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

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ABSTRACT

Aldicarb, a carbamate pesticide, is an acetylcholinesterase (AChE) inhibitor and one of the most toxic pesticides, with oral median lethal dose (LD_{50}) estimates in rats ranging from 0.46 mg/kg to 0.93 mg/kg. Toxicity in humans has been observed for estimated amounts of aldicarb as low as 0.022 mg/kg. A three-phase approach was used to develop a comprehensive threat assessment of aldicarb as an oral-ingestion hazard. First, the solubility of aldicarb in popular consumer beverages (bottled water, apple juice, and 2% milk) was assessed. Lethality was then assessed by administering aldicarb in bottled water via gavage. A probit model was fit to 24-hour survival data and predicted a median lethal dose of 0.83 mg/kg (95% confidence interval (CI): 0.54 – 1.45 mg/kg; slope: 4.50). 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 as well as liquid eggs adulterated with aldicarb at various concentrations. This organoleptic assessment determined that aldicarb was readily consumed at lethal and supra-lethal doses. Overt toxic signs presented within 5 minutes post-ingestion, and all rats died within 20 minutes after consuming the highest concentration (0.542 mg/mL), regardless of amount consumed. Because rats have more developed chemoreceptive capabilities than humans, these results suggest that aldicarb may be consumed in toxic or even lethal concentrations by humans in a variety of beverages or foods.

INTRODUCTION

Aldicarb (O-(Methylcarbamoyl)-2-methyl-2-(methylthio)propionaldehyd-oxime), a carbamate pesticide developed in 1962 by Union Carbide [1], was previously sold in the U.S. under the tradename Temik (registrant: Bayer CropScience) and is now marketed as Meymik/AgLogic (registrant: AgLogic Chemical LLC) [2]. It is registered for agricultural use as solid granules applied to the soil to protect cotton, dry beans, peanuts, soy beans, sugar beets, and sweet potatoes against insects, mites, and nematodes [3]. The U.S. Environmental Protection Agency has classified aldicarb as a category 1 toxin due to its extreme oral toxicity [4], so certification is required for its purchase and application. Despite the ban on household use in the U.S., aldicarb is sold in the Dominican Republic and Mexico under the tradename Tres Pasitos (Three Little Steps; named for the number of steps taken before an animal dies following ingestion) [5, 6] and sold illegally in South Africa as a rodenticide named Two Step [7].

Aldicarb, like all carbamate pesticides, is an acetylcholinesterase (AChE) inhibitor, and its toxicity manifests as muscarinic hyperactivity (e.g., bradycardia, salivation, lacrimation, bronchorrhea, emesis, and miosis) and nicotinic stimulation (e.g., ataxia, sweating, muscle weakness, tachycardia, fasciculation, tremor, and convulsions) [5, 6, 8, 9]. Unlike organophosphate pesticides, which are also AChE inhibitors, the binding of aldicarb to AChE (as well as to butyrylcholinetserase, BuChE) is reversible [9, 10]. Aldicarb also undergoes rapid oxidization to aldicarb sulfoxide and aldicarb sulfone, which are then hydrolyzed to other non-toxic compounds [9, 11]. Like most carbamate pesticides, aldicarb's half-life is short; approximately 90% of aldicarb is excreted in the urine within 24 hours, and AChE inhibition can recover within 6 hours of exposure [11]. Despite this, the detection of aldicarb-inhibited BuChE following oral ingestion by a human (suicide attempt) proved to be a useful method of poison identification 7 hours after ingestion [10].

Aldicarb is primarily an oral hazard and is rapidly and almost completely absorbed in the gastrointestinal tract. The onset of toxicity following ingestion can occur as rapidly as 5 minutes in rats [12] and 15 to 30 minutes in humans [13]. Aldicarb is one of the most toxic pesticides, with oral median lethal dose (LD_{50}) estimates in rats ranging from 0.46 mg/kg [14] to 0.93 mg/kg [9]; toxicity in humans has been observed for estimated amounts of aldicarb and aldicarb sulfoxide as low as 0.022 mg/kg [15] and 0.0011 mg/kg [16], respectively. The rat oral LD_{50} estimate of aldicarb sulfoxide is 0.88 mg/kg and aldicarb sulfone is 25 mg/kg [9].

The first reported poisoning was a woman who consumed mint grown near an aldicarb-treated rose bush [cited in 17]. Since that time, aldicarb has been found in groundwater in Arizona, California, Florida, Maine, New York, North Carolina, Virginia, and Wisconsin [18, 19], despite the laboratory and field data collected prior to its registration that suggested aldicarb would not reach groundwater [18]. There have also been multiple poisoning incidents due to aldicarb-treated cucumbers and watermelon [16]. In the most severe incident, over 1000 people were poisoned after eating watermelons grown in a field treated with aldicarb [20]. Aldicarb has been used maliciously to injure or kill pets in South Africa [7, 21] and been

implicated in several intentional human poisonings [13, 22]. Aldicarb has also been used for suicide, and several accidental human exposures have required emergency medical treatment [6, 13, 23-25], including two epidemics in New York [26] and Rio de Janeiro [27]. The toxicity of aldicarb is well understood and its potency is cause for concern, but aldicarb's potential to cause widespread harm as a food or beverage adulterant has not been evaluated. The current study assessed the threat of aldicarb as a mass-casualty oral-ingestion hazard using our established solubility, toxicity, and organoleptic assessments, previously used to comprehensively evaluate carfentanil [28].

Assessing oral-ingestion hazards requires the *voluntary* consumption of the chemical threat agent. Many studies investigating oral toxicity use gavage; however, this intra-esophageal administration of a compound bypasses important oral mucosa, preventing possible intra-oral absorption (e.g., buccal absorption) while completely ignoring the importance of a compound's organoleptic properties (e.g., taste, smell, texture). 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. The organoleptic properties of many chemicals are understudied in toxicology, but are critical in determining which chemicals would be consumed in harmful amounts.

Rats were used for the current assessments because they eat many of the same foods that humans eat, so the actual food and/or drink items of interest may be adulterated to determine realistic oral-ingestion threats. Likewise, rats are neophobic [29] 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 [30, 31], so any compound a rat consumes in toxic concentrations would likely be consumed by humans as well. In this study, we leveraged the rat's chemoreceptive capabilities to test the organoleptic properties of aldicarb in several beverages popular in the U.S. (bottled water, apple juice, and 2% milk) as well as in liquid eggs (a liquid food product that has widespread use within commercial bakeries and restaurants). By assessing solubility, oral toxicity, and organoleptics via voluntary oral ingestion, we were able to develop a comprehensive threat assessment of aldicarb as an oral-ingestion hazard.

METHODS

Chemicals and Vehicles

Aldicarb (O-(Methylcarbamoyl)-2-methyl-2-(methylthio)propionaldehyde-oxime; ≥ 98% purity) was obtained from Sigma-Aldrich and stored protected from light at room temperature. All handling of aldicarb 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), Cloverland® 2% milk (1 pint, 473 mL; single bottle), and Egg Beaters® whole liquid eggs (32 oz, 946 mL) 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. The milk and eggs were purchased at the beginning of each week and kept refrigerated at approximately 4 °C.

Subjects

One hundred thirty (130) 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 100 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 under 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. 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 [32-34].

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

Aldicarb 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 aldicarb (4.45 – 7.39 mg; M = 5.65, SD = 1.04) placed into a vial followed by 0.6 – 4.2 mL of a beverage. An incremental volume of 0.025 – 0.05 mL (based on the expected solubility) 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 the solution at the appropriate temperature. This assessment was conducted three times for each beverage. Solubility was not assessed with liquid eggs, as a homogenous suspension met the needs of the organoleptic assessment.

Median Lethal Dose Determination

A stagewise, adaptive dose design [35-37] was used to determine the median lethal dose (LD_{50}) of aldicarb in 21 °C (room temperature) water. Doses for the first stage were selected based on the available literature [38]. 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, then checked hourly thereafter until 5 hours post-exposure. A final observation occurred at 24 hours post-exposure, and survivors were 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 all rats met the criterion.

The vehicles were adulterated with aldicarb 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 0.083 mg/mL (LD_{50} equivalent), 0.271 mg/mL (LD_{99} equivalent), and 0.542 mg/mL (2x LD_{99} equivalent). The LD equivalents were calculated assuming a 300-gram rat consumed the entirety of the 3.0 mL adulterated beverage. The 0.083 mg/mL concentration was the first to be assessed for all beverages. Subsequent concentrations were increased as a result of the consumption observed with the previous concentration(s). This assessment was repeated with new concentrations when at least 9 out of 10 rats consumed at least 2.5 mL of the adulterated beverage or the maximum concentration (0.542 mg/mL; 2x LD_{99} equivalent) was reached. Liquid eggs were only evaluated at the 0.271 mg/mL (LD_{99}) concentration.

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. If

the stopping criteria were not met and the maximum number of animals was reached, no further animals were used. The estimated LD_{50} at that point was accepted as adequate.

RESULTS

Solubility Determination

The solubility of aldicarb was assessed in multiple beverages, both room-temperature and refrigerated, as shown in Table 1. Refrigeration decreased solubility, as did the dissolved solids and other physical attributes of the juice and milk.

Table 1

Solubility of aldicarb in bottled water, apple juice, and 2% milk. Solubility was assessed three times (indicated by the numbered headings) per beverage, including an additional assessment with room-temperature (21 °C) water. All solubility data are presented as mg/ml.

Beverage	Temp	1	2	3	Mean	SD
Water	21° C	3.96	4.14	3.72	**3.94**	0.21
Water	4° C	2.80	2.59	2.54	**2.64**	0.14
Juice	4° C	1.83	1.64	1.56	**1.68**	0.14
Milk	4° C	1.68	1.56	1.45	**1.56**	0.12

Median Lethal Dose Determination

A probit model was fit to 24-hour survival data and predicted a median lethal dose of 0.83 mg/kg (95% CI: 0.54 – 1.45 mg/kg; slope: 4.50). 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. Ataxia and a loss of posture (or lying prone) were the most common initial signs, although the 0.576 mg/kg and higher doses commonly (~50%) did not produce ataxia before the rats began exhibiting more severe toxic signs. Fasciculation was not observed at the lowest dose (0.309 mg/kg), but was observed in at least half of the rats at all other doses. Tremor was observed at all doses and was more frequent with higher aldicarb doses. Gasping was either not observed or infrequently observed (1 out of 4 rats) at the 0.795 mg/kg dose and below. Higher doses usually produced more frequent gasping, with the exception of the 1.048 mg/kg dose (1 out of 4 rats exhibited gasping). All rats that died (n = 12) had at least one convulsion, though not all convulsive episodes led to death (n = 4). The 0.550 mg/kg dose was the lowest that produced convulsions, and higher doses typically produced more frequent and severe convulsions.

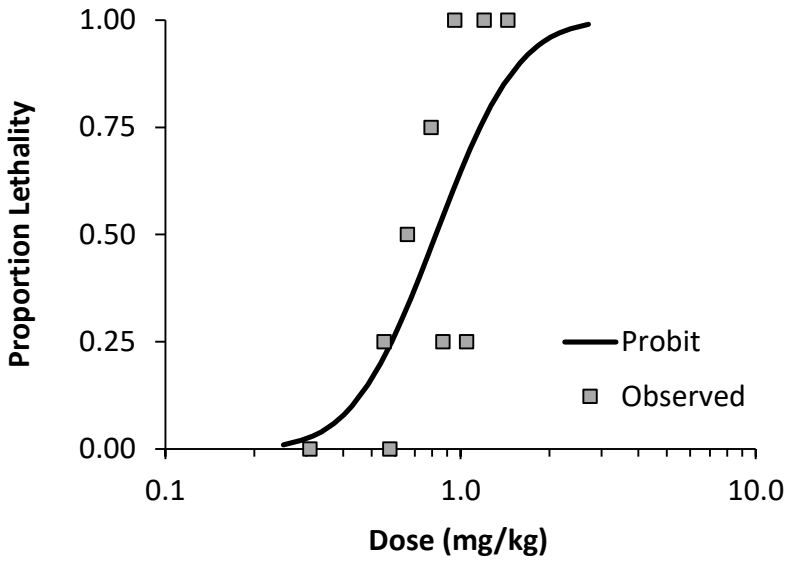

Figure 1. Probit model of 24-hour survival as a function of aldicarb dose (mg/kg). Observed survival rates at each dose are shown as gray squares, and the fitted model is shown as a black line. The estimated median lethal dose was 0.83 mg/kg (95% CI: 0.54 – 1.45 mg/kg; slope: 4.50).

Organoleptic Assessment

Rats were given the opportunity to voluntarily consume water, apple juice, milk, or liquid eggs adulterated with aldicarb 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 number of rats that accepted the adulterated vehicles is shown in Table 2. The 0.083 mg/mL (LD_{50}) concentration was the first to be assessed, and rats consumed the entirety of the adulterated beverages (eggs were not assessed at this concentration). Volumes were mistakenly recorded as "3.0" to represent the entirety of the volume, though in reality the volumes likely varied from 2.60 to 3.00 mL, as evidenced by the volumes consumed at higher concentrations. Based on these results, the concentration was increased to 0.271 mg/mL (LD_{99}), and once again all of the rats consumed ≥ 2.5 mL of the adulterated beverages. The concentration was then increased to 0.542 mg/mL (2x LD_{99}), and rats consumed ≥ 2.5 mL in almost all cases. Three rats (1 juice and 2 milk) stopped drinking prior to the 2.5 mL cutoff, suggesting that the juice and milk may have failed to mask the taste of aldicarb. However, it seems more likely that rapid intoxication occurred prior to completing consumption, because overt toxic signs were noted within 2 minutes in some subjects and all subjects exhibited overt intoxication within 5 minutes at this concentration of aldicarb. As discussed below, the three rats that failed to reach the 2.5 mL "acceptance" criterion still consumed a lethal dose of aldicarb.

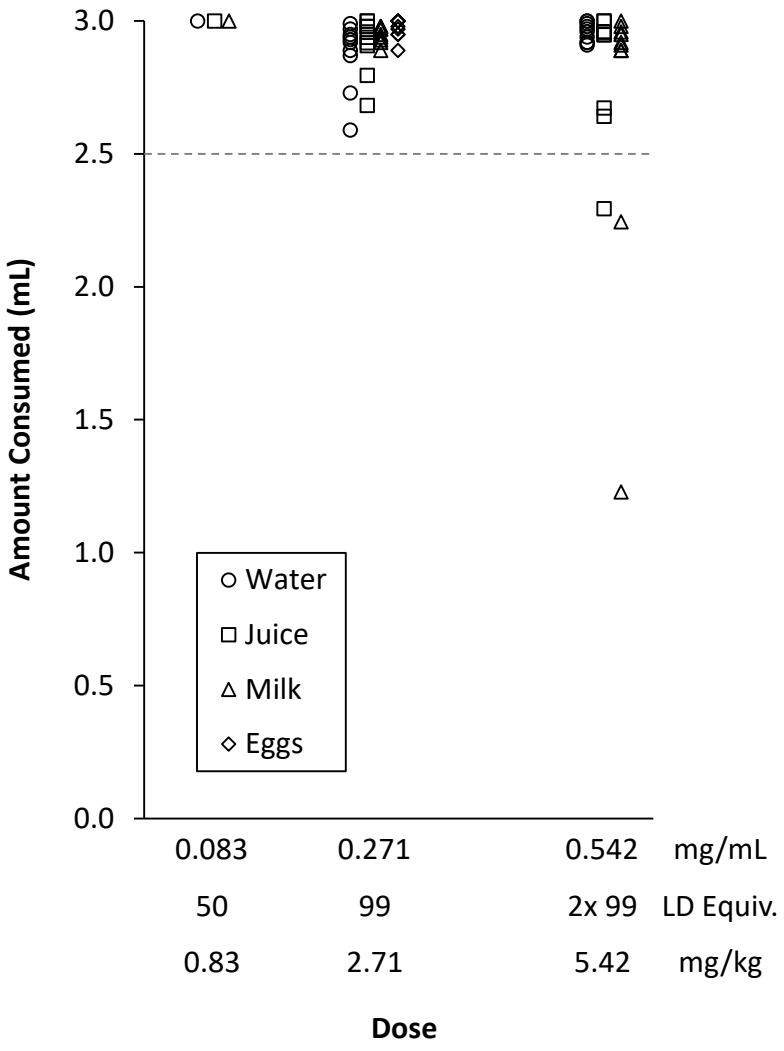

Figure 2. Amount of adulterated vehicle consumed for all concentrations assessed as a function of vehicle. Water is shown as circles, juice is shown as squares, milk is shown as triangles, and eggs are shown as diamonds. Each data point represents an individual subject's volume consumed. The gray, dashed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

Table 2

The number of rats that "accepted" (i.e., consumed at least 2.5 mL) the adulterated vehicles as a function of concentration (shown as LD equivalents).

	Concentration (LD Equivalent)		
Vehicle	**LD_{50}**	**LD_{99}**	**2x LD_{99}**
Water	10/10	10/10	10/10
Juice	10/10	10/10	9/10
Milk	10/10	10/10	8/10
Eggs	-	10/10	-

As shown in Table 3, 24-hour lethality following voluntary consumption of aldicarb-adulterated beverages was exactly as predicted in water (5 of 10 rats died), but lower than expected in juice (3 of 10 died) and milk (1 of 10 died) at the 0.083 mg/mL (LD_{50}) concentration based upon the median lethal dose determination using gavage. All rats that consumed the adulterated vehicles at the 0.271 mg/mL (LD_{99}) and 0.542 mg/mL (2x LD_{99}) concentrations died within an hour, regardless of the vehicle used. Liquid eggs also served as a suitable vehicle for aldicarb at the 0.271 mg/mL (LD_{99}) concentration, as all rats readily consumed the adulterated eggs and died. At the highest dose assessed (across all three beverages), 19 out of 30 rats were dead within 10 minutes, 27 out of 30 rats were dead within 15 minutes, and all rats were dead within 20 minutes, demonstrating the rapid lethality of this aldicarb concentration.

Table 3

The number of rats that died within 24 hours of consuming an adulterated vehicle as a function of concentration (shown as LD equivalents).

	Concentration (LD Equivalent)		
Vehicle	**LD_{50}**	**LD_{99}**	**2x LD_{99}**
Water	5/10	10/10	10/10
Juice	3/10	10/10	10/10
Milk	1/10	10/10	10/10
Eggs	-	10/10	-

Changes in body weight 24 hours after exposure were also recorded as a secondary measure of intoxication (or recovery). The body weight changes are shown in Figure 3 for any rat that survived to 24 hours. Rats typically gain 1-3% of their bodyweight per day prior to exposure. As no rats survived following the consumption of the two highest concentrations, those groups are excluded from the x-axis. All rats given adulterated water lost weight overnight and also lost more weight on average than rats given juice or milk. These results corroborate the survival data, in that rats given water were more likely to die and those that survived also appear to have been more severely intoxicated or for a longer duration. Some of

the rats given adulterated juice and milk showed weight gain overnight, though the group average weight change was around 0% for both groups, suggesting that mild to severe intoxication occurred in some rats, whereas a few rats recovered in time to eat and drink during the 2-hour window that water was made available.

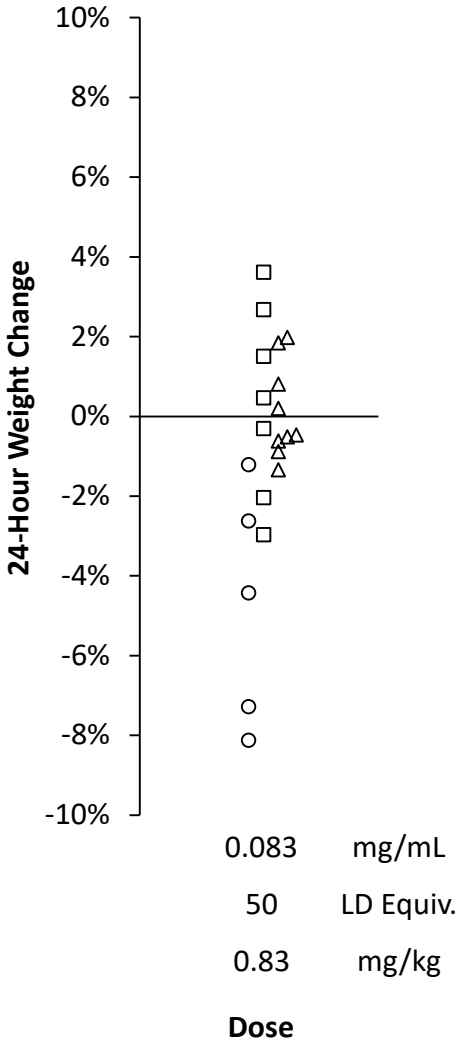

Figure 3. Change in body weight 24 hours after consumption of adulterated beverages for the 0.083 mg/mL (LD_{50}) concentration. There were no survivors in the higher concentration groups. Water is shown as circles, juice is shown as squares, and milk is shown as triangles. Each data point represents 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 aldicarb as an oral-ingestion hazard. First, the solubility of aldicarb in room-temperature water and three refrigerated beverages was assessed. Solubility was decreased by refrigeration and also varied by beverage type, as shown in Table 1. Solubility was highest for water, followed by juice, and finally milk. Solubility was not assessed for liquid eggs, although a homogenous suspension was sufficient for use in the organoleptic assessment. The thorough mixing used to create the suspension, lack of visible residue, and resulting toxicity that matched the beverage vehicles suggest that the suspension was homogenous, and the rats consumed the intended amounts of aldicarb. The toxicity of aldicarb was then assessed by delivering adulterated room-temperature water via gavage. The median lethal dose (LD_{50}) was estimated to be 0.83 mg/kg, which approximated previously reported estimates [9, 38]. Based on the solubility and toxicity data, we were able to assess up to our pre-determined maximum of 2x the LD_{99} concentration in all beverages. However, a maximum of 5.75x the LD_{99} concentration was attainable with these refrigerated beverages, which could be increased to 9.5x the LD_{99} in refrigerated water or 14.5x the LD_{99} using room-temperature water.

The organoleptic assessment occurred after the median lethal dose determination, wherein rats were given the opportunity to voluntarily consume (or reject) aldicarb-adulterated water, apple juice, 2% milk, and liquid eggs at various concentrations corresponding to estimated doses from the probit function. The 0.083 mg/mL (LD_{50}) concentration was the first to be assessed in the refrigerated beverages, and all rats consumed the entirety of the adulterated beverages. Based on this obvious lack of rejection, the concentration was increased to 0.271 mg/mL (LD_{99}), and again all rats accepted (consumption ≥ 2.5 mL) the adulterated beverages, as well as the liquid eggs. The concentration was then increased to the 0.542 mg/mL (2x LD_{99}) maximum, and all but three rats (90%) drank more than 2.5 mL of the adulterated beverages. The three rats that did not meet the 2.5 mL criterion may have detected (and subsequently rejected) the aldicarb or may have become intoxicated during consumption. Previous research has shown that intoxication can occur within 5 minutes [12] (the consumption duration allowed in the current experiment), and toxic signs noted during the median lethal dose determination were similarly rapid. Therefore, it is possible that aldicarb is not detectable (based upon the lack of rejection) at the 0.542 mg/mL (2x LD_{99}) concentration and incomplete consumption was in fact due to rapid onset of intoxication.

Intoxication in the organoleptic assessment was quantified by 24-hour lethality and weight change. In animals that survive, weight change serves as a good indicator of the duration of intoxication, as animals that recover sooner are more likely to consume food and gain weight overnight. This is particularly true of the current experiment, as water access is scheduled and occurs relatively soon after aldicarb ingestion. Any rats that were intoxicated for extended periods may fail to drink their daily allotment of water. This measure successfully quantified intoxication of carfentanil in this same model [28], though the exceptionally high rates of

lethality with aldicarb make the analysis more difficult. Only rats given 0.083 mg/mL (LD_{50}) aldicarb survived to 24 hours, so no dose-dependent weight changes could be evaluated. However, beverage-dependent intoxication may be evident as rats that consumed water typically lost more weight than those ingesting juice or milk. Lethality was also higher for rats that drank water (50%) compared to juice (30%) and milk (10%). A similar trend was found following carfentanil ingestion in this same model: lethality was highest for the water group compared to milk and juice [28]. The solids and other nutritive properties of the beverages (e.g., milk's fat content) could have altered aldicarb's toxicity, although these differences might simply reflect normal between-subject variation, as only 10 rats were assessed with each beverage at this concentration, and additional subjects would be required to rigorously test this hypothesis. Although decreased lethality was observed for both carfentanil and aldicarb when ingested in juice or milk, the low number of subjects and survivors within each group precludes any definitive statements about beverage-dependent toxicity. These results underscore the value of evaluating different beverages and suggest potential avenues for future research. Additionally, toxicity and lethality may in fact vary as a function of the beverage adulterated, which would clearly have implications for modeling large-scale attacks.

The absorption of ingested aldicarb is rapid and nearly complete, as demonstrated when radio-labeled aldicarb and aldicarb sulfoxide were ingested by female rats: 80-90% was excreted in urine and very little was found in the feces (2-5%) within the first 24 hours [39]. This finding was replicated in male rats, wherein several aldicarb radio-labeled isotypes were all primarily excreted via the urine within 24 hours and aldicarb recovery was up to 95% [11]. Both of these studies also revealed that aldicarb was generally distributed around the body and sequestration by a tissue or tissue group did not occur [11, 39]. The rapid absorption and excretion of aldicarb was also found in cows: 92%, 3%, and 1% of the ingested aldicarb was eliminated in the urine, feces, and milk, respectively [40]. The fact that very little aldicarb is found in the feces and is primarily excreted in the urine demonstrates the near complete absorption that occurs in the gastrointestinal tract. This rapid absorption can also produce rapid intoxication. Rats that ingest high doses of aldicarb can show signs of intoxication within 5 minutes, as found in the current experiment and in previous research [12], and intoxication has been reported to occur within 15 minutes for human exposures [13].

Aldicarb is an AChE inhibitor, and the toxic signs reported in human cases are typical for cholinergic crisis, including both muscarinic and nicotinic overstimulation [6, 13]. These toxic signs are sometimes summarized using the mnemonics SLUDGE (salivation, lacrimation, urination, defecation, gastric distress, and emesis) or DUMBBELLS (defecation, urination, miosis, bronchorrhea, bradycardia or body tremors, emesis, lacrimation, lethargy, salivation) [5, 41]. The toxic signs observed in the rat do not directly replicate those seen in humans; here, rats ingesting aldicarb primarily exhibited ataxia, lethargy, mastication, lacrimation (porphyrin excretion), fasciculation, tremor, and convulsion. These toxic signs were dose-dependent, with the most severe signs occurring more commonly at higher doses of aldicarb.

The toxic signs observed in the current experiment differed from the human response in several key ways. Unlike humans, rats are unable to vomit, so emesis cannot be observed in this species. Urination and defecation often occurred post-exposure, but it was difficult to compare to pre-exposure and was therefore inconclusive. We also lacked the ability to systematically score bronchorrhea, and while salivation was sometimes noted post-exposure, there was no clear dose-dependent effect. However, both bronchorrhea and salivation could have manifested and simply not been observable given our methodology. Despite these differences to human clinical signs, clear dose-dependent toxicity was observed in this species, and the toxic signs observed in the current experiment provide valuable comparisons to those same clinical signs in humans.

The primary purpose of the current experiment was to establish a median lethal dose and assess the organoleptic profile of aldicarb. Thus, toxic signs were a secondary measure and could be significantly improved upon with a different approach. Previous research quantifying the resulting toxicity of ingested aldicarb produced a list of toxic signs similar to those seen here in addition to other important signs (i.e., decreased body temperature, motor response, and pupillary response) [8]. Large doses of aldicarb given to Nubian goats also produced hemorrhage and congestion in several organ systems, including the brain, gastrointestinal tract, kidneys, liver, and lungs. This was comorbid with pulmonary edema, hepatic fatty changes, and renal degeneration [42], demonstrating the systemic distribution of aldicarb as well as its profound toxicity.

Treatment of aldicarb poisoning is mostly supportive and combinatorial atropine sulfate and oxime therapy is recommended, as would be expected for an ongoing cholinergic crisis. Atropine treats the toxic signs produced by muscarinic overstimulation, but nicotinic overstimulation (e.g., fasciculation, tremor, and convulsion) will likely persist [5, 43]. Atropine therapy has been successfully used in humans, typically with adjunct pralidoxime (2-PAM) therapy [6, 13, 22]. Benzodiazepine therapy is also commonly recommended and used for controlling convulsions [44]. Gastric lavage and activated charcoal have been used to treat ingested poisons, depending on the time since ingestion, though the efficacy and appropriateness of both methods for acute poisonings have come into question [45-47]. Ventilation is also commonly used for human poisonings [22, 48], though typically in response to bronchial secretions rather than muscular weakness [6]. Although these therapies are efficacious, they require prompt administration following ingestion, especially given aldicarb's rapid absorption and extreme oral toxicity. These facts combined with the present data raise a deep concern that, in the absence of rapid medical management and treatment, aldicarb poisoning could prove promptly fatal.

The current experiment demonstrated that rats would readily and voluntarily consume lethal amounts of aldicarb, indicating that aldicarb is a clear oral-ingestion hazard. This is further confirmed by the fact that, in 2001, Bayer CropScience added extremely bitter substances to the pesticide to prevent its use in suicides and homicides [cited in 7]. The

necessity of adding a bitter substance to prevent its voluntary consumption in humans suggests that aldicarb either has an undetectable organoleptic profile or at the very least is not aversive. Rats have more developed chemoreceptive capabilities than humans, so the voluntary consumption of aldicarb in the current experiment suggests that aldicarb may be consumed in toxic or lethal amounts by humans in a variety of foods and beverages.

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