

**Experiment Number:** K61541E  
**Route:** Dosed Feed  
**Species/Strain:** Mouse/B6C3F1

**Toxicokinetics Data Summary**  
**Compound:** Codeine/ **Analyte:** Codeine  
**CAS Number:** 76-57-3

**Request Date:** 7/11/2023  
**Request Time:** 10:03:16  
**Lab:** Microbiological Assoc, Inc  
Burroughs Wellcome Company

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Male

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Treatment Group (ppm)

---

(Day 7)

750 Dosed Feed Plasma<sup>a</sup>

3000 Dosed Feed Plasma<sup>a</sup>

6000 Dosed Feed Plasma<sup>a</sup>

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AUC_0-T (ng/mL*hr)	4990	13110	19774
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**Male**

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**Treatment Group (ppm)**

---

**(Day 21)**

**750 Dosed Feed Plasma<sup>a</sup>**

**3000 Dosed Feed Plasma<sup>a</sup>**

**6000 Dosed Feed Plasma<sup>a</sup>**

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AUC_0-T (ng/mL*hr)	3526	13954	16482
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Burroughs Wellcome Company

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Male

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Treatment Group (ppm)

---

(Day 90)

750 Dosed Feed Plasma<sup>a</sup>

3000 Dosed Feed Plasma<sup>a</sup>

6000 Dosed Feed Plasma<sup>a</sup>

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AUC_0-T (ng/mL*hr)	2214	9928	17342
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**Female**

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**Treatment Group (ppm)**

---

**(Day 7)**

**750 Dosed Feed Plasma<sup>a</sup>**

**3000 Dosed Feed Plasma<sup>a</sup>**

**6000 Dosed Feed Plasma<sup>a</sup>**

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AUC_0-T (ng/mL*hr)	9434	16518	25152
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**Female**

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**Treatment Group (ppm)**

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**(Day 21)**

**750 Dosed Feed Plasma<sup>a</sup>**

**3000 Dosed Feed Plasma<sup>a</sup>**

**6000 Dosed Feed Plasma<sup>a</sup>**

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AUC_0-T (ng/mL*hr)	1654	11590	12442
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**Request Time:** 10:03:16  
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Burroughs Wellcome Company

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**Female**

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**Treatment Group (ppm)**

---

**(Day 90)**

**750 Dosed Feed Plasma<sup>a</sup>**

**3000 Dosed Feed Plasma<sup>a</sup>**

**6000 Dosed Feed Plasma<sup>a</sup>**

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AUC_0-T (ng/mL*hr)	3128	10050	13856
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**Experiment Number:** K61541E  
**Route:** Dosed Feed  
**Species/Strain:** Mouse/B6C3F1

**Toxicokinetics Data Summary**  
**Compound:** Codeine/ **Analyte:** Norcodeine  
**CAS Number:** 76-57-3

**Request Date:** 7/11/2023  
**Request Time:** 10:03:16  
**Lab:** Microbiological Assoc, Inc  
Burroughs Wellcome Company

---

Male

---

Treatment Group (ppm)

---

(Day 7)

750 Dosed Feed Plasma<sup>a</sup>

3000 Dosed Feed Plasma<sup>a</sup>

6000 Dosed Feed Plasma<sup>a</sup>

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AUC_0-T (ng/mL*hr)	5914	24142	58122
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**Toxicokinetics Data Summary**  
**Compound:** Codeine/ **Analyte:** Norcodeine  
**CAS Number:** 76-57-3

**Request Date:** 7/11/2023  
**Request Time:** 10:03:16  
**Lab:** Microbiological Assoc, Inc  
Burroughs Wellcome Company

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Male

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Treatment Group (ppm)

---

(Day 21)

750 Dosed Feed Plasma<sup>a</sup>

3000 Dosed Feed Plasma<sup>a</sup>

6000 Dosed Feed Plasma<sup>a</sup>

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AUC_0-T (ng/mL*hr)	4940	27966	45892
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Male

---

Treatment Group (ppm)

---

(Day 90)

750 Dosed Feed Plasma<sup>a</sup>

3000 Dosed Feed Plasma<sup>a</sup>

6000 Dosed Feed Plasma<sup>a</sup>

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AUC <sub>0-T</sub> (ng/mL*hr)	3568	21860	43942
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**Female**

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**Treatment Group (ppm)**

---

**(Day 7)**

**750 Dosed Feed Plasma<sup>a</sup>**

**3000 Dosed Feed Plasma<sup>a</sup>**

**6000 Dosed Feed Plasma<sup>a</sup>**

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AUC_0-T (ng/mL*hr)	20710	41490	71098
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**Female**

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**Treatment Group (ppm)**

---

**(Day 21)**

**750 Dosed Feed Plasma<sup>a</sup>**

**3000 Dosed Feed Plasma<sup>a</sup>**

**6000 Dosed Feed Plasma<sup>a</sup>**

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AUC_0-T (ng/mL*hr)	3088	32702	56178
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Male

---

Treatment Group (ppm)

---

(Day 90)

750 Dosed Feed Plasma<sup>a</sup>

3000 Dosed Feed Plasma<sup>a</sup>

6000 Dosed Feed Plasma<sup>a</sup>

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AUC_0-T (ng/mL*hr)	3852	26160	47604
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**Experiment Number:** K61541E  
**Route:** Dosed Feed  
**Species/Strain:** Mouse/B6C3F1

**Toxicokinetics Data Summary**  
**Compound:** Codeine/ **Analyte:** Morphine  
**CAS Number:** 76-57-3

**Request Date:** 7/11/2023  
**Request Time:** 10:03:16  
**Lab:** Microbiological Assoc, Inc  
Burroughs Wellcome Company

---

Male

---

Treatment Group (ppm)

---

(Day 21)

750 Dosed Feed Plasma<sup>a</sup>

750 Dosed Feed Plasma<sup>a</sup>

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AUC_0-T (ng/mL*hr)	90.6	8418
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**Species/Strain:** Mouse/B6C3F1

**Toxicokinetics Data Summary**  
**Compound:** Codeine/ **Analyte:** Morphine  
**CAS Number:** 76-57-3

**Request Date:** 7/11/2023  
**Request Time:** 10:03:16  
**Lab:** Microbiological Assoc, Inc  
Burroughs Wellcome Company

---

Male

---

Treatment Group (ppm)

---

(Day 21)

3000 Dosed Feed Plasma<sup>a</sup>

3000 Dosed Feed Plasma<sup>a</sup>

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AUC_0-T (ng/mL*hr)	254	30576
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**Experiment Number:** K61541E  
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**Species/Strain:** Mouse/B6C3F1

**Toxicokinetics Data Summary**  
**Compound:** Codeine/ **Analyte:** Morphine  
**CAS Number:** 76-57-3

**Request Date:** 7/11/2023  
**Request Time:** 10:03:16  
**Lab:** Microbiological Assoc, Inc  
Burroughs Wellcome Company

---

Male

---

Treatment Group (ppm)

---

(Day 21)

6000 Dosed Feed Plasma<sup>a</sup>

6000 Dosed Feed Plasma<sup>a</sup>

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AUC_0-T (ng/mL*hr)	316	37528
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**Experiment Number:** K61541E  
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**CAS Number:** 76-57-3

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**Lab:** Microbiological Assoc, Inc  
Burroughs Wellcome Company

---

**Female**

---

**Treatment Group (ppm)**

---

**(Day 21)**

**750 Dosed Feed Plasma<sup>a</sup>**

**750 Dosed Feed Plasma<sup>a</sup>**

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AUC_0-T (ng/mL*hr)	80.6	7948
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**CAS Number:** 76-57-3

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**Lab:** Microbiological Assoc, Inc  
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---

**Female**

---

**Treatment Group (ppm)**

---

**(Day 21)**

**3000 Dosed Feed Plasma<sup>a</sup>**

**3000 Dosed Feed Plasma<sup>a</sup>**

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AUC_0-T (ng/mL*hr)	261	26622
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**CAS Number:** 76-57-3

**Request Date:** 7/11/2023  
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**Lab:** Microbiological Assoc, Inc  
Burroughs Wellcome Company

---

**Female**

---

**Treatment Group (ppm)**

---

**(Day 21)**

**3000 Dosed Feed Plasma<sup>a</sup>**

**3000 Dosed Feed Plasma<sup>a</sup>**

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AUC_0-T (ng/mL*hr)	340	27276
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**Species/Strain:** Mouse/B6C3F1

**Toxicokinetics Data Summary**  
**Compound:** Codeine/ **Analyte:** Codeine/Norcodeine/Morphine  
**CAS Number:** 76-57-3

**Request Date:** 7/11/2023  
**Request Time:** 10:03:16  
**Lab:** Microbiological Assoc, Inc  
Burroughs Wellcome Company

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## LEGEND

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### MODELING METHOD & BEST FIT MODEL

<sup>a</sup>AUC values calculated using trapezoidal rule

### ANALYTE

Codeine  
Morphine  
Norcodeine

### TK PARAMETERS

AUC<sub>0-T</sub> = Area under the plasma concentration versus time curve, AUC, from time  $t_i$  (initial) to  $t_f$  (final), AUC<sub>last</sub>

### TK PARAMETERS PROTOCOL

#### ANALYSIS METHOD

Unconjugated and total (unconjugated plus conjugated) concentrations of morphine, a metabolite of codeine, were determined in day 21 plasma samples by radioimmunoassay (RIA). Determination of total levels involved assay after hydrolysis by a beta-glucuronidase preparation. Due to interference in the codeine RIA for norcodeine, unconjugated (free) concentrations of codeine and its metabolite, norcodeine, were determined using high performance liquid chromatography (HPLC, 210 nm UV detection) in plasma samples of mice on days 7, 21, and 90. Insufficient sample volumes prevented using both HPLC and RIA techniques for codeine samples. Approximate area under the curve (AUC) values over the 12-hour blood sampling period were determined by the trapezoidal rule.

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TK PARAMETERS PROTOCOL (cont'd)

TK\_DOSED FEED PLASMA

750 ppm, 3000 ppm, 6000 ppm

In order to elucidate the disposition of codeine and/or some of its metabolites in the dose ranges studied in the full toxicology study (2-year dosed feed rat and mouse study Related Study--(C61541C) NTP Technical Report on the Toxicology and Carcinogenesis Studies of Codeine (CAS NO. 76-57-3) in F344/N Rats and B6C3F1 Mice (Feed Studies). August 1996. NTP TR 455. NIH Publication No 96-3360.), plasma concentrations of these compounds were determined on three days during the first 90 days of the study in a special toxicokinetic study. Codeine base was administered in the feed (NIH-07) to male and female B6C3F1 mice ad libitum. The mice received 750, 3000 and 6000 ppm of codeine base in the diet for up to 90 days. Blood samples were taken on study days 7, 21 and 90 from 5 mice from each treated group at each of 4 timepoints (0, 4, 8, and 12 hours) over the 12-hour dark cycle. Plasma was analyzed for unconjugated morphine, and total morphine (unconjugated plus conjugated) concentrations by radioimmunoassay (RIA). Plasma was analyzed for unconjugated codeine and unconjugated norcodeine by high performance liquid chromatography (HPLC, 210 nm UV detector) due to interference of norcodeine with the codeine RIA. Only area under the curve (AUC) values were calculated. The animals were dosed and blood collected at one laboratory and the plasma samples shipped to a different laboratory for analysis. Start date is the date the original protocol was signed.