SURVIVING THE JOURNEY: COMPARISONS OF TEMPERATURE-STABILIZING MATERIALS FOR LIVING ARTHROPOD SHIPMENTS

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ABSTRACT. Shipments of living mosquitoes and other arthropods require temperatures that are within a range that is compatible with their health and survival. In addition to express shipping and insulated containers, shipments often include materials that either store heat (i.e., have thermal mass) or otherwise stabilize the temperature. In this paper, we present the results of comparisons of thermal mass and phase change materials to stabilize the temperature under various conditions. We compared a rigid foam refrigerant and a number of phase change materials to bubble wrap for their capacity to moderate temperature change by measuring the temperatures in standard uninsulated shipping containers during exposure to high (37°C), cold (4°C), and freezing (−20°C) temperatures. We make recommendations for shipments depending on the ambient conditions that are expected to be experienced en route.

KEY WORDS Arthropods, perishable, phase change materials, shipping temperature

INTRODUCTION

In research laboratories, being able to study strains of arthropods that cannot be collected locally is made possible by exchanges of samples and strains between labs or from the field to a distant laboratory. Some species, such as ticks and Aedes aegypti (L.) eggs, can be shipped under relaxed conditions due to the resistance of the material to drying conditions or temperature extremes. Thousands of such exchanges of living arthropods certainly occur every year; the Malaria Research and Reference Reagent Resource Center (MR4) alone makes hundreds of shipments of various species of living mosquitoes to laboratories with appropriate containment annually, typically in the form of eggs (Dotson, unpublished).

Exposure to temperature extremes impacts mosquitoes and other arthropods in a number of ways; typical responses have been described by Block et al. (1990) and include stupor, coma and death (see Figure 1 of Block et al. 1990) outside a range of approximately 7–37°C. It is not only essential that arthropods survive in a cold environment, but that they develop through each life stage successfully and are able to reproduce. As arthropods are exposed to cooler temps (e.g. < 10°C), mobility, feeding and metabolism are reduced. These factors lead to an extended life cycle producing adults with smaller body size, reduced wing length, and poor reproduction (Block et al. 1990). Both cold-tolerant and intolerant species exist, but the concern addressed in this study is toward species that do not tolerate extremes well. We were particularly concerned about the viability of tropical mosquitoes exposed to colder temperatures.

Ensuring the viability of biological material is facilitated by express courier shipments, but delays due to inclement weather, customs clearance, holidays, etc., can interfere with rapid delivery. During this time, shipments of arthropods also often face exposure to extreme temperatures that directly affect their viability. While there are materials such as insulated foam chests and packing peanuts included in most shipments, these materials might not stabilize the shipment within an acceptable temperature range for the necessary amount of time. It may, therefore, be necessary to include appropriate temperature-stabilizing material based on the temperatures that a shipment is anticipated to experience. Some thermal protection is often provided by including freezer cold packs in the shipping container. The latter can provide thermal mass to buffer fluctuations in temperature experienced.

Phase change materials (PCMs), classified as latent heat storage units, are materials capable of storing and releasing large amounts of energy (Sharma et al. 2007). This exchange of energy takes place when the PCM undergoes a phase change, typically solid to liquid or liquid to solid. Because PCMs use latent heat, they are able to maintain the temperature of their environment until their capacity is exhausted. Therefore, as the temperature in an environment falls, PCMs alleviate the cold by releasing heat, and they absorb heat when the temperature rises, thus maintaining a steadier temperature.

Re-Freez-R-Brix™ (Polar Tech Industries Inc., Genoa, IL) refrigerant packs are commonly available, reusable packages of rigid foam that can protect shipments from thawing or freezing but do not undergo a phase change. When used in a standard insulated shipping container, prewarmed Re-Freez-R-Brix release heat into cooler surroundings, delaying the shipment from chilling or freezing. Alternatively, chilled Re-Freez-R-Brix that are cooler than the surroundings absorb heat, protecting the shipment from excess heat. Unlike PCMs, Re-Freez-R-Brix
attenuate temperature change rather than maintaining
their environment at a steady temperature until a
phase change; therefore, they are classified as
sensible heat units (Pfleger et al. 2015).

In this paper, we explore the effectiveness of
various types of PCMs (melting temperatures of
15°C, 22°C, and 28°C) and Re-Freez-R-Brix and
make recommendations based on the ambient
temperature a shipment is expected to experience.
As a baseline comparison that was expected to
provide little temperature protection, we included
containers internally packed with bubble wrap.

MATERIALS AND METHODS

Three types of materials were tested in these
experiments: common packing bubble wrap, Re-
Freez-R-Brix foam refrigerant packs, and PCMs with
varying melting temperatures. The PCMs selected
were PCM 15 pouches (savENRG™, Arden, NC),
Phase 22™ Flex Packs (Cryopak™, Edison, NJ), and
MatVesl 28 mats (Pure Temp LLC, Minneapolis,
MN). We will refer to these simply as PCM-15, PCM-
22, and PCM-28 respectively. With one exception, the
materials were tested under three different environ-
mental conditions, −20°C, 4°C, and 37°C, to simulate
shipments that are expected to experience freezing,
cold, or hot conditions en route. Unlike the other
PCMs, the PCM-28 material is already solid at the
initial ambient temperature and would not undergo
phase change by solidifying in the colder ambient
conditions; therefore, it was tested only at the 37°C
ambient temperature. A Fisherbrand™ freezer was
used for all −20°C conditions, and a Fisherbrand
general-purpose lab refrigerator (both from Fisher
Scientific International Inc., Waltham, MA) was used
for all 4°C conditions. A Percival™ environmental
control system incubator with 80% relative humidity
(Percival Scientific, Perry, IA) was used for all 37°C
conditions. The PCMs were suitably prepared for
each environmental condition as follows: PCM-15 and
PCM-22 designated for −20°C and 4°C were fully
liquefied by incubation at room temperature (ca.
23°C), and PCM-28 designated for the same temper-
atures was fully liquefied by incubation at 37°C for 24
h prior to testing. All PCMs designated for 37°C were
fully solidified by cooling at 10°C for 24 h prior to
testing. The Re-Freez-R-Brix and bubble wrap were
held at room temperature prior to testing in −20°C and
4°C chambers and chilled at 10°C prior to testing in
the 37°C chamber. A Fisherbrand low-temperature
incubator was used for all 10°C incubations. Each type
of material was wrapped around a LogTag™ data
recorder (LogTag Recorders Ltd, Auckland, NZ) to
record the internal temperature every 5 min and placed
in a 1.5 liter BioJar™ container inside an AirSea™
shipping box (AirSea Containers, McDonald, PA).
The LogTag™ devices are 86 × 54.5 × 8.6 mm, which
is similar to a pouch of mosquito eggs or a vial of
ticks, and was considered to reasonably represent the
size and temperatures that would be experienced by
one of these. A comparable amount of each stabilizing
material was used in all experiments: 1 Re-Freez-R-
Brix (429 g), 3 PCM-22 flex packs (170 g each), 4
PCM-15 pouches (102 g each), or 3 PCM-28 mats
(145 g each). The bubble wrap cell sizes were
approximately 2 × 2 cm. Two 50.8 × 15.2 cm sheets of
bubble wrap were used, which weigh much less
than the other materials. The boxes were taped closed
and placed in the appropriate environmental condition.
An additional data recorder was placed outside the
boxes to record the external temperature at 5 min
intervals. The containers were left in the environmen-
tal conditions for 4 days. The containers were then
removed, and the temperature data were downloaded
using LogTag Analyzer for Windows™ (LogTag
Recorders Ltd, Auckland, NZ).

We determined the time that containers tested
under each condition would maintain temperatures
within a range that might permit survival of various
arthropods (Block et al. 1990). For cold and freezing
conditions, these were the amounts of time during
which the logger measured temperatures above 5°C
or 10°C. For hot conditions, these were times when it
was lower than 30°C or 35°C. The analysis of two
values for the cold and freezing ambient temperature
data sets and the hot data used different calculations
based on the same data sets. Each combination of
shipping material and environmental condition tested
was measured three times.

The time taken to reach these predetermined and
biologically significant temperatures was considered
primary data. Analysis of variance with Tukey HSD
post hoc tests were used to estimate difference as a
function of the insulating material used. Throughout,
statistical analyses were performed using R 3.5.1 (R
Core Team 2018).

RESULTS

Typical temperature log results of two trials are
shown (Fig. 1). Although data were collected for 4
days, in these uninsulated boxes, all of the containers
used reached the ambient temperature within 2 days.

Ambient colder: There were differences in the
stabilizing capacity of these materials, though the
pattern was similar at both cold and freezing external
temperatures (Table 1). The PCM-22 outperformed
all other materials (P < 0.001 in all between-
temperature comparisons) and delayed the tempera-
ture decrease below the chosen critical limits longest
(Figs. 2 and 3). At 4°C all two-way comparisons were
significant (P < 0.001, Fig. 2). At −20°C ambient
conditions (Fig. 3), PCM-15 provided no benefit over
bubble wrap to 10°C (P = 0.34) though PCM-15
delayed chilling for longer than bubble wrap to 5°C
(P < 0.05). All other two-way comparisons indicated
statistically significant differences in performance (P
< 0.001).

Ambient warmer: When exposed to warmer
external temperatures, there were differences in
performance in the stabilizing capacity of these
materials (Table 1, Fig. 4). Phase change materials, PCM-22 and PCM-28, did not differ in time required to exceed 30 or 35°C ($P = 0.90$ and $P = 0.86$, respectively) and delayed the temperature rise the longest. To 30°C, PCM-15 outperformed bubble wrap ($P < 0.05$), and all other two-way comparisons were significant ($P < 0.001$). To 35°C, PCM-22 outperformed Re-Freeze-R-Brix ($P = 0.02$), though PCM-28 did not ($P = 0.11$). All other two-way comparisons indicated a clear difference in performance ($P < 0.001$). In Table 2 we summarize these results and thus our recommendations for the convenience of the reader.

**DISCUSSION**

Most live arthropod shipments are made using express courier services such as FedEx, UPS, and DHL, and a sender might reasonably be concerned about the conditions experienced by their material both on the ground and in the air. Depending on the perishability of the material, next day or priority overnight delivery might be chosen when available. At the origin where the package is prepared, the temperatures experienced are under the control of the sender, but once the package leaves their hands, it will be exposed to the ambient temperatures of loading docks, trucks, shipping containers used for airfreight, and airplanes. In a study sharing similar concerns that stimulated the study reported here, Schallenberger et al. (2016) determined the temperatures experienced by courier shipments from Belgrade, MT, to five warm locations within the USA during August and September. They observed that the package experienced temperatures consistent with the destinations and no cold extremes. Singh et al. (2010) observed the temperatures experienced during FedEx™ Priority Overnight™ shipments that included both short feeder and regular pressurized flights, also within the USA. The minimum and maximum temperatures experienced in flight were 13.9°C and 26°C, respectively, and the averages ranged from 21.4°C to 26.9°C. Placement location of the package within aircraft along with the duration of the flight can also affect the package. Emond et al. (1999) recorded temperatures ranging from 10°C to 31°C in different sections of the holds of Boeing 747-400 combi aircraft set up for cargo transport with substantial variation within short distances in the cargo hold (17°C over 2 m). Generally, though, the mostly moderate temperatures experienced in flight are within typical biological limits, but the temperatures and times on the ground are perhaps even more important to consider. In a recent study of conditions met by perishable horticultural crops during international air transport (Pelletier et al. 2018), extended transfer times were witnessed; for three shipments observed, the total time outside on the tarmac exceeded 11 h.

Table 1. Summary ANOVA statistics for variation in the capacity of the tested materials to buffer temperature change under a range of conditions as determined by the time taken to reach each critical temperature.

| Material starting temperature (°C) | Ambient temperature (°C) | Critical temperature (°C) | F     | df  | P     |
|-----------------------------------|--------------------------|---------------------------|-------|-----|-------|
| 23                                | 4                        | 10                        | 233.2 | 3, 8 | <0.001|
| 23                                | -20                      | 10                        | 273.9 | 3, 8 | <0.001|
| 10                                | 37                       | 30                        | 651.2 | 3, 8 | <0.001|
|                                  |                          | 35                        | 345.8 | 4, 10| <0.001|
|                                  |                          |                          | 295.9 | 4, 10| <0.001|
Fig. 2. Time taken, from a starting temperature of 23°C, for containers to reach 10°C (left) and 5°C (right) at ambient conditions of 4°C. Error bars are 95% CI, n = 3 per material. Bubble, bubble wrap; RFR-Bx, Re-Freez-R-Brix

Fig. 3. Time taken, from a starting temperature of 23°C, for containers to reach 10°C (left) and 5°C (right) at ambient conditions of −20°C. Error bars are 95% CI, n = 3 per material. Bubble, bubble wrap; RFR-Bx, Re-Freez-R-Brix

Fig. 4. Time taken, from a starting temperature of 10°C, for containers to reach 30°C (left) and 35°C (right) at ambient conditions of 37°C. Error bars are 95% CI, n = 3 per material. Bubble, bubble wrap; RFR-Bx, Re-Freez-R-Brix
In this study, we compared temperature stabilizing materials for the practical purpose of protecting arthropods by buffering temperatures during ship-ments in the absence of significant insulation. The effect of any of these materials will be extended if the shipping containers are well insulated, a practice that provides both temperature and physical protection. The conditions that were chosen were those that we believed represented critical stresses depending on the location from and to which the shipment is being made. Overall, PCM-22 provided the best protection regardless of the conditions. In the absence of this product, Re-Freeze-R-Brix is widely accessible and could be used as a substitute that, while not as effective, still provides good protection.

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**Table 2. Summary of materials that provided the longest temperature stabilization among those tested.**

| Expected ambient conditions | To maintain the temperature above 5°C or 10°C | To maintain the temperature below 30°C or 35°C |
|----------------------------|---------------------------------------------|----------------------------------------------|
| 4°C                        | PCM*-22                                     | NA                                           |
| −20°C                      | NA                                          | PCM-22 or PCM-28                             |
| 37°C                       | PCMB-28 or PCMB-28                          | NA                                           |