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Organoleptic Assessment and Median Lethal Dose Determination of Oral Aniline in Rats

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The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

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Aniline, an aromatic amine, is used as a reagent or precursor in many chemical and industrial processes, including pesticide and pharmaceutical creation. A three-phase approach was used to comprehensively assess aniline as an oral-ingestion hazard. First, the solubility of aniline in popular consumer beverages (bottled water, apple juice, and 2% milk) was assessed, but aniline proved to be poorly soluble and typically formed a suspension at projected lethal concentrations. Lethality was then assessed by administering aniline via gavage in bottled water across varying concentrations. A probit model was fit to 24-hour survival data and predicted a median lethal dose of 762.15 mg/kg (95% CI: 550.30 – 983.97 mg/kg; slope: 7.05). Intoxication was rapid, with lethargy and ataxia typically occurring within 5 minutes. Tremor and rapid breathing were observed within 30 minutes post-exposure at moderate doses. High doses of aniline produced cyanosis that was often followed by death. Finally, the organoleptic properties (i.e., taste, smell, texture, etc.) were assessed by allowing rats to voluntarily consume 3.0 mL of the above beverages adulterated with aniline at various concentrations. This organoleptic assessment determined that aniline was readily detected at toxic concentrations and rats failed to consume significant amounts of the adulterated beverages except at concentrations equivalent to and lower than the LD01. These results suggest that aniline is not likely to be consumed in toxic or lethal concentrations by humans and primarily remains an occupational hazard and environmental pollutant.

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ABSTRACT

Aniline, an aromatic amine, is used as a reagent or precursor in many chemical and industrial processes, including pesticide and pharmaceutical creation. A three-phase approach was used to comprehensively assess aniline as an oral-ingestion hazard. First, the solubility of aniline in popular consumer beverages (bottled water, apple juice, and 2% milk) was assessed, but aniline proved to be poorly soluble and typically formed a suspension at projected lethal concentrations. Lethality was then assessed by administering aniline via gavage in bottled water across varying concentrations. A probit model was fit to 24-hour survival data and predicted a median lethal dose of 762.15 mg/kg (95% CI: 550.30 – 983.97 mg/kg; slope: 7.05). Intoxication was rapid, with lethargy and ataxia typically occurring within 5 minutes. Tremor and rapid breathing were observed within 30 minutes post-exposure at moderate doses. High doses of aniline produced cyanosis that was often followed by death. Finally, the organoleptic properties (i.e., taste, smell, texture, etc.) were assessed by allowing rats to voluntarily consume 3.0 mL of the above beverages adulterated with aniline at various concentrations. This organoleptic assessment determined that aniline was readily detected at toxic concentrations and rats failed to consume significant amounts of the adulterated beverages except at concentrations equivalent to and lower than the LD_{01}. These results suggest that aniline is not likely to be consumed in toxic or lethal concentrations by humans and primarily remains an occupational hazard and environmental pollutant.

INTRODUCTION

Aniline is a common industrial chemical used in the manufacture of pigments, dyes, pesticides, pharmaceuticals, and rubber products, as well as a chemical precursor in the production of many polymers [1]. It is the simplest of the aromatic amines and is easily modified to create substituted aniline compounds for specific manufacturing uses. Aniline is a highly toxic compound that can be absorbed into the body via the dermal, inhalation and oral routes [2-4] and is primarily excreted through urine [5, 6]. Genotoxicity and damage to multiple organ systems have been observed following acute aniline exposure [7-15]. There are numerous cases of accidental, intentional, and occupational exposures to toxic amounts of aniline [1, 16-18]. Due to its wide use and relative ease of availability, aniline presents a potential mass-casualty threat in the event of intentional or accidental contamination of food or commercial products.

Aniline is a flammable, colorless-to-brown oily liquid with a sweet, amine-like odor [19]. Chronic exposure to sub-acute levels of aniline can cause decreased hematopoiesis leading to hemolytic anemia, as well as detrimental changes to the spleen, including enlargement, siderosis (deposition of iron), sarcomas, and fibrosis [7, 20, 21]. Acute exposure to aniline can induce fatal methemoglobinemia, in which methemoglobin (which cannot bind to oxygen) is present in such high amounts in the blood that it prevents the adequate formation of oxyhemoglobin from hemoglobin during oxygen transport throughout the vascular system, leading to cyanosis and hypoxia [22].

The exact mechanism through which aniline produces methemoglobinemia is unclear, but some studies suggest it is chiefly caused by a toxic metabolite of aniline, phenylhydroxylamine, which oxidizes hemoglobin into methemoglobin [23]. The standard treatment for aniline-induced methemoglobinemia is methylene blue, though numerous studies have indicated that over-administration of methylene blue may have deleterious hemolytic effects [24, 25]. Methylene blue's therapeutic effect in treating methemoglobinemia is via the reduction of methemoglobin to hemoglobin, essentially the opposite mechanism of action of aniline's chief toxic metabolite, phenylhydroxylamine [26].

While an exact toxic or fatal dose of aniline in humans is difficult to predict, severe toxic symptoms have been noted in adult patients who have consumed as little as 4 ml of aniline (if pure, this would equate to 4.08 g of aniline). In this case, the patient had a blood methemoglobin concentration of over 74% even after multiple treatments of methylene blue. The blood samples drawn from the patient had the characteristic chocolate brown color associated with high blood levels of methemoglobin [17]. Human aniline consumption studies have shown doses as small as 25-65 mg can significantly increase blood levels of methemoglobin, albeit without overt signs of toxicity [27]. In a separate incident, a 25-year-old male was exposed while unloading large containers of aniline. He was splashed on the face and upper body with approximately 200 mL of aniline. Interestingly, no immediate symptoms presented other than burns around the exposed areas, but cyanoderma eventually did manifest following oxygen-inhalation treatment. The patient had a methemoglobin concentration of 26.3%, though respiration was normal and no abnormalities were evident on ECG or chest radiography. Methylene blue was administered and decreased the methemoglobin concentration to 6.6%

after six hours. The patient eventually made a full recovery and was discharged seven days after admission [28].

In the event of an intentional or accidental contamination of food with aniline, the taste or smell of aniline in the food product may be what alerts a potential victim to its presence. However, despite reports that aniline has a sweet, amine-like odor [19], little information is available in the literature about its taste. In one case of intentional poisoning, a woman noted a "bitter soapy taste," as well as a burning and numbing sensation on her lips while drinking aniline-tainted breakfast coffee [18]. In that case, she immediately discarded the remainder of the coffee upon noticing the odd sensation on her lips, but had already consumed a toxic amount of aniline and required hospitalization. Tolonium chloride and ascorbic acid were used to treat her cyanosis, and she made a full recovery by the third day. With estimated mean human lethal doses of aniline ranging from 15-30 grams (approximately 15-30 ml of pure aniline) [29], determining the taste threshold concentrations of aniline in various liquids and food items would offer important data when assessing its risk as a toxic food adulterant.

Assessing oral-ingestion hazards requires the *voluntary* consumption of the chemical adulterant. Chemical threats that are tasteless and odorless are more likely to cause harm than those that are easily detected and therefore able to be rejected prior to the consumption of toxic or lethal amounts. A rat model established for assessing oral chemical threats [30, 31] was used here to evaluate aniline as a mass-casualty consumer beverage adulterant. Rats are neophobic [32] and will tend to refuse new items, making them a conservative model when testing the organoleptics of chemical threats. Rodents also have chemoreceptive capabilities superior to humans [33, 34], so any compound a rat consumes in toxic concentrations would likely be consumed by humans as well. Additionally, rats are more resistant to aniline toxicity than humans [35], so any toxicity observed in the rat model would translate to more substantial effects in humans. In this experiment, we leveraged the rat's chemoreceptive capabilities to test the organoleptic properties of aniline in several beverages popular in the U.S. (bottled water, apple juice, and 2% milk). By assessing solubility, oral toxicity, and organoleptics via voluntary oral ingestion, we were able to develop a comprehensive threat assessment of aniline as an oral-ingestion hazard.

METHODS

Chemicals and Vehicles

Aniline (≥ 99% purity) was obtained from Alfa Aesar and stored protected from light at room temperature. Preparation of aniline prior to being placed into solution occurred within the confines of a certified chemical fume hood.

Aquafina® purified drinking water (16.9 oz., 500 mL; 24 pack), Mott's® 100% apple juice (8 oz., 237 mL; 6 pack), 2% milk (1 pint, 473 mL; single bottles purchased from local grocery stores) were purchased from local vendors. The water and apple juice were purchased and stored at room temperature for up to several weeks prior to being placed in a refrigerator at least 24 hours prior to use. Milk was purchased at the beginning of each week and kept refrigerated at approximately 4 °C.

Subjects

One hundred ninety (190) male Sprague-Dawley rats (SAS SD 400) were obtained from Charles River Laboratories (Wilmington, MA, USA). Thirty (30) rats were assigned to the median lethal dose determination, and 160 rats were assigned to the organoleptic assessment. All rats weighed between 226-250 g at the time of shipping and were allowed five days (under group housing) to acclimate to our facility. All subjects were housed individually thereafter in a vivarium with a 12-hour light/dark cycle (lights on at 0600). All rats had free access to food and water during acclimation, after which water regulation was implemented and maintained for the remainder of the study (food remained freely available). Water regulation was implemented by pulling the cages of the rats outward several inches, removing the ability to drink from the water valve [36]. When water was made available, the cages were pushed back several inches until the water valve was inserted into the home cage. Water access was limited to 2 hours per day (typically from 1230 to 1430) and occurred at least one hour after the organoleptic assessment training. This 2-hour duration was sufficient for daily water needs, and similar durations have been used in other experimental procedures [37-39].

The experimental protocol was approved by the Institutional Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense (USAMRICD), and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The USAMRICD is a research facility fully accredited by AAALAC International.

Solubility Determination

Aniline solubility was assessed in room-temperature (21 °C) water as well as refrigerated (4 °C) water, juice, and milk. Each assessment began with a known amount of aniline placed into a vial followed by 2-4 mL of a beverage. An incremental volume of 1 mL was then repeatedly added until solubility was achieved, and the final concentration was recorded. Mechanical agitation (5-second duration) with a Vortex-Genie 2 laboratory mixer (Daigger Scientific Inc., Vernon Hills, IL) followed each incremental addition, and a 10-second partial submersion in an ice-water bath followed every third incremental addition to keep refrigerated beverages at the appropriate temperature. Whenever solubility was achieved, it was replicated an additional two times for each beverage.

Median Lethal Dose Determination

A stagewise, adaptive dose design [40-42] was used to determine the median lethal dose (LD_{50}) of aniline in 21 °C (room temperature) water. Doses for the first stage were selected based on available literature [4]. Doses for the second and all subsequent stages were based on 24-hour lethality observed from the previous stage(s). Doses were administered via gavage in 2.5 – 3.0 mL of 21 °C water, and all subjects were observed continuously for the first hour and then checked hourly thereafter until 5 hours post-exposure. A final observation occurred at 24 hours post-exposure, and survivors were humanely euthanized. One subject received a partial dose, so was excluded from study and humanely euthanized. Doses were selected such that the

entire range of lethality (0% to 100%) was observed. Probit models using maximum likelihood estimates were fitted to the combined data for all stages.

Organoleptic Assessment

The organoleptic assessment occurred in a polycarbonate rodent cage (45.7 cm × 24.1 cm × 20.3 cm) with an air-filtered lid. A polycarbonate insert was placed into the bottom of the cage that had a cutout that held a 5.72 cm diameter smooth tempered glass condiment dish. The glass dish was at a comfortable height from which the rats could drink without tipping the dish. All vehicles used in the organoleptic assessment were refrigerated (4 °C). Training for the assessment occurred for 7 sessions (one session per day) prior to exposure to the adulterated vehicles. The first two training sessions allowed the rats 10 min to consume up to 10 mL. The following three sessions gave the rats 5 min to consume 5 mL, and the final two training sessions provided 5 min to consume 3 mL. Rats had to consume at least 2.5 mL during the session prior to exposure to be included in the analysis, and 129 out of 130 rats (99.2%) met the criterion.

The vehicles were adulterated with aniline immediately prior to being distributed to the glass dishes on the day of exposure. The volume of the adulterated vehicle was 3.0 mL. The concentrations of the adulterated vehicle were 76.21 mg/mL (LD_{50} equivalent), 61.14 mg/mL (LD_{25} equivalent), 50.14 mg/mL (LD_{10} equivalent), 35.64 mg/mL (LD_{01} equivalent), and 17.82 mg/mL (½ LD_{01} equivalent). The LD equivalents were calculated assuming a 300-gram rat consumed the entirety of the 3.0 mL adulterated beverage. The 76.21 mg/mL concentration was the first to be assessed for all beverages. Subsequent concentrations were decreased as a result of the consumption observed at the previous concentration(s). This was repeated until a suitable range of concentrations, to include the minimum concentration (17.82 mg/mL; ½ LD_{01} equivalent), was assessed. The concentrations used here were above the determined solubility limit, so homogenous suspensions were created and then immediately dispensed into the dishes.

Statistical Analysis

The median lethal dose estimate and associated 95% confidence interval were obtained using methods similar to those described by Feder et al. [25-27] with IBM SPSS Statistics 22. After each stage, probit dose response models using maximum likelihood methods were fitted to the combined data from all stages. A stopping criterion was used and defined as (95% upper confidence interval of the LD_{50} – 95% lower confidence interval of the LD_{50}) / (2× LD_{50}) < 0.40.

RESULTS

Solubility Determination

Aniline solubility was assessed in room-temperature (21 °C) water as well as refrigerated (4 °C) water, juice, and milk. Room-temperature water was assessed first, and estimates provided in the open literature [43] were confirmed by starting with 0.1 mL of aniline (1.02 g/mL) placed into a vial and adding 3.0 mL of water. A solution was created (34 mg/mL) and then repeated two additional times as confirmation. Refrigerated water was assessed in the same manner, starting with 0.1 mL aniline in 3.0 mL of water. The mixture was an obvious suspension, as

aniline created large globules within the water. Two 1 mL additions of water were needed before a solution was created (20.4 mg/mL), which was replicated an additional two times.

No solubility estimates from the literature were available for apple juice, so the first assessment began with 1 mL of aniline in 4 mL of juice. The mixture turned milky white and looked like a suspension. The second assessment was 0.25 mL of aniline in 4 mL of juice, which was similar to the previous assessment, but less white in appearance. The third assessment, 0.1 mL of aniline in 4 mL of juice, also produced a suspension but was approaching the solubility limit. The fourth assessment, 0.8 mL of aniline in 4 mL of juice, produced a solution (20.4 mg/mL) and was replicated twice. Increasing the amount of aniline to 0.9 mL in 4 mL of juice produced a suspension, demonstrating that the solubility limit had been reached.

As was the case for apple juice, no solubility estimate was available for aniline in milk. Two assessments were conducted concurrently, 2 mL of aniline in 2 mL of milk and 1 mL of aniline in 4 mL of milk, and then allowed to sit on ice (to remain cold) to determine if separation occurred. It was assumed that both of these mixtures would be suspensions based on the solubility estimates from water and juice, but it was too difficult to visually determine the outcome of these assessments. Although the solubility of aniline in milk is likely just below that for aniline in apple juice, it was unable to be accurately determined given the opaqueness of milk.

Median Lethal Dose Determination

A probit model was fit to 24-hour survival data and predicted a median lethal dose of 762.15 mg/kg (95% CI: 550.30 – 983.97 mg/kg; slope: 7.05). The combined probit function and the observed survival proportions are shown in Figure 1. Subjects were continuously observed for the first hour following exposure, and the general progression of toxic signs was noted. Lethargy was the most common sign across all doses, though rats exposed to 400 mg/kg and higher also shook their heads several times in the first few minutes post-exposure and then eventually demonstrated ataxia when ambulating about the cage. Tremor also appeared in the 400 mg/kg group (40% of rats), but was more common at 533.40 mg/kg and higher (75-100% of rats). A loss of posture (or lying prone) was typically comorbid with tremor, though tremor did occur without a loss of posture. Rapid breathing was noted only in rats exposed to ≥711.30 (50-100% of rats), which typically progressed to cyanosis (17-100% of rats). All fatalities but one occurred overnight, so toxic signs (like cyanosis) were likely present prior to death, but were not observed given the timeframe during which they occurred.

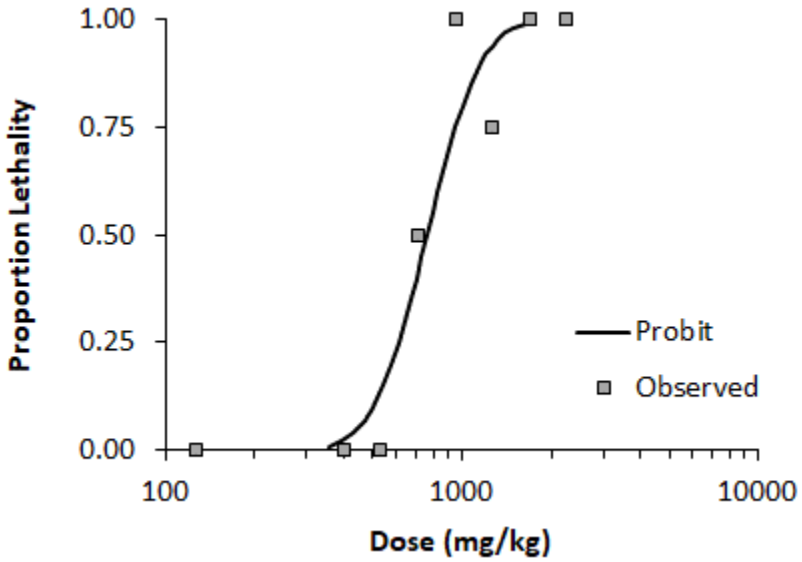

Figure 1. Probit model of 24-hour survival as a function of aniline dose (mg/kg). Observed survival rates at each dose are shown as gray squares, and the fitted model is shown as a black line. The estimated median lethal dose was 762.15 mg/kg (95% CI: 550.30 – 983.97 mg/kg; slope: 7.05).

Organoleptic Assessment

Rats were given the opportunity to voluntarily consume water, apple juice, and milk adulterated with aniline at various concentrations, shown in Figure 2. If the volume consumed was at least 2.5 mL, the adulterated liquid was scored as "accepted" and considered to be generally palatable. The 76.21 mg/mL (LD_{50}) concentration was the first to be assessed, and all rats rejected the adulterated beverages (i.e., consumption volumes were less than 2.5 mL; Water: *M* = 0.52 mL, *SD* = 0.49 mL; Juice: *M* = 0.13 mL, *SD* = 0.23 mL; Milk: *M* = 0.11 mL, *SD* = 0.11 mL). Based on these results, the concentration was decreased to 35.64 mg/mL (LD_{01}), and half of the rats given adulterated water and nine out of ten of the rats given adulterated juice and milk consumed ≥ 2.5 mL of the beverages (Water: *M* = 1.97 mL, *SD* = 1.19 mL; Juice: *M* = 2.87 mL, *SD* = 0.25 mL; Milk: *M* = 2.83 mL, *SD* = 0.32 mL). The concentration was then increased to 61.14 mg/mL (LD_{25}), and none of the rats consumed ≥ 2.5 mL (Water: *M* = 0.19 mL, *SD* = 0.09 mL; Juice: *M* = 0.05 mL, *SD* = 0.05 mL; Milk: *M* = 0.10 mL, *SD* = 0.06 mL). The concentration was then decreased to the minimum concentration (17.82 mg/mL; ½ LD_{01}), and all rats consumed ≥ 2.5 mL (Water: *M* = 2.96 mL, *SD* = 0.03 mL; Juice: *M* = 2.95 mL, *SD* = 0.03 mL; Milk: *M* = 2.95 mL, *SD* = 0.05 mL). A final assessment using 50.14 mg/mL (LD_{10}) was conducted to determine if intermediate consumption volumes could be obtained, relative to the 35.64 and 61.14 mg/mL conditions; only one rat consumed more than 2.5 mL, but mean consumption volumes were marginally higher than at the 61.14 mg/mL concentration (Water: *M* = 0.46 mL, *SD* = 0.82 mL; Juice: *M* = 0.09 mL, *SD* = 0.09 mL; Milk: *M* = 0.25 mL, *SD* = 0.14 mL).

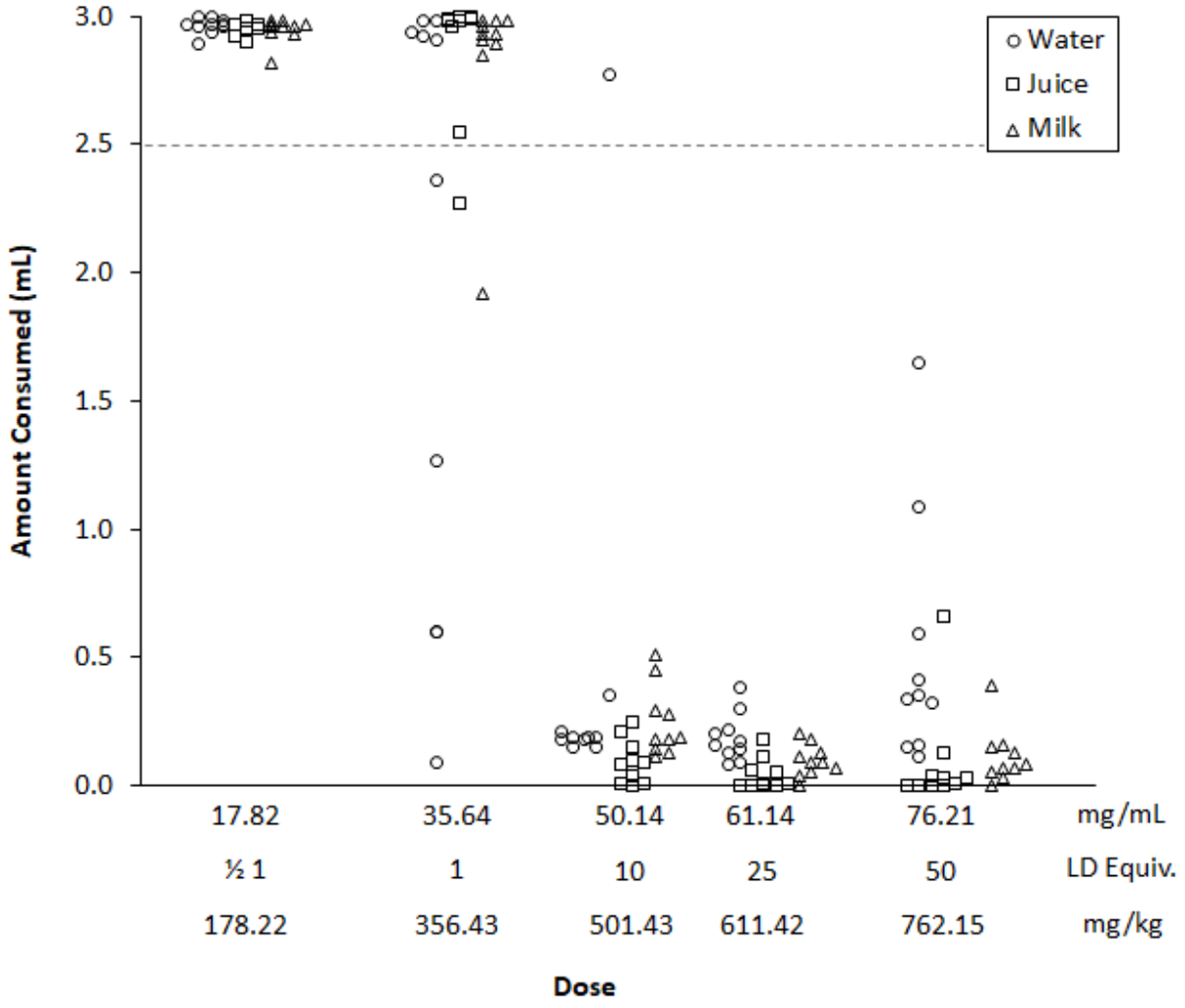

Figure 2. Amount of adulterated vehicle consumed for all concentrations assessed as a function of vehicle. Water is shown as circles, juice is shown as squares, and milk is shown as triangles. Each data point represents an individual subject's volume consumed. The gray, dashed line represents the 2.5 mL threshold to be counted as "accepted."

Concentrations were calculated based on a 300-g rat consuming all 3.0 mL of the adulterated beverage. However, rats vary in weight and consumption values were always less than 3.0 mL. Figure 3 shows actual consumed amounts of aniline, calculated based on each rat's pre-exposure weight and recorded consumption volume. The lowest doses (mg/kg) were from the highest concentrations (50.14 – 76.21 mg/mL), as consumption volumes were lowest in those conditions. The highest obtained doses were from 35.64 mg/mL concentration (*M* = 314.76 mg/kg, *SD* = 101.54 mg/kg), as consumption was markedly higher. However, despite this increase in obtained dose, no animals died. In fact, no lethality was observed in any rat across all conditions of the organoleptic assessment.

Changes in body weight 24 hours after exposure were also recorded as a secondary measure of intoxication and recovery. On average, rats at this stage of development are

expected to gain 1-3% of their body weight overnight. The majority of rats that consumed 17.82 mg/mL (*M* = 1.40%, *SD* = 1.09%) and 50.14 mg/mL (*M* = 1.69%, *SD* = 1.22%) aniline were within this range. The highest consumed doses were from the 35.64 mg/mL concentration, and as expected, weight loss was most pronounced in that condition (*M* = -0.69%, *SD* = 0.68%). The two highest concentrations also had lower weight gain than expected (61.14 mg/mL: *M* = 0.20%, *SD* = 0.76%; 76.21 mg/mL: *M* = -0.13%, *SD* = 0.69%). The consumed doses for the 50.14 and 61.14 mg/mL concentrations appeared very similar, but post-exposure weight gain appeared lower for the 61.14 mg/mL groups. No consistent differences in intoxication or recovery as a function of beverage were observed, but were predicted by the amount of aniline consumed. The small differences in 24-hour weight changes between some of these concentrations were likely not functionally important as rats appeared normal (not intoxicated) by 24 hours post-exposure and would likely recover fully.

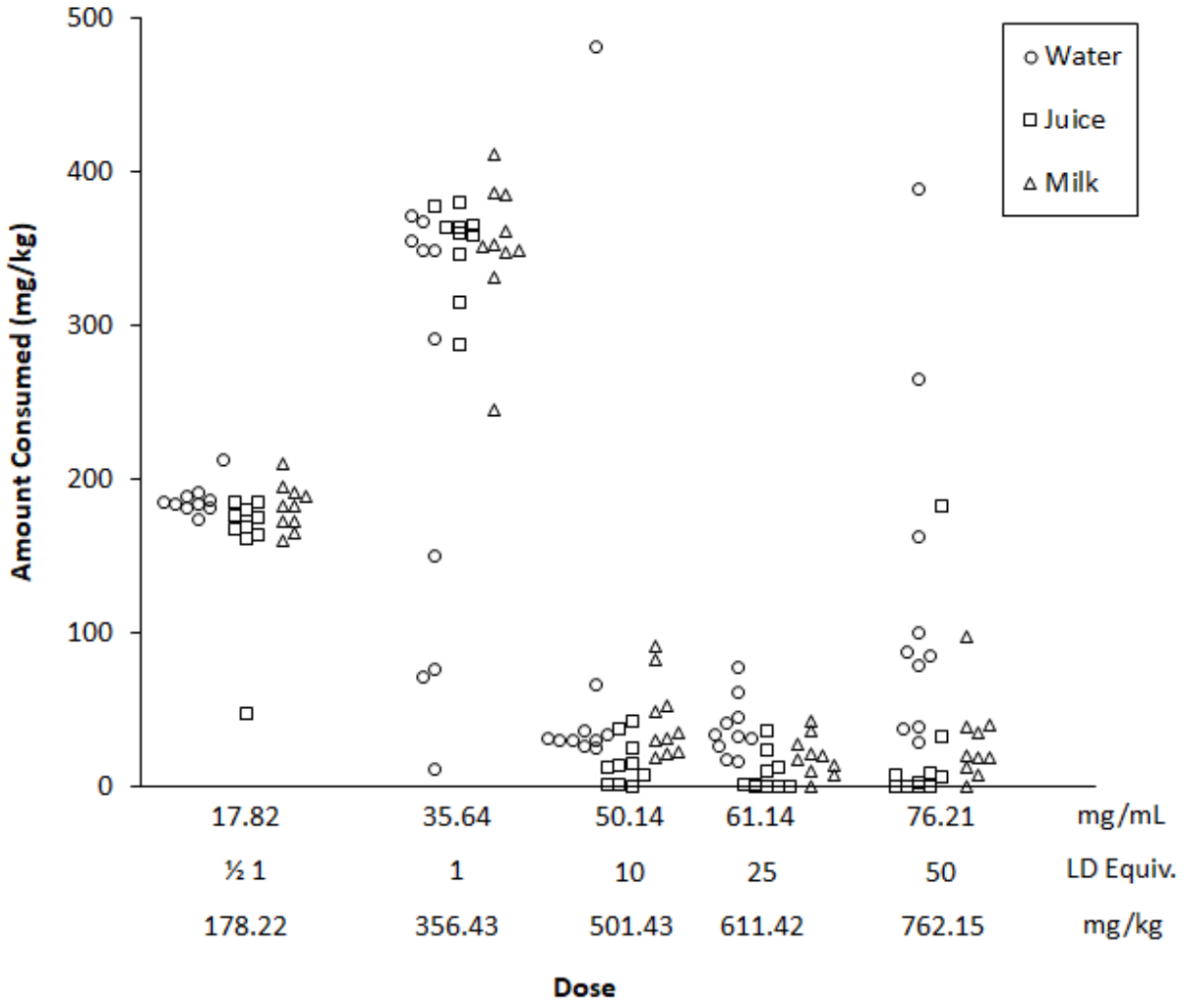

Figure 3. Amount of aniline consumed (mg/kg) calculated from the volume of adulterated beverage consumed, the concentration of the adulterated beverage, and the individual subject's weight. Water is shown as circles, juice is shown as squares, and milk is shown as triangles.

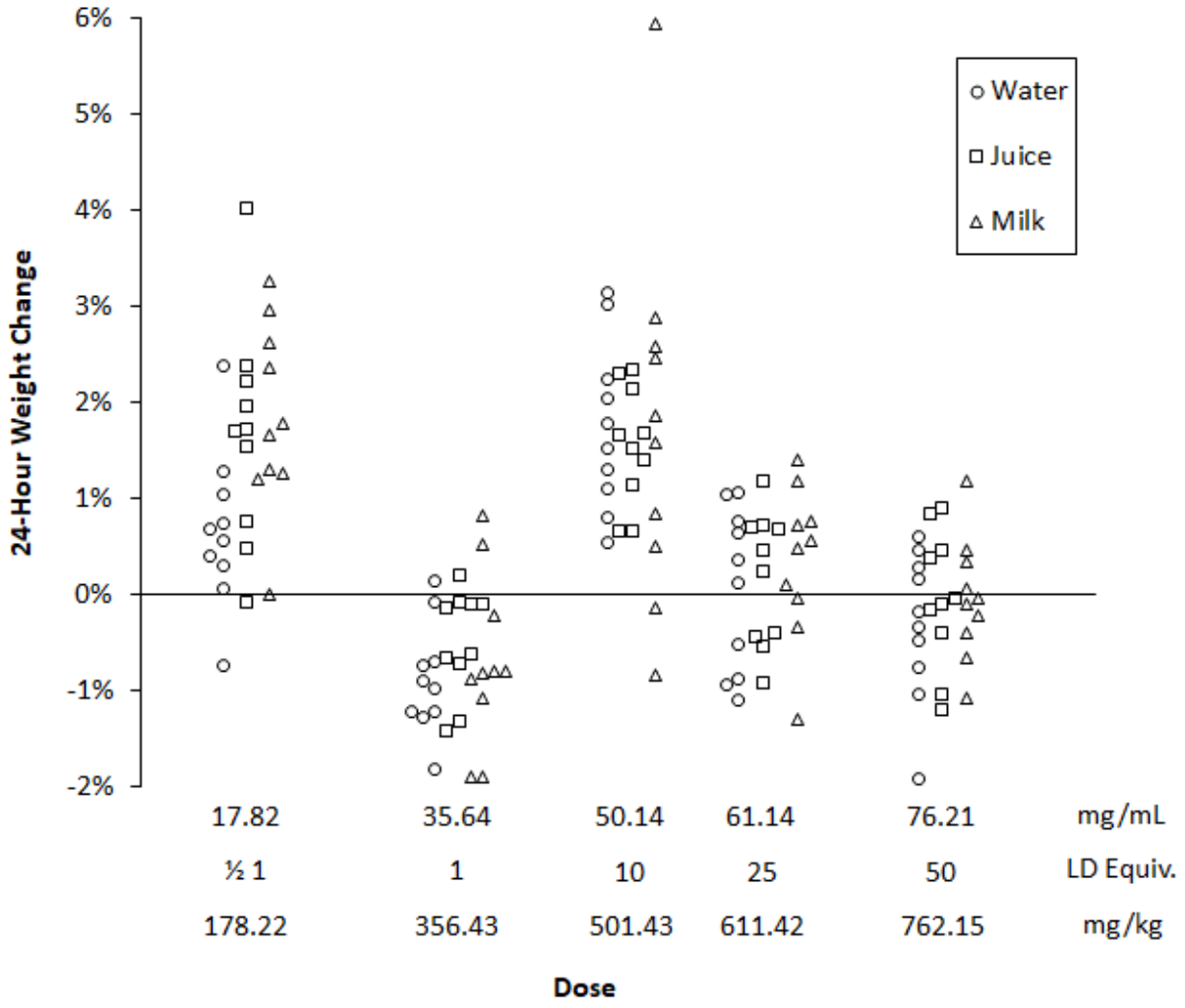

Figure 4. Change in body weight 24 hours after consumption of adulterated beverages for the 0.083 mg/mL (LD_{50}) concentration. There were no survivors in the higher concentration groups. Water is shown as circles, juice is shown as squares, and milk is shown as triangles. Each data point represents an individual subject that survived to 24 hours.

DISCUSSION

A three-phase approach was used in the current experiment to develop a comprehensive threat assessment of aniline as an oral-ingestion hazard. Solubility, acute toxicity, and organoleptics of aniline were assessed and together suggest that aniline is not a high-priority chemical for additional research. The solubility assessment revealed that while aniline was soluble in room-temperature water, it was insoluble in refrigerated beverages at toxic concentrations, but it could be made into a suspension. The use of a solution in the organoleptic assessments is obviously preferred, but because aniline was insoluble we opted to continue the assessment with the aniline suspensions. The careful handling and constant mixing of the aniline suspensions still produced clear dose-dependent changes in consumption volumes. The

required use of a suspension also indicates that aniline is likely a poor adulterant, especially since higher concentrations are more likely to be noticed by manufacturers and consumers.

The toxicity of aniline was then assessed by delivering adulterated room-temperature water via gavage. The median lethal dose (LD_{50}) was estimated to be 762.15 mg/kg, which was 1.7 to 3 times higher than previously reported estimates [4, 44]. The wide range of reported median lethal doses suggests that further experimentation is required, but the dose obtained within this experiment correlates directly to those used in the organoleptic assessment, as the same personnel, rat strain, and chemical batch/lot were used throughout. The median lethal dose obtained here obviously precluded the use of aniline solutions, so suspensions had to be used to assess organoleptics of potentially toxic concentrations.

After the median lethal dose determination, the organoleptic assessment occurred wherein rats were given the opportunity to voluntarily consume (or reject) aniline-adulterated water, apple juice, and 2% milk at various concentrations corresponding to estimated doses from the lethality probit function obtained via gavage. The 762.15 mg/mL (LD_{50}) concentration was the first to be assessed in the refrigerated beverages, and all rats failed to consume a significant portion (2.5 mL) of the adulterated beverages. Based on this obvious rejection, the concentration was decreased to 61.14 mg/mL (LD_{25}), and again all rats rejected (≤ 2.5 mL) the adulterated beverages. The concentration was decreased again to 50.14 mg/mL (LD_{10}), and all rats rejected the adulterated juice and milk, and 9 out of 10 rats rejected the adulterated water. The concentration was again decreased to 35.64 mg/mL (LD_{01}), and acceptance occurred for 9 out of 10 rats in both the juice and milk conditions, but only 5 out of 10 rats when aniline was given in water. The final assessment occurred with 17.82 mg/mL (1/2x LD_{01}), and all rats consumed at least 2.5 mL of the adulterated beverages. Thus, high levels of consumption/ acceptance occurred only at the very low concentrations, relative to the estimated lethal dose. No rats died following the voluntary consumption of any adulterated beverage. At toxic concentrations, rats immediately rejected the beverages and frequently consumed less than 0.20 mL. Rats only began to drink the adulterated beverages at concentrations equal to or lower than the LD_{01}, so the lack of lethality was expected given the small amounts of aniline consumed. Although mild and transient toxicity was observed in the organoleptic assessment, no rats ever appeared to be in any danger of dying. Weight changes appeared normal or near-normal for most groups, with the largest weight loss occurring after rats consumed the 35.64 mg/mL concentration corresponding to the highest obtained dose of any group. However, even the amount of weight loss observed in that condition was mild compared to other, more toxic adulterants [30, 31].

The current experiment demonstrated that rats readily detected and rejected aniline when it was presented in popular consumer beverages. While rats have better chemoreceptive capabilities than humans [33, 34] and are more resistant to aniline toxicity [35], the data collected here suggest that aniline is not a high-priority adulterant. Aldicarb and carfentanil, two other chemicals assessed in this model, produced significant toxicity and lethality and were more soluble [30, 31]. Although aniline may not be a high-priority threat, it could still cause significant harm to consumers if used to adulterate food and beverage products. Lethality is of obvious significance, but hospitalizations and depletion of medical resources could occur

following a mass-casualty, but non-lethal, adulteration event. Aniline may still be able to cause harm, but it is clearly not as dangerous as other chemicals assessed in this model and therefore need not be prioritized for additional sensor and medical countermeasure development.

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