Mice are subject to temperature extremes during air shipment

Eric Syversen[a], Fernando J. Pineda[b], Julie Watson[c *]

[a]*Johns Hopkins University, Research Animal Resources*[b]*Johns Hopkins Bloomberg School of Public Health*
[b]*Dept. of Molecular Microbiology & Immunology & Dept. of Biostatistics*
615 N. Wolfe St., Baltimore Maryland 21205-2179

[c]*Johns Hopkins University School of Medicine*
Dept. of Molecular & Comparative Pathobiology

Introduction

Institutions commonly ship mice nationally and internationally in support of biomedical research. Transport is governed by the IATA Live Animal Regulations, the Guide for the Care and Use of Laboratory Animals (*Guide)*, and the Animal Welfare Act Regulations. The *Guide* recommends that unadapted mice and rats be maintained between 64-79^{o}F. World Courier and Marken follow commercial passenger airline restrictions and ship when predicted temperature maxima and minima at en route airports for 24h after departure fall within a range of 45-85^{o}F. Other couriers do not place temperature restrictions on shipments. We are not aware of any published information on actual temperatures experienced by mice shipped under these conditions. In this study we monitored ambient temperatures during 127 routine mouse air shipments from Johns Hopkins University to other institutions over a 12m period. We assumed that temperatures above 85^{o}F or below 45^{o}F for ≥10 minutes, or large (>20^{o}F) temperature excursions, would be stressful for rodents. Therefore we evaluated various factors for their effect on these parameters. Temperature plots also provided interesting preliminary data on the typical pattern and duration of national and international mouse shipments departing from Johns Hopkins University.

Materials & Methods

All procedures were approved by the Johns Hopkins University Institutional Animal Care and Use Committee. Before shipment, mice were housed in individually ventilated racks (Allentown caging, Allentown, PA) in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care International under conditions compliant with the *Guide*. Mice were adults of unique strains shipped for research collaboration. 127 international and domestic mouse shipments were monitored. Shipments for the study were selected based on commitments by consignees to return the monitors. Study shipments constituted approximately one third of all outgoing shipments. Mice were shipped in autoclaved plastic shipping crates (Jackson Labs, Bar Harbor, ME) containing 64 oz autoclaved Beta Chips (7090M) corncob bedding (Harlan Teklad, Indianapolis, IN), 8-16 oz. of autoclaved 2019SX rodent chow (Harlan Teklad, Indianapolis, IN) and 16 oz of irradiated gel as a water source (Clear H2O, Bar Harbor,

Corresponding author. Tel.: 410 889 8173 fax:410-502-5068; e-mail: Julie.watson@jhu.edu.

ME). Each crate contained up to 10 mice in 1 or 2 compartments. Mice were typically placed in the crates the morning of shipment.

Temperatures were recorded at one minute intervals throughout transport using TransiTempRH multi- and single-use monitors (MadgeTech, Warner, NH). Monitors were wrapped in a single layer of bubble wrap (Office Depot, Delray Beach, FL) for protection and taped to the outside of the shipping crates. Holes were cut in the bubble wrap to expose the sensors. For return of the monitors, pre-printed Federal Express waybills and envelopes were attached to the short side of the crate away from ventilation ports. Monitors were turned on at least ten minutes prior to pick-up from JHU and returned to JHU by priority overnight shipping. On their return, monitors were turned off and data downloaded using the supplied software.

Shipments were handled by five couriers: World Courier (New Hyde Park, NY), AirNet (Columbus, OH), BAX Global (Irvine, CA), UPS (Atlanta, GA), and Marken (Lynbrook, NY). All international shipments were handled by World Courier. All couriers, except for World Courier and Marken, use their own aircraft. World Courier and Marken place shipments in the cargo holds of commercial passenger airlines. Mice were transported from JHU to the departure airport by TransporTech (Brockton, MA) and from the arrival airport to the consignee either by the main courier or local ground carriers subcontracted by the main courier. Detailed shipping data was collected from couriers. This included the airline or ground shipper used in each leg of a shipment, drop-off and pick-up times and airport arrivals and departures. All times were reported as local time, but standardized for subsequent analysis by applying GMT time-zone offsets followed by conversion to GPS epoch seconds. Finally, receiving institutions were contacted to determine if any mice died during shipment.

For practical reasons, temperature sensors were placed on the outside of crates. To determine whether exterior temperature sensors would provide suitable estimates of interior temperatures, a crate with both internal and external sensors was stabilized at an ambient temperature of 78^{o}F and then placed in an oven at 220^{o}F. The crate was removed after reaching an external temperature of 161^{0}F and re-equilibrated to the ambient temperature. Comparison of internal and external temperature traces revealed that the interior sensor equilibrated via conductive heat transfer with a characteristic time scale of 10-15 minutes. There was also evidence for more rapid heat transfer. On cool down, internal temperatures closely approximated external temperatures, probably due to rapid convective air exchange through ventilation ports. We concluded that exterior temperature sensors provided a good representation of the environment experienced by the rodents.

Results

A total of 127 shipments were monitored. Of these 23 were rejected because of missing or malfunctioning monitors. Of the remaining 104 shipments, 76 were domestic and 28 were international. An example temperature time-series is shown in Fig. 1.

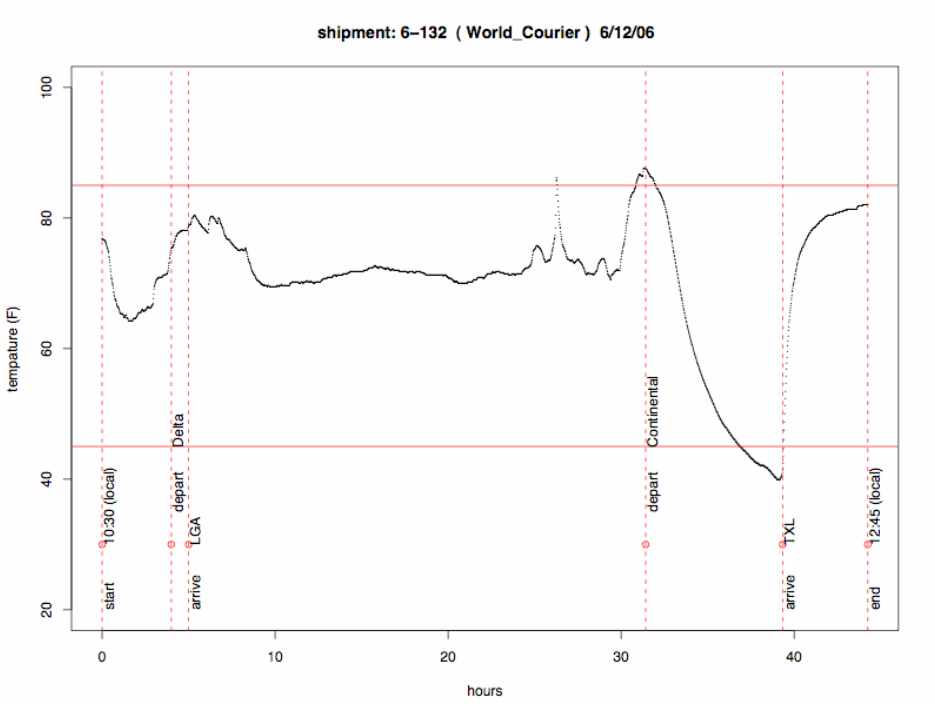

Fig. 1 Example temperature time-series illustrating high-temperature excursion and a low temperature excursion within in a single shipment.

Table 1 shows duration of domestic and international shipments broken down by courier. All international shipments were handled by World Courier. Most such shipments were driven to the World Courier rodent holding facility ("Mouse House") in Long Island, NY while waiting for flights or document/customs clearance. Domestic shipments had an average of 2.3 flight legs and a median duration of 23.2 hours, while international shipments had an average of 2.2 flight legs and a median duration of 48.3 hours. Canadian shipments were the shortest (mean 38.4h) while Australian shipments were the longest (mean 59.6h). Shipments to West Asia (mean 47.3h), East Asia (mean 53.5h), and Europe (mean 55.1h) fell in between. Fig. 2 shows the distribution of shipment duration for both international and domestic flights. Note that the longest international shipment took 3.4 days, while the longest domestic shipment took 2.1 days.

Courier	Domestic				International			
	N	median	mean	std. dev.	N	median	mean	std. dev.
AirNet	28	23.1	27.1	9.5				
BAX	5	24	23.7	2.0				
Marken	1	24.5	24.5					
UPS	1	28.6	28.6					
World Courier	42	22.8	23.6	2.7	27	48.3	52.4	12.8

Table 1. Shipment Duration (Hours). Comparison of Domestic and International Flights by Courier.

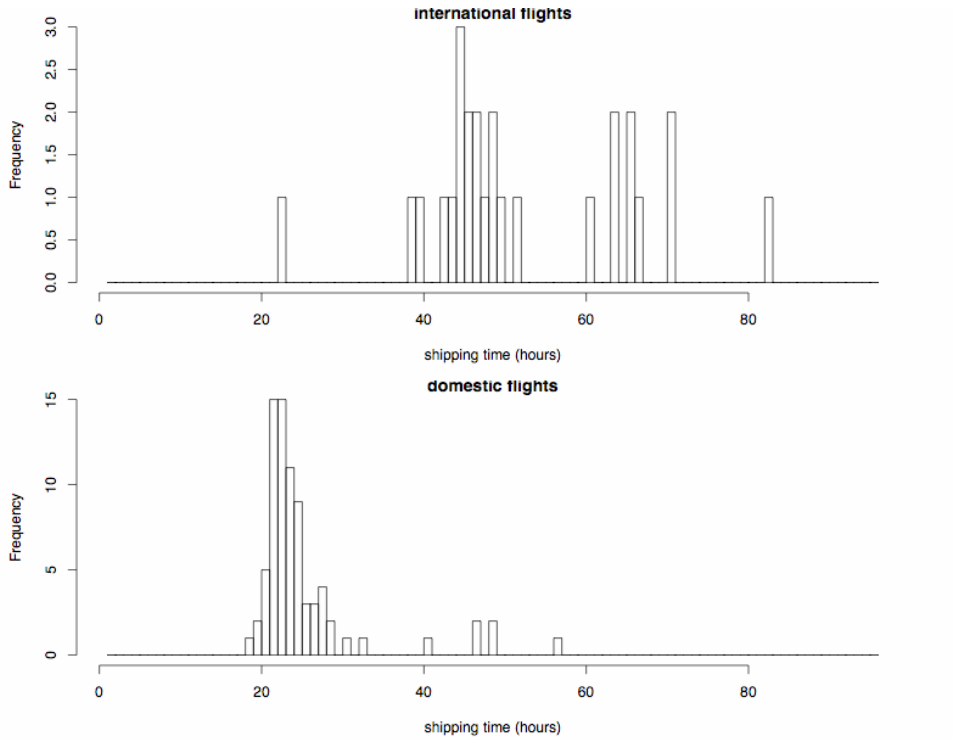

Fig. 2 Histograms of shipment duration in hours for international and domestic flights.

To understand the shipments in more detail, each shipment was segmented into legs according to their characteristics as shown in Table 2. There were 580 total legs.

Leg	N	Characteristics
Start	104	Collection from JHU through departure of first flight
Flight	238	Flight departure through flight arrival
Stopover	134	Flight arrival at airport through next flight departure
Final	104	Arrival of final flight through delivery to consignee

Table 2. Number and characteristics of shipment legs

A summary of the time statistics broken down by leg is shown in Table 3. A surprisingly large proportion of the total journey time for domestic shipments was occupied by start and final legs (63.5% for domestic shipments vs. 24.1% for international shipments). Start and final legs included both travel and airport holding. Start legs for couriers that fly at night (Airnet, UPS and BAX) were generally long because JHU initiated all airport transfers in the morning, thus requiring shipments for overnight flights to wait all day at the airport. In contrast, international shipments transiting through NY rarely experienced a departure delay because the air shuttle from Baltimore to NY leaves every half hour. Final legs were also prolonged when shipments arrived at night and were held at the airport overnight for delivery the following morning.

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Leg	Domestic					International				
	N	max	median	mean	std. dev.	N	Max	median	mean	std. dev.
Start	75	40.9	5.08	6.81	5.52	29	16.9	4.75	6.90	4.63
Flight	75	31.7	1.75	3.05	4.30	29	8.42	2.02	2.54	1.93
Stopover	78	37.6	2.51	7.02	9.89	31	51.3	1.92	6.94	11.26
Final	78	25.3	8.5	9.05	5.99	31	28.4	11.1	10.3	6.90

Table 3. Breakdown of leg time statistics by leg type.

Analysis of temperature excursions

Incidence of temperature excursions

We defined high- or low- temperature excursions as more than 10 minutes above 85°F or below 45°F respectively. The 45–85°F range is the temperature range used by couriers to accept unacclimated rodents on passenger flights, while 10 minutes was the characteristic time we estimated for equilibration of internal shipping crate temperatures to ambient temperature. 40% of domestic shipments and 70.4% of international shipments, experienced high-temperature excursions. 15.6% of domestic shipments and 40.7% of international shipments, experienced low-temperature excursions.

Low-temperature excursions

5% of legs experienced low-temperature excursions. We were unable to identify any factors upon which low-temperature excursions might depend. In particular, no category of leg experienced a statistically significant excess of excursions. Nor was there a statistically significant dependence on choice of courier or airline.

High-temperature excursions

22% of legs experienced high-temperature excursions. These excursions occurred in all leg categories and there was no evidence for a leg-category dependence. On the other hand, there was evidence for a courier dependence in both flight and stopover categories ($p=2x10^{-5}$ and $p=1x10^{-5}$ respectively, via chi squared test). In particular, World Courier shipments were significantly more likely to experience high temperature excursions in flight and stopover legs than other couriers. World Courier does not use its own airplanes instead using commercial passenger carriers to handle the shipments in transit. There was no courier dependence on start legs. This was expected since JHU uses a separate company for delivery to the airport independent of the courier used for the shipment. There was also no courier dependence on finish legs. Again this was unsurprising because a number of different ground carriers apart from the main courier delivered mice to consignees.

Effect of Courier on Temperature Range Excursions

Large temperature swings are presumed stressful for rodents. We arbitrarily defined a swing of greater than 20^{o}F during a single leg as a temperature-range excursion. Overall, 22.9% of legs experienced temperature-range excursions. There was a significant ($p=0.00001$) carrier dependence, with World Courier experiencing most incidents in both flight and stopover legs.

Effect of Airline on Temperature Range Excursions in Flight

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Four commercial airlines (American, Delta, Continental and Northwest) used by World Courier and Marken, and 3 courier-owned airlines (AirNet, BaxGlobal and UPS) were used for domestic flights including the domestic portion of international shipments. 21.8% of flight legs experienced $\geq 20^{o}$F temperature variations. There was a significant ($p=10^{-05}$) effect of airline, with American and Continental experiencing the most incidents.

Discussion

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It should be emphasized that all but one shipment in this study arrived at their destination safely. Two categories of courier handled JHU mouse shipments, those using passenger airlines and those using their own freight airplanes. World Courier handled the majority of shipments and used commercial passenger airlines. Marken (1 shipment) also fell into this category. All other shipments were handled by freight carriers, with the majority handled by Airnet. Despite the fact that World Courier imposes temperature restrictions, shipments they carried were significantly more likely to experience both high temperature excursions (>85°F for >10m) and temperature range excursions (>20°F within a travel leg). This can be attributed to the fact that the commercial passenger airlines used by World Courier travel by day, while the freight aircraft used by other couriers travel at night. World Courier also handled all the international flights, which had longer flight durations and more in flight low-temperature excursions than domestic flights.

Mice in this study were often exposed to temperatures outside the range 45-85°F during routine air shipments. High temperature excursions (>85°F) were most common and were often observed during transitions (loading and unloading) and short daytime flights. The highest recorded temperature in our study was 105°F (experienced during a stopover). These were the only mice that did not survive. Mice do not tolerate high temperatures well: they have no sweat glands, do not pant and have limited ability to salivate. They usually cope with high temperatures behaviorally by escaping to an underground burrow. Above 98.7°F they begin to die. We suggest that high temperature excursions could be minimized by shipping mice at night during hot weather.

Low temperature excursions (<45°F) were less common, and were most evident during transitions in cold weather and during longer flights. Decreasing temperatures were observed during many flight legs, suggesting those cargo holds were not maintained at room temperature. Ambient temperatures fall 5.4°F for every 1000' of altitude, so even hot weather flights are likely to experience low in-flight temperatures if there is sufficient time for the cargo hold to cool down. Both high and low temperature excursions could be reduced by minimizing exposure to ambient temperatures during loading and unloading. For longer flights, low temperatures and large temperature drops in flight could be avoided by controlling temperatures in airline cargo holds within narrower limits. Our lowest recorded temperature was 18°F (experienced during in flight and stopover segments of one flight), and these mice survived in good condition. Mice are better able to withstand low temperatures: they maintain their body heat through huddling and by generating heat through non-shivering thermogenesis.

Temperature plots suggested there were discrepancies between actual and reported take off and arrival times. Unless there were obvious problems with the data analysis, times were not corrected. However incorporation of a pressure monitor would have provided accurate in-flight travel time. All reported times were local and had to be adjusted to a

standard time. Because countries vary in the date they implement daylight savings time, it was only possible to ensure international times were correct to one hour, and this could have been the source of some discrepancies.

We were only able to ensure return of the monitors if they were attached to the outside of the crate, so all our data referred to outside temperatures. However we concluded that this was a good approximation for temperatures experienced by the mice. Our comparison between internal and external temperatures of a shipping crate during heating and cooling showed that temperatures inside rapidly equilibrated to those outside, probably due to convective air exchange through ventilation ports.

Although we undertook this study to determine temperatures mice were exposed to during shipment, our results also provided valuable preliminary information on the nature and duration of mouse shipments. Shipments were surprisingly complicated, often involving several couriers, more than one airline, and more than one flight even for East Coast shipments. The median number of flights for domestic and international shipments was similar (2.2 and 2.3). Most domestic shipments took at least 24h, with international shipments taking 2 or 3 days. Long waits of many hours were common in ground legs, usually to accommodate airline schedules.

During shipping, mice are exposed to loss of the normal light cycle, jet lag, pressure variations, and in all likelihood loud noises, vibrations and unusual odors. We have documented they are also exposed to high and low temperatures and wide temperature variations. This would indicate the need for a generous acclimation period after arrival.

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