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Temperature Variations Recorded During Interinstitutional Air Shipments of Laboratory Mice

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Despite extensive guidelines and regulations that govern most aspects of rodent shipping, few data are available on the physical environment experienced by rodents during shipment. To document the thermal environment experienced by mice during air shipments, we recorded temperatures at 1-min intervals throughout 103 routine interinstitutional shipments originating at our institution. We found that 49.5% of shipments were exposed to high temperatures (greater than 29.4 °C), 14.6% to low temperatures (less than 7.2 °C), and 61% to temperature variations of 11 °C or more. International shipments were more likely than domestic shipments to experience temperature extremes and large variations in temperature. Freight forwarders using passenger airlines rather than their own airplanes were more likely to have shipments that experienced temperature extremes or variations. Temperature variations were most common during stopovers. Some airlines were more likely than others to experience inflight temperature extremes or swings. Most domestic shipments lasted at least 24 h, whereas international shipments lasted 48 to 72 h. Despite exposure to high and low temperatures, animals in all but 1 shipment arrived alive. We suggest that simple measures, such as shipping at night during hot weather, provision of nesting material in shipping crates, and specifying aircraft cargo-hold temperatures that are suitable for rodents, could reduce temperature-induced stress. Measures such as additional training for airport ground crews, as previously recommended by the American Veterinary Medical Association, could further reduce exposure of rodents to extreme ambient temperatures during airport stopovers.

Abbreviations: GPS, global positioning system; IATA, International Air Transport Association

Institutions typically ship research mice by air to other institutions throughout the world in support of biomedical research. Multiple regulations and guidelines govern various aspects of shipping that could affect rodent welfare, including temperature control, food, water, container size and ventilation,[6,8,12] air pressure,[6] and training of air carrier employees.[18] The Animal Welfare Regulations[16] and Lacey Act Amendments[10] address animal welfare during shipment but do not include research rats and mice. The International Air Transport Association (IATA) Live Animals Regulations[8] lists standards for rodent shipments that are applicable to member airlines. Despite these regulations and guidelines, little published information is available regarding the actual conditions that animals experience during shipment. Indeed, so few relevant published data are available regarding these shipments that an Institute for Laboratory Animal Research committee, which was convened in 2004 to address problems encountered during the transportation of research animals, could not draw any conclusions about the quality of air transportation for research animals.[13] However, this committee did document a low incidence of problems with animal shipments in general.

A particular concern when shipping rodents is temperature control. Research mice typically are housed in animal facilities that maintain temperatures within narrow limits.[12] However, shipping creates the potential for exposure to extreme ambient temperatures. Recognizing this potential, freight forwarders (couriers) often restrict shipments when extreme temperatures are predicted. In our experience, couriers that use passenger airlines typically accept unacclimated rodents for shipment only when the temperature maxima and minima at en route airports for 24 h after departure are predicted to be between 7.2 and 29.4 °C. Couriers using freight airlines vary with respect to the temperature restrictions they place on domestic shipments. Passenger airlines that are members of the IATA are subject to the IATA Live Animals Regulations,[8] which specify an effective temperature range (that is, the temperature experienced, not the ambient temperature) of 5.0 to 35.0 °C for rodent shipments. To our knowledge, no published information is available that documents the pattern of temperatures experienced during air shipments, although 1 previous study recorded maximal and minimal temperatures for a single air route.[11] The goal of the present study was to provide data regarding the actual temperatures to which laboratory mice are exposed during routine interinstitutional shipments and to identify any areas of particular concern. In addition, temperature plots provided preliminary data on the typical pattern and duration of national and international mouse shipments departing from our institution.

Materials and Methods

We monitored ambient temperatures during 103 routine mouse shipments from our institution to multiple institutions over a 12-mo period. An additional 24 shipments were discarded because the temperature monitors malfunctioned or were destroyed. Shipments were selected based on commitments by consignees to return monitors and constituted more than one third of all outgoing shipments. Mice (*Mus musculus*) were of

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multiple unique genetically manipulated strains being shipped for research collaboration, and all mice were included on protocols approved by the Johns Hopkins University Institutional Animal Care and Use Committee. All mice were from colonies that routinely tested free of mouse hepatitis virus, epidemic diarrhea of infant mice, Theiler murine encephalomyelitis virus, mouse parvovirus 1, minute virus of mice, murine adenovirus 2, *Ectromelia virus*, lymphocytic choriomeningitis virus, *Mycoplasma pulmonis*, pneumonia virus of mice, reovirus, Sendai virus, mouse cytomegalovirus, and ecto- and endo-parasites.

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 for the Care and Use of Laboratory Animals*.[12] Mice were shipped in autoclaved plastic shipping crates (Jackson Labs, Bar Harbor, ME) containing 1.9 l of autoclaved corncob bedding (Beta Chips 7090M, Harlan Teklad, Indianapolis, IN), 240 to 475 ml of autoclaved rodent chow (2019SX, Harlan Teklad), and 475 ml γ-irradiated gel as a water source (Clear H_2O, Schlotterbeck and Foss, Portland, ME). Each crate contained as many as 10 mice in 1 or 2 compartments. Mice typically were placed in crates on the morning of shipment.

Temperatures were recorded at 1-min intervals throughout transport by using multi- and single-use monitors (TransiTempRH, 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, preprinted overnight-shipping waybills and envelopes were attached to the short side of the crate away from ventilation ports. Monitors were turned on at least 10 min prior to shipment pick up from our institution and returned to our institution by priority overnight shipping. On their return, monitors were turned off and data downloaded by using the vendor-supplied software.

Shipments were handled by 5 couriers, identified in this manuscript by the letters A through E. Couriers A and E used the cargo holds of passenger airlines to transport mice. Couriers B, C, and D used their own freight aircraft. Courier B additionally restricted freight to a maximum of 31.75 kg or combined dimensions (length + width + height) of 228.6 cm. All international shipments were handled by courier A. Mice were transported from our institution to the departure airport by TransporTech (Brockton, MA) and from the arrival airport to the consignee either by the main courier or by local ground carriers subcontracted by the main courier. Detailed shipping data were collected from couriers. These data included the airline or ground shipper used during each segment of a shipment, drop-off and pick-up times at airports, and flight arrival and departure times. Consignees were contacted to obtain information regarding safe arrival of the mice.

To ensure their return, 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 25.6 °C and then placed in an oven at 104.4 °C. The crate was removed after reaching an external temperature of 71.7 °C and re-equilibrated to the ambient temperature. Comparison of internal and external temperature traces during heating and cooling revealed that the interior equilibrated to the exterior by means of conductive heat transfer, with a characteristic time scale of 10 to 15 min (data not shown). In addition, there was evidence of a much faster equilibration component due to convection, presumably due to air exchange through ventilation ports (data not shown). We concluded that exterior temperature sensors provided a good representation of the environment experienced by the rodents.

Table 1. Percentage of shipments experiencing high and low temperature excursions by season

	No. of shipments	% with high-temperature excursions	% with low-temperature excursions
Spring	30	43	13
Summer	40	75	5
Fall	15	40	20
Winter	17	12	35

Time-series data downloaded from temperature sensors were standardized for subsequent analysis by applying Greenwich Mean Time time-zone offsets followed by conversion to global positioning system (GPS) epoch seconds. Conversion of dates, days, hours, minutes, and seconds to a standard unit (in this case, GPS epoch seconds) was necessary to allow data comparison and analysis. In addition, pick-up, departure, and arrival times provided by the main couriers were converted to GPS epoch seconds. Therefore, the time-series data could be divided into segments, and temperature events in the time-series data could be associated with events reported by the main couriers. Data standardization and other preprocessing were performed with Perl scripts.[19] Statistical analysis was performed by using the R statistical programming environment.[15] Statistical significance was tested by using the Pearson χ^2 test. P values were calculated with 10^5 Monte Carlo simulations.[4] For the purposes of this study, high-temperature excursions were defined as periods of at least 10 min during which temperatures exceeded 29.4 °C, low-temperature excursions as periods of at least 10 min during which temperatures were lower than 7.2 °C, and temperature-range excursions as a difference of at least 11.1 °C between the maximal and minimal temperatures during a single segment of the journey.

Results

Of the 127 shipments monitored, 24 were rejected because of missing or malfunctioning monitors. Of the remaining 103 shipments, 77 were domestic, and 26 were international. Further, 30 shipments were shipped in spring (Mar 21 to Jun 20), 40 in summer (Jun 21 to Sep 20), 15 in fall (Sep 21 to Dec 20), and 18 in winter (Dec 21 to Mar 20). All shipments arrived safely, except for 1 that was exposed to temperatures of 40.6 °C and greater.

Table 1 shows the incidence of high- and low-temperature excursions, sorted according to season. High-temperature excursions were most frequent in summer, spring, and fall, and low-temperature excursions were most common in winter. However, both high- and low-temperature excursions occurred during all seasons.

Temperature–time-series examples (Figures 1 through 4) demonstrate various problems that occurred in temperature control. Figure 1 shows a domestic shipment during cold weather that encountered substantial reductions in temperature during flight segments. Figure 2 shows an international shipment during hot weather during which both heating problems (during airport handling) and cooling problems (during flight) occurred. In contrast, Figure 3 illustrates an international cold-weather shipment for which the cargo-hold temperatures were well regulated. Figure 4 shows a domestic shipment during

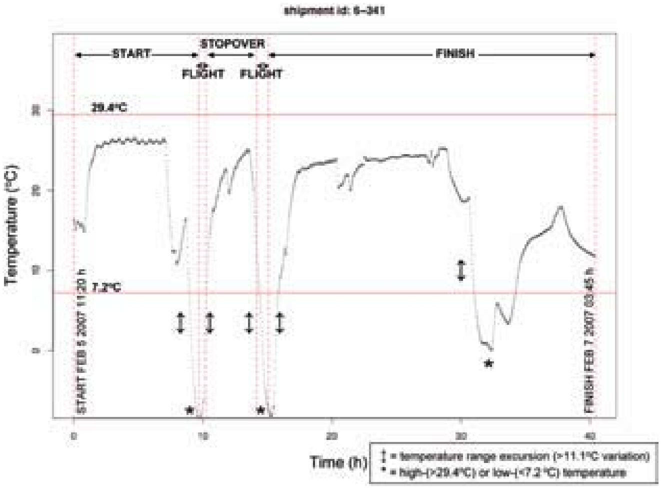

Figure 1. Domestic shipment during cold weather with substantial reductions in temperature during flight and final segments of transport.

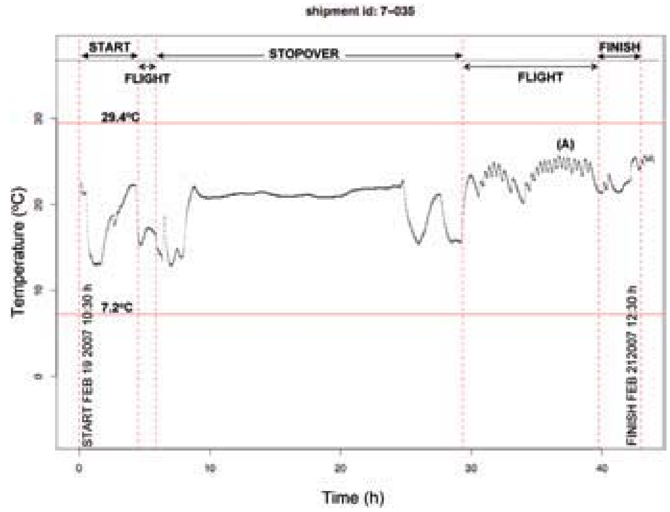

Figure 3. International shipment during cold weather, demonstrating use of heated cargo hold (A).

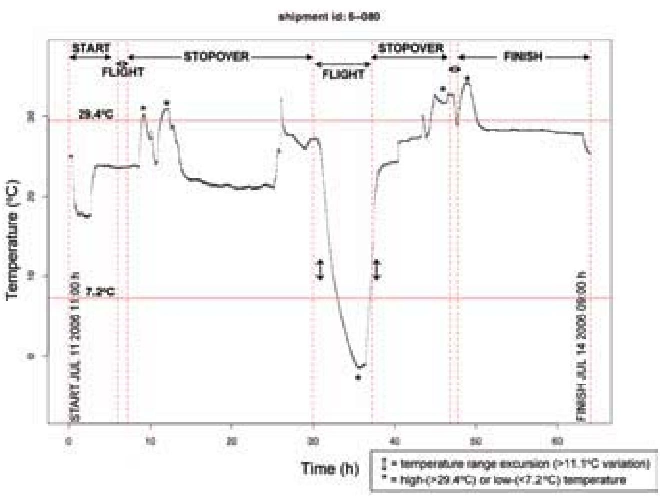

Figure 2. International shipment during hot weather with both high-temperature excursions (during stopovers) and low-temperature excursions (in flight).

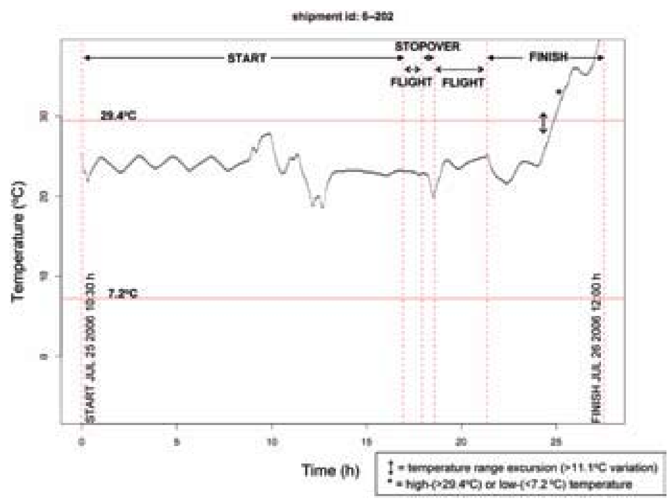

Figure 4. Domestic shipment during hot weather with high-temperature excursion during final segment.

hot weather that encountered poor heat control during ground transport. Many of the marked changes in several plots could be attributed to loading and unloading, for example, during the stopover in Figure 3. In addition, small, regular temperature oscillations representing thermostatic control during holding periods occurred in several plots.

Table 2 shows the duration of domestic and international shipments sorted according to courier. All international shipments were handled by courier A. Most international shipments were driven from one of the New York airports to a rodent holding facility in Long Island, NY, to await flights or document or customs clearance. Domestic shipments had an average of 2.2 flight segments and a median duration of 23.2 h, whereas international shipments had an average of 2.4 flight segments and a median duration of 48.7 h. European shipments were the shortest (mean, 34.5 h), whereas Australian shipments were the longest (mean, 59.6 h). Shipments to Canada (mean, 38.4 h), West Asia (mean, 47.3 h), and East Asia (mean, 53.5 h) had intermediate durations. Figure 5 shows the distribution of shipment durations for both international and domestic flights. Note that the longest international shipment took 3.4 d, whereas the longest domestic shipment took 2.1 d.

To understand the shipments in more detail, we divided each shipment into segments according to their characteristics: start, stopover, flight, and final (Table 3). There were 575 segments in total. A summary of the time statistics broken down by segment is shown in Table 4. A surprisingly large proportion of the total journey time for domestic shipments was accounted for by the start and final segments (63.5% for domestic shipments versus 24.1% for international shipments), which included both ground travel and airport holding. Start segments for freight couriers that fly at night (couriers B, C, and D) were generally long because our institution 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 New York rarely experienced a departure delay because the air shuttle from Baltimore to New York leaves hourly. International shipments did have relatively long stopovers in NY while awaiting clearance for international flights (for example, Figures 2 and 3). Final segments were prolonged when shipments arrived at night and were held at the airport overnight for delivery the following morning.

Analysis of temperature excursions. High- and low-temperature excursions were defined as periods of at least 10 min during which the recorded temperature exceeded 29.4 °C or was lower than 7.2 °C, respectively. The 7.2-to-29.4°C range is the ambient enroute temperature range frequently used by couriers for accepting unacclimated rodents on passenger airlines;

Table 2. Shipment duration: comparison of domestic and international shipments by courier

	No. of shipments	Duration (h)		
		Median	Mean	1 SD
Domestic shipments				
courier A	42	22.8	23.6	2.7
courier B	28	23.1	27.1	9.5
courier C	5	24.0	23.7	2.0
courier D	1	28.6	28.6	not applicable
courier E	1	24.5	24.5	not applicable
International shipments				
courier A	26	48.7	52.7	13.0

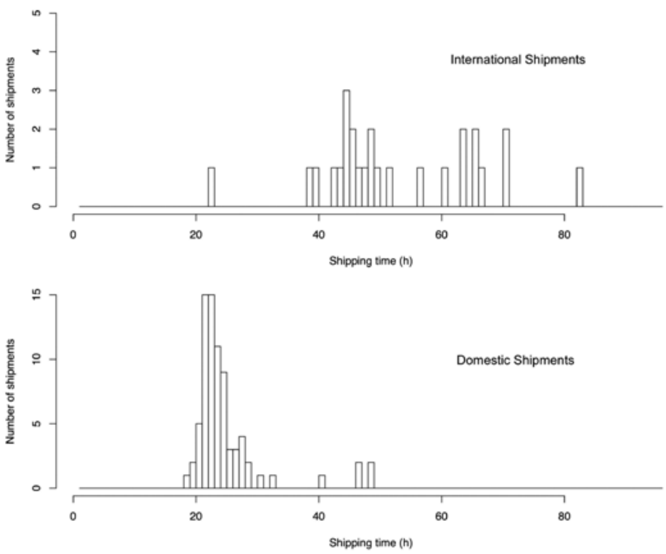

Figure 5. Distribution of shipment duration (h) for international and domestic flights.

therefore, we expected the external temperatures of shipping crates to remain within this range. The interval of 10 min was the characteristic time delay we estimated for equilibration of internal shipping crate temperatures to external temperatures; therefore, the mice in the crates likely actually were exposed to the temperatures recorded on the outside of the crates.

High-temperature excursions. Overall, 40% of domestic shipments and 77% of international shipments experienced high-temperature excursions. When analyzed by segment category, 22% of segments experienced high-temperature excursions. These excursions occurred in all segment categories, with no evidence of segment–category dependence.

When analyzed by courier, both flight and stopover categories showed courier dependence ($P = 2 \times 10^{-5}$ and 1×10^{-5}, respectively). In particular, courier A shipments were significantly more likely to experience high temperature excursions during flight ($P = 1 \times 10^{-5}$) and stopover ($P = 1 \times 10^{-5}$) segments than were shipments by other couriers. However, courier A uses commercial passenger carriers rather than its own aircraft to handle shipments in transit. Start segments were not influenced by courier because our institution uses a single company for delivery to the airport. Similarly, ground carriers other than the main courier sometimes delivered mice from the airport to consignees, and courier dependence was not a factor during finish segments.

Low-temperature excursions. Low-temperature excursions occurred during 16% of domestic shipments and 12% of international shipments. When analyzed by segment category, 5% of the segments experienced low-temperature excursions. No category of segment experienced significantly more excursions than other categories, nor was the number of excursions significantly related to courier or airline.

Temperature-range excursions. Temperature variation of at least 11.1 °C during a single segment was defined as a temperature-range excursion. Overall 61% of shipments experienced temperature-range excursions, with a greater proportion of international flights affected than domestic flights (81% versus 55%). When analyzed by segment, range excursions were most frequent during stopovers (42% of international shipments versus 31% of domestic shipments) and were less common during flight segments (22% of international versus 21% of domestic). However, within flight segments, airline was a significant factor ($P = 1 \times 10^{-5}$), with 3 of the 5 airlines represented experiencing more excursions in proportion to the number of segments they flew (55%, 53%, and 33% respectively) than did the other 2. The incidence of range excursions during the start segment was higher for international shipments (43%) compared with domestic shipments (12%). Final segments were the least likely to experience range excursions (11% international and 18% domestic). Temperature-range excursions showed significant courier dependence ($P = 1 \times 10^{-5}$), with courier A experiencing the most incidents during both flight and stopover segments.

Discussion

We documented temperatures during 103 air shipments of mice and evaluated the effect of segment category, courier, and airline on high and low temperatures and large temperature variations. The National Research Council[13] has recommended that the temperature range for safe transportation of adult mice is 4 to 34 °C (39 to 93 °F), based on the range of temperatures to which mice have been successfully acclimated. Our data show that mice shipped when ambient temperatures remain within this range arrive safely. None of our mice were acclimated to high or low temperatures; therefore, we analyzed our data for excursions outside the ambient temperature range of 7.2 to 29.4 °C often used by couriers for accepting shipments.

Although, in our experience, couriers using passenger airlines attempt to prevent exposure to high and low temperatures by accepting animal shipments only when temperatures en route are predicted to be between 7.2 and 29.4 °C, mice in this study often were exposed to temperatures beyond this range. Further the actual temperatures experienced by the mice likely were as much as 5 °C higher than recorded temperatures, due to the heat generated by the mice themselves.[8]

High-temperature excursions (exceeding 29.4 °C) were most common. Examination of temperature traces revealed that ground transitions (presumed to reflect loading and unloading) were at high risk for poor temperature control. The IATA

Table 3. Number and characteristics of shipment segments

Segment	No.	Characteristics
Start	103	Collection from our institution through departure of first flight
Flight	236	Flight departure through flight arrival
Stopover	133	Flight arrival at airport through next flight departure
Final	103	Arrival of final flight through delivery to consignee

Table 4. Time statistics according to segment type

	Domestic shipments					International shipments				
	No. of segments	Duration (h)				No. of segments	Duration (h)			
Segment		Maximum	Median	Mean	1 SD		Maximum	Median	Mean	1 SD
Start	77	40.9	6.9	7.9	5.7	26	6.9	3.2	3.8	1.1
Flight	173	8.4	1.6	2.0	1.3	63	15.6	2.1	4.4	4.3
Stopover	96	14.7	0.9	2.4	3.3	37	51.3	23.5	20.1	11.6
Final	77	25.3	9.8	9.4	6.4	26	28.4	8.2	9.6	5.9

specifies that animals should be loaded as close to departure time as possible,[8] compartment doors should be opened during prolonged transit stops at ramp temperatures exceeding 20 °C, and ground equipment should be used to condition cargo holds during extreme temperatures.[8] However, the definition of 'extreme temperature' is open to interpretation. Consequently high cargo hold temperatures can result when doors are held open for long periods during temperatures that are higher than 20 °C but still interpreted as less than 'extreme.' Further, aircraft-generated temperature control is not available unless aircraft engines are running, and power may not be available for heating or cooling during full-throttle take-off.[8] Shipments with courier A (which uses passenger airlines) were more likely to experience high temperatures than were shipments with freight couriers, probably because passenger airlines fly during the daytime, whereas freight airlines travel at night. The highest temperature during our study was 65.6 °C (recorded during a daytime stopover); the mice in this shipment were the only ones in our study that did not survive. Mice do not tolerate high temperatures well; unlike some other mammals, they have limited ability to regulate their body temperature by sweating or panting.[5] Rodents can use evaporative heat loss through saliva spreading; however, that mechanism has limited efficacy at high humidity or when water is restricted.[5] The upper limit of the mouse's thermoneutral zone (33.9 °C) is close to the maximal temperature they can tolerate (37.1 °C), above which they begin to die.[9] Mice usually cope with high temperatures behaviorally by escaping to underground burrows.[5,9] High temperatures were the most common temperature excursion in this study and potentially are the most problematic. We suggest that high-temperature excursions might be minimized during hot weather by shipping and transferring mice at night, using ground transport instead of air transport where practical, and using routes through airports that are not experiencing extreme temperatures.

Low-temperature excursions (less than 7.2 °C) were less common than were high-temperature excursions in our study. Inspection of temperature traces revealed that mice were at greatest risk for exposure to cold temperatures during stopovers. The lowest temperature during the study was –7.8 °C (recorded during flight and stopover segments of 1 flight). These mice arrived safely. Mice are better able to withstand low temperatures than high temperatures: they maintain their body heat through huddling and by generating heat through nonshivering and shivering thermogenesis.[5,9] The temperature the study mice actually experienced was likely higher than the recorded temperature,[8] particularly with group-housed mice. We suggest that in addition to the measures suggested for high temperatures, including nesting material in shipping crates to provide additional insulation during cold weather might mitigate the impact of low temperatures. Both high- and low-temperature excursions might be reduced by minimizing the time of exposure to ambient temperatures during loading and unloading, as required by the IATA Live Animals Regulations.[8] Our results are consistent with previously identified problems during airport ground handling. In their 2002 report on animal air transportation,[1] the American Veterinary Medical Association identified weaknesses in the ground handling of animals during their transfer from one airplane to another, particularly when subcontractors are used. Airlines may provide some training of ground personnel regarding animal shipments, and training materials are available via the IATA Training and Development Institute (www.IATA.org). Nevertheless the American Veterinary Medical Association recommended establishment of a formal training program for airport ground crews, with consistent standards and recurrent training.

Inflight temperature traces often revealed large progressive temperature differences, which occurred significantly more often with 3 particular passenger airlines. Atmospheric temperatures fall on average 6.5 °C for every 1 km increase in height,[3] so even hot-weather shipments are likely to experience low in-flight temperatures if unheated cargo holds have sufficient time to cool down. Large temperature variations in flight might be avoided by specifying appropriate cargo hold temperatures. The courier is responsible for requesting transport in cargo holds with temperatures suitable for live animals; otherwise airlines may ship mice in holds maintained, for example, at the lower temperatures suitable for perishable goods but still within the effective temperature range listed in the IATA's Live Animals Regulations (5 to 35 °C).[8]

Some temperature traces suggested differences of as much as 1 h between actual and reported take-off or landing times. These times were not corrected. In most cases the reason was probably inaccurate reporting of take-off or landing. However another possible source for these discrepancies is incomplete correction for Daylight Savings Time. All reported times for international flights were local and should have been adjusted to compensate for differences in the implementation date of Daylight Savings

Time before conversion to GPS epoch seconds. However, ensuring correct conversion was difficult when multiple time zones were involved. In future experiments, including a pressure monitor would provide an independent and accurate measure of take off and landing times.

Although we undertook this study to determine the temperatures to which mice were exposed 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, multiple airlines, and multiple flights, even for regional shipments (US East Coast). International shipments flying from New York often waited hours or days for document clearance or flights, for reasons beyond courier or airline control. International shipments require delivery of cargo with completed paperwork at least 4 h before departure, and shipments often are presented 5 h in advance to provide a safety buffer. In addition, animals may be held at a site other than the airline's freight warehouse for 1 to 2 h to allow customs brokers to complete paperwork. Not all carriers or airports may have conditioned space available for live-animal shipments during waiting periods. In our study, waits of many hours also were common during stopover segments, probably to accommodate airline schedules. Rodent shipments are also subject to being 'bumped' for higher-priority cargo, such as cadavers, mail, and domestic animals.

During air shipment, mice are exposed to multiple stressors: absence of the normal light cycle, jet lag, pressure variations, and, in all likelihood, loud noises, vibrations, and unusual odors. After arrival, mice must adapt to a new environment. We have documented mice also are exposed to high and low temperatures and wide temperature variations during shipment. Previous studies have shown that physiologic responses to transport stress return to baseline within 1 to 7 d.[14] However disruptions in the glucocorticoid circadian rhythm in mice can require more than 2 wk to return to baseline,[17] and reproduction may take more than 1 mo to normalize.[7] In some species, the magnitude of the stress response increases with travel time.[2] Additional effects of temperature-related stress have not been evaluated. Therefore acclimation periods after arrival should be generous to ensure that research parameters have returned to normal.

The present study demonstrated that mice were exposed often to high and low temperatures and large temperature variations during air shipment from our institution. Nevertheless, the mice almost always arrived safely. Changes such as night shipment and substitution of ground transport or cooler airports during hot weather, inclusion of nesting material during cold weather, and specification of appropriate temperatures for aircraft cargo holds could further reduce temperature stress. In addition, implementation of the American Veterinary Medical Association recommendations for more thorough training of airport ground crews might reduce exposure to extreme ambient temperatures during airport handling.

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