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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

**SUBJECT:** Review of a Rat Metabolism Study Conducted With Naptalam, Sodium salt.

**EPA I.D. Numbers:** P.C. Code: 030703
Submission: S499879
Reregistration Case # 0183
DP Barcode: D222683
MRID # 438818-01

**TO:** Linda Propst / Susan Cerelli
Product Manager # 73
Special Review and Reregistration Division (7508W)

**FROM:** Timothy F. McMahon, Ph.D. 7/2/96
Pharmacologist, Review Section I
Toxicology Branch II, Health Effects Division (7509C)

**THRU:** Yiannakis M. Ioannou, Ph.D. Y.M. Ioannou 7/3/96
Section Head, Review Section I
Toxicology Branch II, Health Effects Division (7509C)

and

Stephanie R. Irene, Ph.D. Y.M. Ioannou for 7/3/96
Acting Chief, Toxicology Branch II
Health Effects Division (7509C)

**Registrant:** Uniroyal Chemical Co, Inc.

**Action Requested:** Review of a rat metabolism study conducted with technical grade Naptalam.

**Recommendations:** Toxicology Branch II has reviewed a rat metabolism study with naptalam conducted for the purpose of satisfying data gaps from review of an earlier metabolism study (MRID # 41860003). The present study is

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considered unacceptable, and the §85-1 data requirement is still considered a data gap, based on the lack of identification of fecal metabolites from the previous study. The requirement for a repeated oral dose metabolism experiment is waived, based on the lack of specific toxcity concerns for naptalam.

## Executive Summary


The present study (MRID # 438818-01) was conducted to determine the absorption, distribution, and elimination of radiolabeled naptalam in male and female Sprague-Dawley rats following intravenous administration of a 5 mg/kg radiolabeled dose. Urinary and fecal residues comprising more than 5% of the administered dose were subjected to procedures to characterize and identify these residues. For male rats, the excretion of naptalam derived radioactivity was approximately equivalent in urine and feces (46.9% and 48.8% of the dose, respectively), but in female rats, the excretion was predominantly in urine (85.4% of the dose in urine, 13.8% of the dose in feces).

In female rats, 4 hour urinary excretion showed 70.6% of the dose eliminated compared to 29.5% in male urine. Fecal excretion for 12-24 hours in male rats showed approximately 45% of the dose eliminated during this time compared to 12% in female rats. Based on these data and the lack of a sex-related difference in elimination after oral dosing (MRID # 41860003), the enterohepatic cycling which appears to occur also appears more significant to male rats. The major metabolite identified in urine of male and female rats was unchanged naptalam. Female rats urine was observed with a greater percentage of this metabolite (68.7%) than male rat urine (36.4%). In feces of male rats, the major product of naptalam biotransformation was identified as 5-hydroxy naptalam, constituting approximately 8% of the dose (this metabolite constituted only 1.74% of the dose in female rat feces). In female rats, naptalam was again the major compound observed, constituting 2.66% of the administered dose. Overall, this study addresses the deficiencies pointed out from review of the earlier metabolism study on naptalam, but this study does not address the repeated dosing experiment nor were fecal metabolites from the previous metabolism study identified.

The present study is classified as **unacceptable**, and the §85-1 data requirement is still considered a data gap, based on the lack of identification of fecal metabolites from the oral dosing metabolism study.

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Reviewed by: Timothy F. McMahon, Ph.D. 7/2/96
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 7/3/96
Section I, Toxicology Branch II (7509C)

## Data Evaluation Record

Study type: Metabolism (§85-1) P.C. Code.: 030703

EPA identification numbers: EPA MRID number: 438818-01
DP Barcode: D222683
Submission: S499879

Laboratory Project numbers: Uniroyal Project No. 9534; Corning Hazleton Project No. CHW 6111-151.

Test materials: Sodium Naptalam; [14-C] Sodium Naptalam

Chemical name: 2-[(1-naphthylenylamino)carbonyl] benzoic acid, sodium salt.

Testing Facilities: Uniroyal Chemical Company, Inc., Middlebury, CT and Corning Hazleton, Inc., Madison, Wisconsin

Sponsor: Uniroyal Chemical Company, Inc.

Title of report: Disposition of Naptalam Following Intravenous Administration to Rats

Author(s): M.H. Gay

Study Date: December 18, 1995

**Executive Summary:**

The present study (MRID # 438818-01) was conducted to determine the absorption, distribution, and elimination of radiolabeled naptalam in male and female Sprague-Dawley rats following intravenous administration of a 5 mg/kg radiolabeled dose. Urinary and fecal residues comprising more than 5% of the administered dose were subjected to procedures to characterize and identify these residues. For male rats, the excretion of naptalam derived radioactivity was approximately equivalent in urine and feces (46.9% and 48.8% of the dose, respectively), but in female rats, the excretion was predominantly in urine (85.4% of the dose in urine, 13.8% of the dose in feces).

In female rats, 4 hour urinary excretion showed 70.6% of the dose eliminated compared to 29.5% in male urine. Fecal excretion for 12-24 hours in male rats showed approximately 45% of the dose eliminated during this time compared to 12% in female rats. Based on these data and the lack of a sex-related difference in elimination after oral dosing (MRID # 41860003), the enterohepatic cycling which appears to occur also appears more significant to male rats. The major metabolite identified in urine of male and female rats was unchanged naptalam. Female rats urine was observed with a greater percentage of this metabolite (68.7%) than male rat urine (36.4%). In feces of male rats, the major product of naptalam biotransformation was identified as 5-hydroxy naptalam, constituting approximately 8% of the dose (this metabolite constituted only 1.74% of the dose in female rat feces). In female rat feces, naptalam was again the major compound observed, constituting 2.66% of the administered dose. Overall, this study addresses the deficiencies pointed out from review of the earlier metabolism study on naptalam, but this study does not address the repeated dosing experiment nor were fecal metabolites from the previous metabolism study identified. The requirement for a repeated dosing experiment is waived, based on the lack of specific toxicity concerns for naptalam. However, the present study is classified as **unacceptable**, and the §85-1 data requirement is still considered a data gap, based on the lack of identification of fecal metabolites from the oral dosing metabolism study.

Compliance: Signed and dated statements of Compliance with Good Laboratory Practice Standards, No Data Confidentiality, and Quality Assurance were provided.

# I. MATERIALS

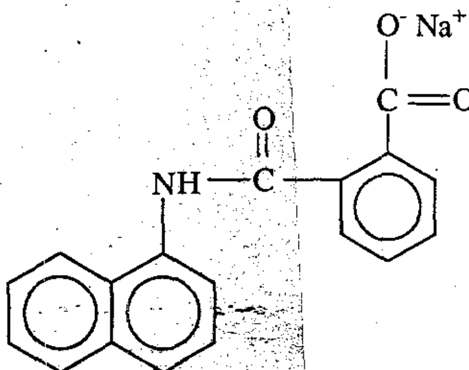
## A. Test Materials

[1]: $^{14}C$-Naptalam, radiochemical purity > 95%.

[2]: Unlabelled Naptalam. Lot number DIR 1471-136. Purity: 98.2%
Storage: frozen.

[3]: Reference standards, including: phthalic acid, lot # AC1304-147, 100% purity; naptalam imide, lot # DIR1471-140, 99.9% purity; naphthylamine, lot # AC1442-39, 99.9% purity; N-acetyl-1-naphthylamine, lot # AC1251-1B; 5-hydroxy-1,4-naphthoquinone, lot # AC1251-2J; and 5-hydroxy-naphthylamine, lot #P4951.

Structure of Naptalam:




Note: Radiolabel was stated as at position 1 of the naphthyl ring.

## B. Vehicles:

dose solution formulated as a water solution for intravenous administration.

C. Test Animals: Species: rat, male and female
Strain: Sprague-Dawley
Source: Charles River Laboratories (Portage, WI).
Housing: The report stated that rats would be housed in a facility where "temperature, humidity , and ventilation are controlled," although the specific conditions were not mentioned. On page 18 of the report, it was stated that "during acclimation and study, animals were housed in plastic metabolism cages which permitted collection and immediate freezing of urine and feces." However, on page 151 of the report, it was stated that animals were transferred to individual metabolism cages at 24 to 48 hours prior to dosing with naptalam. Prior to this, rats were housed individually in stainless steel or polycarbonate cages for a one week quarantine period. During quarantine, each rat was examined by a veterinarian to verify general health and appropriateness for the study. Temperature was set to maintain between 19-25 $^{O}C$ and relative humidity at 50±20%, with a 12 hour light/dark cycle.

Food: Stated on page 151 that food will consist of "certified rodent chow in meal form." Available *ad libitum.*
Water: available *ad libitum.*

## II. METHODS

### A. Study Design

Five male and 5 female rats were originally assigned to this study. . Rats were stated as young adult, with initial weights of 165-179 grams for males and 144-161 grams for females. According to data presented on page 130, data for collection of radioactivity in female rats used only 4 females, as there was a "sample collection problem" with one of the female rats.

The study design was simple: Rats received formulated 14-C naptalam by the intravenous route at a dose of 5.0 mg/kg and a mean dose volume of 0.954 ml/kg. Animals received test chemical through the lateral tail vein using a 5/8 inch, 25-gauge needle. Doses were based on individual body weight. Actual dose was determined by weighing the syringe before and after dosing. Duplicate pre-dose and post-dose aliquots were taken for determination of dose and homogeneity. Urine and feces were collected 48 and 24 hours prior to dosing, and again at 4, 8, 12, 24, 32, and 48 hours post-dose. Daily collection of urine and feces was made after 48 hours up to 7 days post-dose, at which time rats were humanely sacrificed and a gross necropsy conducted. Collection for expired volatiles and carbon dioxide was not performed, as a pilot study (MRID # 40274502) indicated no expiration of carbon dioxide.

C. Experimental

1) Animal Husbandry

Animal husbandry was described above.

2) Dosing

As stated, rats received an intravenous dose via the tail vein of 5 mg/kg. Analysis of the dosing solution was presented on page 182 of the report. This analysis presented only the radioactivity present in the dosing solution, which was stated as 93, 040,000 DPM/g, or 41.91 µCi/g. The radioactivity of the solution was apparently stable, as pre-dose and post-dose analyses showed no significant loss of radioactivity from the solution. On page 185, the actual doses received ranged from 4.93-5.05 mg/kg in males, and 4.94-5.11 mg/kg in females.

3) Sample Collection and Analysis

Following the injection, the site was wiped with gauze sponge to collect any test material that may have leaked from the puncture. Urine and feces were collected in plastic containers surrounded by dry ice from all rats 48 and 24 hours preceeding treatment, at 4, 8, 12, 24, 32, and 48 hours spot-treatment, and daily thereafter through 7 days post-dose. Immediately prior to sacrifice, rats were made to urinate and this sample was combined with the 168-hour sample. At sacrifice, cages were rinsed with 1% aqueous trisodium phosphate and then wiped with gauze pads moistened with 1% aqueous trisodium phosphate. All samples were stored at greater than -10 $^{0}$C before and after analysis.

Urine samples were homogenized, and aliquots analyzed for radioactivity by LSC. Feces were homogenized ib 2-3x their weight in deionized water, and duplicate aliquots combusted in a sample oxidizer and analyzed by LSC. Small tissue samples (heart and spleen) were minced, split in to two weighed aliquots, combusted, and analyzed by LSC. Ovaries were combusted whole and analyzed by LSC. Remaining tissues (brain, kidneys, liver, lungs, muscle, and testes) were homogenized with a probe-type homogenizer, and duplicate aliquots weighed for combustion followed by LSC. Bone samples were cut in to pieces, and split in to 4 weighed aliquots. Aliquots were combusted and then counted by LSC. Residual carcass was cut into small pieces which were frozen in liquid nitrogen. Pieces were placed in a Wiley Mill, pre-cooled with dry ice, then ground with more dry ice. When the dry ice had sublimed (in the freezer), duplicate samples were oxidized and then counted by LSC.

Metabolite identification in urine and feces was accomplished by the use of HPLC. Effluent from the HPLC was split to a radioactivity monitor and a mass spectrometer such that the time at which radioactive metabolites were introduced into the mass spectrometer could be determined accurately.

4) Statistics

According to the report, all data manipulation, rounding, and calculations were performed using Lotus 123 Release 2.3. Statistical analysis in this study was limited to calculations of the mean and standard deviation.

III. RESULTS

A. Pharmacokinetic Studies

1) In-Life Observations

According to the report (pages 27 and 122), there were no unusual observations noted throughout the quarantine, acclimation, and treatment periods. Rats were considered normal, and tissues harvested at necropsy appeared normal. All rats gained weight between the dosing and termination periods.

2) Disposition of Radioactivity

A summary of the routes of elimination for naptalam derived radioactivity was presented on page 41 of the report, and is reproduced below.

**Dispostion of Intravenously Administered 14-C Naptalam in Male and Female Rats at 168 Hours Post-Dose[a]**
(% Administered Dose)

| Matrix | Males | Females |
|---|---|---|
| Urine | 46.9 ± 7.33 | 85.4 ± 2.69 |
| Feces | 48.8 ± 6.11 | 13.8 ± 1.39 |
| Cage Wash | 0.39 ± 0.21 | 0.54 ± 0.60 |
| Cage Wipe | 0.11 ± 0.06 | 0.24 ± 0.15 |
| Total Tissues | 0.26 ± 0.03 | 0.28 ± 0.24 |
| Total Recovery | 96.4 ± 1.84 | 100 ± 0.52 |

[a]data from page 41 of the report.

As shown, recovery of administered radioactivity was essentially quantitative in both male and female rats. However, a significant difference was observed in the percentage of an administered dose of naptalam derived radioactivity excreted in urine and feces of male vs female rats. Radioactivity was excreted largely in the urine of female rats, while in males, a significant percentage was excreted in the feces. As there is no absorption phase to consider in this study, sex-related differences in metabolic rate, elimination, or enterohepatic cycling of radioactivity

emerge as possibilities for the sex-related difference in elimination of radioactivity. The contribution of gut microflora cannot be ruled out either. The time course of elimination of radioactivity in urine and feces (pages 42 and 43 of the report) suggest that there is more naptalam derived radioactivity available for urinary excretion at 4 hours post-dose in female rats (70.6%) as compared to males (29.5%). By 24 hours post-dose, feces of male rats are detected with 38.8% of the administered dose, while only 7.4% of the administered dose is dectected in female rats. The nature of the radioactivty which is unavailable for urinary excretion in male rats is radioactivity which is obviously contained within another compartment of the male vs female rat. This could be in the form of metabolite differences, differences in biliary excretion and/or gut microfloral metabolism, or a combination of both.

3) Tissue Residues

Data on the distribution of naptalam derived radioactivity at 168 hours post-dose in male and female rat tissues was presented for the individual animals on pages 135-138 of the report. Data of this nature are usually limited in their interpretation, especially in light of the relatively rapid elimination of radioactvity. Under the revised Subdivision F §85-1 guideline, data of this type are no longer required unless requested by the Agency. The data show that as a percentage of the administered dose, extremely small percentages ($\leq$ 0.005) of radioactivity were present in most of the tissues of male and female rats. Te highest percentages were observed in the liver, kidneys, and residual carcass of both sexes. Of interest in light of the sex-related difference in disposition is the higher percentage of radioactivity observed in the liver of male rats (0.07%) vs female rats ( 0.02%). On a µg/g tissue basis, male rats also showed a higher amount of residual radioactivity (0.065 µg/g) than females (0.028 µg/g). However, this result is not corroborated by the result obtained in the kidney, in which male rats also showed a higher level of radioactivity (0.090 µg/g) than females (0.045 µg/g).

B. Metabolite Characterization

1) Identification of Metabolites in Matrices

For identification of metabolites in excreta, pooling of samples was performed to minimize bias of results from individual samples. In urine, samples were pooled by time point of collection as well as for the entire elimination period. For example, the 5 samples from the 4 hour urine time point were thawed from each rat. Approximately 10% of the volume of each sample was transferred to a vial which became the representative sample for 4 hour urinary elimination from male rats. Similarly, for the elimination of naptalam during the entire study period, a "total elimination pooled sample" was prepared for each sex.

Urine samples were passed through a 0.2 µm filter prior to analysis by HPLC. Feces were homogenized in cold solvent, homogenates centrifuged, and pellets re-extracted with the same solvent until minimal quantities of radioactivity were present in the supernatants. Supernatants were pooled, volumes measured, and levels of radioactivity determined by LSC. Extracts were then concentrated by vacuum rotary evaporation.

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The post-extraction solids were subjected to enzymatic hydrolysis by sulfatase, glucuronidase, and glucosidase. The post-enzyme hydrolysis solids were subjected to chemical hydrolysis by 1N HCl or 1N NaOH.

Separation of metabolites was achieved on a Hewlett-Packard ODS Hypersil 5 µm 200 x 4.6 cm column. Separation of urinary metabolites was conducted by a 5 minute isocratic phase of 80% solvent A (0.1% aqueous trifluoroacetic acid [solvent B was 0.1% aqueous trifluoroacetic acid in acetonitrile]. A linear gradient followed for 20 minutes to 60% solvent A which was then maintained for 10 minutes. A 10 minute linear gradient followed to 10% solvent A which was maintained for 20 minutes and was also used to clean the column. Separation of fecal metabolites from extracts and hydrolysates used initial conditions of 100% solvent A followed by a linear gradient to 40% A in 60 minutes. Flow rate was 1 ml/min for both gradients. Metabolites in feces were separated using a Phenomenex Spherisorb ODS semiprep column 10 µm particle size, 250 x 10 mm, protected by a Brownlee RP18 Newguard cartridge. The separation solvents were the same for feces, but three different elution schemes were used, depending on the time of retention of the metabolite as shown by TLC analysis. Isolated peaks were further purified using a Supelco Supelcosil LC-DP column, 5 µm particle size, 250 x 4.6 mm and a Brownlee phenyl Newguard Cartridge.

Mass spectrometry of metabolites was accomplished using initially HPLC separation, of which 4 different methods were employed, depending on the matrix. These methods are listed on page 23 of the report.

With the gradient used for analysis of urine samples, retention times were: phthalic acid, 4.5 min.; N-acetyl-1-naphthylamine and 1-naphthylamine, 17 and 20 minutes, respectively; 5-hydroxy-naphthoquinone, 23 minutes; and naptalam, 25 minutes. For the gradient used for analysis of feces, phthalic acid showed a retention time of 20 minutes, with the subsequent order of elution the same as for urine. Naptalam imide, not previously used, had a retention time of 55 minutes.

Profiling of metabolites was achieved by collection of 1 minute fractions. Radioactivity in each fraction was determined by LSC. Levels of radioactivity in at least one fraction in a peak had to exceed 2 times background or blank values to define a peak. A peak was defined as the sum of the radioactivity in the collected fractions from a valley to the fraction preceding the next valley.

Summary of urinary metabolites observed in male and female rats covering the entire study period was presented on page 44 of the report, and is reproduced below:

**Urinary Metabolites in Male and Female Rats Administered Naptalam by the Intravenous Route[a]**

**Percent Administered Dose**

| Metabolite | Males | Females |
| --- | --- | --- |
| Naptalam | 36.4 | 68.7 |
| Unknown 1 | 3.03 | 3.39 |
| Unknown 2 | 3.03 | 3.39 |
| Unknown 3 | 0.872 | 2.28 |
| Unknown 4 | 0.854 | 1.89 |
| Baseline[b] | 2.60 | 7.37 |
| Total accounted for | 46.8 | 87.1 |

[a]data from page 44 of the report. [b]baseline was defined as column recovery - $\Sigma$defined peaks.

From the total elimination samples, the data above indicate that naptalam represented the major component of the urine in male and female rats. Of a total of 46.9% of the dose eliminated in urine of male rats, 36.4% was eliminated as naptalam, representing 77.7% of the chromatographed radioactivity. A similar finding was observed for female rats. The time course of elimination for urinary metabolites was presented in Figure 6, page 54 of the report showing time course in pooled samples from male and female rats. At 4 hours, naptalam was the prominent component in urine of both male and female rats. By 8 hours, naptalam was still the major component, but the size of the peak was greatly diminished. By 24 hours, the naptalam peak was barely detectable. The naptalam peak was identified by co-chromatography with an authentic standard.

The extraction of feces is reproduced from page 45 of the report:

**Quantitative Extraction of Feces in Naptalam Treated Rats[a]**

| | Male | | Female | |
|---|---|---|---|---|
| Fraction | % Fecal RA | % Admin. Dose | % Fecal RA | % Admin. Dose |
| Aqueous Methanol | 67.0 | 32.7 | 71.3 | 9.84 |
| 0.1N Acid/Base[b] | 9.92 | 4.84 | 7.72 | 1.07 |
| Enzyme hydrolysate[c] | 4.11 | 2.01 | 3.48 | 0.480 |
| Acid hydrolysate[d] | 5.14 | 2.51 | 5.82 | 0.803 |
| Base hydrolysate[e] | 6.35 | 3.10 | 6.52 | 0.899 |
| Bound[f] | 2.30 | 1.12 | 5.42 | 0.748 |
| Total | 94.9 | 46.3 | 100 | 13.8 |

[a]data from page 45 of the report. [b]combined 0.1N HCl and NaOH extracts. [c]data for combined hydrolysates [d]neutralized 1N HCl hydrolysates [e]neutralized 1N NaOH hydrolysates
[f] determined by combustion and LSC of the final post-enzyme hydrolysis solids.

As shown in the above table, extraction with aqueous methanol released 67 and 71% of the radioactive residue from feces of male and female rats, respectively. Sequential dilute acid and base extraction recovered an additional 8-10% of the residue. Approximately 20% of the total residue was not extractable and required hydrolysis.

HPLC analysis of the concentrated aqueous methanol extracts of the total elimination pooled samples from male and female rats treated with naptalam showed several peaks. A summary of the components observed in male and female rats is shown below, reproduced from pages 46 and 47 of the report.

**Fecal Metabolites in Male Rats Administered Intravenous Naptalam[a]**
% Administered Dose

| Metabolite | Methanol Fraction | Acid/Base Extracts | Hydrolysates | Total |
|---|---|---|---|---|
| Naptalam | 4.00 | 0.675 | - | 4.68 |
| 5-OH naptalam | 7.44 | 0.559 | 0.195 | 8.19 |
| 5-OH naptalam imide | 4.79 | 0.318 | 0.309 | 5.42 |
| Unknown 1 | 0.548 | 0.352 | 0.194 | 1.09 |
| Unknown 2 | | 0.163 | | 0.163 |
| Unknown 3 | 1.33 | 0.140 | 0.249 | 1.72 |
| Unknown 4 | 1.69 | 0.152 | 0.222 | 2.06 |
| Unknown 5 | 4.06 | 0.156 | 0.102 | 4.32 |
| Unknown 6 | | 0.448 | | 0.448 |
| Baseline[b] | 4.73 | 0.323 | 0.836 | 5.89 |
| Total | 28.6 | 3.29 | 2.11 | **34.0** |

[a]data from page 46 of the report. [b]baseline = column recovery - $\Sigma$defined peaks

**Fecal Metabolites in Female Rats Administered Intravenous Naptalam[a]**
% Administered Dose

| Metabolite | Methanol Fraction | Acid/Base Extracts | Hydrolysates | Total |
|---|---|---|---|---|
| Naptalam | 2.63 | 0.0349 | - | 2.66 |
| 5-OH naptalam | 1.54 | 0.150 | 0.0534 | 1.74 |
| 5-OH naptalam imide | 0.428 | | 0.0293 | 0.457 |
| Unknown 1 | 0.323 | 0.0225 | 0.0164 | 0.362 |
| Unknown 2 | 0.363 | 0.115 | 0.0574 | 0.535 |
| Unknown 4 | 0.551 | | 0.0270 | 0.578 |
| Unknown 5 | 1.12 | | | 1.12 |
| Unknown 6 | | 0.0421 | 0.0462 | 0.0883 |
| Unknown 7 | 0.806 | | | 0.806 |
| Unknown 8 | | | 0.0595 | 0.0595 |
| Baseline[b] | 1.00 | 0.264 | 0.267 | 1.53 |
| Total | 8.79 | 0.629 | 0.630 | **10.0** |

[a]data from page 47 of the report. [b]baseline = column recovery - $\Sigma$defined peaks

The quantitative extraction of feces as presented on page 11 of this review shows good recovery of the radioactivity in the various fractions when compared to the total percentage of the dose excreted in feces by each sex. However, when these various fractions were analyzed for fecal metabolites and the total radioactivity identified summed, only approximately 70% of the total radioactivity was accounted for. This loss of radioactivity possibly reflects the difficulty of extraction; however, column recovery was stated to be between 92-103% for the methods employed in HPLC analysis (page 32 of the report). Thus, in the absence of an explanation, the radioactivity must be considered as unaccounted for.

In male rats, approximately 8% of the dose was excreted in feces as 5-hydroxy naptalam. Much less of a precentage of this metabolite (1.74%) was excreted in feces of female rats. The 5-hydroxy naptalam imide metabolite constituted 5.42% of the dose in male rat feces, and only 0.457% in female rat feces. Naptalam was also present in feces of both sexes (4.68% in males, 2.66% in females). Compared to males, where the 5-hydroxy metabolite was the major residue, naptalam was observed as the major residue in the feces of female rats. These differences in fecal metabolite profiles point to sex-related differences in metabolism. It is possible that male and female liver produce similar metabolites but in differing quantity.

The report, in addition to evaluating the metabolite profile in feces over the entire study period, examined changes in the pattern of naptalam residues in feces over the time period of collection. Figure 13, page 61 of the report, summarized observed metabolites in feces at 12, 24, and 48 hours post-dose. At 12 and 24 hours post-dose, naptalam and a metabolite with a retention time of 22 minutes (apparently not identified) were observed as the most prominent metabolites in feces of male and female rats. By 48 hours post-dose, only the peak at 41 minutes (naptalam) appeared visible to any extent, and only in female rats.

## IV. DISCUSSION

The present study was performed at the request of the Agency, based on review of previously submitted metabolism data (MRID # 418600-03). From that study, no conclusions could be drawn regarding sex- or dose-related differences in metabolism of naptalam. Furthermore, no metabolic pathway could be determined. The study was classified as core supplementary, but it was possible that the study could be upgraded. The present study was submitted supposedly in response to the Agency's request. The Agency had requested identification of metabolites in feces, intravenous dosing to evaluate fecal elimination, and repeated dosing metabolism data. The present study addressed the fecal metabolite and intravenous dosing issues, but did not address the repeated dosing request.

In the present study, male and female rats (5/sex) were administered an intravenous dose of $^{14}C$ sodium naptalam at a dose of 5 mg/kg to both sexes. The disposition of the chemical was monitored by quantitation of excreted radioactivity in urine, feces, tissues, and residual carcass, as well as separation and identification of metabolites comprising $\geq$ 5% of the dose in urine and feces.

Examination of the disposition of radioactivity showed that for male rats, the excretion of naptalam derived radioactivity was approximately equivalent in urine and feces (46.9% and 48.8% of the dose, respectively), but in female rats, the excretion was predominantly in urine

(85.4% of the dose in urine, 13.8% of the dose in feces). In the previously conducted naptalam study (MRID # 41860003), oral dosing at 250 and 1000 mg/kg as a single oral dose to separate groups of rats resulted in excretion in urine and feces that showed no significant sex- or dose related differences. The presence of a distinct sex-related difference in excretion in the present study suggests a difference in metabolism, biliary excretion, enterohepatic cycling, elimination kinetics, or a combination of these. Urinary excretion data (page 42 of the report) showing the time course of elimination for naptalam derived radioactivity and similar data for fecal excretion (page 43 of the report), indicate that in both sexes, the largest percentage of urinary radioactivity is excreted at 4 hours post-dose, and in feces, the largest percentage is excreted 12-24 hours post-dose. However, in female rats, 4 hour urinary excretion showed 70.6% of the dose eliminated compared to 29.5% in male urine. Fecal excretion for 12-24 hours in male rats showed approximately 45% of the dose eliminated during this time compared to 12% in female rats. Based on these data and the lack of a sex-related difference in elimination after oral dosing, the enterohepatic cycling which appears to occur also appears more significant to male rats. The lack of plasma data demonstrating an increased half-life in male rats hampers this interpretation. It is noted that the fecal metabolites, while qualitatively similar between male and female rats, showed that female rats contained less of the same metabolites. This indicates that the processes involved in production of these metabolites are either operative to a lesser degree in female rats, or that the process of enterohepatic cycling operates to a lesser degree in female rats. The basis for this is not easy to determine based on the available data.

The major metabolite identified in urine of male and female rats was unchanged naptalam. Female rat urine was observed with a greater percentage of this metabolite (68.7%) than male rat urine (36.4%). In feces, the major product of naptalam biotransformation was identified as 5-hydroxy naptalam, constituting approximately 8% of the dose (this metabolite constituted only 1.74% of the dose in female rat feces). In female rats, naptalam was again the major compound observed, constituting 2.66% of the administered dose.

On the basis of the data in the present study, a metabolic scheme for naptalam biotransformation was proposed. In this scheme (reproduced below), the initial step in naptalam biotransformation is hydroxylation at the 5 position to form the 5-hydroxy naptalam, followed by dehydration to form the 5-hydroxy naptalam imide. As mentioned in the report (page 38), previous work has shown that naptalam is hydrolyzed to naphthylamine and phthalic acid. No naphthylamine was detected in this study. While 5-hydroxy-naphthylamine was isolated and identified, the level was very low, and it appears to be artefactual, as this metabolite was only observed following severe treatment of samples.

Overall, this study addresses the deficiencies pointed out from review of the earlier metabolism study on naptalam, but this study does not address the repeated dosing experiment. It is also pointed out that with regard to metabolite identification, only metabolites at the low intravenous dose were identified. The request to identify fecal metabolites in the previous study was not fulfilled. The lack of specific toxicity concerns for naptalam would appear to make the repeated dose experiment unnecessary at this time. However, the lack of identification of fecal metabolites from the oral dose study as requested appears to be a deficiency which must be addressed.

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The present study is classified as **unacceptable**, and the §85-1 data requirement is still considered a data gap, based on the lack of identification of fecal metabolites from the oral dosing metabolism study. In order for this study to be considered acceptable, the fecal metabolite identification from samples obtained in the previous study should be performed.

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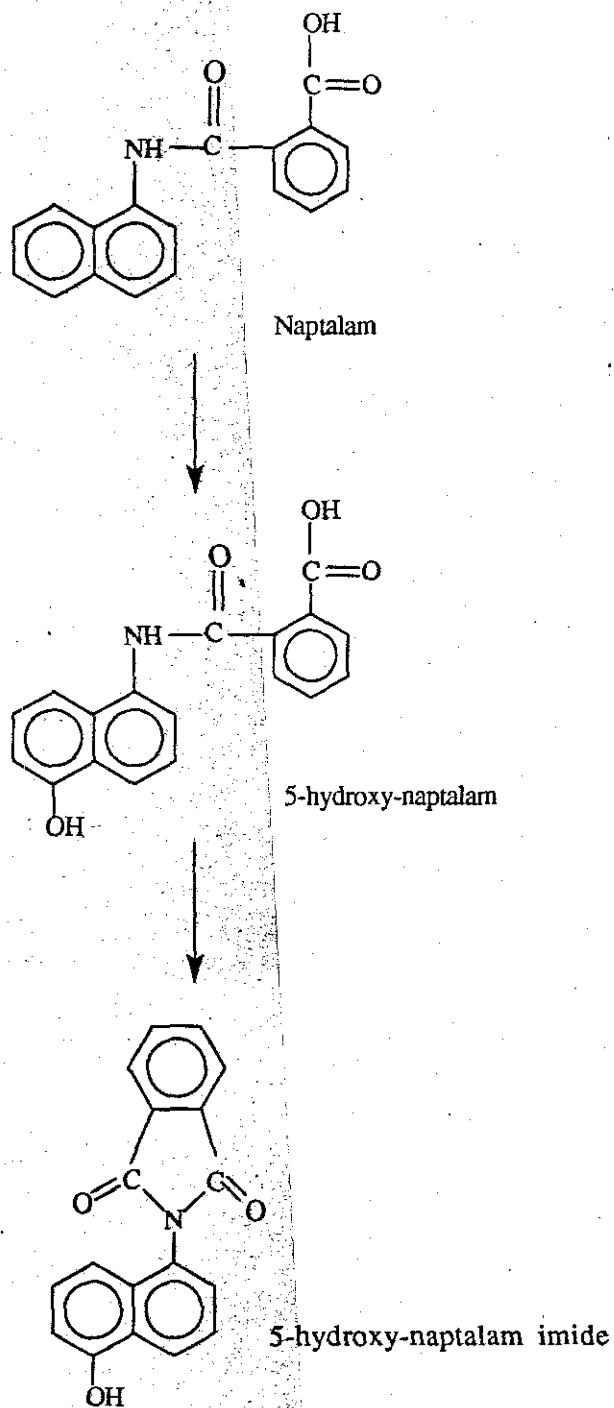
## Scheme for Biotransformation of Naptalam



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|---|---|
| **Chemical:** | **Benzoic acid, 2-((1-naphthalenylamino)ca** |
| **PC Code:** | **030703** |
| **HED File Code** | **13000 Tox Reviews** |
| **Memo Date:** | **07/05/96** |
| ***File ID:*** | ***TX011972*** |
| **Accession Number:** | **412-02-0280** |

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