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GUIDELINE SERIES 85-1: Metabolism

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DATA EVALUATION REPORT

STUDY TYPE: Metabolism in rats (Guideline Series 85-1)

EPA IDENTIFICATION NUMBER:

PC Code: 014002

Tox Chem Number: 140

MRID Number: 429064-01

TEST MATERIAL: Hydrogen cyanamide

SYNONYM: None

STRUCTURE:

$$H_2\text{–}N\text{–}C^{*}\equiv N$$

* denotes the position of the ^{14}C label

SPONSOR: SKW Trostberg Aktiengesellschaft, Trostberg, Germany

PERFORMING LABORATORY: Hazleton Wisconsin, Inc., Madison, WI

AUTHOR: Craig B. Struble, Ph.D.

REPORT: Metabolism of [^{14}C]-Hydrogen Cyanamide in Rats (Preliminary and Definitive Phases). Laboratory Project ID# HWI 6265-101.

STUDY COMPLETION DATE: August 19, 1993

CONCLUSIONS: The absorption, distribution, metabolism, and excretion of hydrogen cyanamide were studied in groups of rats administered a single oral gavage dose of 1 or 20 mg/kg ^{14}C-hydrogen cyanamide or administered daily oral doses of 1 mg/kg/day unlabeled hydrogen cyanamide for 14 days followed by a single dose of 1 mg/kg ^{14}C-hydrogen cyanamide on day 15. An additional group received a single intravenous (i.v.) dose of 1 mg/kg ^{14}C-hydrogen cyanamide.

The study demonstrated that ^{14}C-hydrogen cyanamide is rapidly absorbed, distributed, metabolized, and excreted following oral and i.v. administration in rats. Total recoveries of the radioactivity were high for all oral and i.v. groups (98.7-105.0% of administered dose); urine was the major route of excretion (66.9-97.7% of administered dose). The elimination of hydrogen cyanamide was sex-, dose-, and route-related. The recovery of radioactivity in the feces was higher in the i.v. group (13.2-15.0% of administered dose) compared to oral groups (2.76-4.15% of administered dose), suggesting that biliary excretion is an important route following i.v. administration. Recoveries in expired $^{14}CO_2$ were higher in the males than in the females and were higher in the i.v. and single low-dose oral groups compared to the repeated-dose and high-dose groups. These findings suggest that there is a depletion of metabolic enzymes at the high-dose group, and therefore, only a limited amount of the hydrogen cyanamide is metabolized to CO_2. Tissue bioaccumulation of hydrogen cyanamide was low for all groups. Most of the ^{14}C-hydrogen cyanamide is *N*-acetylated to form *N*-acetylcyanamide, which is eliminated in the urine and feces.

CORE CLASSIFICATION: Minimum. This study satisfies the minimum requirements set forth under Guideline Series 85-1 for metabolism studies.

A. MATERIALS

1. Test Substance

The nonradiolabeled test material (lot number 111701) had a purity of 99.7% and specific activity of 14.8 mCi/mmol. The ^{14}C-radiolabeled test material (lot number 061H9212) had a radiochemical purity of >98% (determination by thin-layer chromatography [TLC]).

2. Test Animals

Male and female HSD:SD rats (5/sex/group) were obtained from Harlan Sprague Dawley, Inc., Madison, WI. The animals weighed 133-313 g. A single oral gavage dose of 1 mg/kg (low-dose group) or 20 mg/kg (high-dose group) ^{14}C-hydrogen cyanamide was administered to rats. Another group received daily oral gavage doses of 1 mg/kg unlabeled hydrogen cyanamide for 14 days followed by a single oral dose of 1 mg/kg ^{14}C-hydrogen cyanamide on day 15 (repeated-dose group). The fourth group received a single i.v. dose of 1 mg/kg ^{14}C-hydrogen cyanamide (i.v. group).

In a preliminary study, 4 male rats received a single oral dose of 20 mg/kg ^{14}C-hydrogen cyanamide, and urine, feces, and expired air were collected. Bile was also collected from two of these animals. Two additional male rats received an i.v. dose of 1 mg/kg unlabeled hydrogen cyanamide to determine if the dose would be tolerated by the test animals. The basis for the dose selection was not reported.

B. METHODS

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1. Acclimation

Rats were acclimated to the laboratory environment for at least 1 week prior to study initiation. Animals were individually housed in stainless steel cages and transferred to metabolism cages prior to dosing.

Animals were provided Purina Certified Rodent Lab Chow #5002 (St. Louis, MO) *ad libitum* throughout the study, except overnight prior to radioactive dosing until 4 hours after dosing. Water was given *ad libitum*. There were no known contaminants in the food or water that would interfere with the study.

In the preliminary study, two of four male rats receiving oral dosing were acclimated to restraining cages 3 hours prior to placement of bile duct cannula. These animals were fasted overnight prior to surgery.

2. Dosing Solutions

The oral radiolabeled dose solutions of hydrogen cyanamide were prepared by mixing nonradiolabeled hydrogen cyanamide and ^{14}C-hydrogen cyanamide in ammonium phosphate buffer. The intravenous radiolabeled dose solution was prepared in sodium chloride and administered into the tail vein of the rats. The radiolabeled dose solutions were prepared up to 2 days before dosing while the nonradiolabeled solution (for the repeated-dose group) was prepared up to 7 days before dosing. Dose solutions that were refrigerated were stable for at least 7 days.

3. Sample Collection

Urine and feces were collected at 12 and 24 hours postdosing and daily thereafter for 7 days postdosing. Cage wash and wipes were collected at 7 days postdosing. After animals were sacrificed at 7 days postdosing, tissues (femur, blood, brain, fat, ovaries, testes, heart, thyroid/parathyroid, liver, kidneys, lungs, muscle, spleen, uterus, and residual carcass) were collected for radioanalysis. Expired $^{14}CO_2$ was trapped in 2-ethoxyethanol:ethanolamine (1:1), and expired organic volatiles were trapped in activated charcoal at 12 and 24 hours postdosing and daily thereafter for 7 days postdosing.

The radioactivity of the collected samples was quantitated by liquid scintillation counting using a Model 4640 or Model 1500 liquid scintillation counter (Packard Instrument Co.), in duplicate. Samples of feces, blood, CO_2, cage wash, and some tissues were homogenized prior to radioanalysis. The expired volatiles were combusted and oxidized prior to radioanalysis.

In the preliminary experiment, urine, feces, and $^{14}CO_2$ were collected from the orally dosed animals for 4 days postdosing. Bile was

collected for 4 days postdosing in rats implanted with bile-duct cannulae. Radioanalysis was not performed on the i.v.-dosed animals.

4. Metabolite Analysis

Urine samples from selected animals of each group and each collection interval (up to 48 hours) were analyzed by TLC for characterization and quantitation of urinary ^{14}C-labeled metabolites. Urine samples (pooled by sex and by dose groups) were also prepared; 0-48-hour samples were pooled for the 1-mg/kg groups (oral and i.v.) and 0-24-hour samples were pooled for the 20-mg/kg group. The pooled samples were analyzed by TLC.

To isolate the ^{14}C-labeled urinary metabolites, urine samples were applied to a C18 solid phase extraction (SPE) cartridge using water and methanol. The eluted radioactivity was chromatographed by preparative TLC, and the isolated radioactive band from TLC was extracted with chloroform:ethanol.

Four radioactive bands were detected, isolated for extraction with methylene chloride, and analyzed by electron impact mass spectrometry. The major urinary metabolite, *N*-acetylcyanamide (UM-2), was isolated from the urine of 20-mg/kg males and then derivatized with *p*-nitrobenzyl bromide for analysis by TLC. A minor polar urinary metabolite (UM-1) was isolated from the 0-24-hour urine of 20-mg/kg males by preparative TLC.

Fecal samples from the i.v.-dosed animals were pooled by sex. The pooled samples contained 63.7% and 72.7% of the total radioactivity excreted in feces of males and females, respectively. Samples were extracted with methanol and analyzed by TLC, or eluted through C18 SPE cartridges and then analyzed by HPLC.

Statistical analyses were limited to means and standard deviations.

5. Regulatory Compliance

The quality assurance statement and the statement of compliance with Good Laboratory Practices for the study were signed on August 19 and August 28, 1993, respectively.

C. REPORTED RESULTS

1. Preliminary Study

The 4-day postdosing recovery was 91.0-97.1% of the administered dose in male rats orally dosed with 20 mg/kg ^{14}C-hydrogen cyanamide. Most of the administered dose was recovered in the urine (92.6% of administered dose) at 4 days postdosing. Radioactivity in the expired air was a minor route of elimination; 2.04% of the administered dose was detected in $^{14}CO_2$ and <0.01% in organic volatiles. In the bile-cannulated male rats, recovery was 86% and 2.57% of the administered dose in the urine and bile, respectively. The feces contained about 2.45% of the administered dose. Bile was

not collected in the definitive study since this was considered a minor route of hydrogen cyanamide elimination.

The i.v. dose was well tolerated, and therefore, 1 mg/kg was the i.v. dose level for the definitive study.

2. Definitive Study/Elimination and Recovery

No signs of toxicity were observed in the dosed animals.

Total recoveries were 98.7-105.0% of the administered dose for all oral dose groups at 7 days postdosing (Table 1); most of the radioactivity was recovered within the first 24 hours after dosing. The highest recoveries were in the urine (79.0%, 82.6%, and 95.3% of the administered dose in males of the single low-dose, repeated low-dose, and high-dose groups, respectively; 86.2%, 90.7%, and 97.7% in females of the same respective groups). Recoveries in the feces were highest in the low-dose groups (4.15%, 4.14%, and 2.76% of the administered dose in males of the single low-dose, repeated low-dose, and high-dose groups, respectively; 4.07%, 2.86%, and 3.26% in females of the same respective groups). Most of the radioactivity expired as CO_2 was eliminated by 12 hours postdosing. Recovery of expired $^{14}CO_2$ was slightly higher in males than females at 7 days postdosing; 10.00%, 7.08%, and 2.31% of the administered dose in males of the single low-dose, repeated low-dose, and high-dose groups, respectively, compared to 5.77%, 3.78%, and 1.45% in females, respectively. Radioactivity eliminated in expired organic volatiles was negligible at 7 days postdosing (<0.01% of the administered dose from all dose groups).

In the i.v. group, recoveries in the urine, feces, and expired $^{14}CO_2$ were 66.9%, 15.0%, and 10.5% of the administered dose, respectively, in males and 77.6%, 13.2%, and 5.47%, respectively, in females at 7 days postdosing (Table 1). Total recovery of the administered i.v. dose was 98.7-101.0%.

There was a low amount of radioactivity in the tissues (0.45-2.31% of administered dose) and carcass (2.26-4.36% of administered dose) for oral and i.v. groups at 7 days postdosing. No tissue contained ≥1.2% of the administered dose; the highest radioactivity was found in the liver (0.027-1.18%). The blood contained 0.051-0.257% of the administered dose.

3. Metabolism

TLC profiles of the pooled and individual urine samples indicated that the metabolite pattern in the urine was similar by sex and by dosing groups. Four radioactive bands (UM-0, UM-1, UM-2, UM-3) were isolated from urine samples. The major radioactive band, UM-2 (≈32-63% of administered dose in the 0-48-hour or 0-24-hour samples), was identified as *N*-acetylcyanamide by TLC and confirmed by derivatization, isotope dilution, and mass spectral analysis.

A minor band (UM-1; 4-14% of administered dose for the 0-48-hour or 0-24-hour samples) was characterized. TLC was used to isolate UM-1 from the 0-24-hour pooled urine samples from 20-mg/kg males. The eluted radioactive component was chromatographed two more times, and three radioactive bands were detected. Two of the bands (Rf0.77 and UM-1C) were further analyzed. Rf0.77 contained two radioactive bands while UM-1C contained five bands. None of these radioactive bands accounted for >4.8% of the radioactivity in the pooled urine sample. The study author concluded that UM-1 was formed as a result of the decomposition of *N*-acetylcyanamide, and therefore, they did not attempt to perform further analyses. It was noted that the relative amount of UM-1 increased with storage time (from 5.2% to 14.1% of administered dose from the 20-mg/kg male urine samples), while the amount of metabolite UM-2 decreased. UM-0 and UM-3 in pooled urine samples were not characterized because of the low amount in the samples (<10% of the administered dose).

In the pooled fecal samples, <10% of the administered dose was recovered from the oral dose groups; therefore, no attempt was made to analyze these samples for metabolites. Metabolite analysis was conducted on pooled fecal samples of the i.v. group (≈9.5% of administered dose). The major radioactive band in the fecal samples (nearly 8% of administered dose) was identified as *N*-acetylcyanamide by TLC and confirmed by HPLC. No radioactive band was detectable for the parent compound in the urine or feces.

The study author concluded that the metabolic pathway for ^{14}C-hydrogen cyanamide in rats involves acetylation of the nitrogen to form *N*-acetylcyanamide (58-74% of the radioactivity in pooled urine samples and over 80% in pooled fecal samples).

D. CONCLUSIONS BASED ON REVIEWERS' DISCUSSION AND INTERPRETATION OF DATA

The study demonstrated that ^{14}C-hydrogen cyanamide is rapidly absorbed, distributed, metabolized, and excreted (primarily in the urine) following oral and i.v. administration in rats. Total recoveries of the radioactivity were high for all groups (98.7-105.0% of the administered dose) after 7 days postdosing. Oral absorption was almost complete as indicated by high recoveries in the urine of the oral dose groups and in the urine and feces of the i.v. dose group. The urine was the major route of excretion (79.0-97.7% of administered dose in oral dosing groups and 66.9-77.6% in the i.v groups). The recovery of radioactivity in the feces was higher in the i.v. group (13.2-15.0% of administered dose) compared to oral groups (2.76-4.15% of administered dose), suggesting that biliary excretion is an important route following i.v. administration. However, there were large standard deviations in the radioactivity recovery values for the feces and urine of the i.v. group. The slightly greater urinary recovery of radioactivity in the oral high-dose groups compared to the oral low-dose and repeated-dose groups suggests a slight dose-related difference in elimination of ^{14}C-hydrogen cyanamide.

The recovery of expired $^{14}CO_2$ was dose- and sex-related; recoveries were higher in the males (2.31-10.5% of administered dose) than in the females (1.45-5.77% of administered dose) and were higher in the i.v. and single low-dose oral groups (5.47-10.5% of administered dose) than in the repeated-dose group (3.78-7.08% of administered dose) and the high-dose group (1.45-2.31% of administered dose) at 7 days postdosing. These findings suggest that there is a depletion of metabolic enzymes at the high-dose group, and therefore, only a limited amount of the hydrogen cyanamide is metabolized to CO_2. Tissue bioaccumulation of hydrogen cyanamide was low for all groups since the administered radioactivity was <1.2% or not detectable in tissues at 7 days postdosing. Hydrogen cyanamide is metabolized to *N*-acetylcyanamide, which is eliminated in the urine and feces. $^{14}CO_2$ in the expired air is probably a minor byproduct of hydrogen cyanamide metabolism or degradation.

TABLE 1. Recovery of Radioactivity at 7 Days Postdosing in Rats Administered ^{14}C-Hydrogen Cyanamide[a]

Dose Group	Sex	Percentage of Radioactive Dose[b]						
		Feces	Urine[c]	Carcass	Tissues	CO_2	Volatiles	Recovery
1 mg/kg (low dose)	M	4.15±0.968	79.0±2.54	4.36±0.482	2.31±0.198	10.0±0.85	<0.01	99.9±0.98
	F	4.07±0.648	86.2±1.57	3.30±0.373	1.37±0.163	5.77±0.851	<0.01	101±0.5
1 mg/kg (repeated dose)[d]	M	4.14±1.134	82.6±2.93	4.00±0.261	1.53±0.282	7.08±0.548	<0.01	99.3±3.94
	F	2.86±0.503	90.7±1.14	2.90±0.167	1.00±0.149	3.78±0.624	<0.01	101±0.4
20 mg/kg (high dose)	M	2.76±0.608	95.3±2.03	2.26±0.179	0.85±0.031	2.31±0.558	<0.01	103±1.5
	F	3.26±1.352	97.7±6.02	2.40±0.414	0.45±0.095	1.45±0.607	<0.01	105±4.8
1 mg/kg (i.v. dose)	M	15.0±8.06	66.9±8.74	4.31±0.278	1.89±0.238	10.5±0.49	<0.01	98.7±1.10
	F	13.2±9.54	77.6±10.81	3.24±0.390	1.23±0.162	5.47±0.604	<0.01	101±1.2

[a]Data were extracted from Table 8, p. 47
[b]Means of 5 animals/sex/group.
[c]Includes cage wash and cage wipe.
[d]Rats administered a single oral dose of 1 mg/kg ^{14}C-hydrogen cyanamide (1 mg/kg) after 14 daily oral doses of unlabeled 1 mg/kg hydrogen cyanamide.

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DER #17

Hydrogen Cyanamide: Metabolism in Rats
Sponsor Name: SKW Trostberg, AG, Trostberg, Germany. Year of Study - 1993.
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FINAL

DATA EVALUATION REPORT

HYDROGEN CYANAMIDE

Study Type: Metabolism

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