Evaluation of Consumption of Self-Administered Acetaminophen in Drinking Water and Two Gel Delivery Systems in C57BL/6 Mice

M K Brunell, C H Olsen, A C Christy, B M Maxwell, D E Bentzel

Abstract

Acetaminophen is frequently administered to rodents in the drinking water to provide analgesia, although the effectiveness is often questioned. Gel products are now available to facilitate delivery of oral medications to rodents. We measured consumption of non-medicated and medicated water and gels, alone or in combination, in unmanipulated and post-operative male and female C57BL/6 mice to determine if these are effective delivery methods for acetaminophen. Unmanipulated male and female mice consumed similar amounts overall. The model showed a significant interaction between sex and treatment indicating that male and female mice had different preferences. Consumption was highest for a water/gel combination, regardless of gel type, in both males and females. In males, consumption was significantly higher for MediDrop® than either water or MediGel® in both the medicated and non-medicated conditions. In females, consumption was significantly lower for water than for either gel in both the medicated and non-medicated conditions. In both males and females, all treatment groups were estimated to be within or exceed the recommended dose range of 110-305mg/kg. In post-operative male and female mice, females consumed less than males. Combination water and gel resulted in the highest consumption and estimated doses, in both males and females. Additionally, all treatment groups were estimated to be within or exceed the recommended dose range. There was no change in activity level or body weight regardless of delivery method in either normal or postsurgical mice. Our results indicate that acetaminophen-medicated water or gels, alone or in combination, may serve as reliable delivery methods for oral analgesic administration.

INTRODUCTION

There are regulatory, scientific and ethical reasons to provide postoperative analgesia and minimize pain in animals used in biomedical research. The Guide for the Care and Use of Laboratory Animals mandates that “unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals”. As a prey species, rodents may not display obvious signs of pain which complicates postsurgical veterinary management and contributes to underuse of postoperative analgesics. Therefore, alleviating pain especially in the postoperative period must be considered essential.

Pain and surgical stress trigger the release of corticosteroids from the adrenal gland. Elevated corticosteroid levels are associated with impairment of the immune system as well as altered physiological and endocrine functions. Pain can lead to lack of appetite, hypothermia, decreased gut motility and cause urinary retention. Therefore, if pain will potentially inhibit recovery or alter the experimental model, it should be controlled as part of good experimental design.

Research personnel and veterinary staff have an ethical obligation to alleviate or minimize postoperative pain unless pain is itself the topic of the study or there is strong justification that administration of analgesics are contraindicated because they may compromise the outcome of a research protocol. Administration of analgesics to relieve postoperative pain makes a substantial contribution to refinement of animal experiments and is a cornerstone of experimental animal welfare.

Acetaminophen is frequently administered in the drinking
water of rodents to provide analgesia after surgery or other painful procedures(2,4,22), although the effectiveness is often questioned. The literature has conflicting results to whether acetaminophen is an effective analgesic for rodents.(1,5,6,8,10-15,19-21) Injection of analgesics provides the most reliable means to administer drugs. However, self-administration of analgesics has the advantage of stress reduction due to decreased handling, restraint and injection pain. Other advantages include no disruption of the diurnal rhythm of the rodents being treated and no disruption of the other animals in the room. In addition, administration of individual doses can be time consuming, especially when a large number of animals need to be treated. Acetaminophen produces analgesia and antipyresis by inhibition of cyclooxygenases (selective inhibitor of COX 3) and does not possess anti-inflammatory activity.(3,19,21) Acetaminophen is readily available and has low toxicity compared to opioids.(2) The recommended oral dose of acetaminophen in mice is 110 to 305 mg/kg.(4) Gel products are available to facilitate delivery of oral medications to rodents. When analgesics are orally self-administered, the animals must consume a sufficient volume to provide an efficacious dose of the analgesic. Because an animal's intake of fluids may be diminished during recovery from anesthesia and further reduced by post-operative malaise, it is important to determine whether animals consume a sufficient dose to provide adequate post-operative analgesia.

The goal of this study was to evaluate consumption of self-administered acetaminophen in drinking water and two gel delivery systems by mice. The focus of the study was not to determine whether acetaminophen provides sufficient postsurgical pain relief, but rather to determine if there was a preference for delivery system and whether the addition of medication altered that preference in male and female mice. Mice that underwent a laparotomy were compared to non-surgical animals to evaluate the impact on delivery system preference. The amount consumed was used to estimate the dose of acetaminophen received. The information obtained in this study may be used by investigators to refine painful procedures and improve animal welfare.

MATERIALS AND METHODS
The following study was conducted in accordance with the guidelines set forth by the Guide for the Care and Use of Laboratory Animals.(9) All procedures were approved by the Institutional Care and Use Committee of the Uniformed Services University of the Health Sciences, which is an AAALAC-accredited facility.

ANIMALS
The study was conducted in two separate experiments. Experiment 1 was an acetaminophen consumption evaluation utilizing 40 male and 40 female adult C57BL/6. Experiment 2 was an acetaminophen consumption evaluation using 80 male and 80 female mice following a surgical procedure (laparotomy). C57BL/6 mice (weight, 17 to 33 g; age, 9 to 12 weeks) were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were housed in groups of 5 (Experiment 1) or 2 (Experiment 2), in static, polycarbonate shoebox-type cages with filter tops (Ancare, Bellmore, NY) on rodent hardwood bedding (Maple SaniChips, Envigo, Indianapolis, IN). Shredable nesting material (Nestlets, Ancare) and a polycarbonate igloo (BioServ, Flemington, NJ) were provided as environmental enrichment. Mice were fed pelleted-rodent food ad libitum (Teklad Global 18% Protein Rodent Diet, Envigo, Indianapolis, IN). Hydration was provided according to experimental group.

The room was kept on a 12:12 hour light:dark cycle (lights on, 0600; lights off, 1800), temperature was maintained between 68 to 79°F (20-26°C), and relative humidity was 30% to 70%. Only the investigators changed the cages and fed animals. Husbandry and veterinary staff were instructed not to manipulate the racks or cages to minimize water loss from the drinking bottles.

Sentinel mice were used to monitor the health status of the experimental animals. Every week, CD-1 mice were directly exposed to dirty bedding from all colony animals. Every quarter, blood and fur swab samples were collected and every 6 months the sentinel mice were euthanized using CO2. Serology and PCR samples were sent to Idexx Bioresearch (Columbia, MO) for ELISA (Mouse Assessment Profile) and PCR (pinworm/fur mite PCR). In addition, tissues were submitted semi-annually to the Joint Pathology Center (Silver Spring, MD) for histopathology. Colony surveillance testing was performed quarterly by collecting samples from the pre-filter of the Animal Bedding Disposal Center located in cage wash and submitting for PCR (Mouse Opti-XXPress Profile-Basic Antimortem) to Idexx Bioresearch (Columbia, MO). At the time of this study, all mice were negative for pinworms, fur mites, Sendai Virus, pneumonia virus of mice, mouse hepatitis virus, minute virus of mice, mouse parvovirus, mouse...
norovirus, Theiler murine encephalomyelitis virus, reovirus, mouse rotavirus, lymphocytic choriomeningitis virus, ectromelia virus, mouse adenovirus, polyoma virus, and Mycoplasma pulmonis. Mice were not tested for Helicobacter spp.

**EXPERIMENT 1. ACETAMINOPHEN CONSUMPTION IN ADULT MALE AND FEMALE C57BL/6 MICE.**

Eight cages of male mice (n=40) were randomly assigned to 1 of 8 experimental conditions (A-H) for one week. Every week the cages rotated through the experimental conditions (A-H) until each cage had experienced all eight conditions over an 8-week period. The experiment was repeated with 40 female mice.

Experimental Conditions:

A – Non-medicated water provided in a drinking bottle

B – Non-medicated MediGel® provided in a 2oz cup

C – Non-medicated MediDrop® gel provided in a drinking bottle

D – Medicated (Acetaminophen) water provided in a drinking bottle

E – Medicated (Acetaminophen) MediGel® provided in a 2oz cup

F – Medicated (Acetaminophen) MediDrop® gel provided in a drinking bottle

G – Both Medicated (Acetaminophen) MediDrop® gel provided in a drinking bottle AND

   Medicated (Acetaminophen) MediGel® provided in a 2oz cup

H – Both Medicated (Acetaminophen) water provided in a drinking bottle AND

   Medicated (Acetaminophen) MediGel® provided in a 2oz cup.

For the first four days of the week (Mon -Thurs) animals were provided hydration in one of the methods described above. Filtered tap water was provided for water. MediGel® is a flavored gel available in 2 oz cups (MediGel Sucralose, Clear H20, Westbrook, ME). One 2oz cup provides hydration for 5 mice for up to 2 days. MediDrop® is a fluid gel designed to go into a drinking bottle (MediDrop Sucralose, Clear H20, Westbrook, ME) and suspends medications allowing for constant delivery.

For the last three days of the week (Fri - Sun) animals were provided non-medicated water in a drinking bottle as a wash out period before the animals rotated to the next condition. It also served to rehydrate animals if they were dehydrated, for example, if they did not consume as much water because they did not prefer a delivery system and/or the medication taste.

The recommended oral dose of acetaminophen is 110-305 mg/kg. At an anticipated total daily consumption of 4-6 ml per mouse, the desired concentration of the water and gel is approximately 1.1 mg/ml to achieve a dose within the recommended range (approximately 200mg/kg).

To prepare the acetaminophen-treated water, 6.9ml of cherry-flavored acetaminophen liquid (Mapap™ Liquid, Major Pharmaceuticals, Livonia, MI) was mixed with 200ml filtered, tap water. The acetaminophen concentration was 160mg per 5ml, resulting in drinking water containing acetaminophen at 1.1 mg/ml.

To prepare the acetaminophen-treated MediGel®, 2.1ml of cherry-flavored acetaminophen liquid was added to each 2oz gel cup. The resulting concentration of medicated gel was 1.1mg/ml. According to manufacturer’s recommendations, to thoroughly mix the acetaminophen throughout the gel, the unopened gel was warmed in a water bath until the gel become a liquid; 2.1ml acetaminophen liquid was placed in a 3ml syringe with an 18-gauge needle, the needle was inserted through the lid of the cup, and the acetaminophen injected into the cup. The needle and syringe were removed, and a piece of tape placed over the hole made by the needle. The cup was shaken for approximately 10 seconds to ensure a homogeneous color (indicating even distribution of the acetaminophen) and then placed in a refrigerator to allow the gel to reform. The gel was brought to room temperature before being placed in the cage with the mice.

To prepare the acetaminophen-treated MediDrop®, 6.9ml of cherry flavored acetaminophen liquid was added to 200ml of MediDrop® gel and mixed thoroughly. The acetaminophen concentration was 160mg per 5ml, resulting in MediDrop® containing 1.1mg/ml.
Animals were weighed mid-week. The weights of animals in each cage were averaged to represent the weight of an individual mouse in that cage. Drinking bottles with medicated water were shaken daily to mix acetaminophen. Acetaminophen is stable in aqueous solutions at room temperature at neutral pH and, therefore, was not expected to degrade in drinking water.(5) Gel delivery systems do not require daily mixing because according to the manufacturer, both MediGel® and MediDrop® suspend non-soluble medications uniformly. Gel cups were replaced every two days. Drinking bottles with water, drinking bottles with MediDrop®, and cups with MediGel® were weighed at initial placement and every subsequent day to determine amount consumed from each. The amount ingested (ml) was multiplied by the concentration (mg/ml) of acetaminophen in each delivery system. This was then divided by the weight of the mouse (g) and multiplied by 1000 to get an estimated dose of acetaminophen (mg/kg) received by each mouse. Calculating the estimated dose received allowed an approximation of whether animals were ingesting a therapeutic dose or being under/overdosed in medicated treatment groups (D-H).

EXPERIMENT 2. ACETAMINOPHEN CONSUMPTION IN ADULT MALE AND FEMALE C57BL/6 MICE FOLLOWING SURGERY.

Eight cages each with two male mice were placed in one of five medicated groups, (D-H) described in Experiment 1, after undergoing a laparotomy (n=80). Surgery was performed early in the morning on the first day and mice were in one of the five treatment groups for four days. The experiment was repeated using 80 female mice. Non-medicating control groups were not used for mice that underwent surgery to prevent unalleviated pain and distress. Currently at our facility, oral medication is typically first provided to animals the day of surgery after recovery. Therefore, oral medication was provided in the cage immediate after surgery to mimic and therefore assess current practices.

SURGERY

Mice were anesthetized with 87.5 mg/kg ketamine hydrochloride (Mylan Institutional LLC, Galway, Ireland) and 12.5mg/kg xylazine (Anased, Akorn Animal Health, Lake Forest, IL) IP. Once the mice were sufficiently sedated, the hair on the abdomen was clipped, and the skin was cleaned by using Betadine solution (Purdue Products, Stamford, CT) followed by isopropyl alcohol 70% (HUMCO, Texarkana, TX). Aseptic techniques were used for all surgical procedures. Sharp scissors were used to make a 1-cm incision through the abdominal skin and then through the abdominal musculature. The laparotomy was to mimic insertion of an abdominal telemetry implant. The muscular layer and skin edges were closed with 5-0 absorbable suture on a tapered needle (Vicryl, Ethicon, San Lorenzo, Puerto, Rico) in a single cruciate suture pattern. Skin edges were opposed using tissue glue (Vetbond, 3M, St. Paul, MN). For anesthetic recovery, mice were placed in a cage containing 1-inch paper cellulose bedding (Alpha-dri, Shepherd Specialty Papers, Kalamazoo, MI) which was placed atop of a Hotdog heating pad (Augustine Biomedical & Design, Eden Prairie, MN) and remained until mice were ambulatory. Following anesthetic recovery, mice were maintained in a cage with paper cellulose bedding.

STATISTICAL ANALYSIS

For Experiment 1, an 8x8 Latin square was designed with the 8 cages of animals and 8 week period. Each experimental condition occurred once in each cage and once each week. Data were analyzed using analysis of variance for a Latin square design, which compares treatment groups after adjusting for main effects of cage and week. Tukey’s post-hoc test was used for pair wise comparisons among treatment groups, with a family wise 5% error rate. Data were analyzed using SPSS version 22. Consumption was compared between males and females after adjusting for week, cage and treatment using ANOVA. Interaction terms were added to the ANOVA model to test whether the differences in consumption across weeks for among treatment groups varied with sex.

For Experiment 2, which does not follow a Latin square design, a one-way ANOVA was used to compare conditions. Pair wise comparisons among treatments were conducted using Tukey’s multiple comparisons adjustment. The proposed study design had 80% power to detect overall differences among treatments of 1.4 standard deviations; after Tukey’s adjustment, pair wise differences of 2 standard deviations could be detected. Power calculation was conducted using the following software: Lenth, R. V. (2006-9). Java Applets for Power and Sample Size [Computer software]. Retrieved 19 March 2015 from http://www.stat.uiowa.edu/~rlenth/Power. All calculations were based on a 5%, two-sided significance level.
RESULTS

Experiment 1

Consumption. Male and female mice consumed similar amounts overall. The average daily consumption was 5.8ml for male and 5.7ml for female mice ($F_{1,105} = 0.306, P = 0.581$), after adjusting for week, cage and treatment using ANOVA. The model showed a significant interaction between sex and treatment ($F_{7,1,05} = 8.72, P < .001$), indicating that male and female mice had different preferences. Results are presented in tabular (Table 1) and graphical (Figure 1) form.

Table 1

Mean consumption per mouse (ml/day) by treatment group (A-H) and gender, listed from highest to lowest preference. Probability value between treatment groups A-H.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Male</th>
<th>Probability</th>
<th>Female</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.3 (0.7)</td>
<td>&lt;0.01</td>
<td>6.1 (0.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B</td>
<td>6.0 (1.0)</td>
<td>0.05</td>
<td>4.8 (0.8)</td>
<td>0.06</td>
</tr>
<tr>
<td>C</td>
<td>4.8 (0.9)</td>
<td>0.08</td>
<td>4.4 (0.9)</td>
<td>0.12</td>
</tr>
<tr>
<td>D</td>
<td>3.8 (1.0)</td>
<td>0.10</td>
<td>3.0 (0.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>E</td>
<td>2.6 (0.8)</td>
<td>0.21</td>
<td>2.0 (0.8)</td>
<td>0.30</td>
</tr>
<tr>
<td>F</td>
<td>1.8 (0.6)</td>
<td>0.32</td>
<td>1.6 (0.6)</td>
<td>0.42</td>
</tr>
<tr>
<td>G</td>
<td>1.5 (0.5)</td>
<td>0.43</td>
<td>1.3 (0.5)</td>
<td>0.52</td>
</tr>
<tr>
<td>H</td>
<td>1.3 (0.4)</td>
<td>0.53</td>
<td>1.0 (0.4)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Differences between water, MediGel® and MediDrop® consumption in males. Consumption was significantly higher for MediDrop® (C,F) than either water (A,D) or MediGel® (B,E) in both the medicated and non-medicated conditions. There was no significant difference in consumption between MediGel® (B,E) and water (A,D) in either the medicated or non-medicated condition.

Differences between medicated and non-medicated consumption in males. Consumption of medicated water (D) was significantly greater than non-medicated water (A), but there was no significant difference between medicated and non-medicated MediGel® (E,B), or between medicated and non-medicated MediDrop® (F,C).

Differences between medicated and non-medicated consumption in females. Consumption was significantly lower for water (A,D) than for either MediGel® (B,E) or MediDrop® (F,C).
MediDrop® (C,F) in both the medicated and non-medicated conditions. However, for medicated water (D) versus medicated MediDrop® (F), the difference did not reach statistical significance. There was no significant difference in consumption between MediGel® (B,E) and MediDrop® (C,F) in either the medicated or non-medicated condition.

**Differences between medicated and non-medicated consumption in females.** There was no significant difference in consumption between medicated and non-medicated versions of either water (A vs D), MediGel® (B vs E) or MediDrop® (C vs F).

**Consumption of water/gel combinations in females.** Consumption was highest in the conditions that included both medicated water and gel, for both MediGel® (H) and MediDrop® (G). Differences were significant versus all other conditions except medicated MediGel® (E).

**Estimated Dose.** Results are presented in tabular (Table 2) and graphical (Figure 2) form. Among males, there was a significant difference among treatment groups ($F_{4,21} = 28.6, P < 0.001$). Combination medicated MediGel® and medicated MediDrop® (G) yielded the highest estimated dose, significantly higher than all other treatments. Medicated water (D) and medicated MediGel® (E) yielded the lowest estimated dose, significantly lower than all other treatments, but not significantly different from each other. All treatment groups were estimated to be within the recommended dose range of 110-305mg/kg.

**Table 2**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean Estimated Dose (mg/kg/day)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L: Both medicated MediDrop® and medicated MediGel®</td>
<td>297.3 (26.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>H: Both medicated water and medicated MediGel®</td>
<td>277.5 (20.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>F: Medicated MediGel®</td>
<td>233.3 (22.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>D: Medicated water</td>
<td>216.6 (20.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E: Medicated MediGel®</td>
<td>193.4 (19.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G: Both medicated MediDrop® and medicated MediGel®</td>
<td>404.7 (34.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>H: Both medicated water and medicated MediGel®</td>
<td>379.6 (32.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E: Medicated MediGel®</td>
<td>319.4 (31.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>D: Medicated water</td>
<td>292.4 (27.5)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Female**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean Estimated Dose (mg/kg/day)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>L: Both medicated MediDrop® and medicated MediGel®</td>
<td>297.3 (26.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>H: Both medicated water and medicated MediGel®</td>
<td>277.5 (20.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>F: Medicated MediGel®</td>
<td>233.3 (22.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>D: Medicated water</td>
<td>216.6 (20.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E: Medicated MediGel®</td>
<td>193.4 (19.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G: Both medicated MediDrop® and medicated MediGel®</td>
<td>404.7 (34.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>H: Both medicated water and medicated MediGel®</td>
<td>379.6 (32.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E: Medicated MediGel®</td>
<td>319.4 (31.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>D: Medicated water</td>
<td>292.4 (27.5)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

In females, there was significant difference among treatment groups ($F_{4,21} = 16.8, P < 0.001$). Combination medicated MediGel® and medicated MediDrop® (G) yielded the highest estimated dose, significantly higher than all other treatment groups except combination medicated water and medicated MediGel® (H). Medicated water (D) yielded the lowest estimated dose, significantly lower than all other treatment groups except medicated MediGel® (F). Groups D and F were estimated to be within the recommended dose range (110-305mg/kg) while groups E, G and H were above it.

**EXPERIMENT 2**

**Consumption.** The average daily consumption was 6.4ml for male and 4.9ml for female mice ($F_{5,70} = 38.6, P < 0.001$), after adjusting for treatment using ANOVA. Among males, there was a significant difference among treatment groups ($F_{4,35} = 3.3, P = 0.021$). Results are presented in tabular (Table 3) and graphical (Figure 3) form. Consumption was significantly higher in combination medicated water and medicated MediGel® (H) than in medicated water (D) treatment. No other significant differences were found. Among females, overall there was a significant difference among treatment groups ($F_{4,35} = 24.7, P < 0.001$). Combination medicated MediDrop® and medicated MediGel® (G) treatment yielded significantly
higher consumption than treatments D, E and F and treatment H yielded significantly higher consumption than all other treatments. No significant differences were observed between treatments D, E and F.

Table 3
Mean consumption per mouse (ml/day) by medicated treatment group (D-H) and gender, listed from highest to lowest preference. Probability value between treatment groups D-H.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Male</th>
<th>Female</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>H: Both medicated water and medicated MediGel®</td>
<td>8.6(4.3)</td>
<td>2.0(1.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G: Both medicated MediDrop® and medicated MediGel®</td>
<td>7.6(4.2)</td>
<td>3.0(0.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>F: Medicated MediDrop®</td>
<td>7.4(4.1)</td>
<td>2.9(0.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E: Medicated water</td>
<td>7.3(4.1)</td>
<td>2.8(0.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>D: Medicated water</td>
<td>7.2(4.1)</td>
<td>2.8(0.9)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Estimated Dose. Results are presented in tabular (Table 4) and graphical (Figure 4) form. Among males, the average estimated dose for combination medicated water and medicated MediGel® (H) treatment was significantly higher (F[4, 35] = 4.04, P = 0.009) than for treatments D and E, and no other significant differences among treatment groups were observed. Among females, overall there was a significant difference between treatment groups (F[4, 35] = 29.4, P <0.001). No significant differences were observed between treatments D, E and F. Treatment G yielded a significantly higher average estimated dose than treatments D, E and F, and treatment H yielded a significantly higher estimated dose than all other treatments.

Table 4
Mean estimated dose per mouse (mg/kg/day) by medicated treatment group (D-H) and gender, listed from highest to lowest preference. Probability value between treatment groups D-H.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Male</th>
<th>Female</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>H: Both medicated water and medicated MediGel®</td>
<td>33.6(16.8)</td>
<td>3.9(3.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G: Both medicated MediDrop® and medicated MediGel®</td>
<td>35.4(10.8)</td>
<td>4.4(3.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>F: Medicated MediDrop®</td>
<td>34.3(10.8)</td>
<td>3.8(3.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E: Medicated water</td>
<td>34.2(10.8)</td>
<td>3.8(3.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>D: Medicated water</td>
<td>33.2(10.8)</td>
<td>3.8(3.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G: Both medicated water and medicated MediGel®</td>
<td>35.7(10.8)</td>
<td>4.4(3.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>F: Medicated MediDrop®</td>
<td>34.6(10.8)</td>
<td>3.9(3.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E: Medicated water</td>
<td>34.5(10.8)</td>
<td>3.9(3.0)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Figure 3
Average daily consumption per mouse according to gender and by treatment group D-H (ml; mean ± SEM). The expected daily consumption is 4 – 6 ml (dotted lines).

Figure 4
Average estimated acetaminophen dose per mouse according to gender and treatment group D-H (mg/kg; mean ± SEM). The recommended therapeutic dose is 110 – 305 mg/kg (dotted lines).

Activity level. Subjectively, all mice were bright, alert, and active by the following morning, approximately 24h after surgery. Activity level and ability to ambulate throughout the cage were observed and demonstrated no apparent difference between groups.
EXPERIMENT 1 VS EXPERIMENT 2

In females, consumption was significantly lower after Experiment 2 (surgery) than Experiment 1 (no surgery) \( (F_{4,70}=6.893, P <0.0001) \). Post-hoc comparisons using Sidak’s multiple comparisons test found significant differences for treatments E, G and H but not for D and F. The results are presented graphically (Figure 5).

**Figure 5**
Average consumption per female mouse in surgical and non-surgical treatment groups D-H (ml; mean ± SEM). The expected daily consumption is 4 – 6 ml (dotted lines).

In males, there was no significant difference in consumption between experiments \( (F_{1,70} = 0.13, P = 0.72) \). No post-hoc comparisons were performed. The results are presented graphically (Figure 6).

**DISCUSSION**

The purpose of the study was to evaluate consumption of self-administered acetaminophen in water and two gel delivery systems (MediGel®, MediDrop®) by mice. The focus of the study was not to determine whether acetaminophen provides sufficient postsurgical pain relief, but was to evaluate consumption of acetaminophen in males and females and determine if there is a preference for delivery system and if the presence of medication altered this preference. Consumption following a surgical procedure (laparotomy) was also evaluated to determine if preference was affected.

Experiment 1 determined consumption (ml/day/mouse) of eight delivery conditions and estimated dose (mg/kg/day/mouse) of the five delivery conditions containing acetaminophen, in both male and female mice that did not have surgery. Overall, males and females consumed similar amounts. No delivery system was refused by the animals. At an anticipated total daily consumption of 4-6ml per mouse most delivery conditions worked well with mice consuming adequate amounts. Combination medicated MediDrop® and medicated MediGel® (G) treatment group followed by combination medicated water and medicated MediGel® (H) had the highest consumption for both males and females and all were over 6ml/day. Higher consumption was evident when offered two delivery systems, similar to
previous findings by Christy et al, 2014,(4) suggesting that access to more delivery systems improves the opportunity to ingest acetaminophen. Males had a high preference for MediDrop®, both plain and medicated, consuming over 6ml in both groups. Females had lower preference for water, both plain and medicated, consuming less than 4ml in both groups. Therefore, all three delivery systems appear to be suitable in males, however, MediDrop® or MediGel® may be better delivery systems for female mice.

Treatment group G followed by H had the highest estimated doses for both male and female mice, which is logical since they were the most consumed. As previously mentioned, consumption amounts were similar between males and females, therefore it is likely that females received higher doses because they weigh less than males. All male treatment groups were estimated to be within the recommended dose range of 110-305 mg/kg.(4) In females, several delivery conditions (E,G,H) were estimated to be above the recommended dose range. Although female mice consumed less medicated water (D), they were still in the recommended dose range. In groups where mice were given two delivery systems, male estimated doses were close to the upper end of the dose range and females exceeded it, therefore we recommend providing only one delivery method to avoid potential acetaminophen toxicity.

Experiment 2 determined consumption (ml/day/mouse) and estimated dose (mg/kg/day/mouse) of five medicated delivery conditions in both male and female mice after a surgical procedure. Overall, females consumed less than males. However, no delivery system was refused by either gender. All delivery systems were utilized sufficiently by the mice to provide a total daily consumption of 4-6ml/mouse. The order of consumption preference was the same between males and females, H followed by G then F. Similar to the results from Experiment 1, treatment groups H & G had the highest consumption for both males and females and it appears that when mice have access to more delivery choices, they consume more. Treatment group F had the third highest consumption for both genders. For the remaining treatment conditions males and females differed in their preference. Males preferred E while females preferred D. These results suggest MediDrop®, MediGel® or water appear to be suitable delivery systems for both males and females following surgery.

In treatment groups in which two delivery options were provided, female groups (G,H) were estimated to exceed the dose range, while only the medicated water/gel group (H) was estimated to exceed the recommended dose range in male mice. All treatment groups that provided a single delivery method were within the recommended dose range. Consistent with our recommendation based on Experiment 1 results, we recommend providing only one delivery system to stay within the dose range and avoid potential acetaminophen toxicity.

Females consumed less after experiment 2 (surgery) than experiment 1 (no surgery) in 4/5 treatment groups with groups E, G and H being significantly lower. There was no significant difference in consumption in males after experiment 2 than experiment 1. Perhaps post-surgical pain decreased consumption in females compared to males.

To avoid having to singly house animals and increase stress due to deprivation of social interaction, we used average water or gel intake for the cage to estimate individual consumption and dose per mouse. We acknowledge that this may have been a limitation in that it is possible that strong individual mouse preferences may have had skewed the overall consumption for the cage, although averaging was intended to reduce the effect of any one animal's preference. Additionally, we acknowledge that individual doses may have varied and it is possible individual animals did not receive a therapeutic dose. It should also be noted that mice placed bedding material in the gel cups, although this did not seem to affect consumption. Every attempt was made to remove bedding prior to weighing the cups, but it is possible that bedding may have increased the weights, thereby underestimating the amount of material consumed.

In summary, the results of this study determined both male and female mice will consume adequate amounts acetaminophen-medicated water, MediDrop®, and MediGel® whether these delivery systems are used individually or in combination. We recommend providing acetaminophen only in one delivery system, as providing two sources may lead to overconsumption and potential acetaminophen toxicity. It is imperative that appropriate pain relief be provided to enhance animal welfare and mitigate data variability. It is our intention that investigators use a simple and reliable delivery method to provide self-administered acetaminophen to laboratory mice for pain reduction.

ACKNOWLEDGEMENTS

We especially thank Mike Junio, Cara Reiter, Andrew
Brown, and Clayton Gerrian for their technical assistance in completing this project. The views in this article are those of the authors and do not necessarily reflect the views, official policy, or position of the Uniformed Services University of the Health Sciences, the Department of Defense, or United States Federal Government.

References

Author Information

**Marla K. Brunell**
Center for Laboratory Animal Medicine, Uniformed Services University of the Health Sciences
Bethesda, Maryland

**Cara H. Olsen**
Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences
Bethesda, Maryland

**Amanda C. Christy**
Center for Laboratory Animal Medicine, Uniformed Services University of the Health Sciences
Bethesda, Maryland

**Branden M. Maxwell**
Center for Laboratory Animal Medicine, Uniformed Services University of the Health Sciences
Bethesda, Maryland

**David E. Bentzel**
Center for Laboratory Animal Medicine, Uniformed Services University of the Health Sciences
Bethesda, Maryland