EXHIBIT 4

To Plaintiffs' Motion For Sanctions For Spoliation and Discovery Violations Civ. No. 03-2006 (EGS/JMF)

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Circus Vargus

Objectives

- 1. Characterize the environment within truck and rail cars that are typically used for transporting circus elephants during hot and cold climatic conditions.
- 2. Characterize the reaction of circus elephants to transport in trucks and rail cars during hot and cold conditions.

Introduction

Transportation of livestock has occurred for over 3500 years (Cregier. 1982). Contemporarily, animals are transported for a variety of reasons including for agriculture, athletic events, and entertainment. A vast amount of research has demonstrated that transportation negatively affects animals and severely compromises welfare. Broom (1998) defines the term welfare as the state of an animal as it works to maintain homeostasis within its environment. Research of animal transportation has largely focused on livestock species in which transport is essential for moving animals from the producer to slaughter facilities, between units in a production system, etc. No work has been published investigating the transportation of elephants used by circuses and other exhibitor groups, both of which undergo quite rigorous and lengthy travel schedules through extreme weather conditions. Thus, the effects of transport on circus elephants and the conditions experienced by these animals are relatively unknown. However, growing public concern over the lifestyle of circus animals has generated a need for investigation into the management practices of circuses and animal exhibitors.

Research has shown that livestock species are negatively affected by transport through a variety of means that suggest compromised welfare of these animals (e.g., Eicher, 2000; Swanson and Morrow-Tesch, 2000; Zanella, 2000). Transportation of healthy horses for 24 hr resulted in weight loss and raised body temperature, among other factors indicating compromised welfare (Friend et al., 1998; Stull and Rodiek, 2000). Swine transported for 16- and 24-hr periods suffered weight loss, raised cortisol concentrations, and had indications of muscle fatigue (Brown et al., 1999). Research by Knowles et al. (1995) demonstrated that sheep undergoing transport for periods of three, nine, 15-, 18- and 24-h experienced increased plasma cortisol and heart rates, though apparently adapted after nine hours of transport. Knowles et al. (1999) also found that transported cattle for 14-, 21-, 26-, and 31-h suffered from weight loss, increased plasma cortisol concentrations, as well as increased concentrations of plasma creatine kinase. Increased plasma creatine kinase (CK) suggests that tissue damage occurred during travel. Transport of steers for 24-h compromised immune function and resulted in elevated plasma cortisol and plasma glucose concentrations (Tarrant et al., 1992).

Though transported livestock are generally believed to suffer from compromised welfare, the sources of these effects are complex. An understanding of these stress-producing elements or stressors would allow transporters to eliminate or reduce the stress placed on the animal.

Density

Density is considered one of the most important factors when transporting livestock. Density was determined to be proportional to the total number and severity of injuries horses received and the time to regain balance once they fell during a 30-min transit session (Collins et al., 2000). Indication of muscle fatigue was seen in lambs transported at high density (Knowles et al., 1998). General fatigue was also seen in sheep during the post transport period (Cockram et al., 1996). Cattle transported at comparatively high densities had elevated plasma cortisol and glucose, as well as elevated concentrations of plasma CK, thought to be a result of the animals' inability to

adopt a preferred orientation and/or adjust to changes in the vehicle's motion (Tarrant et al., 1992). Eldridge and Windfield (1988b) determined that high density conditions significantly increased the general occurrence of injuries in transported cattle.

Additionally, high densities are associated with increased loss of balance, considered the major risk for injury in cattle transport (Tarrant, 1990; Tarrant and Grandin. 1993).

Investigations of densities in swine transport revealed that select tissues had elevated pH during the highest treatment density indicating muscle fatigue (Lambooy and Engel, 1991). This fatigue is most likely a result of laying pigs being disturbed by those attempting to lie or pigs not being able to lie at all (Lamboy and Engel, 1991; Warriss, 1998).

Duration of Transport

The duration that horses and livestock are transported has become an increasingly important topic as the number of processing and slaughtering plants has decreased. Transporters are forced to make longer trips during which animals are typically not provided water and/or food during transit. Particularly during hot weather and longer trips, access to water is essential to maximize the body's cooling efforts. Friend et al. (1998, 2000) determined that healthy horses transported during hot, humid conditions (>90°F) for longer than 24 hr without access to water showed signs of extreme dehydration. Friend also found that that providing horses with water during the trip delayed the onset of dehydration indefinitely, although fatigue became limiting factor after 28 hours. Lambs undergoing trips greater than 15 hr benefited from a twohour stop during which they received food and water. In trips longer than 24 hr. an eight-hour rest before 10 additional hours of transport proved beneficial to the lambs (Knowles et al., 1996). Knowles et al. (1999) transported cattle for 31 hr with water provided at 14 h and found the trips not extremely physically demanding, though signs of fatigue were evident after 24 hr. However, the researchers observed that many animals (42%) did not drink when offered water. External temperature during the trip was moderately warm with a high of 84.2°C. The researchers also suggested that

towards the end of the journey, additional fatigue may have been caused by sleep deprivation as the animals had to remain awake to maintain balance. The density of the animals transported in the trailer most likely made lying down difficult as discussed above. Hoffman et al. (1998) observed a proportional increase in the severity of bruises in beef cows with varying transport distances. It was concluded that this was most likely due to fatigue from withholding food, among other factors. Brown et al. (1999) determined that pigs transported at a low stocking density with access to food and water did not show elevated plasma cortisol, CK or other signs of stress despite a 24-hour transit time.

Unfamiliar Animals

Individual livestock typically develop relationships over time with herdmates that reflect that particular specie's social organization (Craig, 1986). Mixing unfamiliar animals during transport where these relationships have not developed is considered a major stressor in the transport of horses (Grandin et al., 1999; Friend, 2000), calves (Trunkfield and Broom. 1990), and swine (Kilgour and Dalton, 1984). Kenny and Tarrant (1987) found that incidences of fighting and elevated plasma CK cortisol increased in young bulls when reorganized in unfamiliar groups and transported for one hour. Mixing conspecifics reared in isolation will generally increase this problem (Trunkfield and Broom. 1990).

Noxious Gases

Concentrations of noxious gases in transportation situations and the resulting effects are not well researched, though a six-day exposure to constant concentrations of ammonia in swine production systems was shown to compromise immune resistance in a rate proportional to exposure (Urbain et al., 1994). Animals were noticed to be lethargic within two days of 50-ppm exposure. Swine on a restricted diet avoided food when subjected to short bursts of ammonia indicating that the gas's presence may interfere with the expression of feeding activity (Jones et al., 1997).

Specific Events

In addition to certain characteristics of transport increasing fatigue or other measures of stress, specific events can also be stressful. Waran and Cuddeford (1995) investigated loading in horses and concluded that multiple evasive behaviors during loading of yearlings suggested a clear desire not to enter the trailer. The behavior was most probably related to a horse's fear of dark, closed areas (Cregier, 1982). Knowles (1998) suggested that the first few hours of sheep transport were the most stressful as demonstrated by a sharp rise in heart rate and cortisol concentrations during the first four hours of transport. Tennessen et al. (1984) found the loading process in steer transportation to be the most stressful component of transport as suggested by increased heart rates. Kent and Ewbanks (1983) reported elevated cortisol concentrations of sixmonth-old calves immediately after loading and again before departure. Tarant (1990), in a review of cattle transport, suggests that loading in particular is stressful, though she advised that similar results of similar investigations were variable. The variability may be a result of the overall stresses imposed by transport rather than loading procedures alone (Kent and Ewbanks, 1983; Tarrant, 1990). Loading was found to be the most stressful and physically demanding event in swine transport (Augustini and Fischer. 1982) when substantially increased heart rates and rectal temperatures were recorded. In general, livestock, with the exception of horses, are usually herded onto transport vehicles using a dog or person as a driver, a process that inevitably will cause a fear response.

Environmental Characteristics

The environmental characteristics in the transport vehicle can be the most important factor in assessing conditions that will affect welfare. Temperature extremes can impose their own stresses such as hypo- and hyperthermia associated with impaired thermoregulation and increased sweating. Increased sweating will speed the onset of dehydration or potentially exacerbate the aforementioned factors. For instance, consider

a group of cattle transported in highly dense numbers during hot weather. In addition to the stressors associated with high density, the animals will have reduced ability to dissipate heat to the surrounding air as a result of the decreased space provided for each animal. In certain trailers, where ventilation is solely through vents in the side of the trailer, animals in high density conditions will be pushed against the sides and cover the vent holes, reducing ventilation rates (Randall, 1993). Conditions that cause the animals to be more active, such as mixing unfamiliar conspecifics (Kenny and Tarrant, 1987), will worsen conditions through increased heat production and water loss associated with physical activity. Additional water loss through excessive urination and highly liquid feces, observed in calf transport (Kent and Ewbanks, 1983), is likely to be a debilitating factor as well. Increased defecation will make the trailer floor slippery, compounding problems. Similar results would be expected with other livestock. Rare cases of large numbers of livestock deaths associated with high external temperatures have been reported (Knowles, 1998).

Habituation

Despite the stressors and rigors associated with transport, research has shown that animals can be habituated to the process. Often this is not practical in a production situation, particularly when animals that are brought to slaughter might spend their entire or a large part of their life in a range setting without any exposure to humans or confinement. However animals transported for other reasons, such as horses used in athletic events and circus animals, undergo regular and routine transportation.

Adams (1994), in a review of animal transportation and welfare, suggested that the capacity of animals to adapt to the conditions of transport is essential in minimizing negative impacts on welfare. Grandin (1997) conducted a similar review of adaptation in travel and recommended acclimating animals to stressors early in life to reduce the stress response during exposure as an adult. The capacity to adapt is dependent on a variety of factors, such as the fearfulness of the individual and the species as a whole, and the nature of previous experiences. Waran and Cuddeford (1995) studied the effects

of loading on several groups of horses, each with a different level of experience ranging from yearlings with no experience to adult mares with numerous experiences. The yearling group took significantly more time to load and displayed the most struggle through a multitude of evasive maneuvers. This distinctly contrasted with the other groups; researchers observed cases of older individual horses pulling the handler into the trailer at times, indicating willingness to load. Knowles et al. (1995) observed that after transporting sheep for nine hours in a 24-hr journey, measurements of cortisol, glucose, and heart rate had returned to pre-transport levels indicating that the animals no longer perceived transport as stressful. Similar decreases in heart rate were reported by Tennessen et al. (1984) after loading steers in preparation for transport.

Tarrant (1990) suggested that cattle almost always exhibit signs of stress through altered behavioral and physiological parameters when being transported. However, in the absence of poor conditions, cattle will adapt to the journey and avoid distress, a state characterized by the development of radical behavioral changes and pathological disorders. Other evidence of habituation to transport in cattle has been observed by Eldridge and Winfield (1988a) and reviewed by Trunkfield and Broom (1990). Crookshank et al. (1979) noted increased habituation in calves during handling, an effect possibly due to the young age of the animals. Additionally, Friend (1991) suggested in a review of animal adaptation that after an initial response, often characterized by hyperactivity, escape attempts, and other behavioral signs of acute stress, the animals may adapt and as a result cease exhibiting signs of stress.

Summary

In summary, livestock transport is generally considered a stressful process. A better understanding of the factors which cause the stress can allow for improved travel methods. Some of the factors that have been identified are travel density, trip duration. access to food and water, mixture of unfamiliar animals, concentration of noxious gases, specific events such as loading, and high environmental temperatures. Lastly, adaptation to travel can reduce the stresses imposed on the animal during transit.

Relevancy and Background of Methods

In the following sections, the relevancy of certain factors to animal welfare is considered. These factors include environmental conditions, physiological, psychological, and behavioral elements. Environmental conditions can provide information as to whether the animal's environment compromises welfare from a causal perspective. For example, welfare can be assumed to be compromised in animals being transported where ammonia concentrations exceed an amount regarded as safe. The latter three factors can indicate compromised welfare by providing a partial picture of an animal's internal state. For instance, extreme fluctuations in body temperature indicate the animal is not successfully coping with temperature loads and potentially suffering.

Environmental Conditions

Determining environmental temperature and humidity within the transport vehicle during transport is important to insure that interior temperatures do not become extreme or dangerous. Maintaining numerous mammals in a small, enclosed space with inadequate ventilation while external temperatures reach critically high levels could create an excessive temperature buildup inside the vehicle. Conversely, extreme cold temperatures can create an environment where animals can suffer from excessive discomfort or worse, hypothermia and/or frostbite. Maintaining animals at extreme temperatures to which they are not adapted can have severe, if not fatal, effects. Thus, the internal temperature of the transport vehicle can provide crucial information in regards to the quality of transport.

Instrumenting transport vehicles with equipment to record temperature and humidity parameters can verify the temperature inside the vehicle and whether the conditions are hazardous. A set of continuous readings can suggest correlations with ambient parameters as well as specific events during transport. Furthermore, information regarding attempts by transport personnel to maintain interior conditions

within certain ranges, i.e., insulation, ventilation fans, can potentially be evaluated. If successful, certain procedures can benefit any industry that transports animals under similar conditions.

A series of three articles (Baker et al., 1996; Hoxey et al., 1996; Kettlewell et al., 2000) investigated the ventilation, heat production, aerodynamic characteristics, and effects of a forced ventilation system involved in transporting broilers by truck and attempted to correlate these parameters with indicators of welfare and stress. Results showed that: interior temperatures were affected by vehicular speed, ventilation was affected by a variety of factors including structural design and position, among other details which potentially provide a model for improved transport of broilers. Randall (1993) and Schrama et al. (1996) reviewed comparative research for livestock and suggested environmental parameters and other details necessary for comfortable and safe travel of livestock. Similarly, Hahn (1999) reviewed efforts to define appropriate thermal heat loads for cattle and their effects on measures of animal health and productivity. He recommends that the response of these measures during heat loads can be used to establish management strategies to effectively deal with high temperatures.

Noxious Gases

Exposure to noxious gases is another critical concern during transport (Randall, 1993). With proper ventilation and/or containment of the gases in question, problems can be eliminated. Concentrations of ammonia under 25 ppm should be maintained; exposure to greater concentrations can elicit irritation and in larger quantities are associated with breathing difficulties (CDC, 2001). In transportation, the principle source of ammonia is from urine, where urea will degrade to ammonia over time. In terms of carbon monoxide, concentrations of 25 ppm are the maximum level of safe exposure; effects include headache, nausea, and dizziness (CDC, 2001). The principal source of carbon monoxide during transportation is from vehicular emissions.

Body Temperature

Proper thermoregulation is essential to homeotherms, a class of animals that must maintain a constant body temperature in the face of changing external and internal conditions. Under ideal functioning, the body's temperature is maintained at a specific level in thermal equilibrium where heat production is balanced with heat loss (Bligh, 1973; Schrama et al., 1996). If environmental temperatures exceed body temperature (warmer environments) or if heat production from the body exceeds the organism's ability to dissipate heat to the environment, heat will be stored in the animal's body, raising body temperature. The animal, in an attempt to prevent elevated body temperature, maximizes heat loss through processes such as perspiration, panting, or other species-specific methods (Bligh, 1973). If possible, the animal can also decrease its own heat production through reduced activity and feed intake. The animal may also move to a cooler environment to minimize heat flow to the organism from the environment.

Contrasting this, in conditions where the environmental temperature is less than body temperature (colder environments), the surrounding environment will act as a sink and draw heat from the body (Bligh, 1973), lowering the body temperature of the animal. In response, the animal alters its behavior by moving to a warmer environment in an effort to reduce heat loss from the body. If this proves ineffective, the animal increases heat production through increased activity, metabolic rate, shivering, or other physiological and physical responses depending on the severity of the difference.

Animals can tolerate a range of environmental temperatures over which their bodies can safely maintain a required body temperature. This range is dependent on species, prior exposure, and type of housing, among other factors which can either raise or lower the upper and lower limits of this range (Randall, 1993). Environmental temperatures outside of this range can disrupt thermoregulation processes (Hahn, 1999) and cause the animal to suffer from hypothermia/hyperthermia because the animal does not possess the mechanisms to maintain an appropriate body temperature through heat loss/production under such extreme conditions. Thus, fluctuations in body temperature

in response to environmental temperatures can be used to indicate heat or cold stress (Schrama et al., 1996) and impacts on animal welfare. Raised body temperature or fever indicates other ailments.

Fluctuations in body temperature can be used to determine acceptable temperature ranges during transportation. Friend et al. (1998) and Stull and Rodiek (2000) found body temperature measurements to be a useful indicator of health during transportation of horses under relatively hot conditions where a maximum of 95°F was reached. Tennnessen et al. (1984) detected significantly raised body temperatures in bulls transported for two hours where external temperature reached a maximum of 82.4°F. Under simulated conditions, Lambooy et al. (1981) used heat production and environmental temperatures to determine acceptable temperature ranges in swine transport. Becker et al. (1985) correlated increased body temperature and concentrations of serum cortisol with increased heat exposure. Augustini and Fischer (1996) also found body temperature as a useful indicator of stressful conditions in transported swine.

Cortisol

Cortisol, classified as a glucocorticoid, is a hormone that has a variety of effects on the body's metabolic functions including gluconeogenesis and the breakdown of proteins, carbohydrates and fats. Cortisol is released from the adrenal cortex in a series of processes that begins with stimulation of the hypothalamus to release corticotropin-releasing hormone (CRH) which acts upon the anterior pituitary (AP) gland to cleave proopiomelanocortin (POMC). Adrenocorticotropic hormone (ACTH), a product of cleaving POMC, is released from the AP and activates the adrenal cortex to release cortisol which then binds to target tissues within several hours (Norris, 1980; Guyton, 1991). This series of glands, responses, and hormones is often referred to as the hypothalamic pituitary adrenal (HPA) axis or system.

The role of cortisol in the stress response was first described by Selye's (1936) General Adaption Syndrome (GAS) which involved the release of ACTH and resulting elevated plasma cortisol concentrations in response to stressors imposed on the animal.

Since then, knowledge of cortisol and the processes that affect it has grown tremendously though questions remain in interpreting fluctuations in concentrations. For instance, Moberg (1987) cites the difficulty in interpreting varying levels of cortisol concentrations and their inconsistency with other stress indicators. Furthermore, the activation of the adrenal cortex, a system that has evolved to aid organisms to adjust to stressors, may falsely imply the animal was affected negatively by the stimulus.

Nonetheless, cortisol concentrations remains a powerful tool in determining stimuli/situations that an animal perceives as stressful and is widely used in behavioral research (e.g., Becker et al., 1985; Dathe et al., 1992). Cortisol is generally considered an indicator of psychological stress (Mason, 1971; Dantzer and Mormede, 1983), though it can be used to detect stress in a broad range of situations because the psychological component is often present (Dantzer and Mormede, 1983). For instance, the presence of a dominant pig in the pen of a subordinate raised cortisol concentrations significantly whether fighting occurred or not (Arnone and Dantzer, 1980).

Cortisol has been used to indicate the affect of stressors that are applied or perceived over various durations, from a few seconds (Lay et al., 1992), several hours (Becker, 1985), and days (Dathe et al., 1992). Exposures to acute heat and cold temperatures have been shown to elevate cortisol concentrations (Alvarez and Johnson, 1973; Blatchford et al., 1978), illustrating the diverse range of stimuli to which the hypothalamic pituitary axis is capable of reacting.

Cortisol has been used to measure stress during transport in horses (Friend et al., 1998), sheep (Knowles, 1998), cattle (Crookshank et al., 1979), and swine (Brown et al., 1999). In a study (Kenny and Tarrant, 1987) examining a variety of factors affecting hour long transportation sessions in young bulls, cortisol was found to be a superior physiological measure of stress during transport compared with non-esterified fatty acids (NEFA), plasma CK, and glucose. However, the researchers suggested that during longer trips cortisol concentrations measured towards the conclusion of the journey might be reduced as the animals adapted to transport. This scenario could falsely imply the animal was not affected by travel as earlier events could have been very stressful.

Direct and Video Observations

Direct and video observations can provide valuable information in determining an animal's perception of its environment and whether its behavioral needs are satisfied. However, Duncan (1981) recommends caution in behavioral observations, mainly regarding attempts by humans to distinguish normal behaviors in non-human animals. Friend (1991) reviewed several vital concepts regarding normal behaviors that are important to consider when conducting such observations. Firstly, the absence of grooming and other self-maintenance behaviors indicate a stressful environment. Secondly, exploratory behavior is normally exhibited only when an animal is in an environment perceived as safe. Lastly, failure to participate in group herd behaviors and/or altered eating patterns may indicate the animal is under stress.

The occurrence of stereotypic behavior can also be useful when considering an animal's internal state. Stereotypic behavior is defined as a repetitive action that occurs relatively unchanging over time and without apparent function (Dantzer, 1986). Typically performed in animals in confinement, stereotypies are believed to suggest that the animal exhibiting the behavior finds its current environment stressful (Mendl, 1991). The actions may be a means for the animal to provide itself stimulation in a "dull" environment or to dissipate tension or frustration caused by the environment's inadequacies (Dantzer, 1986; Dantzer and Mormede, 1981). Often the stereotypic behavior itself may result in secondary problems such as foot injuries from excessive pacing.

Though stereotypic behavior normally infers a poor environment, the potential causes and effects of the behavior must be considered before accurate conclusions regarding impacts on welfare can be drawn. For instance, animals using stereotypic behavior as release of anticipation may not suffer from compromised welfare if the "built-up emotion" is released within an appropriate amount of time, indicated by the halting of the behavior. Stereotypic behaviors that develop in a lacking or stressful environment may also persist after the stressor is removed (Mason, 1991).

Methods

Overall Procedure

The researchers, with suggestions from circus management, attempted to identify at least two occasions when participating circuses or private exhibitors with elephants were transporting animals under relatively hot and cold conditions reflective of typical seasonal conditions. Due to the rapid and inflexible travel schedule circuses follow, some facets of our procedure could not be performed for some participating circuses. During each trip, one or more vehicles (either truck or railcar) were instrumented to characterize environmental conditions (temperature, relative humidity) of the transport environment. Ambient conditions (temperature, relative humidity, and solar radiation) were also monitored concurrently. Air samples were taken when possible to determine levels of ammonia and carbon monoxide.

The researchers measured body temperature continuously during transport by using ingested temperature loggers as an indication of the elephants' physiological response. To estimate the psychological response of elephants to transport, blood was collected before and after transport to determine cortisol concentrations. This measure was collected only during hot weather surveys because obtaining blood samples from elephants via venipuncture during cold weather is extremely difficult. During cold weather, elephants will typically direct blood away from their peripheral veins in an attempt to conserve body heat. Direct and/or video observations of the animals during transport were also gathered when possible.

Environmental Measures

To record environmental conditions of the railcar or truck, environmental dataloggers (HOBO-H8, Onset Computers, Pocasset, MA) with the capacity to record

temperature and relative humidity were mounted throughout the transport vehicle. Additional channels for temperature probes could be used. Probes consisted of a thermocouple at the end of a wire lead of varying length. This specific group of loggers are referred to as environmental loggers throughout this report. The environmental loggers were mounted to the wall of the transport vehicle on a piece of styrofoam (4' X 4' X 0.75" wide) that insulated the logger from the wall. The environmental loggers were factory tested and found to be accurate within ± 1.8°F. Spreading the environmental loggers over multiple sites in the transport unit (when possible) was done to investigate temperature gradients within the trailer/railcar and increase the chances that environmental loggers would be recovered after transit. Due to the substantial reach an elephant has with its trunk, the loggers were generally in an area behind the elephant and significantly in front of other elephants was sought to place the environmental loggers. The environmental loggers recorded temperature and relative humidity in the vehicle at 5-minute intervals. Another environmental logger of the same type was mounted outside of the truck/railcar to determine external temperature. relative humidity, and solar radiation. Solar radiation was estimated using a copper black globe fitted with a temperature probe inside the globe and then sealed with duct tape. The globe was then attached to the vehicle's exterior in an area that would receive direct sunlight. Solar radiation values quantify radiant heat from the sun, i.e. high black globe temperature indicates the globe was receiving direct sunlight. Comparisons of interior and exterior measures were made to determine whether adequate ventilation was occurring and to establish the relationship between the two environments.

Noxious Gases

Ammonia and carbon monoxide concentrations were determined using a toxic gas detection kit (80140-KA, Matheson-Kitagawa, Parsippany, NJ). Our equipment was capable of detecting concentrations of ammonia between 10-260 ppm and carbon monoxide concentrations of 1 –1000 ppm. In cases where trips lasted less than three hours, measurements were taken immediately upon arrival at the destination before the

doors were opened using a flexible plastic tube 3.0 ft in length inserted into a ventilation opening. In cases of transport over several days, recordings were made when possible every 24 h during transport and upon arrival.

Body Temperature

In order to measure body temperature, our lab developed a procedure using a miniature datalogger (DS1921-F5, Dallas Semiconductor, Dallas, TX) encased in a biologically inert epoxy (EPO-TEK T905, Epoxy Technologies, Bourne, MA) to record continuos body temperature by having the animal ingest the unit. These particular loggers are referred to as body temperature loggers in this report. The body temperature loggers are factory tested and claimed by the manufacturer to be accurate within ±1.8°F and exhibit a ±0.9°F level of precision. Each body temperature logger was a round disc with an approximate diameter of 0.64" and 0.23" thick. When the epoxy potting process was completed, the logger was 0.8" wide and 0.50" thick. The end of a ripstop nylon ribbon approximately 0.75" in width and 6" long was embedded in the epoxy to make the body temperature loggers more visible during recovery. Each recorded temperature reading was time/date stamped, allowing fluctuations in body temperature to be correlated with transportation events (i.e. loading) and/or changes in environmental parameters. A similar procedure using a transmitter encased in a plastic shell approximately twice the size as that used in the present study was used by Dr. Michael Schmidt (personal communication) and was found to be safe and accurate. In our own test of the body temperature logger's accuracy and precision, two random assortments of a total of 33 unused body temperature loggers were put in a water bath for 30 min during which four readings were made with a thermometer. The body temperature loggers read an average of $0.59^{\circ}F \pm 0.13^{\circ}F$ less than the thermometer reading. The largest average difference for a single body temperature logger over the four thermometer readings was -1.82°F.

The normal Asian Elephant body temperature is 95°F to 98.6°F (AAZK, 1992), though several ranges have been reported (Benedict, 1936; Altevogt, 1990), that have

slightly higher minimum value for the range. Body temperature data were examined to detect increases of individual elephant temperatures greater than 3.0°F. Fluctuations in body temperature greater than 3.0°F indicate the animals were experiencing a significant heat load. Body temperature was also examined for temperatures greater than 100°F. Actual temperatures greater than 100°F indicate considerable fever (Benedict, 1936) or general thermoregulatory difficulties (Schmitt, personal communication).

Cortisol

When possible trips during the hot weather surveys, circus personnel took blood samples from the elephants for plasma cortisol analysis. Pre-transport samples for the Carson and Barnes circus were taken an hour before and then immediately before loading. Post transport samples were taken immediately after unloading and then again an hour later. During trips for Ringling Red and Ringling Blue, the first sample was taken immediately before the animals were led to the train and the second immediately after loading. The third sample was taken after the walk to the animal compound and the fourth an hour after that. During the trip for Ringling Blue, an early sample was taken approximately four hours before the one-hour pre-shipment sample.

To serve as a comparison and demonstrate fluctuations in cortisol in response to a positive stimulus, blood samples were taken an hour before. a half-hour before, immediately after, and a half-hour after being brought to their exercise pen were they were turned loose and provided watermelons.

At each sampling, approximately 5 ml of blood were collected using a vacutainer containing lithium-heparin. The samples were placed on ice until the samples were centrifuged, the plasma collected, and frozen. Cortisol concentrations were determined using a cortisol assay kit (DPC, Los Angeles, CA). To control for diurnal effects, a second set of control blood samples was obtained from each of the elephants in the study. The goal was to collect control blood samples at the same time of day as the samples obtained during transport but during days when the elephants were idle.

Direct and Video Observation

Video footage were made of the animals whenever possible during transport. This record allowed the researchers to determine whether the animals were performing abnormal behaviors, principally weaving, the most commonly observed stereotypic behavior in circus elephants when not being transported (Friend, 1999). Weaving was defined as a continous shifting of the animal's weight from either side to side or to-and-fro for a period greater than 30 seconds, a definition adapted from Schmid (1995). If steps were taken in this described fashion repeatedly, this was also considered weaving. Animals were also observed for the occurrence of normal behaviors that are commonly seen when not being transported or in a natural setting, i.e. throwing hay on their backs, drinking, and eating. Video data were collected by mounting a video camera (Panasonic WV-BP312 CCTV, Seacaucus, NJ) with a light source in the vehicle. The video was recorded on a time-lapse video recorder (Panasonic VHS AG-1070, Seacaucus, NJ) and analyzed for the percentage of time spent weaving, laying down, and standing using Etholog (Etholog 2.2, Sao Paolo, SP, Brazil), a software program for the timing of behavior observation sessions (Ottoni, 2000).

Direct observations were also made by the researcher whenever possible. The researcher rode in the actual elephant compartment of the transport vehicle during transport when possible.

Statistical Analysis

Results from blood collection were divided into two categories: short (Carson and Barnes) and long trips (Ringling Red, Ringling Blue). For short trips, sources of variation between sample times were tested by analysis of variance using the general linear model repeated measures procedures of SAS (SAS, 1999) where sample time was the independent variable and cortisol concentration was the dependent variable.

Repeated measures procedures are required when successive samples are taken from the same animal during the same test period. Tukey's test, a statistical test that minimizes

both Type I and Type II errors, was used to detect significant differences between each sampling time if the sample time variable was found significant (α <.05). Comparisons with controls were not possible due to an unsuccessful attempt to get accurate control samples.

During longer trips, a student's t-test was used to detect significant differences between means of control and transport cortisol concentrations for each sampling time. Use of ANOVA repeated measures was considered for long trip analysis, but rejected because only two means (transport and control means for each sample time) were to be compared during each of the five sample times instead of five means as in the short trip analysis. Thus, under these conditions, ANOVA did not provide any statistical benefit and a student's t-test was used.

Transport Vehicle Descriptions

To determine ventilation and design characteristics of each vehicle, elevations detailing overall dimensions, windows, doors, ventilation equipment (i.e. fans), and other particulars were developed for each vehicle using computer aided design software (AutoCad, 1997).

Results

General

A plot of the environmental and body temperature data is provided for each trip that a circus made which we surveyed. The legend codes for each parameter displayed, which vehicle the environmental logger was recording in, and whether a probe recorded the measure or not. For example, with "temp (60p)", "temp (60*)", and "temp(62), each of the three indicated environmental loggers recorded temperature. The first and second environmental loggers recorded in vehicle "60" and the third in vehicle "62". The first temperature was recorded by a probe as indicated by the "p"; the remaining two were recorded by the environmental logger itself as indicated by the absence of a "p".

Body temperatures are labeled to indicate duplicates (i.e. Miniak and Miniak(*)) when more than one body temperature logger was recovered from a single animal during the same time frame. Loggers that were given in sequential time frames during the course of the trip are labeled (i.e. Miniak(1), Miniak(2), etc.).

Hot Weather

Ringling Brothers, Barnum and Bailey (Red Unit)

The elephants with Ringling Red travel throughout North America in railcars that are part of a train that is nearly a mile long. They typically begin take down and prepare to leave an arena at 17:00 on Sunday nights. After the last show, generally 22:00, the animals are walked one to three miles to the railcar and then loaded. The various sections of the train are then assembled, if not done already. The train then departs the next morning for a trip that usually lasts two to three days. Upon arrival, the animals are walked to the arena where a crew that travels by ground has already set up the animal areas. If the train arrives late at night, the animal walk is done at approximately 8:00 the

next morning. Most of the equipment, personnel, and circus-owned animals are transported by train, but some acts and equipment are transported over land using a variety of trucks, trailers, and recreational vehicles.

Ringling Red transported 14 Asian elephants and a variety of hoofstock using railcars that have insulated walls. The elephants are chained diagonally using the standard method of one forward and one rear leg. Six windows with expanded metal screens were built into each side of the elephant cars (Figure 1). Sixteen vents, each with an electric exhaust fan and covered with expanded metal, were spaced in two rows down the roof of each car. Each vent had an exterior cover that prevented sun and rain from entering into the car through the vent. The entire area provided to the elephants was approximately 6593 cubic ft. The ceiling of the railcar was rounded (the height provided was the highest point in the car). Twelve drains were spread equidistantly along the floor of the railcar. One elephant car had a dropped floor and raised roof that increased the height 1'4". The extra height was needed to accommodate their largest elephants. These elephant cars did not have accommodations for a caretaker to observe the elephants during transport and no misting system existed at the time of the survey. The elephants were divided six into one car and eight in another. During travel, the doors of the Ringling Red elephant cars remained closed due to fear that an elephant could injure its trunk by placing it out the door of the moving train.

Lafayette, LA. to San Antonio. TX. During the Lafayette, LA. to San Antonio, TX, trip (Figure 2), three environmental loggers (one with a probe) were divided among the three cars. One environmental logger was placed in the midsection of car 60 6' 5" high. Two environmental loggers were placed in car 63, one on an end wall 6' 8" high and the other 7' 3" high with the probe mounted 2' below the environmental logger. External relative humidity could not be obtained due to an equipment shortage.

The railcars' interior reached a maximum of 99.5°F though never exceeded the external temperature more than 5.04°F. This variation tended to be greatest during the period after loading (#2) and before departure (#3). Comparison of the stationary (#1 -

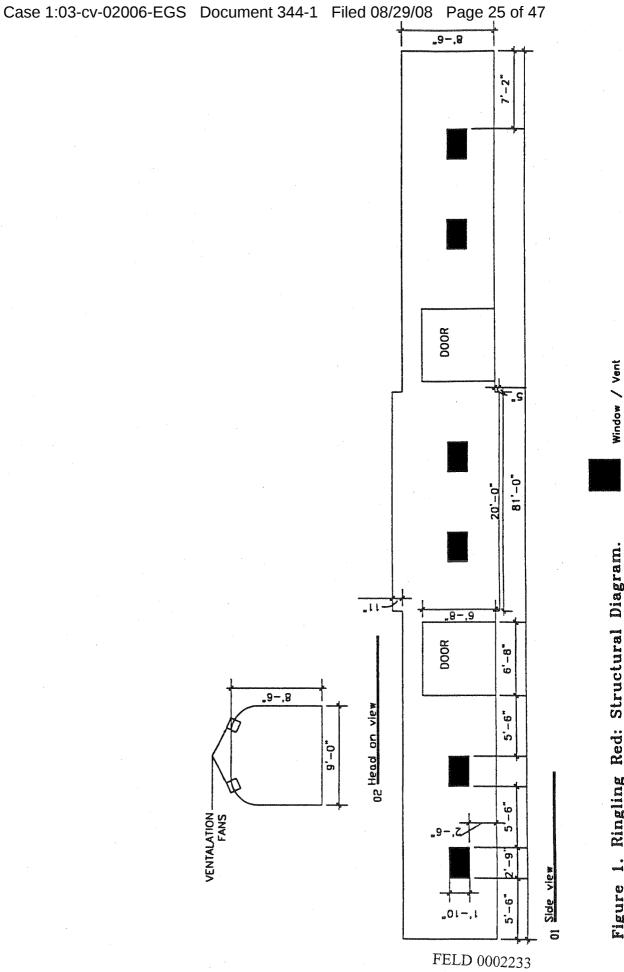
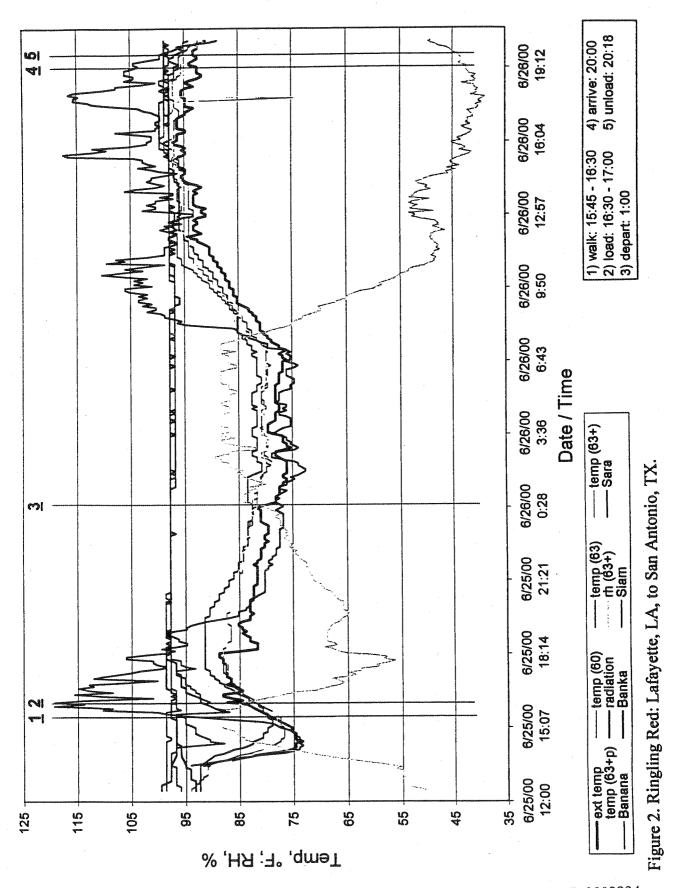


Figure 1. Ringling Red: Structural Diagram.



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#3) and non-stationary (#3 - #4) periods suggests that motion decreased variation between environmental loggers.

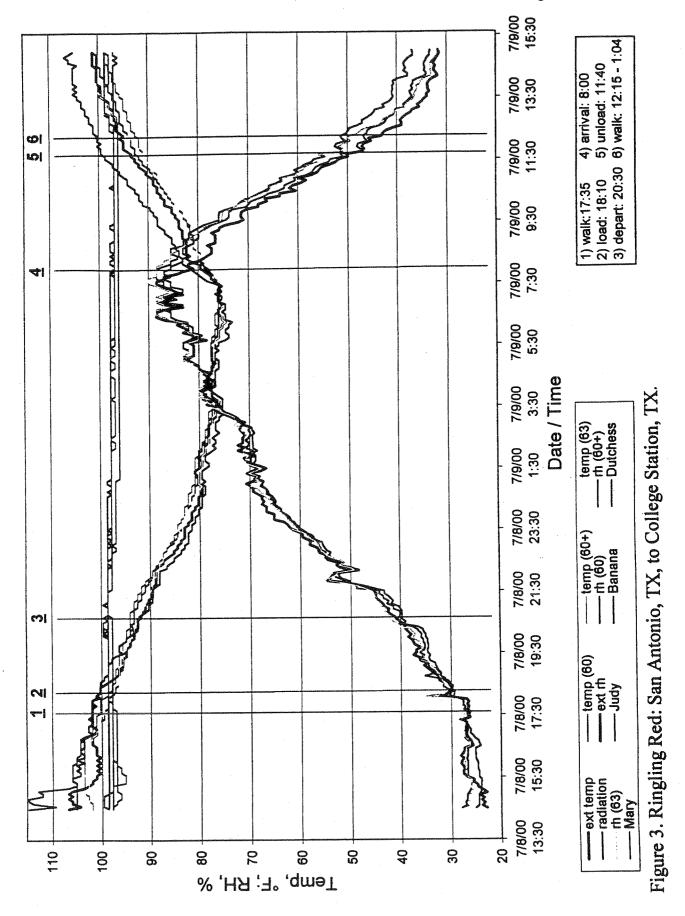
A large dip in car 63's interior temperature occurred at 6/26/00 17:47, though the dip spanned less than 10 minutes. We are unsure of the cause, though it was most likely a result of the elephants spraying water on the environmental logger during drinking. The drop was recorded by both the environmental logger and attached probe.

Ringling attempted to cool the railcar before loading the elephants by running cool water over the roof of the stockcars for several hours utilizing a large soaker hose. The attempt lowered the interior temperature a considerable amount although it had little long-term effect. The interior temperature had returned to within 5.0°F of the prewatering temperature by loading time because the hose had to be turned off prior to loading the elephants. Unfortunately, our environmental loggers recording ambient measures (including radiation) were also cooled in the process making accurate comparisons difficult. The sudden rise in radiation after 6/25/00 14:37 indicates that the late afternoon sun would have created high external temperatures.

Tests for the presence of noxious gases were made upon arrival. Ammonia concentration was 17 ppm, but the plastic collection tube had fallen directly into a pile of feces. There was no detectable ammonia once the problem had been corrected. No carbon monoxide was detected.

Of eight body temperature loggers given to elephants, four were recovered. Body temperatures were maintained fairly stable between 95.9 °F and 98.6°F with individual loggers for each elephant reading within a 1.9°F temperature range. A large dip in temperature coinciding with watering sessions occurred in Siam's body temperature at 14:27 and 15:52 6/25/00. A similar dip occurred in Banka's body temperature during the same periods.

San Antonio, TX, to College Station, TX. During the San Antonio, TX, to College Station, TX, trip (Figure 3), a total of three environmental loggers were mounted in the cars. The two environmental loggers in car 60 were placed at opposite sides of the



midsection of the car 4' 9" and 7' 6" from the floor. The third environmental logger was placed on an end wall of car 63 6' 6" from the floor.

The cars' interior temperatures never exceeded 98.1°F while the elephants were in the cars. During the entire course of the trip, differences between interior and exterior temperatures were negligible (0.18°F \pm 1.3°F). Variation between interior environmental loggers was also very slight indicating uniform ventilation.

Relative humidity during both trips was maintained at or below 65% when the interior temperature was greater than 90°F allowing evaporative cooling to function at a high rate.

Tests for the presence of noxious gases were made upon arrival. Within our range of detection, no ammonia or carbon monoxide was detected.

As in the previous trip, eight body temperature loggers were given to the elephants and four were recovered. With the exclusion of what appears to be drinking related dips, body temperatures ranged from 95.9°F to 99.5°F. The sudden drop in temperature for Dutchess at 7/9/00 13:00 indicates when the body temperature logger was expelled.

A minor sustained body temperature increase was seen during this trip. As mentioned in the travel description. Ringling's units typically walk their elephants to the train for loading and departure within an hour after their last show. During the pre-walk period on 7/8/00 17:25, body temperature for Judy was 96.8°F. Approximately 10 minutes after the walk began, body temperature rose to 97.7°F and remained at that temperature for several hours afterward. Another example of activity related temperature change occurred after arrival on 7/9/00 11:45 when Mary's pre-walk temperature of 96.8°F rose to an eventual 99.5°F after the walk had begun. It began to drop within an hour after peaking.

Ringling Brothers, Barnum and Bailey (Blue Unit)

Ringling Blue's travel routine was identical to the Red unit, typically walking their elephants on a Sunday night, after the last show, from the venue to the train.

Ringling Blue transported 10 Asian elephants and a variety of other hoofstock utilizing four stock cars structurally similar to Ringling Red. There are special cases where over-land transportation is used. For instance, three juvenile elephants were transported by truck over land for four months to get the animals acclimated to transport and shorten the overall duration of the trip. During transport, each elephant had one forward and one rear leg chained in the standard diagonal fashion. The elephants were divided among three cars: the adult animals in a whole car (#1), two to three adults in a half car (often referred to as the "alpaca car") (#2), and three juveniles in one half car (#3). The two half cars (#'s 2 and 3) have a climate-controlled room in the car's center from which one or more handlers (and researchers) observed the elephants during transport. At the time of the survey, one set of handlers had the sole responsibility for the juveniles while another set cared for the seven adults. The handlers left the observation room many times to remove feces, spread fresh bedding, feed, water, and observe the animals as needed. Sections of car #3 were used for storage of fresh hay and grain. Water tanks on the train hold a day's supply of water.

The dimensions of the car are structurally similar to Ringling Red, though Ringling Blue did not have a raised ceiling or dropped floor (Figure 4). During trips, Ringling Blue would ventilate the cars by opening two large doors on each side during transport. When the doors are opened, two horizontal pipes block the openings ensuring that a loose elephant could not get out of the car. The ceiling of the railcar was rounded (the height given in this report was the highest point in the car) and had a high-pressure mister system with a separate control system for each car along the length of the ceiling.

Los Angeles, CA, to San Diego, CA. During the Los Angeles, CA, to San Diego, CA, trip (Figure 5), two environmental loggers were placed in the full (#1) car (one with a probe) and two in the alpaca car (#2), though only a single environmental logger was

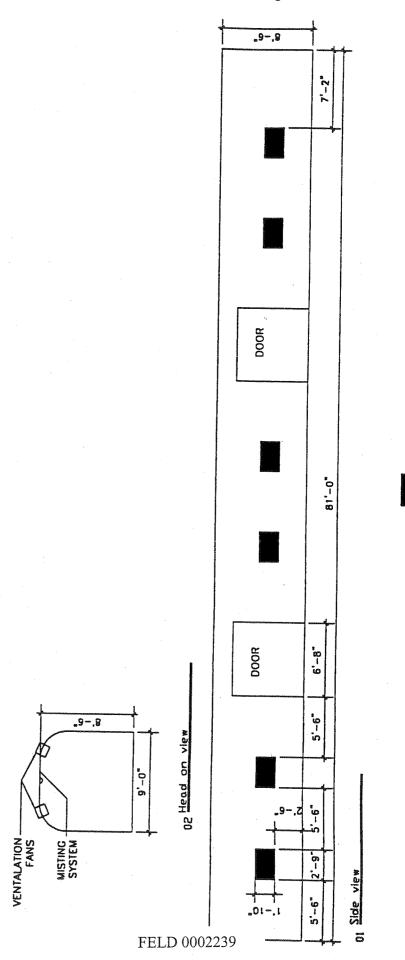
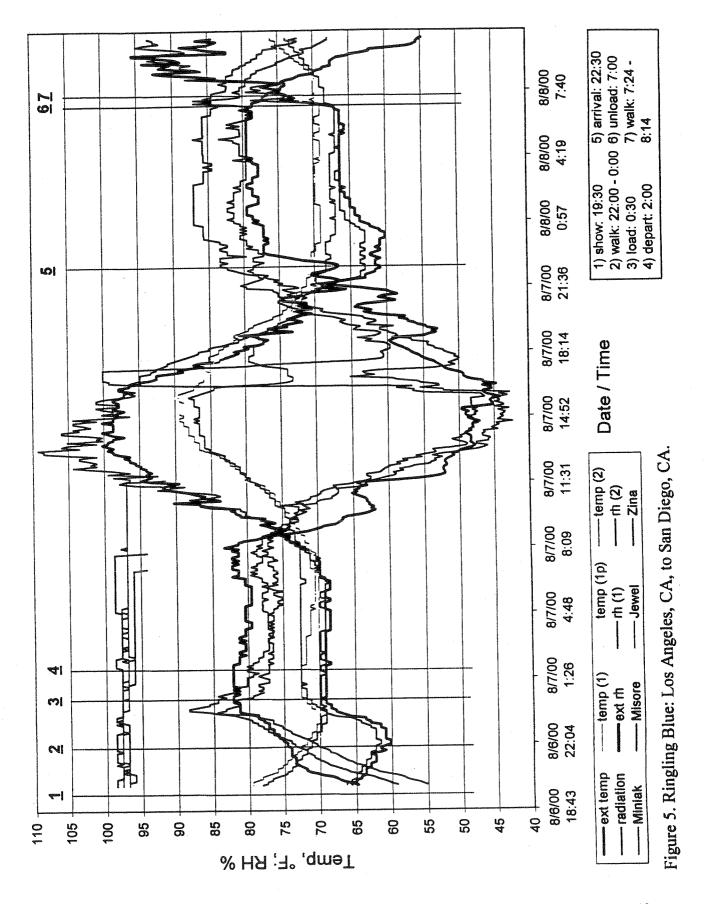


Figure 4. Ringling Blue: Structural Diagram.

Window / Vent



Of the three juvenile elephants which the researcher spent the preponderance of time during the trip observing, one elephant weaved nearly 80% of the time while standing. Despite this near constant motion while standing, she did lay down and rested during the trip for several hours each night.

Hawthorn Corporation #2. During this trip, the researcher did not travel in the elephant trailer as in previous instances but in the cab of the truck. As seen in Figure 30, stops were made frequently to allow for feeding of the animals and staff breaks. The researcher was not able to see the animals in the trailer, though the head manager did not believe that the elephants could lie down during transit due to lack of room. For this reason, he would take a minimum of breaks to reach the destination as soon as possible.

During stops, the animals would be provided water and/or feed using a bucket slid in through the access panels on the side. The morning after the overnight stop, feces and urine that had collected at the rear of the animals were shoveled out through the entrance/exit doors. This procedure most likely had a large impact on the absence of ammonia when the air was sampled.

Discussion

Extreme environmental temperatures can result in harmful effects on animals being transported if precautions are not taken to insure that conditions inside the transport vehicle are kept within a safe range. Animals that respond poorly to transport or find the transport environment inadequate or stressful will suffer from compromised welfare if their response or the environment does not change. The present project was undertaken to survey the environmental conditions that circus and other performing elephants experience while being transported during relatively extreme temperatures, evaluate methods to maintain appropriate conditions within the transport vehicle, and characterize the response of the elephants to transport. Measurements of interior and exterior temperature and relative humidity data, as well as concentrations of noxious

gases during transit, were obtained to fulfill the first two objectives. Measurements of body temperature, direct and video observations, and plasma cortisol concentrations were collected to accomplish the third objective. Although our findings lead to the formation of new questions and suggest areas for additional research, the results suggest overall positive transport conditions.

The internal and external environmental temperatures of the transport vehicles observed during transport were all within a range of temperatures not considered unusual for non-circus populations of Asian elephants. Asian elephants in their native habitat experience temperatures ranging from below 32°F to 104°F (Sukumar, 1989). Elephants in zoological parks of the northern United States commonly experience temperatures as low as 38°F (Keele, personal communication). Thus, the temperatures observed during transport were within the range that elephants have the physiological and behavioral ability with which to cope.

As seen in surveys of transport conditions in livestock, interior temperatures were strongly influenced by exterior conditions. Results of the present study indicate that during relatively hot weather the temperature within the transport vehicles was generally maintained below 100.4°F. Measurements greater than this value did occur but they were short-lived and variable among environmental loggers within the same vehicle suggesting that these occurrences were related to equipment malfunctions, such as spray from an elephant creating a short, rather than hazardous conditions. As demonstrated in a survey of Ringling Blue's trip from San Diego, CA to Oakland, CA, two separate temperature spikes were recorded at approximately 8/15/00 8:15 and 9:25, the second of which reached a maximum of 104.2°F. The lack of a correlating fluctuation in the external temperature or the third environmental logger measuring interior temperature suggests that the cause of the temperature spikes were not related to external conditions and not an accurate description of the overall environment within the railcar.

The circuses surveyed during hot weather utilized several methods to avoid relatively hot temperatures during the summer touring season. The two circuses

surveyed that travel over rail lines – Ringling Blue and Ringling Red - typically have trips that might span days. These circuses may experience periods where environmental conditions reach extreme temperatures. Both circuses respond to this challenge by utilizing railcars outfitted with insulated walls, high capacity ventilation fans, and other structural and environmental enhancements that can effectively maintain internal temperatures below or within a relatively safe range of the external temperature. During Ringling Red's trip from San Antonio, TX to College Station, TX (Figure 3), internal temperatures did not exceed the external temperature despite external temperatures above 98.6°F. During the Lafayette, LA, to San Antonio, TX (Figure 2), trip, internal temperatures were only several degrees above the external temperature during similar extremely high external temperatures.

The other circus and exhibitors surveyed did not have the structural and environmental enhancements (e.g., high capacity ventilation fans) that Ringlings' units possessed. Instead, these groups avoided high daytime temperatures by traveling late at night (i.e., Clyde Beatty, Circus Vargas) or in the early morning (i.e., Carson and Barnes). This method proved extremely effective; the highest external temperature measured in all surveys for these three latter circuses while the animals were in the transport vehicles was 87.1°F, though it was often below 80.1°F. Since these circuses avoided high external temperatures and solar radiation, their ability to maintain low internal temperatures and prevent a buildup of temperature inside the vehicle was dependent on adequate ventilation achieved through vents in the sides of the trailers. This objective was also met with success as demonstrated during Clyde Beatty's trip from Staten Island, NY, to Forest Park, NY (Figure 15). The difference between internal temperatures and external temperatures reached a maximum of 7°F, a value typical of the other circuses that traveled during periods of low external temperatures.

Other methods were used by individual circuses in an attempt to reduce the internal temperature of the transport vehicle. For instance, Ringling Red ran a soaker hose connected to a fire hydrant over the entire length of elephant cars several hours prior to loading for their trip from Lafayette, LA, to San Antonio, TX (Figure 2),

successfully lowering the internal temperature 14°F. Though this change was substantial, the effect was short-lived and thus not a viable alternative to cool the railcar during hot weather conditions unless performed throughout travel. Hence, this is not a feasible option.

Internal temperatures sometimes did change over a rather brief period of time. Though changes were within ranges easily tolerated by healthy elephants, a temperature telemetry device with high and low temperature alarms to alert handlers of the temperature conditions inside the transport vehicle could prove invaluable. For instance, during Circus Vargas's trip from Santa Barbara, CA, to San Luis Obispo, CA (Figure 17), a temperature increase of 25.2°F occurred over a 90-min period the morning after travel, most likely a result of rising external temperature and radiation. Though the elephant was removed before the temperature the temperature became a concern, a warning device would alert the handlers to extremes in temperature within the trailers could be very useful.

During cold weather transport, internal temperature of the transport vehicle was generally kept above 77°F, though lesser values did occur. Internal temperature exceeded the external reading 5.4°F to 39.6°F. This value was dependent on the type of trailer and the procedures used to control the internal temperature. For instance, before Humps and Trumps departed from Cut 'n' Shoot, TX, to College Station. TX (Figure 33) an electric heater was utilized to keep the pre-departure internal temperature at roughly 64.9°F while the external temperature was 36.9F, a difference of 28.1°F. In comparison. Carson and Barnes did not use an electric heater in their uninsulated trailer during their trip from Rockdale, TX, to Tomball, TX (Figure 31), but reduced ventilation and relied on heat production from the elephants as the sole heat source. During the pre-transport period, both Carson and Barnes' trailers maintained an internal temperature between 51.8°F and 62.6°F during which the external temperature was 45.0°F. In both situations the measures taken were successful in moderating the temperature within the vehicle despite relatively cold exterior temperatures.

Relying solely on the heat produced from the elephants in the transport vehicles to maintain an adequate internal temperature proved particularly effective with the Hawthorn Corporation during the Chicago, IL, to Nashville, TN, trip (Figure 30). Separate environmental loggers recorded internal temperatures of 55.0°F and 64.9°F while the external temperature was below freezing (27.1°F). Though variable between the different environmental loggers, this was the largest difference between the external and internal temperatures recorded and a likely result of the insulated trailer used by Hawthorn Corporation. The dense travel conditions, 15.7 cubic meters per elephant for Hawthorn Corporation as compared to Carson and Barnes's density of 27.0 cubic m per elephant, undoubtedly also contributed to the difference between external and internal temperatures.

Ringling Blue had distinct differences in exterior and interior temperatures between their three cars used to transport elephants, most likely due to the presence of a thermostat-controlled heating unit in car 2 which held three adult elephants and car 3 which held three juveniles. Particularly during the trip from Chicago, IL, to Savannah, GA (Figure 28), car 2's minimum temperature during transport was 50.9°F.

Comparatively, temperatures in car 1, where five elephants were held, reached 32.5°F several times, equaling the external temperature during this cool period. The elephants did not seem to be adversely affected as indicated by stable body temperatures.

Temperatures of this range are also common in the natural habitat of Asian elephants (Sukumar, 1989). However, with the potential for the temperature of the railcar to reach such low values, the installation of a heating unit in car 1 may warrant consideration.

No carbon monoxide or ammonia gases were detected for any of the surveys except for an isolated incident when the plastic collection tube fell directly into a pile of feces. The buildup of both gases during transit was most likely avoided due to high ventilation, particularly during the summer when fans are used in Ringlings' units which travel over several days. The removal of feces during longer trips as well as the use of hay and sawdust to lessen the spread of urine undoubtedly had a positive effect on

reducing ammonia. The absence of ammonia can also be attributed to insufficient time for urine to breakdown to ammonia.

The third objective of our study was an attempt to characterize the response of the elephants using physiological and behavior parameters. In contrast to the environmental conditions discussed above, the collected physiological and behavioral data provide a view of the internal state of the animal.

Body temperature was possibly the most difficult measurement to obtain as each stage of the process presented several obstacles. Having the elephant ingest the body temperature logger without chewing it proved to be difficult in itself, as was retrieving the logger. In one case, it was discovered that one elephant was eating the feces of another which had been fed a body temperature logger. The logger was not found. Our original estimate of a five-day passage time proved close to the actual average of three days, though times were extremely variable ranging from 12 hours to over four months. The four-month duration occurred when the logger became separated from the epoxy encasement and nylon flag possibly resulting in the body temperature logger being retained in the cecum. The cecum is an area not subject to peristalsis. In a study using ingested loggers to determine body temperature in elephants. loggers were commonly found in this area during extended passage times (Schmidt, personal communication).

Recorded body temperature for hot weather and cold weather trials averaged 97.2°F and 97.3°F, respectively. Body temperature ranged from 95°F to 98.6°F, similar to the reported basal body temperature of 95°F to 98.6°F for an elephant (AAZK, 1997). Temporary drops in temperature were recorded, but appear to be a result of drinking water. Often, within three hours after the body temperature loggers were ingested, a sudden decline of 5.0°F to 15°F from regular body temperature would occur followed by a slow return to normal temperature, e.g., Misore and Jewel's body temperature before the last show in Figure 6. These drops result from the animals drinking cold water while the body temperature logger was still in the stomach of the animal before entering the small intestine. Occasionally, drops are seen later than the first few hours after the body temperature logger was ingested. These later dips were usually much less than the early

dips, indicating that the body temperature logger was possibly entering the intestine, or in the transverse colon or cecum. The transverse colon and cecum are areas close to the stomach and could be affected by the animal taking in water (Hauk, personal communication).

Elevation in body temperature of two to three degrees is not considered a problem in elephants or most other mammals. Body temperature fluctuations within a range of 2.7°F were seen throughout the study at various points. This raised temperature was at times sustained during certain periods of increased activity (i.e., walking to the train) as would be expected. The lack of a larger increase in body temperature in the elephants clearly indicates that they can easily cope with daily environmental temperatures that approach 100°F in the shade as well as travel related activity. Additionally, we think that the exercise and stimulation the elephants receive during the walk is very beneficial for the overall health of the animals.

Tennesenn et al. (1984) observed an average rise of 0.9°F in rectal temperature of steers transported for a two-hour session. They concluded this and other statistically detectable variations in physiological measures to be slight, indicating a relatively stress free response to transit. The researchers noted that transportation with familiar penmates and other efforts to minimize stress during transport had a large impact on this result. Friend et al. (1998) attempted to characterize physiological parameters of horses during relatively long transit distances under hot conditions with a maximum of 95.0°F and found body temperature to be a useful indicator. Of 20 horses that were transported for 24 h with limited access to water, one animal that received water every five hours recorded an elevated rectal temperature of 105.1°F while another horse not provided water had a slight fever of 103.3°F. These temperatures are substantially greater than 100.8°F, the high end of normal body temperature range for a horse (Anderson, 1984). Horses may experience a 3.6°F temperature increase with moderate exercise (Friend, unpublished data). However, the decision was made not to transport these animals further based on the rise in body temperature and a visual evaluation of the animal.

In comparison, the highest body temperature recorded during our survey was only 0.9°F above the normal body temperature range in an elephant (Mary) in Ringling Red's trip from San Antonio, TX, to College Station, TX. Previous to the walk to the train, Mary's body temperature was 97.7°F; thus the respective rise to 99.5°F from this elephant's particular baseline was only 1.8°F. In addition to being minor, the most extreme fluctuations were less than an hour in duration and associated with increased physical activity rather than changes in environmental conditions as seen by Friend et al. (1998). The relatively small increases seen in the present study suggest that transport during extreme temperatures did not affect ability to thermoregulate. Lastly, our procedure for collecting body temperature provided continuous readings throughout the transport session versus periodic measurements during travel or at the trip's conclusion as done in other studies (e.g., Friend et al., 1998; Stull and Rodiek, 2000). Thus, the lack of substantial fluctuations in the present study applies to particular events during transport (e.g. loading, changes in environmental temperature) as well as the body temperature upon arrival.

Due to the loggers' accuracy range of ± 1.8 °F, the body temperature loggers may not have been reporting the true body temperature at that time. As noted earlier, reports of body temperature ranges vary widely in reported literature, particularly in terms of a lower value. However, for the purpose of detecting effective thermoregulation, our primary interest, the occurrence of fluctuations in body temperature for a particular elephants is more important than achieving an overall accurate temperature. Based on the manufacturer's reported precision (± 0.9 °F) and our own tests, we feel that body temperature fluctuations in this study were detected with confidence.

Direct and video observations provide a second important parameter to indicate an animal's internal state. As described in Friend et al. (1991), the performance of certain abnormal and normal behaviors can be used to suggest the status of the animal's welfare. The stereotypic behavior of weaving, considered an abnormal behavior, was observed in several elephants during transit. However, a definitive conclusion regarding their cause is difficult given the speculation for the underlying reasons behind

stereotypic behavior. Normal behaviors, i.e. eating and exploration, were also displayed at the same time as the abnormal behaviors, further impeding conclusions.

Stereotypic behavior has several potential functions that serve the animal exhibiting them. The behaviors may function to help animals cope with little or no variation in their environment by increasing physical and sensory stimulation (Friend and Dellmeier, 1986; Friend, 1991). They may also serve as a replacement for species-specific behaviors, such as foraging, which cannot be performed under the current given environment (Gruber et al., 2000). In both these cases, stereotypic behavior may help the animal deal with situations it finds as "boring". Stereotypic behavior has also been shown to serve as a release of tension or anxiety (Dantzer and Mormede, 1983). Despite the prevalence of stereotypic behavior in restrictive agricultural environments. no published research exists reporting occurrences of the behavior in transported livestock. This observation suggests that the causes of weaving in elephants during travel might be different from the causes that are seen in agriculture, i.e., a poor environment.

Weaving in elephants has been shown to reflect the occurrence of arousal in response to a positive event. Friend (1999) reported weaving in circus elephants to significantly increase immediately before receiving water and hay. The behavior may have been a means of releasing excitement in response to the prospect of the respective stimuli. Weaving in this circumstance should not be interpreted as an indication of a poor environment because watering and performing are not events that would be considered stressful stimuli. Benedict (1936) observed that elephants tended to begin weaving when excited, though did not explain his rational or observations.

Cronin et al (1986) suggested a neurological basis for the cause of stereotypic behavior for animals in an impoverished environment and reported the release of endorphins, an endogenous hormone with effects similar to morphine with the occurrence of stereotypic behavior. In his study, tethered sows reduced or eliminated the stereotypic behavior when an opiate antagonist was administered to prevent the binding of endorphins. Hence, the performance of stereotypic behavior may not reflect problems with the present environment.

The performance of stereotypic behavior in impoverished environments has been shown to lower cortisol concentrations (Dantzer and Mormede, 1981) and reduce the effects of a low stimulus environment on the health of the animals in it (Wiepkema et al., 1987). Thus, though the environments where the behavior is being performed may be inadequate, stereotypic behavior may help to reduce the impact of those inadequacies and eliminate a state of compromised welfare. In circumstances where stereotypies are indicative of a positive stimulus, the behavior may be a release of excitement or anticipation in response to the stimulus. In both circumstances of negative and positive environments, the performance of stereotypic behavior, barring that the behaviors do not cause secondary problems, i.e., foot injuries from excessive pacing, does not indicate a state of compromised welfare.

During this study, weaving was observed in both short and long trips during direct and video observations. All observed animals, whether weavers or not, exhibited some or all of the general normal behaviors described in Friend (1991) including grooming and self-maintenance, exploratory behavior, regular eating, and participation in herd behaviors. For instance, as an example of a maintenance behavior seen in transit, elephants would typically throw hay on their backs. This behavior was also commonly seen during non-transport times in the animal compounds. During travel, elephants would also use their trunk to explore the walls of the train in the area that they were held. During trips in which the elephants were fed and watered, the animals were observed to readily eat and drink, suggesting they maintained a feeling of security.

These results make interpretation of elephants' behavior during transport complicated. The performance of normal behaviors suggests the animal is not afraid or insecure in the transport environment. During short trips where weaving was exhibited, the brief period of several hours the animals were in the restricted transport environment contrasts the long-term impoverished conditions in agriculture which are related to stereotypic behavior. During longer trips where the restricted environment was imposed for a longer period of time, the observed stereotypic behavior was likely related to a barren environment. The animals still maintained elements of a normal behavior routine

in addition to stereotypic behavior. Lastly, the proposed release of endorphins in response to weaving may have an addictive quality causing the animal to weave despite the removal of the stressor, i.e., transport before the animal had adapted. Thus, though stereotypic behavior often implies negative connotations, the incidence of stereotypic behavior in transported elephants does not indicate a state of compromised welfare.

Plasma cortisol concentrations were the third parameter examined to provide an indication of the animals' internal state. Significant differences between cortisol concentrations in response to treatments are typically used in stress research to indicate a perceived stress to the treatment or stressor (Dantzer and Mormede, 1983). Conclusions of this nature must be carefully made and take into account several details.

Heightened cortisol concentrations do not invariably indicate compromised welfare. The hypothalamic pituitary adrenal axis is an evolved system that aids the animal in coping with stressors and other changes to its environment. Activation of this axis is not necessarily synonymous with a harmful stimulus being applied to the animal (Moberg, 1987). The activation of the adrenal cortex functions in an effort to allow the animal to cope with the stressor before a state of compromised welfare is reached. Thus, though fluctuations in cortisol can prove an accurate indicator of compromised welfare, the connection between altered cortisol concentrations and reduced welfare must first be established (Moberg, 1987). Otherwise, false conclusions regarding the impact of stimuli on welfare are possible.

Mendl (1991) theorized whether a definitive point existed at which cortisol concentrations compromised welfare when that point was surpassed. He suggests that such a point does exist if the criterion used in establishing that point is grounded in reliable evidence, such as the animal's reproductive fitness or other long term indicators of health. Moberg (1987) advised a similar examination of long-term measurements of welfare and suggested evidence of compromised immune function as a viable possibility to indicate compromised welfare.

Additionally, although cortisol generally increases with the onset of a negative stimulus, evidence in this study from blood collected during application of a positive

stimulus (free time in an exercise pen) suggests that cortisol may rise in situations perceived as exciting or enjoyable. Researchers can use cortisol as an accurate indicator of compromised welfare only when these guidelines and considerations are taken into account.

The results of the present study found a trend towards increasing cortisol concentrations immediately before transport and after. Significant differences between two sets of sample times were detected during Carson and Barnes's trip from Williamstown, NJ, to Chester, NJ (Figure 23) with increases of 190% and 160% between samples one and two and one and three, respectively. Though not significant, the mean difference for the final collection during long trip transport for Ringling Blue was 274% greater than the control value.

Fluctuations of cortisol concentrations in response to transport have varied widely in published research. Kenny and Tarrant (1987) found a 1010% increase in plasma cortisol in young bulls in response to being transported for one hour. During transport, significant increases in pushing and headbutting, principal causes of bruising, was observed as well. Kent and Ewbanks (1983) transported calves for 6 hours and determined serum cortisol had risen over 695% after two hours of transit. The relatively large increases in cortisol were likely related to the animal's youth and lack of transport experience. In comparison, during the short trips surveyed of Carson and Barnes in the present study where trip duration was typically two to three hours, the largest increase during transport was 190% and occurred prior to loading.

Much smaller increases in cortisol fluctuations have been reported in transported livestock though the lower concentrations are most likely a result of the samples being taken 12 or more hours after the start of transport. Using this method of sampling later in the transport session, any elevations in cortisol that occurred in response to the first hours of transit would have cleared the blood by the time of the first sample. Also, the initial drain of cortisol during the beginning of transport may have depleted the animal's storage of cortisol, an effect termed exhaustion (Selye, 1973). The first few hours of transport is considered the most stressful period of transport as a whole in unacclimated

animals (Tarrant, 1990). Thus, studies that base their conclusions in regards to cortisol concentrations on data collected during the latter part or at the conclusion of transport may be overlooking the most critical segment of travel and serve as poor comparisons for the present study. If accurate comparisons are to be made between transport, the length of the trip and the periods between sampling must be taken into account. For instance, Knowles et al. (1999) reported a 32% increase in plasma cortisol over a 31 h transport session of cattle. The first sample was taken after 14 h and showed an increase of 13% from the initial sample. Tarrant et al. (1992) reported a 46% increase in plasma cortisol concentrations of cattle transported for 24 h, though only two samples were taken, before and after travel.

Though not in response to travel, Dathe et al. (1992) reported an increase of 295% in an Asian elephant over a period of two days after introduction of the animal to an unfamiliar captive herd, an increase larger than any of the fluctuations that occurred in the present study.

Under alternative conditions, i.e. long term intensive confinement, the incidence of weaving and statistically significant elevated cortisol concentrations might be interpreted as indications of stress during transport. However, as discussed above, interpretations regarding the results of these tests must be considered carefully. The performance of weaving observed prior to watering and performances as reported in Friend (1999) suggests that the animals are aroused or stimulated by travel during short trips where confinement in the truck is limited to several hours. Similarly, though raised cortisol concentrations were observed, the lack of a precise cut off point makes interpretation difficult, particularly when the occurrence of much larger increases in comparative agriculture research and stress research in elephants is considered. Furthermore, the highest cortisol concentrations measured in this study occurred during the last two sample periods of the short trip control collection when the elephants were not transported. This observation suggests that the event of not being transported, or a break in the routine the circus elephants had become accustomed to, may have imposed more stress than being transported. Dantzer et al. (1980) reported a parallel finding

when swine, trained to expect a routine food reward, no longer received the reward.

Lastly, due to lack of accurate controls, we were unable to control for any effects imposed by diurnal rhythms.

Considering the ambiguity of the results for cortisol and behavior analysis, examination of long term indicators of fitness can provide important information regarding impacts on welfare (Moberg, 1987; Mendl, 1991) and the perception animals have to transport. Theoretically, if transportation were a significant stressor, reproductive fitness and the overall health of the animals would be severely affected considering the extensive travel done by circus elephants. However reproductive successes in the circus industry are considerably high, surpassing zoological parks in the percentage of young surviving their first year (Keele, 2000).

As additional evidence suggesting transport is not an inherently significant stressor to circus elephants, nearly all of the factors considered to negatively affect transported livestock were not present in the surveys of circus elephants made in this study. Moderate temperatures were seen throughout transport and the mixing of unfamiliar animals did not occur. Due to short trip duration, animals were not restricted from feed and water for extended periods of time, did not spend extensive times standing, were not exposed to noxious gases, and did not have to maintain their balance on wet, slippery floors. During longer trips, feed and water were provided at regular intervals and urine and feces were removed efficiently, eliminating problems associated with noxious gases or slippery floors. Animal density was low enough to allow animals to lie down and rest during longer trips with one exception, though elephants are able to sleep while standing (Benedict, 1936; Tobler, 1992) and thus were most likely not very affected in this case. Loading of animals, generally considered the most stressful event in livestock, seemed rather stress free during observations of elephant loading. With Clyde Beatty's elephants, the animals perform the entire loading process by themselves, running to and entering the trailers after a release command from the trainer.

Additionally, transport can occur essentially without stress if efforts are made to minimize stressors during transport (Tennesen et al., 1984) and animals are acclimated

to the events which cannot be altered (Tarrant, 1992). The regular transport schedule that circus elephants undergo throughout the year, the willingness to load, and the occurrence of natural behaviors such as eating, drinking, and self and mutual grooming during transport suggest that the animals have become acclimated to the rigors of transport.

Conclusions

The results of this research do not indicate that transportation of circus elephants is inherently stressful. The lack of any evidence of hyper or hypothermia during travel, the absence of factors known to be stress-inducing in livestock transport, the comparative high reproductive successes of circus elephants, and the appearance that circus elephants are acclimated to transport indicate that the response of elephants to transport is minimal for the subjects in this study and not a detractor of animal welfare.