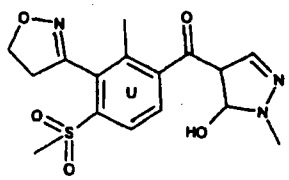
Radiolabeled test material 1: [Pyrazole-4-^{14}C] BAS 670H

Radiochemical purity: >98%
Specific Activity: 5.76 MBq/mg
Batch/Lot No.: 706-1013
Structure:



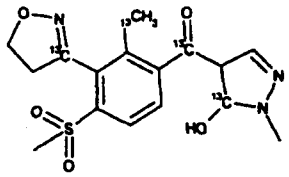
Radiolabeled test material 2: [Phenyl-U-^{14}C] BAS 670H

Radiochemical purity: >98%
Specific Activity: 4.65 MBq/mg
Batch/Lot No.: 714-1026
Structure:



^{13}C-labeled test material: [Isoxazole-3-^{13}C, methanone-^{13}C, 2-methyl-^{13}C, pyrazole-5-^{13}C] BAS 670H

Purity: >94%
Batch/Lot No.: 716-1015
Structure:



Non-radiolabeled test material: BAS 670H

Description: Solid powder
Batch/Lot No.: CP034562; 01311-230; 01586-177
Purity: ≥97.8%
Contaminants: Not provided
CAS # of TGAI: 210631-68-8

2. Vehicle:

0.5% aqueous Tylose (biokinetics study) or
aqueous Cremophor EL and carboxymethylcellulose (metabolism study)

3. Test animals:

Species:	Rat	
Strain:	Wistar Chbb-THOM (SPF)	
Age and weight at dosing:	At least 7-8 weeks 190-280 g	
Source:	Boehringer Ingelheim Pharma KG, Biberach a.d. Riss, Germany	
Housing:	Individually in all-glass Metabowl metabolism cages during mass balance experiments, restriction cages for biliary excretion experiments, or steel wire mesh cages for plasma level and tissue distribution experiments	
Diet:	Granulated Kliba lab diet for rat-mouse-hamster (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	20-27°C
	Humidity:	30-70%
	Air changes:	Not reported
	Photoperiod:	Not reported
Acclimation period:	8 days	

4. Preparation of dosing solutions: In order to achieve the required radioactive dose, the appropriate amounts of unlabeled and radiolabeled test material were mixed together. For the 500 mg/kg dose groups, ^{13}C-labeled test material was also included in the isotope mixture to assist in metabolite identification. The blended test materials were then suspended in 0.5 % Tylose CB 30.000 (biokinetics study) or Cremophor EL and carboxymethylcellulose (metabolite identification study) in water. These suspensions were brought up to the final volume in order to achieve the required concentration. Prior to administration, the preparations were sonicated and stirred to produce homogeneous suspensions. Samples were taken for analyses of the amount of radioactivity and the stability, homogeneity, and concentration of the test substance in the dose suspensions.

It was stated that the analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable. However, data on the homogeneity, stability, and concentration of the dose formulations were not provided. The specific activity of the dose formulations used ranged as follows: (i) 344 to 34,785 dpm/µg in the blood/plasma kinetics experiments; (ii) 1,129-55,245 dpm/µg in the main mass balance/ excretion/metabolism experiments; (iii) 677 to 28,242 dpm/µg in the tissue distribution experiments; (iv) 1,838 to 58,298 dpm/µg in the biliary excretion experiments; and (v) 85 to 628 dpm/µg in the metabolite identification experiments.

B. STUDY DESIGN AND METHODS

1. Group arrangements: In a preliminary study, rats given a single oral dose of 500 mg/kg unlabeled test material showed no clinical signs of toxicity for up to 48 hours. For the blood and plasma kinetics phase of the study (MRID 45902305), animals were assigned to the test groups noted in Table 1a. It was stated that doses of 10, 100, and 500 mg/kg bw were selected based upon previously performed subacute and subchronic toxicity studies in rats; however, no further information was provided. As blood/plasma kinetics (e.g. AUC) were non-linear in initial experiments in this dose range (Groups 1, 2, and 3), blood/plasma kinetics were reexamined at 100, 200, and 400 mg/kg bw (Groups 3A, 3B, and 3C). Based upon the results from these

experiments, doses of 10 and 300 mg/kg were selected for the main mass balance/ excretion/metabolism tests (Groups 4-7), tissue distribution tests (Groups 8 and 9) and bile excretion test (Groups 10 and 11). The first in-life study was started on January 21, 1999, and the final in-life study was terminated on April 17, 2000.

To further characterize metabolism (MRID 45902306; Table 1b), two additional groups of rats (Group DX) were dosed at 500 mg/kg once with [^{14}C-pyrazole]- or [^{14}C-phenyl]-BAS 670H to examine metabolites in excreta, and another two groups (Groups V and W) were dosed at 10 or 300 mg/kg to examine the metabolite profile in plasma, liver, and kidneys.

Table 1a. Dose groups for [^{14}C] BAS 670H rat pharmacokinetics study [a]

Dosing	Group # [b]	Nominal dose (mg/kg)	Mean achieved dose (mg/kg) M/F	# animals/ group	Comments
Blood/Plasma Kinetics					
single oral	1	500	516.9/521.3	4/sex	Blood samples were collected at 1, 2, 4, 8, 24, 48, 72, 96, and 120 hours. Total radioactivity was measured in whole blood and plasma samples.
	2	100	102.6/104.1		
	3	10	10.3/10.1		
	3A	100	103.9/106.2		
	3B	200	207.1/217.2		
	3C	400	413.4/422.0		
Mass Balance/Excretion/Metabolism					
single oral	4 (D)[c]	300	305.7/309.6	4/sex	Excreta were collected after 6, 12, and 24 hours and every 24 hours thereafter up to 168 hours. Urine and feces were used for metabolite identification and quantification. Rats were sacrificed after 168 hours, and total radioactivity was measured in collected organs and tissues.
	5 (B)	10	10.1/10.4		
	6 (D)[c]	300	298.0/302.6		
Repeated dose	7 (C)[d]	300	318.5/295.4		
Tissue Distribution					
single oral	8	300	288.4/282.5	3/sex/time point	Rats were sacrificed at 1, 2, 4, or 12 hours. Total radioactivity was measured in collected organs and tissues.
	9	10	10.1/10.3		Rats were sacrificed at 1, 8, 18, or 22 hours. Total radioactivity was measured in collected organs and tissues.
Biliary Excretion/Metabolism					
single oral	10 (R)	300	304.8/304.2	4/sex	In each rat, the bile duct was cannulated, and bile was collected at 3-hour intervals up to 48 hours. Bile was used for metabolite identification and quantification.
	11 (S)	10	10.3/10.5		

a Data were obtained from pages 23-27, 47-66, 68-71, 79-82, and 88-89 of MRID 45902305. Rats were dosed with [pyrazole-4-^{14}C] BAS 670H in all experiments except Experiment #6, in which rats were dosed with [phenyl-U-^{14}C] BAS 670H.

b Dose group nomenclature from p. 16 of the metabolism study (MRID 45902306) is included in parentheses.

c Exhaled air was collected over 96 hours from 2 males in Experiment 4 (results not reported because of poor recovery of radioactivity) and 2 males in Experiment 6.

d Animals were treated once a day for 14 days with an oral dose of unlabeled BAS 670H followed by a single oral dose of [Pyrazole-4-^{14}C] BAS 670H on Day 15.

Table 1b. Dose groups for additional [^{14}C] BAS 670H rat metabolism studies [a]

Dose group	Nominal dose (mg/kg)	Radioactive label [b]	Mean achieved dose (mg/kg) M/F	# animals/ group	Comment
DX	500	pyrazole	441.0/417.3	10/sex	Urine and feces were collected in 24 hr intervals for up to 4 days for the identification and quantification of metabolites
		phenyl	404.0/425.7	10/sex	
V	10	pyrazole	10.26/10.42	4/sex	Plasma, liver, and kidney were sampled one hour post-dose (presumed peak plasma level) for identification and quantification of metabolites.
		phenyl	10.19/10.34	4/sex	
W	300	pyrazole	312.15/313.15	4/sex	
		phenyl	321.60/318.06	4/sex	

a Data were obtained from pages 20 and 41 of MRID 45902306.
b Rats were dosed with a single oral dose of [pyrazole-4-^{14}C] BAS 670H or [phenyl-U-^{14}C] BAS 670H.

2. **Dosing and sample collection:** Animals received via gavage a single dose of approximately 1 mL radiolabeled dose preparation/100 g bw for the dose groups indicated in Tables 1a and 1b. Actual administered radioactive doses and the number of animals treated in each study are reported in Tables 1a and 1b. It was not stated if the animals were fasted prior to dosing or how the dose actually administered was determined.

a. **Pharmacokinetic studies:** All samples were collected from individual animals. In the blood/plasma kinetics experiments, rats were given a single oral dose of radiolabeled test suspension and placed in steel wire mesh cages. Total radioactivity was determined in whole blood and plasma from blood samples collected from the retroorbital venous plexus of each rat after 1, 2, 4, 8, 24, 48, 72, 96, and 120 hours (Groups 1, 2, 3, 3A, 3B, and 3C).

In the main mass balance/excretion/metabolism experiments, rats were given either a single oral dose of radiolabeled test suspension (Groups 4, 5, and 6) or a single oral dose of radiolabeled test suspension after 14 consecutive days of oral dosing with unlabeled test suspensions (Group 7). Rats were placed in metabolism cages, and urine and feces were collected separately at 6, 12, and 24 hours post-dose and thereafter at 24 hour intervals up to 168 hours. Two male animals from Group 4 were placed in closed metabolism cages in order to collect exhaled air over 96 hours; however, these data are not reported due to insufficient recovery of radioactivity. Exhaled air was collected from 2 males in Group 6 for 96 hours, and recovery of radioactivity was acceptable. Animals were sacrificed at 168 hours post-dose, and the following tissues were collected for radioanalysis:

heart	carcass	adipose tissue
liver	muscle	stomach & stomach contents
spleen	kidney	thyroid gland
bone	testes	adrenal glands
skin	brain	bloodcells and plasma
lung	pancreas	gut and gut contents
ovaries	uterus	bone marrow

In the tissue distribution experiments (Groups 8 and 9), rats were given a single oral dose of radiolabeled test suspension and then placed in steel wire mesh cages. Three rats/sex/time point

were sacrificed at time points, which were selected based on the results from the blood kinetic tests. For the low dose (10 mg/kg bw), the time points corresponded to C_{max} (1 hour) and to approximately ½, $^1/_4$, and $^1/_8C_{max}$ (8, 18, and 22 hours). For the high dose (300 mg/kg bw), the time points corresponded to C_{max} (1 hours), and to ½, $^1/_4$, $^1/_8$ and $^1/_{16}C_{max}$ (2, 4 and 12 hours). After sacrifice, the same organs were collected for radioanalysis as those listed above.

In the biliary excretion experiments, the bile duct of each rat was cannulated. Rats were given a single oral dose of radiolabeled test suspension, and bile was collected at 3-hour intervals up to 48 hours, depending on the health status of the animals and the excretion rate.

All samples (urine, homogenized fecal suspensions, plasma, liver, and kidney) were stored frozen (-18°C) until metabolites could be quantified and identified.

For radioanalysis, liquid samples (plasma, urine, bile, $C0_2$-trap fluid, and cage wash) were mixed with scintillation cocktail and analyzed directly by liquid scintillation counting (LSC). The femur was solubilized in 4 N HCl, and scintillation cocktail was added prior to LSC. Feces, gastrointestinal tract (G.I. tract) and contents, and the carcass were homogenized with water and lyophilized. The resulting solids and the homogenates of the other tissues were solubilized in SOLUENE, bleached using a isopropanol and H_20_2-solution, and left for 24 hours at room temperature. After addition of scintillation cocktail, the samples were analyzed by LSC.

b. **Metabolite characterization:** For determination of metabolites in excreta from the main mass balance/excretion dose groups (Groups 4-7), urine samples from up to 24 hours post-dose were pooled by sex and dose group and analyzed directly by HPLC with radio-detection, using two HPLC systems. Fecal samples from up to 48 hours post-dose were pooled by sex, dose group, and collection interval and were extracted three times with methanol/0.1 M NaOH. Fecal extracts were combined, concentrated, diluted with water and analyzed by HPLC with radio-detection, using two systems. Extractability of radioactivity from the feces (unadjusted for recovery) ranged from 87.2-124.1%. Compounds were identified by comparison with retention times of reference standards.

Bile samples (0-24 hours) from the 300 mg/kg and 10 mg/kg dose groups (Groups 10 and 11) were pooled by sex and dose group and analyzed directly by HPLC with radio-detection.

For more detailed analyses of metabolites in excreta, an additional 10 rats/sex/dose group were given a single oral dose of [pyrazole-4-^{14}C]- or [phenyl-U-^{14}C]-BAS 670H at 500 mg/kg (Group DX) and placed in metabolism cages. Urine and feces were collected from these animals in 24-hour intervals for up to 4 days. Samples of urine and feces were radioassayed as described above for the other dose group. For identification of metabolites in urine from the [^{14}C-pyrazole]-labeled group (0-48 hours) and the [^{14}C-phenyl]-labeled group (0-24 hours), samples were pooled by sex and dose group and analyzed directly by HPLC with radio-detection. Fecal samples (0-48 hours) were pooled by sex, dose group, and interval and were extracted three times with methanol/0.1 M NaOH and once with 0.1 M NaOH. The extracts were analyzed by HPLC with radio-detection, using two systems. Extractability of radioactivity from the feces (unadjusted for recovery) ranged from 89.6-109.2%. Compounds were identified by comparison with retention times of reference standards for quantitation. For confirmation of metabolite identities, parent

and metabolites fractions were isolated from selected urine samples by solvent partitioning and/or preparative HPLC. The identity of each compound was confirmed by LC/MS, LC/MS/MS, and/or ^{1}H-NMR.

For characterization of metabolites in tissues, additional dose groups (4 rats/sex/dose/^{14}C-label) were given a single oral dose of either [pyrazole-4-^{14}C]- or [phenyl-U-^{14}C]-BAS 670H at 10 mg/kg (Group V) and 300 mg/kg (Group W). These animals were sacrificed at T_{max} (1 hour) and samples of plasma, liver, and kidney were collected.

Liver samples from Groups V and W were pooled by sex and dose group, lyophilized, and homogenized. Subsamples were sequentially extracted twice with methanol and twice with water. The methanol extracts extract were combined, concentrated and re-dissolved in methanol. The combined methanol extracts and the first water extract were then analyzed by HPLC with radio-detection. Residual solids were lyophilized and radioassayed by combustion with LSC. Extractability of radioactivity from the liver (unadjusted for recovery) ranged from 89.0-96.2%.

Kidney samples from Groups V and W were pooled by sex and dose group, lyophilized, and homogenized. Subsamples were sequentially extracted twice with methanol and twice with water. Samples from Group V were additionally extracted twice with 0.1 M NaOH. The methanolic extracts were combined, concentrated, re-dissolved in methanol, and analyzed by HPLC with radio-detection. Residual solids were lyophilized and radioassayed by combustion with LSC. Extractability of radioactivity from the kidney (unadjusted for recovery) ranged from 92.6-104.5%.

Plasma samples from Groups V and W were diluted with acetonitrile, stored for 1 hour at 4°C, and centrifuged. The precipitate was rinsed with water:acetonitrile (1:2) and added back to the supernatant. Aliquots of the supernatant were then analyzed for metabolites using HPLC with radio-detection. The precipitates were lyophilized and radioassayed by combustion with LSC. Extractability of radioactivity from the kidney (unadjusted for recovery) ranged from 92.7-98.2%.

3. **Statistics:** Statistical analysis was not performed. Blood/plasma kinetic parameters were calculated with TOPFIT Version 2.0.

II. RESULTS

A. PHARMACOKINETIC STUDIES

1. **Blood/plasma pharmacokinetics:** The highest plasma concentrations (C_{max}) of radioactivity following single oral dose of ^{14}C-test substance were observed at the first time point measured (T_{max} = 1 hour) and ranged from 0.135 µg Eq/g in the 10 mg/kg group to 25.41 µg Eq/g in the 500 mg/kg group (Table 2). In the 10 mg/kg group, a second smaller peak in plasma concentration occurred at 8 hours post-dose in both sexes (0.054-0.080 µg Eq/g). Plasma concentrations declined with time to ≤0.07 µg Eq/g in all groups by 120 hours post-dose. At 10 and 100 mg/kg, plasma concentrations declined biphasically with an initial half life of 4.0-5.8

hours and a terminal half life of 20.7-38.5 hours. At ≥200 mg/kg, plasma concentrations declined tri-phasically with an initial half life of 0.9-13.5 hours and a terminal half life of 25.5-41.1 hours. The total dose absorbed (µg Eq*hour/g) as indicated by the area under the curve (AUC) was proportional to dose in both sexes at ≤200 mg/kg and overproportional with dose at 400 and 500 mg/kg. Based on these findings, a high dose of 300 mg/kg and low dose of 10 mg/kg were selected for the mass balance/excretion/metabolism studies.

Table 2. Pharmacokinetics of radioactivity in plasma after single oral administration of [pyrazole-4-^{14}C] BAS 670H [a]

Dose [mg/kg bw]	Cmax [µg Eq/g]	Tmax [hour]	initial half life [hour]	terminal half life [hour]	AUC [µg Eq*hour/g]
Males					
500	25.41	1	1.4/5.4	40 .2	94.2
400	16.56	1	1.1/9.6	41 .1	58.4
200	3.56	1	1.1/7.5	36 .3	14.0
100 [1st exp.]	2.81	1	4.0	30 .3	9.7
100 [2nd exp.]	1.70	1	5.3	38 .5	8.4
10	0 .179	1	5.3	32 .7	1.3
Females					
500	15.83	1	2.2/5.7	35.4	69.0
400	19.75	1	1.0/10.5	39 .9	55.9
200	6 .93	1	0.9/13.5	25 .5	24 .8
100 [1st exp.]	2 .73	1	5.0	20.7	8.5
100 [2nd exp.]	2 .48	1	5.8	34 .7	12.4
10	0.135	1	5.7	24 .0	1.0

a Data were obtained from Table 2 on page 41 of MRID 45902305.

2. **Absorption and excretion:** Total recovery of the radioactive dose ranged from 94-103% dose at 168 hours post-dose (Table 3). For each dose group, the majority of the dose was recovered within 48 hours in the feces (73-91%) and urine (8-29%). At 168 hours post-dose, fecal excretion accounted for 73-92% in all groups; urinary excretion accounted for 8-29%; and the cage wash accounted for ≤1.12%. The tissues retained ≤0.12% dose. In each group, urinary excretion of radioactivity was higher in females than males. For the 10 mg/kg group, total urinary excretion was 29.2% of the dose for females and 15.7% of the dose for males. For all three 300 mg/kg groups, total urinary excretion was 10.3-16.0% of the dose for females and 7.9-8.9% of the dose for males

Radioactivity present as CO_2 in exhaled air accounted for less than 0.1% of the dose administered to 2 male rats given either a single oral dose of [pyrazole-4-^{14}C] BAS 670H or [phenyl-U-^{14}C] BAS 670H.

Bile was collected for up to 48 hours from rats in a separate experiment. At the 10 mg/kg dose, bile accounted for 31.46% dose in males and 18.98% of the dose in females. At the 300 mg/dose, bile accounted for 9.42% dose in males and 6.69% of the dose in females. Urine and feces were not collected from bile-duct cannulated rats.

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Table 3. Recovery (% of administered dose) of radioactivity in tissues and excreta of rats following treatment with ^{14}C-BAS 670H [a]

Matrix	10 mg/kg Single dose Pyrazole label		300 mg/kg Single dose Pyrazole label		300 mg/kg Single dose Phenyl label		300 mg/kg Repeated dose [b] Pyrazole label	
	Male	Female	Male	Female	Male	Female	Male	Female
Urine, 0-48 h [c]	15.29	28.58	7.79	15.64	8.39	10.13	8.77	13.67
0-168 h	15.68	29.15	7.91	16.04	8.69	10.33	8.89	14.44
Feces, 0-48 h [c]	79.75	72.77	91.45	86.02	89.40	82.95	84.35	85.02
0-168 h	80.16	73.08	91.99	86.71	89.78	85.48	85.30	86.74
Cage wash	0.15	0.30	0.07	0.10	0.29	0.10	0.04	1.12
Total excreted [d]	95.99	102.53	99.97	102.85	98.76	95.91	94.23	102.30
Tissues [c]	0.88	0.88	0.07	0.06	0.07	0.05	0.12	0.07
Total recovery	96.86	103.40	100.03	102.91	98.82	95.96	94.36	102.37

a Data were obtained from Table 3 on page 42 and Table 7 on page 46 of MRID 45902305.
b In order to determine the effect of previous treatment, rats were given a daily oral dose of unlabeled test formulation for 14 days followed by an oral dose of radiolabelled material on Day 15.
c Calculated by the reviewers
d Calculated by the reviewers as the sum of the radioactivity in the urine, feces, and cage wash

3. <u>Tissue distribution</u>: At 168 hours post-dose (Table 4), the concentration of radioactivity was notably higher in the kidneys (0.56-1.69 µg Eq/g) and liver (2.26-4.21 µg Eq/g) than in blood cells and plasma (i.e., blood, ≤0.38 µg Eq/g) in both sexes and in all groups. Other tissues with concentrations of radioactivity notably higher than blood were observed in the: (i) thyroid of both sexes given either the single or repeated 300 mg/kg dose of the ^{14}C-pyrazole-label (0.65-6.43 µg Eq/g) or males dosed once with the ^{14}C-phenyl-label at 300 mg/kg (0.68 µg Eq/g); (ii) adrenal glands in both sexes given the 300 mg/kg repeated dose or a single dose of the 300 mg/kg ^{14}C-phenyl-label (0.32-1.10 µg Eq/g); (iii) uterus, ovaries, bone marrow, and pancreas in both sexes given the ^{14}C-phenyl-label (0.53-1.52 µg Eq/g); and (iv) pancreas in the repeat dose males (0.42 µg Eq/g).

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Table 4. Mean concentrations of radioactivity (µg Eq/g) in tissues of rats at 168 hours after dosing with [^{14}C] BAS 670H.[a]

Matrix	10 mg/kg Single dose Pyrazole label		300 mg/kg Single dose Pyrazole label		300 mg/kg Single dose Phenyl label		300 mg/kg 14 unlabeled + 1 radiolabeled Pyrazole label	
	Male	Female	Male	Female	Male	Female	Male	Female
Blood cells	0.01	0.01	0.38	0.24	0.26	0.33	0.13	0.32
Plasma	0.00	0.00	0.02	0.02	0.06	0.12	0.03	0.03
Kidney	0.56	0.82	0.84	1.11	1.10	1.69	0.78	1.22
Adrenal glands	0.04	0.03	0.45	0.22	1.10	0.47	0.32	0.42
Ovaries	—	0.00	—	0.08	—	0.90	—	0.14
Uterus	—	0.01	—	0.12	—	0.86	—	0.12
Bone marrow	0.01	0.01	0.14	0.19	0.80	1.52	NS	0.26
Thyroid	0.06	0.04	6.43	3.31	0.68	0.25	0.65	1.08
Pancreas	0.01	0.01	0.09	0.10	0.53	0.72	0.42	0.10
Liver	2.49	2.26	3.30	3.12	3.67	4.21	3.57	2.42
Other tissues[b]	≤0.01	≤0.01	≤0.13	≤0.14	≤0.54	≤0.45	≤0.31	≤0.19

a Data were obtained from Tables 4 and 5 on pages 43-44 and Tables 34 and 35 on pages 73-74 of MRID 45902305.
b Other tissues, excluding the stomach, gut, and contents.
— Not applicable
NS Not sampled

The time course of tissue distribution (Table 5) showed that the concentration of radioactivity was higher in the kidneys and liver than in blood in both sexes at all time points in both the low dose group (0.60-3.26 µg Eq/g vs ≤0.33 µg Eq/g in blood) and high dose group (3.26-66.74 µg Eq/g vs ≤17.44 µg Eq/g in blood). In the adrenal glands, transient increases over blood levels were observed in both sexes in the 300 mg/kg rats at 4 hours (5.24-37.01 µg Eq/g vs ≤1.46 µg Eq/g in blood) and in the 10 mg/kg rats at 8 hours (0.13-0.14 µg Eq/g vs ≤0.03 in blood). Radioactivity was higher in the ovaries and uterus beginning at 1 hour in the 10 mg/kg rats (0.12-0.46 µg Eq/g vs ≤0.24 µg Eq/g in blood) and beginning at 2 hours in the 300 mg/kg rats (7.58-70.96 µg Eq/g vs ≤5.49 µg Eq/g in blood). In the thyroid, increases over blood levels were observed transiently in the low dose at 2 hours (0.13-0.29 µg Eq/g vs ≤0.07 µg /Eq/g in blood) and consistently in the high dose beginning at 8 hours (1.62-27.54 µg Eq/g vs ≤17.44 in blood). All other tissues had concentrations of radioactivity that were comparable to or below the concentrations in the blood throughout the time course.

Table 5. Mean concentrations of radioactivity (µg Eq/g) in selected tissues of rats following a single oral dose of [pyrazole-4-^{14}C] BAS 670H at 10 or 300 mg/kg [a]

Matrix	Time after administration [b]							
	1 h		2h/8h		4h/18h		12h/22h	
	Males	Females	Males	Females	Males	Females	Males	Females
300 mg/kg, single dose								
Blood cells	2.94	2.21	15.50	4.96	1.46	1.30	0.62	0.70
Plasma	7.66	4.70	17.44	5.49	2.46	2.10	0.47	0.94
Kidney	39.24	37.39	66.74	38.07	11.48	13.99	3.26	5.64
Adrenal glands	5.17	2.27	13.99	6.78	5.24	37.01	0.74	1.50
Ovaries	—	4.83	—	70.96	—	9.68	—	7.58
Uterus	—	7.68	—	62.91	—	14.31	—	8.94
Thyroid	7.40	7.64	27.54	9.57	8.58	6.78	1.62	4.51
Liver	39.93	17.14	40.25	26.28	10.80	10.19	5.27	6.50
Other tissues [c]	≤6.01	≤3.43	≤21.62	≤19.90	≤2.48	≤5.16	≤17.58	≤6.57
10 mg/kg, single dose								
Blood cells	0.08	0.03	0.00	0.03	0.02	0.03	0.02	0.03
Plasma	0.33	0.24	0.06	0.07	0.01	0.01	0.01	0.01
Kidney	3.26	2.30	0.80	1.32	0.65	0.98	0.60	0.97
Adrenal glands	0.40	0.18	0.13	0.14	0.04	0.04	0.05	0.02
Ovaries	—	0.44	—	0.42	—	0.13	—	0.34
Uterus	—	0.44	—	0.46	—	0.12	—	0.24
Thyroid	0.20	0.23	0.13	0.29	0.05	0.07	0.04	0.06
Liver	2.56	2.21	1.83	2.11	1.50	1.74	1.48	1.91
Other tissues [c]	≤0.43	≤0.34	≤0.39	≤0.68	≤0.08	≤0.11	≤0.08	≤0.07

a Data were obtained from Tables 4 and 5 on pages 43-44 of MRID 45902305.

b Time after administration is 1, 2, 4, and 12 hours for the low dose and 1, 8, 18, and 22 hours for the high dose.

c Other tissues, excluding the stomach, gut, and contents. Skin was also excluded because radioactivity retained in the skin may have been confounded by contamination with excreta.

--- Not applicable

B. METABOLITE CHARACTERIZATION STUDIES: Parent and up to four metabolites (M670H01, M670H02, M670H05, and M670H13) were identified in the urine, feces, bile, liver, and kidney (Tables 6 through 9). Each compound was quantified and initially identified using radio-HPLC, and compound identities were confirmed by mass spectroscopy and/or NMR. Although analyses were also reportedly conducted on plasma, these data were not included in the study.

Parent and identified metabolites accounted for 91.8-104.5% dose in animals receiving a single dose at 10 or 300 mg/kg, or repeated dose (14 days unlabeled + 1 day radio-labeled) at 300 mg/kg (Table 6). Unidentified compounds accounted for <1% dose. The proposed pathway for biotransformation of ^{14}-BAS 670H in rats is presented Figure 1.

In the main mass balance/excretion studies (Groups 4-7, Table 6), parent was the predominant compound identified in both urine (4.0-21.3% dose) and feces (66.3-91.7% dose) in all groups. In the urine from all groups, the primary metabolite was M670H02 (1.0-5.3% dose), followed by M670H01 (0.3-1.2% dose). Two other minor metabolites were detected in the urine at <0.5% dose: M670H05 was only detected in the urine of rats dosed with 300 mg/kg ^{14}C-phenyl-label; and M670H13 was detected in all groups dosed with ^{14}C-pyrazole-label. In the feces, the primary metabolite was M670H01, with 2.0-6.7% dose found in all groups except those dosed with ^{14}C-phenyl-label and the repeat dosed males. A second fecal metabolite, M670H02, was detected

in the males of all groups and in the single high dose females given the ^{14}C-pyrazole-label (1.3-2.7% dose).

Table 6. Metabolite profile in excreta from rats dosed with [^{14}C] BAS670H [a]

Dose Group	Percent of administered dose							
	Single low dose (10 mg/kg) pyrazole label		Single high dose (300 mg/kg) pyrazole label		Single high dose (300 mg/kg) phenyl label		Repeated high dose (300 mg/kg) pyrazole label	
Metabolite/fraction	Male	Female	Male	Female	Male	Female	Male	Female
Identified Urinary Metabolites	**14.43**	**27.35**	**7.28**	**14.91**	**8.16**	**9.81**	**8.25**	**5.40**
M670H13	0.20	0.19	0.25	0.40	—	—	0.28	0.08
M670H05	—	—	—	—	0.19	0.21	—	—
M670H02	5.33	4.64	1.71	2.21	2.56	2.00	0.97	1.04
M670H01	1.05	1.22	0.43	0.62	0.53	0.40	0.35	0.32
BAS670H	7.85	21.30	4.89	11.68	4.88	7.20	6.65	3.96
Identified Fecal Metabolites	**79.40**	**73.04**	**93.12**	**89.58**	**94.41**	**91.09**	**84.29**	**86.37**
M670H02	1.42	—	1.36	1.25	2.70	—	1.54	—
M670H01	3.16	6.71	2.02	3.00	—	—	—	2.29
BAS670H	74.82	66.33	89.74	85.33	91.71	91.09	82.75	84.08
Total identified Metabolites	**93.83**	**100.39**	**100.40**	**104.49**	**102.57**	**100.90**	**92.54**	**91.77**
Unanalyzed Fractions [b]	1.52	2.26	0.95	1.56	2.32	4.71	1.65	13.4
Urine	0.9	1.6	0.34	0.75	0.47	0.52	0.42	10.56
Feces	0.47	0.36	0.54	0.71	1.56	4.09	1.19	1.72
Cage wash	0.15	0.30	0.07	0.10	0.29	0.10	0.04	1.12
Total accounted for [c]	**95.35**	**102.65**	**101.35**	**106.05**	**104.89**	**105.61**	**94.19**	**105.17**

a Data were obtained from Tables 8 through 11 on pages 47-50 of MRID 45902306.
b Unanalyzed fractions were tabulated by the reviewers from Table 3 on page 42 of MRID 45902305.
c Total accounted for = (Total identified) + (Total unanalyzed). Total unknowns in urine + feces were <1% dose.
— Not detected

Findings similar to the main study were observed in groups given a single dose at 500 mg/kg (Table 7). Parent and identified metabolites accounted for 86.6-102.9% dose. Parent was again the predominant compound identified in both urine (4.2-13.6% dose) and feces (71.2-84.3% dose) in both groups. In the urine in all groups at this dose, the primary metabolite was again M670H02 (1.0-2.9% dose), followed by M670H01 (0.2-0.6% dose). Two other minor metabolites were detected in the urine at <0.2% dose: M670H05 was detected in the urine of males and females dosed with ^{14}C-phenyl-label; and M670H13 was detected in the urine of both sexes dosed with ^{14}C-pyrazole-label.

Table 7. Metabolite profile in excreta from rats dosed with 500 mg/kg [^{14}C] BAS670H [a]

Dose Group	Percent of administered dose			
	Single high dose (500 mg/kg) pyrazole label		Single high dose (500 mg/kg) phenyl label	
Metabolite/fraction	Male	Female	Male	Female
Identified Urinary Metabolites	**14.29**	**16.18**	**5.45**	**9.03**
M670H13	0.12	0.11	—	—
M670H05	—	—	0.03	0.03
M670H02	2.87	2.05	1.03	1.03
M670H01	0.57	0.43	0.18	0.19
BAS670H	10.73	13.59	4.21	7.78
Identified Fecal Metabolites	**88.60**	**73.89**	**81.11**	**80.16**
M670H02	3.13	1.89	1.80	2.47
M670H01	1.16	0.76	0.64	1.12
BAS670H	84.31	71.24	78.67	76.57
Total identified Metabolites	**102.89**	**90.07**	**86.56**	**89.19**

a Data were obtained from Tables 6 and 7 on pages 45-46 of MRID 45902306.
— Not detected

In the bile, parent was the predominant compound identified, accounting for 10.6-13.7% dose in the low dose rats and 3.4-3.7% dose in the high dose animals (Table 8). In both sexes at both doses, M670H02 was the primary metabolite found in the bile (2.2-12.1% dose), followed by M670H01 (0.2-1.3% dose). M670H13 was also detected at both doses in both sexes, but accounted for <0.5% dose.

Table 8. Metabolite profile in bile (0-24 hour) from bile duct cannulated rats dosed with [^{14}C] BAS670H [a]

Dose Group	Percent of total radioactivity in bile			
	Single low dose (10 mg/kg) pyrazole label		Single high dose (300 mg/kg) pyrazole label	
Metabolite/fraction	Male	Female	Male	Female
Identified Metabolites	**27.50**	**17.71**	**7.88**	**5.79**
M670H13	0.49	0.09	0.11	0.02
M670H02	12.08	6.48	3.77	2.16
M670H01	1.28	0.58	0.35	0.20
BAS670H	13.65	10.56	3.65	3.41
Total unanalyzed	**3.83**	**1.00**	**1.50**	**0.93**
Total radioactivity	**31.46**	**18.98**	**9.42**	**6.69**

a Data were obtained from Tables 4 and 12 on pages 43 and 51 of MRID 45902306. Note that total unknowns in bile were <0.3% dose in each group and are not reported in this table because one value was negative, as percent dose was not corrected for recovery.

For the groups dosed with either ^{14}C-label and sacrificed at 1 hour post-dose (Groups V and W), liver accounted for 2.48-3.00% of the dose for the 10 mg/kg groups and 0.94-1.24% of the dose for the 300 mg/kg groups (Table 9). Kidneys accounted for 0.56-1.02% of the dose for the 10 mg/kg groups and 0.36-0.43% of the dose for the 300 mg/kg groups. Extraction and analysis of these tissues identified 83.1-91.5% of the total radioactive residues (TRR) in liver and 91.9-104.7% of the TRR in kidneys (values not corrected for recovery).

In the liver, parent was the predominant compound identified in all groups, accounting for 48.3-61.8% of the TRR. The primary metabolite identified in liver was M670H02 (24.7-34.6% TRR) in all groups, and minor amounts of M670H01 (0.9-3.8% TRR) were detected in all groups, except for the low-dose, ^{14}C-phenyl-label females.

A similar metabolite profile was observed in kidneys. The predominant compound identified kidneys in all groups was parent (53.9-82.5% TRR), and M670H02 (14.5-30.4% TRR) was the primary metabolite. However, in kidney, the relative levels of metabolite M670H01 (2.5-14.0% TRR) were higher than in liver.

Table 9. Metabolite profile in liver and kidney from rats dosed with [^{14}C] BAS670H [a]

Dose Group	Single low dose (10 mg/kg) pyrazole label		Single high dose (300 mg/kg) pyrazole label		Single low dose (10 mg/kg) phenyl label		Single high dose (300 mg/kg) phenyl label	
Metabolite/fraction	Male	Female	Male	Female	Male	Female	Male	Female
Liver								
% Dose in tissue	**2.92**	**2.48**	**0.94**	**1.09**	**3.00**	**2.72**	**1.24**	**1.08**
M670H02 (% dose)	1.01	0.70	0.31	0.28	0.74	0.73	0.39	0.32
(% TRR) [b]	**34.6**	**28.2**	**33.0**	**25.7**	**24.7**	**26.8**	**31.5**	**29.6**
M670H01 (% dose)	0.11	0.05	0.03	0.01	0.09	—	0.03	0.02
(% TRR) [b]	**3.8**	**2.0**	**3.2**	**0.9**	**3.0**	—	**2.4**	**1.9**
BAS670H (% dose)	1.41	1.31	0.52	0.64	1.72	1.68	0.69	0.59
(% TRR) [b]	**48.3**	**52.8**	**55.3**	**58.7**	**57.3**	**61.8**	**55.6**	**54.6**
Total Identified (% dose)	2.53	2.06	0.86	0.93	2.55	2.41	1.11	0.93
(% TRR) [b]	**86.6**	**83.1**	**91.5**	**85.3**	**85.0**	**88.6**	**89.5**	**86.11**
Kidney								
% Dose in tissue	**1.02**	**0.62**	**0.36**	**0.40**	**0.74**	**0.56**	**0.43**	**0.40**
M670H02 (% dose)	0.31	0.09	0.09	0.06	0.22	0.11	0.10	0.07
(% TRR) [b]	**30.4**	**14.5**	**25.0**	**15.0**	**29.7**	**19.6**	**23.3**	**17.5**
M670H01 (% dose)	0.14	0.05	0.02	0.02	0.05	0.03	0.02	0.01
(% TRR) [b]	**13.7**	**8.1**	**5.6**	**5.0**	**6.8**	**5.4**	**4.7**	**2.5**
BAS670H (% dose)	0.55	0.46	0.24	0.33	0.41	0.41	0.34	0.30
(% TRR) [b]	**53.9**	**74.2**	**66.7**	**82.5**	**55.4**	**73.2**	**79.1**	**75.0**
Total Identified (% dose)	1.00	0.60	0.35	0.41	0.68	0.55	0.45	0.38
(% TRR) [b]	**98.0**	**96.8**	**97.2**	**102.5**	**91.9**	**98.2**	**104.7**	**95.0**

a Data were obtained from Table 5 on page 44 and Tables 13 and 14 on pages 52-53 of MRID 45902306.
b Percent total radioactive residue (TRR) identified was calculated by the reviewers from data presented in this table.
— Not detected

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III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: After single oral administration, ^{14}C-BAS 670H was rapidly absorbed from the gastrointestinal tract. Absorption was incomplete at both dose levels amounting to 17-22% at the high dose and 46-47% at the low dose, indicating dose-dependent bioavailability in the range of 17-47%. After absorption, radioactive material was distributed in all organs and tissues. Absorbed BAS 670H does not accumulate but gets effectively excreted via the feces (predominantly), urine, and bile. Biliary excretion amounted to about 7-9% of the applied dose at the high dose and to about 19-31% at the low dose level. Data of plasma kinetics showed that there is a breakpoint in toxicokinetic parameters at a dose of 200 mg/kg bw and above which is reflected in altered plasma half-lives and an overproportional increase of AUC with dose. The parent compound was metabolized by oxygenation of the isoxazole ring moiety, degradation of the isoxazole ring moiety, and hydrolysis of the central methanone bridge. The metabolic pathway in rats covers the relevant metabolites identified in goats and hen. The minor metabolites from plants and soil were not identified.

B. REVIEWER COMMENTS: Plasma kinetics indicated rapid absorption following single oral dose of pyrazole-labeled test substance, with the highest plasma concentrations (0.135 µg Eq/g at 10 mg/kg to 25.41 µg Eq/g at 500 mg/kg) observed at 1 hour (first time point measured). No differences were noted between sexes. In the 10 mg/kg group, a second smaller peak in plasma concentration occurred at 8 hours post-dose (0.054-0.080 µg Eq/g). Plasma concentrations declined with time to ≤0.07 µg Eq/g at 120 hours post-dose, with declines being bi-phasic at 10 and 100 mg/kg and tri-phasic at ≥200 mg/kg. The AUC was proportional to dose in both sexes at ≤200 mg/kg and overproportional with dose at 400 and 500 mg/kg.

In the main mass balance/excretion/metabolism studies, total recovery ranged from 94-103% dose after 168 hours, with ≤0.12% dose remaining in the tissues and <0.1% dose recovered in exhaled air. Fecal excretion was the major route of elimination, accounting for 73.1-92.0% of the dose in all groups, and the majority of the dose was recovered within 48 hours in the feces (73-91%) and urine (8-29%). The pattern of excretion was similar between the sexes and dose groups although urinary excretion was higher at the 10 mg/kg dose (15.7-29.2% dose) than the 300 mg/kg dose (7.9-16.0% dose). Within each dose group, levels of urinary excretion were also higher in females (10.3-29.2% dose) than males (7.9-15.7% dose). Repeated dosing and the position of the ^{14}C-label within the molecule had no effect on the pattern of excretion.

In the biliary excretion experiment, bile accounted for 19.0-31.5% dose at 10 mg/kg and decreased to 6.7-9.4% dose at 300 mg/kg indicating a saturation effect on biliary excretion, as absorption did not appear to be saturated up to 500 mg/kg (i.e, overproportional with dose at ≥400 mg/kg). At both dose levels, biliary excretion was higher in males (9.4-31.5% dose) and than females (6.7-19.0% dose). Because urine and feces were not collected from bile-duct cannulated rats, the reviewers were unable to compare the radioactivity excreted in the bile with the fecal and urine contributions in the same animals to determine the component in the feces that was absorbed and excreted via enterohepatic circulation.

At 168 hours post-dose, concentrations of radioactivity remaining in the tissues were generally similar between the sexes and across the dose groups. Concentrations of radioactivity were consistently highest in liver (2.26-4.21 µg Eq/g) and kidneys (0.56-1.69 µg Eq/g) of all groups, although higher levels were observed in the thyroid (3.31-6.43 µg Eq/g) of rats dosed once with the ^{14}C-pyrazole-label at 300 mg/kg. Concentrations in the thyroid were also above levels in the blood (≤0.32 µg Eq/g) for males dosed once with the ^{14}C-phenyl-label at 300 mg/kg (0.68 µg Eq/g) and for both sexes dosed repeatedly at 300 mg/kg rats (0.65-1.08 µg Eq/g). For the rats dosed with ^{14}C-phenyl-label at 300 mg/kg, concentrations in adrenal glands, ovaries, uterus, bone marrow, and pancreas (0.53-1.52 µg Eq/g) were all above the concentrations remaining in blood (≤0.33 µg Eq/g) and were higher than in the same tissues from rats dosed with the ^{14}C-pyrazole-label at 300 mg/kg (0.08-0.45µg Eq/g). These results suggest some differential compartmentation of metabolites derived from the different parts of the molecule. Repeated dosing at 300 mg/kg had no effect on the concentration of radioactivity in the tissues, and concentration of radioactivity in other tissues were generally lower than in blood.

The time course of tissue distribution (1-22 hours) showed the same pattern of ^{14}C-residue distribution as seen in the tissues sampled at 168 hours. Higher concentrations of radioactivity were observed in the kidneys and liver than in blood in both sexes at all time points in both the low dose group (0.60-3.26 µg Eq/g vs ≤0.33 µg Eq/g in blood) and high dose group (3.26-66.74 µg Eq/g vs 0.47-17.44 µg Eq/g in blood). In the adrenal glands, transient increases over blood levels were observed in both sexes in the 300 mg/kg rats at 4 hours (5.24-37.01 µg Eq/g vs ≤1.46 µg Eq/g in blood) and in the 10 mg/kg rats at 8 hours (0.13-0.14 µg Eq/g vs ≤0.03 in blood). Radioactivity was consistently higher in the ovaries and uterus beginning at 1 hour in the 10 mg/kg females (0.12-0.46 µg Eq/g vs ≤0.24 µg Eq/g in blood) and beginning at 8 hours in the 300 mg/kg females (7.58-70.96 µg Eq/g vs 0.70-5.49 µg Eq/g in blood). In the thyroid, increases over blood levels were observed transiently in the low dose at 8 hours (0.13-0.29 µg Eq/g vs ≤0.07 µg /Eq/g in blood) and consistently in the high dose beginning at 2 hours (1.62-27.54 µg Eq/g vs 0.47-17.44 in blood).

Radio-HPLC identified and quantified parent and up to four metabolites (M670H01, M670H02, M670H05, and M670H13) in the urine, feces, bile, liver, and kidney, and the identity of each compound was confirmed by LC/MS, LC/MS/MS, and /or NMR analysis. In the main study groups, parent and identified metabolites in excreta accounted for 91.8-104.5% dose in animals receiving a single dose at 10 or 300 mg/kg or repeated dose at 300 mg/kg, while unidentified compounds accounted for <1% dose.

In all groups (including the main study and the single dose study at 500 mg/kg), parent was the predominant compound identified in both urine (4.0-21.3% dose) and feces (66.3-91.7% dose). In the urine, the primary metabolite was M670H02 (1.0-5.3% dose), followed by M670H01 (0.2-1.2% dose). Two other minor metabolites were detected in the urine at <0.5% dose: M670H05 was only detected in the urine of rats dosed with the ^{14}C-phenyl-label; and M670H13 was detected only in groups dosed with the ^{14}C-pyrazole-label. In the feces from the main study groups, the primary metabolite was M670H01, with 2.0-6.7% dose found in all groups except those dosed with the ^{14}C-phenyl-label and the repeat dosed males. A second fecal metabolite, M670H02, was detected at 1.3-2.7% of the dose in the males of all main study groups and in the single dose 300

mg/kg females given the ^{14}C-pyrazole-label. In feces from the 500 mg/kg dose groups, the primary metabolite was M670H02 (1.8-3.1% dose) followed by M670H01 (0.6-1.2% dose).

In the bile, parent was the predominant compound identified, accounting for 10.6-13.7% dose at 10 mg/kg and 3.4-3.7% dose at 300 mg/kg. M670H02 was the primary metabolite found in the bile (2.2-12.1% dose), followed by M670H01 (0.2-1.3% dose). M670H13 was also detected at 10 and 300 mg/kg in both sexes, but accounted for <0.5% dose.

In liver and kidneys sampled at T_{max} (1 hour) from both 10 and 300 mg/kg dose groups, the major compound identified was parent (48.3-82.5% TRR) followed by the metabolite M670H02 (14.5-34.6% TRR). The metabolite M670H01 was also detected in liver and kidney at 0.9-14.0% of the TRR.

Regardless of sex, dose level, and the position of the ^{14}C-label, the overall metabolism of [^{14}C]BAS 670H in rats was similar. Absorption of the dosed radioactivity was rapid, but limited. The ^{14}C-dose was excreted primarily as unchanged parent (82.7-98.3% dose), which was recovered primarily in the feces and to a lesser extent in the urine. Biotransformation of ^{14}C-BAS 670H was limited and primarily involved oxygenation of the isoxazole ring to form M670H02 (1.0-5.3% dose) and subsequent ring opening and loss of the acetic acid moiety to yield M670H01 (0.4-7.9% dose). A minor fraction of parent (<1% dose) was also hydrolyzed at methanone bridge to yield M670H13 and M670H05. The proposed pathway for biotransformation of [^{14}C]BAS 670H in rats is shown in Figure 1.

This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OPP 85-1] in rats.

C. <u>STUDY DEFICIENCIES</u>: No deficiencies were noted in this rat metabolism study.

Figure 1. The proposed pathways for biotransformation of BAS 670H in rats.