Effects of Alternate Water Sources on Weight Gain, Blood Chemistries, and Food Consumption in Sprague Dawley Rats and ICR Mice following Ground Transportation

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ABSTRACT

Although there are numerous commercial products sold for water source of laboratory animals during transit, limited information is available on their effects on body weight, blood chemistries, and food consumption of rodents during shipment. To evaluate the effects of either a commercially available water source, hydration gels or autoclaved potatoes on the health of Hsd:ICR (CD-1) female mice (approximately 21 to 24 g of weight) and Hsd:Sprague Dawley (SD) male rats (approximately 100 to 124 g of weight) over 5 d of transportation, we measured body weight change, food consumption, and approximate water source consumption during transit and select clinical pathology parameters following transit (Hct, BUN, ALT, ALP, and TP). Additionally, ambient shipping box temperature was monitored using a temperature logger at intervals of every 6 h over 5 d in transit. We found that both rats and mice provided autoclaved potatoes showed minimal dehydration based upon nonfasting blood parameters compared to animals provided ample hydration gels. Weight gain was noted in both mice and rats provided *ad libitum* hydration gels as a water source. Additionally, rats and mice provided hydration gels as a water source maintained a higher daily feed consumption. Our results suggest that provision of hydration gels minimizes dehydration or anorexia in rats and mice and supports weight gain in transit.

INTRODUCTION

The exposure to different stimuli during transportation such as temperature fluctuation, housing density and water source may influence induced stress in laboratory animals. This transportation stress effecting experimental results has the ability to jeopardize the validity of scientific research and increase the adaptation period of animals following relocation (Van Ruiven *et al*, 1998). Minimizing variables that may influence physiological parameters while in transit is a service that can be provided by vendors and collaborators. Provision of diet and a hydration source is both a standard and regulatory requirement in the shipment of laboratory animals. Our study was completed to evaluate the use of a commercially available water source, hydration gels versus autoclaved potatoes after transportation of both rats and mice in interstate travel. The prevention of dehydration and maintenance of equilibrium of this commercially available water source and potatoes were compared in outbred mice and rats. In addition, the effect of packing density of mice on hydration, weight gain, food consumption, and box temperature were evaluated.

METHODS

Animals

Cohorts of thirty (30) and forty (40) Hsd:ICR(CD-1)® mice approximately 20-23 grams of weight and 16 Hsd:Sprague Dawley®™ SD®™ rats weighing 100-115 grams were randomly selected for shipment. ICR mice and SD rats were produced within maximum security production barriers and provided autoclaved 18% protein Teklad Global Diet 2018S and *ad libitum* water in advance of shipment. Animals were tested and shown to be free of common rodent pathogens.

Experimental Design

Prior to shipment, mice and rats were identified and provided either autoclaved potatoes or different volumes of gel (Hydrogel™, ClearH$_2$O®, Portland, ME), supplied in a kit attached to the side of the box, or on the floor. Animals were placed within shipping containers using the animal density and gel volumes as defined in Table 1 and provided with a measured amount of feed. Temperature monitors were included in representative boxes for determination of animal density conditions. All groups were shipped interstate in environmentally controlled vehicles to mimic a five (5) day transit period. Upon arrival, the conditions of the animals and boxes were evaluated, the body weights of the mice and rats were measured, and feed was weighed. Animals were euthanized using carbon dioxide and cervical dislocation at the end of evaluation. Blood samples were collected by intracardiac puncture and evaluated for serum chemistry and hematology from groups of animals that still had remaining gel and from groups receiving potatoes. Temperature transmitters were removed upon arrival and the data transcribed for analysis.

Table 1
Summary of Experimental Groups

Species/Strain	Number in Box	Water Source
Mouse/ICR	40	Gel, kit, 32 oz
Mouse/ICR	40	Gel, floor, 32 oz
Mouse/ICR	40	Potato
Mouse/ICR	30	Gel, kit, 32 oz
Mouse/ICR	30	Gel, floor, 32 oz
Mouse/ICR	30	Potato
Rat/SD	16	Gel, kit, 48 oz
Rat/SD	16	Gel, floor, 48 oz
Rat/SD	16	Potato
Mouse/ICR	40	Gel, kit, 48 oz
Mouse/ICR	40	Gel, floor, 48 oz
Mouse/ICR	30	Gel, kit, 48 oz
Mouse/ICR	30	Gel, floor, 48 oz
Rat/SD	16	Gel, kit, 64 oz
Rat/SD	16	Gel, floor, 64 oz

Statistical Analysis

For each variable evaluated (location of gel kit, water source, amount of gel, box density), comparisons of percent body weight change before and after shipping, and clinical pathology parameters were made between groups in which the other variables were controlled. Differences in mean box temperature were evaluated between mice at different box densities. Analysis of Variance (ANOVA) was performed on each parameter and if an overall difference between groups was found at p< 0.05, between group comparisons were made using the least squares means analysis and Tukey's test for Honestly Significant Differences (JMP 7.0, SAS Institute, Inc.) Significant differences between groups at p< 0.05 are summarized in the Figures 2, 3 and 4, and Tables 2 and 3. Mean food consumption per animal for each box was calculated, but statistical comparison of means was not performed because only a single data point was available for each condition.

All animals appeared normal after shipping. All potatoes were almost completely consumed, with remaining pieces showing signs of mold. Gel at the lower volumes was completely consumed. Remaining gel at the higher volumes could not be estimated because it was often mixed in with bedding. In general, the appearance of the bedding and animals were cleaner with the use of the gel kit.

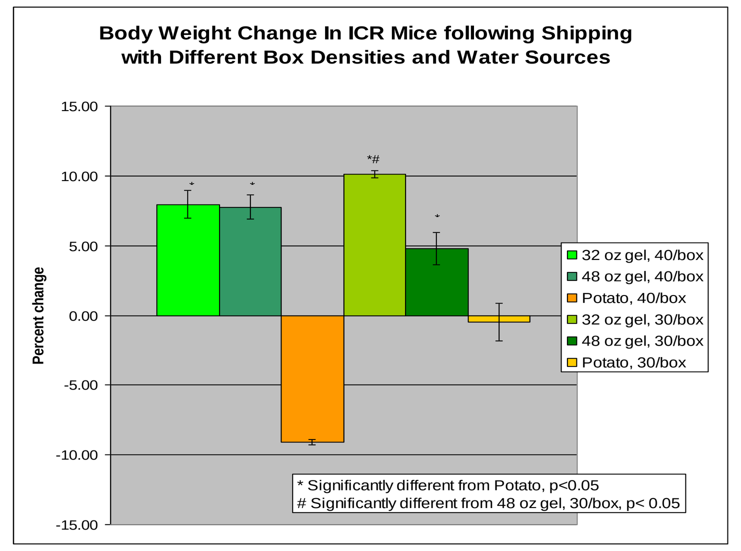

Figure 1
Percent change body weight was higher in mice receiving gel compared to potatoes as a moisture source during shipping. The amount of gel per box and animal density were not significant factors in weight change during shipping. Animal density was a significant variable for animals receiving potatoes.

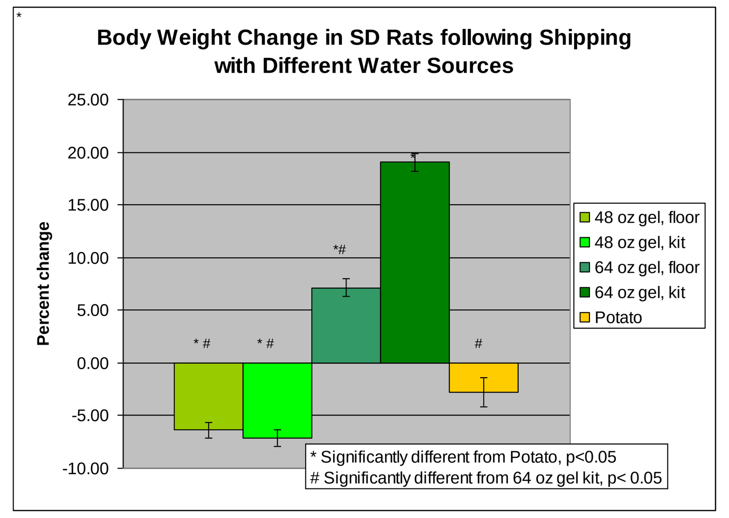

Figure 2
Percent change body weight was significantly higher in rats receiving 64 oz gel in a side-hanging kit compared to placement on the floor or 48 oz gel provided on the floor or in a kit. 64 oz gel resulted in significantly higher weight gain compared to those receiving potatoes, and lower in animals receiving 48 oz gel.

RESULTS

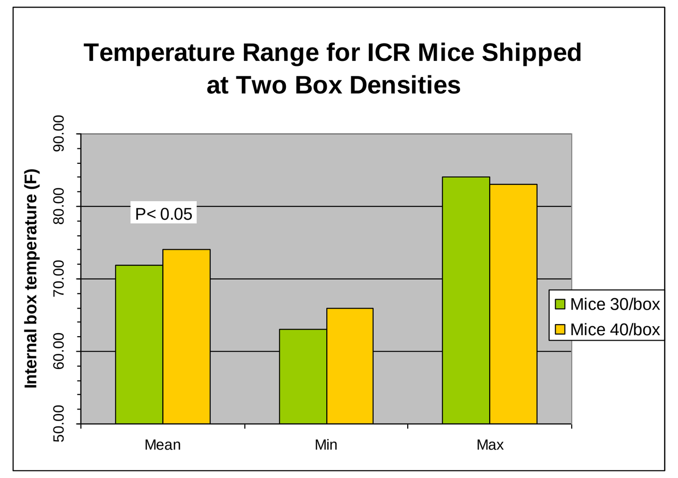

Figure 3
Mean interior box temperature was significantly higher for mice packed at 40/box compared to 30/box (2.15 F). This difference was more pronounced at the lower end of the temperature range compared to the upper end.

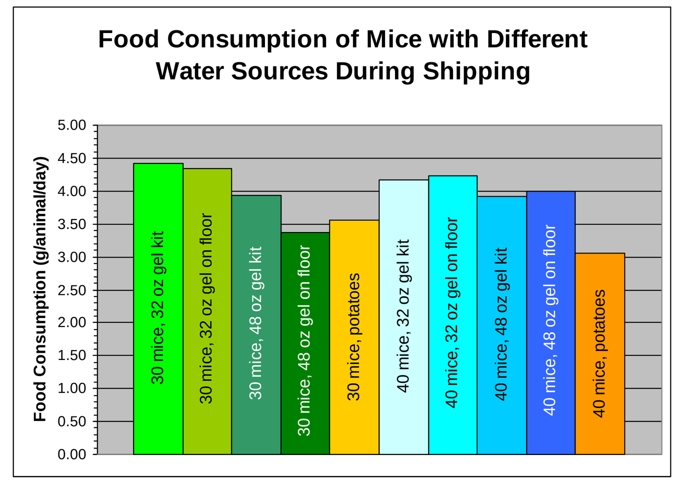

Figure 4
Statistical differences in food consumption among groups could not be evaluated because only a single data point per box was determined. In mice, the use of gel appears to result in higher food consumption in transit, compared to potatoes except at lower box densities.

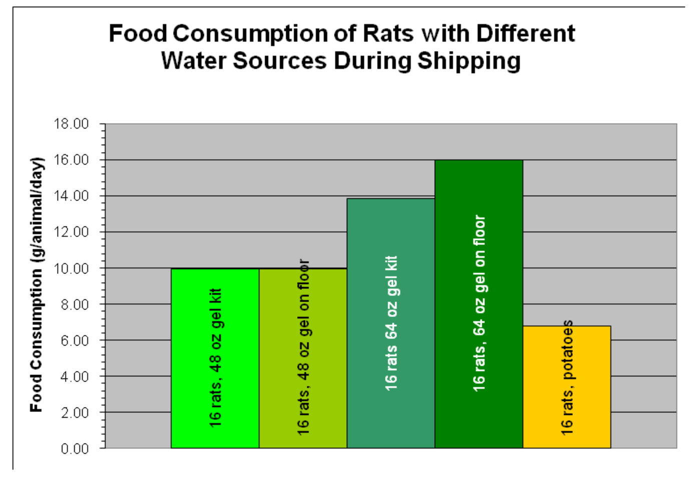

Figure 5
In rats, provision of gel resulted in apparently higher food consumption in all groups compared to potatoes, with the effect being most pronounced at higher gel volumes.

Table 2
Clinical Chemistry Results for Rats and Mice with Different Water Source and Box Density

Red = above laboratory reference range, Blue = below laboratory reference range, * = Significantly different from potato group at same density

Condition	Species		ALT	T bili	ALP	TP	Alb	Glob	Chol	BUN	Creat	Phos	Ca	Gluc	Na	K	Cl	CPK
40/box, Potatoes	Mice	Mean	23.80	0.20	170.33	6.30	3.67	2.70	150.50	32.83	0.50	10.80	11.08	150.60	166.00	6.27	114.83	226.17
		SEM	1.24	0.00	5.05	0.09	0.10	0.04	8.58	1.22	0.00	0.59	0.09	7.12	1.55	0.27	1.22	90.71
40/box, 48 oz gel	Mice	Mean	55.40	0.27	149.00	5.22*	3.13*	2.18*	109.83*	27.00*	0.50	10.64	10.20	156.20	150.00*	7.23	105.83*	219.00
		SEM	18.68	0.07	15.68	0.02	0.10	0.04	11.38	1.69	0.00	0.39	0.22	8.15	1.46	0.47	0.17	69.36
30/box, Potatoes	Mice	Mean	60.17	0.20	200.00	6.17	3.55	2.62	119.83	32.00	0.52	11.07	10.95	149.67	158.33	6.62	110.00	180.00
		SEM	22.73	0.00	11.80	0.06	0.03	0.05	7.68	1.03	0.02	0.45	0.10	8.17	3.07	0.32	1.88	55.31
30/box, 48 oz gel	Mice	Mean	37.67	0.27	136.67	5.60*	3.27	2.37	108.50	25.17*	0.50	9.97	10.23	180.67	151.50	6.93	108.83	149.33
		SEM	4.01	0.04	9.64	0.08	0.05	0.06	8.68	1.11	0.00	0.10	0.06	0.47	1.75	0.14	1.83	18.46
16/box, Potatoes	Rats	Mean	35.67	0.18	286.33	6.53	3.53	3.00	136.17	21.67	0.50	11.45	10.97	122.67	155.33	5.70	105.50	315.33
		SEM	1.58	0.02	10.45	0.06	0.03	0.08	10.49	1.31	0.00	0.41	0.12	5.48	1.45	0.21	0.89	38.03
16/box, 64 oz gel	Rats	Mean	73.33	0.20	503.67*	5.23*	2.85*	2.38*	116.00	20.83	0.53	11.63	11.03	217.17*	142.17*	7.57*	102.00	861.50
		SEM	2.60	0.00	45.89	0.08	0.06	0.05	7.59	1.49	0.03	0.30	0.49	20.88	0.60	0.59	0.89	478.83

Table 3
Hematology Results for Rats and Mice with Different Water Source and Box Density

Red = above laboratory reference range, Blue = below laboratory reference range, * = Significantly different from potato group at same density

Condition	Species		WBC	RBC	Hgb	Hct	MCV	MCH	MCHC	Neut	Lymph	Mono	Eo	Baso	Plt
40/box, Potatoes	Mice	Mean	3.92	9.53	16.40	53.17	55.67	17.27	31.50	73.65	310.27	7.40	3.67	0.00	1008.00
		SEM	0.57	0.14	0.13	2.51	2.17	0.26	1.15	11.71	45.60	2.01	3.67	0.00	34.08
40/box, 48 oz gel	Mice	Mean	5.68	8.63	15.10	46.83	54.17	17.50	32.33	100.63	459.68	8.02	0.00	0.00	1097.00
		SEM	1.10	0.16	0.44	1.25	0.60	0.26	0.21	18.35	90.41	1.92	0.00	0.00	57.26
30/box, Potatoes	Mice	Mean	3.72*	9.07	15.68	48.50	55.17	17.28	32.67	58.63	303.43	9.60	0.00	0.00	1,022.33
		SEM	0.49	0.22	0.41	1.43	1.62	0.23	0.33	11.63	40.27	0.45	0.00	0.00	37.37
30/box, 48 oz gel	Mice	Mean	6.55	8.83	15.52	47.33	53.50	17.53	32.83	130.60	507.33	16.70	3.67	0.00	985.33
		SEM	1.58	0.16	0.41	1.15	0.34	0.19	0.40	31.84	123.41	5.57	3.67	0.00	114.27
16/box, Potatoes	Rats	Mean	8.60	8.25	16.27	50.17	60.67	19.73	32.33	117.12	717.05	23.77	20.67	0.00	919.00
		SEM	1.24	0.10	0.24	1.45	1.23	0.19	0.42	15.15	106.00	3.83	20.67	0.00	65.24
16/box, 64 oz gel	Rats	Mean	11.72	6.73*	13.85*	45.33	67.33*	20.58	30.67	163.77	2480.77	23.70	34.33	0.00	889.00
		SEM	1.43	0.10	0.16	0.33	0.99	0.07	0.33	30.24	1509.57	2.57	34.33	0.00	46.44

Tables 2 and 3
There were no significant differences in any clinical pathology parameters among groups based on location of the gel kit in the box, the volume of gel provided, or the density of mice in the box.

Significant differences were seen in numerous parameters between animals provided with potatoes versus gel. Mean results and Standard Error of the Mean (SEM) are summarized in Tables 2 and 3. Values are highlighted that are above or below reference range.

In general, animals receiving potatoes showed increases in parameters indicative of dehydration, such as serum proteins, blood urea nitrogen (BUN), and electrolytes, and red blood cell indices.

CONCLUSIONS

The results on this study indicate that the use of gel resulted in higher food consumption, higher weight gain, and reduction of blood parameters indicative of dehydration compared to potatoes. Other observations such as rot with the use of potatoes or other vegetables in an alternate water source for animals also make gel an attractive alternative. Gel is recommended over potatoes as a shipping hydration source but sufficient gel must be provided to ensure it is available throughout the transit time.

Some clinical pathology changes were seen in all groups. Changes in glucose are indicative of stress that may be secondary to shipping, or to handling and blood collection. Elevations in ALP and ALT are likely an artifact of sample collection. Although not recorded in the data, low levels of hemolysis could result in these changes. Muscle damage from box inactivity could also result in these changes, but elevations in CPK would also be expected in this case. The elevations in phosphorus and potassium are not understood as they are present in all groups. HydroGel™ does contain potassium and phosphorus as a nutrient, as do potatoes.

Lower box density of mice resulted in higher food consumption for most groups, but only increased weight gain in the group fed potatoes. Mean box temperature was decreased slightly with lower housing density, but the mean, minimum, and maximum values were all within appropriate limits for transportation of mice. There were some indications in the clinical pathology results that mice at higher box density were at higher risk of dehydration. For longer transit times, lower box density should be considered.

References

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