APPENDIX A
DESERT TORTOISE TRANSLOCATION PROGRAM

HYUNDAI MOTOR AMERICA
MOJAVE TEST TRACK SITE

DESERT TORTOISE TRANSLOCATION PROGRAM

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BACKGROUND

There are few well-executed translocation studies on desert tortoises. As such, little is known about the success of desert tortoise translocation. Generally, the studies have been short-distance removal studies, where tortoises are moved immediately off a fenced site but very likely within their home range (e.g., Stewart and Baxter 1987, TRW 1998, Corn, pers. comm. to A. Karl). Furthermore, the studies are not easily accessible as are primarily found in unpublished gray literature reports (e.g., Berry 1975), non-peer reviewed symposium proceedings (e.g., Stewart 1993, Mullen and Ross 1997), or university archives (e.g., Cook 1983) or are not yet fully analyzed [Phil Medica, U.S. Fish and Wildlife Service (USFWS), pers. comm.). Finally, none has included a control group (i.e., a group of tortoises unaffected by translocation) in the study, with the exception of a recent translocation study south of the Las Vegas Valley (Phil Medica, pers. comm., and Saethre *et al.* 2003). Several of the studies have included observations of host, or resident, tortoises but such tortoises were affected by an influx of translocated tortoises, so did not really constitute a valid control group. As such, the conclusions of these translocation efforts have been suggestive, but somewhat limited relative to identifying translocation effects.

While studies have generally had limited utility regarding translocation, their results strongly suggest that translocation, if conducted appropriately and during periods of adequate forage, can result in high survivorship of translocated animals. Stewart (1993) observed that survival rates and average distances moved did not differ between translocated tortoises and resident animals during variable periods of time not exceeding 18 months. Other short-distance translocation studies have resulted in high survival of translocatees for 9-24 months (Stewart and Baxter 1987, TRW 1998). Cook (1983) released 51 tortoises over a two-year period and observed only eight deaths (although monitoring was very limited on more than half of the animals). Not unexpectedly, the season of release had a substantial impact on mortality. Six of the eight known deaths occurred in tortoises released during the summer; these tortoises died of apparent exposure within one day to two weeks of release. The Las Vegas Valley study found that mortality within one year of release was strongly correlated with drought [Phil Medica, U.S. Fish and Wildlife Service (USFWS), pers. comm. and Saethre *et al.* 2003]. Mullen and Ross (1997) observed that there was no difference in survival between resident and translocated tortoises except for tortoises released in a drought year, which also included late spring releases after ambient temperatures exceeded lethal levels daily.

Given the federal and state "threatened" status of this species, translocation should strongly be considered as a tool for saving individual tortoises where their

safety is definitely compromised by localized habitat destruction and/or other factors. A carefully implemented translocation program can not only substantially decrease project impacts to desert tortoises, but also has the potential to provide useful data for application to other analyses and future translocation projects. The Hyundai tortoise translocation study is an opportunity to provide answers to specific questions about translocation. Most of these questions were not previously answered by the Las Vegas study and have never been answered in the western Mojave Desert. The results would provide important management tools for future translocation efforts.

Finally, tortoise populations in the western Mojave Desert have been decimated by a 15-year drought cycle. The translocation program associated with this project will keep the tortoises at the Hyundai site in the population and also assist in replenishing a nearby area.

PROJECT GOALS

The primary goals of translocating tortoises from the Hyundai site are to prevent the mortality of tortoises that reside on the site, to maintain the integrity of the population as much as possible, and to maintain breeding individuals in the population. Secondarily, but importantly, the program will facilitate the repopulation of a site that has experienced tortoise density declines not directly related to habitat loss. The primary goal of the translocation study will be to answer questions about the effects of translocation.

This study intends to address four primary (1º) questions and several secondary (2º) questions. The latter include variables to be analyzed for the primary questions and many of the other secondary questions. Repeated measures analysis, analysis of variance, and analysis of covariance are the basic statistical tests that will be employed for analysis.

1º Question #1 – What is the effect of translocation on survival?

1º Question #2 – What is the effect of translocation on health status, especially (a) exposure to *M. agassizii* and other pathogens, (b) disease expression, and (c) condition indices?

1º Question #3 – Is fencing a translocation site a reasonable procedure for site repatriation of areas that are depauperate due to stochastic climatic events or other factors that have not reduced the habitat quality at the translocation site?

1º Question #4 – How are activity levels affected by translocation?

2º Question #1 – Are there gender effects?

2º Question #2 – Are there size or age effects?

2º Question #3 – Are there effects from forage level variation?

2º Question #4 – Are there effects from precipitation events (i.e. rehydration opportunities)?

2º Question #5 – Could activity level patterns partially explain survival rates and/or health patterns?

2º Question #6 – What is the temporal pattern of the above effects following translocation?

Other factors that are generally considered to be measures of successful translocation, such as reproductive output and age-related recruitment, will not be incorporated into the study because of low anticipated sample size that would result in invalid statistics and meaningless results.

Special Aspects of This Translocation Study not Investigated in Other Translocation Efforts

No study has knowingly translocated tortoises that potentially carried *Mycoplasma agassizii*; nor has any study, with the exception of the Las Vegas study, examined health issues, other than survival. The Las Vegas study specifically did not translocate tortoises that had tested positive for exposure to *M. agassizii* or that had clinical signs of disease. The Hyundai translocation project will incorporate the translocation of seropositive tortoises, animals that have withstood infection by *M. agassizii* and recovered. This will permit the strong physiological and physical attributes that enabled these tortoises to withstand infection to remain in the gene pool. Furthermore, we will not be introducing mycoplasmosis into a "clean" population, as seropositive tortoises or tortoises with clinical signs of infection with *M. agassizii* have been found at the Desert Tortoise Research Natural Area (DTRNA) north of the Hyundai site and at many sites throughout the Mojave desert (Lederle *et al.*, 1997, Homer *et al.*, 1998, Christopher *et al.*, 2002).

The translocation site will be fenced with tortoise-proof fencing for part of the study (see *Translocation Site Fencing*, below). Fencing the translocation site will provide the following benefits:

- Fencing will help contain disease spread. We do not know if seropositive tortoises that host *Mycoplasma* spp. will be under sufficient stress following translocation to enter an acute phase of the infection (i.e., clinical signs). Since tortoises are believed to be contagious when clinically ill (Brown *et al*. 2003), fencing will prevent potential infection of tortoises outside the translocation site.

- Forcing translocated tortoises to remain at the translocation site will result in their becoming accustomed to the site and, thus, maximize the likelihood of their ultimate repopulation of that area, when the fence is

removed. Repatriation of target areas could be an important tool for species recovery.

- Fencing will keep translocated tortoises safe from anthropogenic impacts outside the translocation area (e.g., roads, off-highway vehicles, sheep), which are often considerable in the west Mojave, especially near the DTRNA, the general area targeted for the translocation site.

- While fencing precludes studying long-distance movements, it is already well known from other translocation efforts that a percentage of translocated tortoises move moderate to great distances immediately following release. Fencing will allow us instead to assess fencing as a tool for future translocations to areas of a scale that would accommodate fencing. Other variables that might be studied in an unfenced site can still be studied in a fenced site.

- While fence walking may occur with some translocatees, it is fully possible that the actual hours of fence-walking activity would not exceed the hours of walking away from an unfenced translocation site.

STUDY DESIGN

Translocation and Control Sites

A translocation site must be chosen that will meet the following criteria, a discussion of which follows this list:

- The habitat must be of sufficiently high quality to support both translocated and resident tortoises. Secondarily, acclimation by translocatees would probably be facilitated if habitat elements were sufficiently similar to those at the original capture site.

- The site must be of sufficient size to accommodate an influx of translocated tortoises.

- The site should be within the same population as the Hyundai site, to maintain genetic, morphological and behavioral integrity and facilitate acclimation by translocatees.

- There should be adequate assurance of long-term translocation site protection from development or severe anthropogenic threats. These may be included as part of per-acre dollar amounts for habitat compensation, which include land purchase funds and perpetual management funds.

- Hyundai requires that the translocation site be suitable for compensation lands. As such, the translocation site should be in an area that can add to blocks of land that are already protected for wildlife values or targeted for protection and enhancement.

Carrying capacity at the translocation site is a primary consideration in any translocation effort. During a climatic cycle of average to high rainfall producing abundant forage for many years (e.g., most of the 1970's and 80's), with resultant high tortoise survival and low mortality rates, tortoise densities at carrying capacity for the habitat would be expected. During such a period, areas well below carrying capacity would probably be confined to sites experiencing severe anthropogenic impacts, such as land adjacent to highways. Currently, however, and as a result of the recent ~15-year drought cycle[1], concomitant increases in mycoplasmosis and potentially other diseases, and anthropogenic impacts, tortoise populations are severely depressed throughout the Mojave Desert[2]. While studies are almost entirely unpublished, they are consistent: relatively few live tortoises are found compared to historic or expected counts and mortality rates (based on carcass counts) are high. In the west Mojave alone, for example, Berry (2003) reported that 1996-2002 tortoise densities on the DTRNA were 13-39 tortoises/mi^2, compared to 1979-1982 densities of >175 tortoises/mi^2; Fremont Valley had 2001 densities of 13 tortoises/mi^2, compared to1981 densities of 109 tortoises/mi^2. Karl (2002a, b) observed adult mortality rates of 10.8–46.5% for adults for the preceding two to four years alone (50 % of which were drought years of negligible or highly diminished forage); there were 1.4 times as many dead adult females as adult males. In conclusion, then, it is unlikely that any potential translocation site is currently at, or even near, the average, long-term carrying capacity for the location. As such, exceeding carrying capacity is a consideration for this project that is not critical.

It is likely that only one translocation site, of approximately two square miles in size, will be needed, based on the limited number of tortoises anticipated to be translocated (approximately 30-40 adults based on October 2003 surveys) and the fact that the host population is likely to be depressed well below average carrying capacity. However, the translocation area may be divided into two sites, depending on habitat quality, management potential and availability.

Prior to finalizing the choice of translocation sites, surveys of potentially suitable sites [i.e., approved by the U.S. Fish and Wildlife Service (USFWS) and the California Department of Fish and Game (CDFG) and available for purchase at a reasonable price] will be conducted to ensure that these are appropriate sites for

[1] Seven of the last 15 years have been drought conditions in the western Mojave Desert (United States Department of Commerce, National Oceanic and Atmospheric Administration. 1981-2002)

[2] Drought is both a proximate and ultimate cause of tortoise declines. Proximally, drought results in death and reduced reproduction. Ultimately, dehydration and starvation may engender disease increases due to compromised immune systems. For a review, see Chapter 4 of U.S. Army National Training Center (2003)

translocation. Survey methods will assess tortoise abundance, habitat features, existing anthropogenic influences, and anticipated future impacts. They will generally follow those used by Karl (2002a, b) at the nearby Fort Irwin military reservation because they provide such analyses. The survey will also include a qualitative assessment of the habitat quality for tortoises, based on the expertise of a tortoise biologist extremely experienced with assessing tortoise habitat quality.

Appropriate areas for a control site will be assessed simultaneously, using the same survey methods. The control site will be near (within a couple of miles of) the translocation site, and of similar or identical habitat. If the control site is on the DTRNA or CDFG lands, then data collection associated with the study will provide the Desert Tortoise Preserve Committee (DTPC) or CDFG with a large and useful data set on native tortoises there.

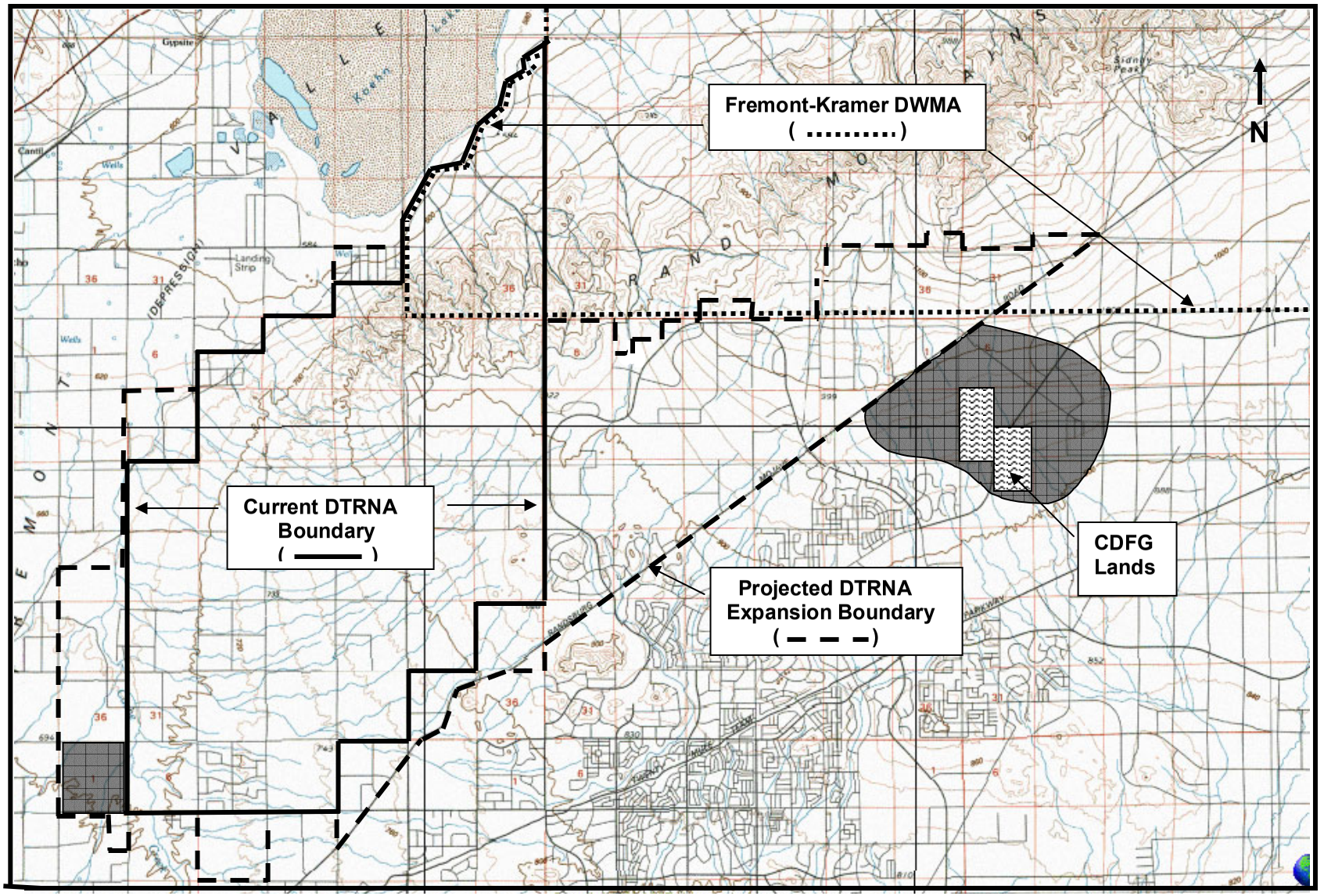
No translocation site has yet been chosen, although this will need to be accomplished in sufficient time to enable the site to be fenced prior to translocating tortoises in April 2004 (Table 1). Two areas currently are under consideration: (1) Section 1 in Township 32S and Range 37E; and (2) around the CDFG-owned lands in Township 32S Range 40E (Figure 1). The first site is adjacent to the DTRNA and within the area identified by the Draft Environmental Assessment/Habitat Conservation Plan (Draft EA/HCP) (Sapphos Environmental, Inc., 2003) as proposed mitigation lands (Figure 4.2.1-1 of the Draft EA/HCP). As land ownership will ultimately be transferred to CDFG, this action would be consistent with Objective No. 1 of the DTRNA Management Plan's *Goals and Objectives* (Appendix E of the Draft EA/HCP), which promotes protection, conservation, and enhancement of habitat in and around the DTRNA. Additionally, translocation of tortoises immediately adjacent to the DTRNA is not inconsistent with the DTRNA Management Plan. Objective No. 3 of *Goals and Objectives* promotes recovery of desert tortoise populations in and around the DTRNA, including a potential head-starting program.

The second potential translocation area would ultimately provide a larger block of protected, state-owned lands in an area targeted as a CDFG ecological reserve. Furthermore, the area is adjacent to both the Fremont-Kramer Desert Wildlife Management Area (DWMA) and the targeted expansion area of the DTRNA, adding to a large block of protected tortoise habitat.

Translocation Site Fencing

The translocation site will be fenced along the perimeter with temporary tortoise-proof fencing. To deter trespass by recreationists or sheep, at least two strands of barbed wire should be strung above the tortoise-proof fencing. The tortoise-proof

Figure 1. Potential translocation areas (▒▒▒) for Hyundai desert tortoise translocation program.



portion of the fencing will be removed during approximately Year 2 of the study (or when animals seem to have settled into the area – i.e., ceased fence-walking and have behaviors similar to the control group and host tortoises) to assess site repatriation and permit tortoises to become elements of the population (i.e., rather than segregated from the population). Site repatriation will be assessed by monitoring subsequent tortoise movements and comparing them to those of control tortoises.

Health Considerations

The transmission of *M. agassizii* is believed to be via direct contact with an infected tortoise (Brown *et al.* 2003). *M. agassizii* can only live outside the tortoise briefly (seconds) (McLaughlin pers. comm. to A. Karl) and a limited experimental study with gopher tortoises (*G. polyphemus*) suggested that it is unlikely to persist on objects or in burrows (McLaughlin 1997 *in* Brown *et al.* 2003). Desert tortoises are believed to be contagious during periods of acute phases, when they have clinical signs (Brown *et al.* 2003). Such signs include a mucous nasal discharge, palpebral edema, wheezing, moist nares or eyes, conjunctivitis, and lethargy. Schumacher *et al.* (1997) observed that positive clinical signs had a high statistical correlation with positive serology (i.e., exposure to *M. agassizii*). A mucous nasal discharge was the clinical sign that was the most reliable predictor (93% of tortoises with a mucous nasal discharge were seropositive), although it could be caused by other pathogens. Other clinical signs were far more subjective, were potentially present for other reasons, and reduced the statistical predictability of positive serology. Positive serology [i.e., a sufficient level of *M. agassizii*-specific antibodies to be detectable by an enzyme-linked immunosorbent assay (ELISA)] indicates that a desert tortoise has been exposed to *M. agassizii* (Schumacher *et al*. 1993). It does not, however, indicate whether the tortoise currently hosts the organism. Evidence of an active infection by *M. agassizii* is currently diagnosed by cultures and polymerase chain reaction (PCR).

For the Hyundai site, only tortoises that are free of definitive clinical signs of disease will be translocated. A mucous nasal discharge will be the threshold criterion to determine clinical illness, although should other signs with extreme severity be present, the tortoise may be considered to be clinically ill. Clinically ill tortoises that test positive for exposure to *M. agassizii* will be placed in the several-hundred acre area in the northwestern portion of the Hyundai site, north of the test track. This area will be fenced with tortoise-proof fencing prior to April 2004 so that clinically healthy tortoises can be translocated from this area to the translocation site prior to ill tortoises being introduced into the enclosure. The fencing will ensure that the sick tortoises cannot escape and will be safe from activities associated with the test track.

All clinically ill tortoises will have ELISA tests for exposure to *M. agassizii* prior to moving them to the disease control area (see Appendix 1- Chart 2). Clinically ill tortoises that are not seropositive for *M. agassizii* will be treated as are clinically healthy tortoises, rather than moving them to the disease control area and potentially exposing them to *M. agassizii*.

The sick tortoises placed in this area will be transmittered and monitored identically to the translocated tortoises (see *Schedule of Clearance and Data Collection*, below). The exception will be adult females. These will be appropriately radiographed for egg production (Karl 1998), confined to a small area when they are nearing oviposition, and their nests collected and moved to the translocation site at the time of oviposition. There appears to be a low probability that infected females can transmit *M. agassizii* to their embryos (Brown *et al.* 2003, Rostal and Lance 2003).

All clinically ill tortoises transported to the disease control area will remain in the enclosure until they exhibit a lack of clinical disease signs over two consecutive weighing/measuring occasions (March, July, and October), in order to reduce transmission of *M. agassizii*. When free of clinical signs, tortoises will be translocated to the translocation site to become part of the study cohort. It is anticipated that few tortoises will exhibit clinical signs because (a) there are few tortoises at the Hyundai site and (b) none exhibited clinical signs during October 2003 surveys. Most tortoises at the Hyundai site will be found by Spring 2004, so it is likely that any with clinical signs at this stage of the study will clear of signs during the study or, possibly, worsen and die. Any that do not clear of signs by the study's end, or are found with clinical signs during Hyundai project operation, after the study is completed, will be used for research or adopted (see Appendix 1-Chart 5).

Study Cohorts and Basic Study Design

Because a major investigation of this study is to examine disease patterns, three cohorts - translocatees, hosts (i.e., tortoises receiving the translocatees), and control tortoises - will be studied. The target size for each cohort will be 15 adult females and 15 adult males (i.e., 30 translocated tortoises, 30 host tortoises, and 30 control tortoises). This number will allow for statistical validity in light of attrition due to lost signals from malfunctioning transmitters and mortality. Study tortoises will be of reproductive size [≥180 mm in median carapace length (MCL)] because it is anticipated that there will be too few smaller tortoises for statistical validity. The exception to this will occur during initial translocation in April 2004, when immature tortoises (at least approximately 428 g, due transmitter size[3]) will also be transmittered to observe early movements following

[3] The smaller transmitters currently in use for the project weigh 25-30 g with epoxy and antenna sheathing. If the units comprise no more than 7 % of a tortoise's mass, the minimum tortoise mass accommodating these transmitters is 428 g.

translocation[4]. Transmitters on the latter tortoises will be removed by Summer 2004.

At the time of capture, all tortoises, including study animals (translocatees, host, and control tortoises) and all other tortoises that will subsequently be removed from the Hyundai site, will be weighed, measured, photographed, sexed, and described. All tortoises will be permanently marked with a unique number, using two of three systems (notching, epoxy numbers, P.I.T. tags) for future identification.

Survival and general health will be monitored through body condition indices (mass to volume ratios), clinical signs, serology and cultures. All tortoises will be examined for clinical signs of disease at the time of capture. Blood samples will be taken to test for the presence of antibodies to *Mycoplasma agassizii*; ELISA for other pathogens will be run as the tests become valid for desert tortoises. Nasal samples will be taken for culturing to detect the presence of *M. agassizii* and other pathogens (e.g., herpesvirus, *M. cheloniae*, iridovirus, *Pasturella testudinis*). The schedule of sampling is discussed below (see *Schedule of Clearance and Data Collection*).

All study individuals will wear appropriately sized transmitters, fitted to insure safety to the individual and lack of interference with growth and behaviors. Transmitters are scheduled to last 18 months and will be changed at appropriate intervals (approximately 16-17 months), or sooner if they exhibit symptoms of malfunctioning.

Activity patterns (i.e. increased aboveground activity levels), which may affect body temperatures and body condition and ultimately health and survival, will be monitored by temperature data loggers (HOBO TidBits), which continuously collect data. These will be mounted on all males in the study cohorts and in sample burrows. (Only males can carry the data loggers without interference with righting or other behaviors.) Data will be downloaded at sufficient intervals to avoid any data loss and identify malfunctioning units. Data loggers have batteries sufficient to last the entire study, without replacement.

The study will last four years, including the translocation year.

Consistency with the Desert Tortoise Recovery Plan

While the choice of translocation sites and the program protocols are largely based on ecological considerations, plus land availability, consistency with the Desert Tortoise Recovery Plan (USFWS 1994) is desirable. The recovery plan offers a brief set of suggestions for translocations that provided some ecological

[4] This part of the study may be re-evaluated since the translocation site will now be fenced, eliminating any long-distance movements of immature animals.

concepts to consider while remaining consistent with the rest of the recovery plan directives (Appendix B of the recovery plan). The Hyundai translocation plan is consistent with these guidelines, with the exception of the first part of Point 7, which reads, "the recipient population should be monitored for at least 2 years prior to the introduction. Necessary data should include the density and age structure of the recipient population, home ranges of resident tortoises and general ecological conditions of the habitat." Home ranges and age structure of the host population are not critical factors for the Hyundai translocation study. (It is assumed that the two-year suggested period of study is for estimating these factors, although the rationale for a two-year study is neither explained nor does it include references). However, the density of the host population and general ecological conditions of the translocation site will be known prior to translocation (see *Translocation and Control Sites*, above). In addition, a control population will be similarly studied to validate study results.

Schedule of Clearance and Data Collection

Based on the current project permitting date of January 2004 and an estimated translocation date of approximately April 7, 2004, the following schedule of activities is offered. The period from October 2003 to October 2004 is shown in Table 1. In addition, decision matrices for various aspects of the entire project period are presented in Appendix 1.

Pre-Translocation, Pre-Permitting and Pre-Construction - October 2003

A survey of the entire Hyundai project site was completed in October 2003 using tortoise searchers spaced at five-meter intervals. The purpose was to conduct blood and nasal sampling for determining current health profiles of tortoises at the Hyundai site. A positive ancillary outcome was that many tortoises were found and transmittered while they were still active. This will permit tortoises to be found more easily during hibernation should construction begin in Winter 2004, hopefully resulting in fewer mortality takes. Tortoises were allowed to enter hibernation naturally.

Pre-Translocation Monitoring During Construction – Through Approximately April 7, 2004

The first priority of monitoring is to avoid killing or injuring tortoises during construction or other project activities. Within this context, the translocation study will be preserved by avoiding disturbance of natural tortoise activities, where possible, and avoiding double-translocation (e.g., moving tortoises to a new area on the site prior to translocating them to the translocation site).

Table 1. Estimated schedule for tortoise translocation program at the Hyundai Mojave Test Track Project - Year 1[1]

Task \ Month	Oct 2003	Nov 2003 - early Mar 2004	Mid-late Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Search Hyundai site to find and transmitter as many tortoises as possible for purposes of conducting blood and nasal analyses for disease										
Survey potential translocation sites and choose final translocation and control sites										
Fence translocation site with tortoise-proof fencing										
Construction of Hyundai project, beginning with fencing, using monitors and fencing as necessary to avoid tortoise losses										
Fence Hyundai site with tortoise-proof fencing, including construction and traffic areas, northwest disease control area, and perimeter										
Find control tortoises and transmitter										
Find host tortoises and transmitter; blood and nasal analyses on all study tortoises and other tortoises										
Second and third passes (minimally) to find all tortoises on Hyundai site, completing northwest disease control area first										
Initial translocation of most tortoises from Hyundai site to translocation site, or to disease control area if clinically ill										

Task \ Month	Oct 2003	Nov 2003 - early Mar 2004	Mid-late Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Continued translocation from Hyundai site - Spring 2004										
Continuous protection of tortoises remaining on Hyundai site during construction (no translocation)										
Translocation of remaining Hyundai tortoises to translocation site, or to disease control area if clinically ill										
Primary production biomass analyses on host and control plots										
Locations of all tortoises 2 times a week[2]										
Locations of all tortoises 2 times a month										
Weigh, measure all tortoises										
Annual blood and nasal sampling of all study tortoises										
Locations of all tortoises 1 time a month										

1. Assumes permits are issued in January 2004.
2. Data loggers will be downloaded periodically, during this and successive locations of tortoises. Transmitters will be changed as necessary.

The activities presented below assume that (a) a permanent tortoise-proof fence will be installed around the perimeter of the Hyundai site, (b) all areas where construction or construction-related travel will occur will be temporarily fenced with tortoise-proof fencing, and (c) translocation will occur in early April (approximately April 7). Fencing of construction zones will be the first construction activity. Permanent tortoise-proof fencing will be ½-inch mesh hardware cloth, as specified by the U.S. Fish and Wildlife Service (Judy Hohman, USFWS, pers. comm. to A. Karl). Vertical burial will be 12-18 inches with an 18-24 inch aboveground extension. Supporting stakes will be sufficiently spaced to maintain fence integrity. Temporary tortoise-proof fencing may be silt fencing or other temporary fencing, buried 12-18 inches with an 18-24 inch aboveground extension. As with permanent fencing, supporting stakes will be sufficiently spaced to maintain fence integrity. Temporary fencing will be in place until it has been determined that all tortoises have been cleared from the project site (see Final EA/HCP). All fence construction will be accompanied by adequate monitoring by qualified tortoise monitors to insure that no tortoises are harmed. All fencing will be monitored on an adequate schedule to ensure fencing integrity (e.g., monthly for permanent fencing, weekly for temporary fencing, and after all storm events that are accompanied by surface water flow).

During construction activities in the winter, all fenced construction and high-traffic zones will be searched for tortoises. The searches will include all burrows that could potentially host a tortoise. These will be excavated with hand tools in the method prescribed by the Desert Tortoise Council (1994). (Note: Any nests found after October are probably infertile and will be examined but not moved.) All tortoises (transmittered or not) will be moved immediately off the construction areas to artificial burrows that provide safe thermal refugia. Artificial burrows will mimic the capture burrows[5]. It is understood that a tortoise generally will not use an artificial burrow readily, so surface soil and scat from the capture burrow will be placed in the artificial burrow to assist with acclimation. The tortoise will also be blocked into the burrow for one-several weeks to promote familiarity, and monitored to insure their safety.

Prior to moving them, untransmittered tortoises will be transmittered, even if the study size cohort is exceeded, to facilitate finding these tortoises again. A combination of monitoring, burrow-blocking, and/or tortoise-proof penning around the artificial burrows will prevent tortoises from re-entering construction or traffic zones until temporary tortoise-proof fences are erected around those zones. All blocks and penning will be removed once construction and high-traffic zones have been fenced.

[5] It is highly unlikely that any tortoises will be found in winter without an association to a burrow. If no burrow is evident, artificial burrows will be a minimum of 1.5 m long and 0.5 m belowground at their interior terminus to insure adequate thermal buffering; the direction will either face north or east to minimize sunlight entering the burrow.

Because small tortoises are difficult to find and are also subject to depredation by a variety of nocturnal and diurnal predators, any juveniles found prior to the estimated translocation date of approximately April 7 that cannot be transmittered (i.e., smaller than approximately 428 g), will be sequestered in a holding pen that is both predator-proof, escape-proof and with sufficient shrub vegetation for ample shade and sun. The size of the pen will depend on the number of tortoises found, but will start at 20 feet in diameter and be extended to approximately 50 feet if more than three tortoises are contained. Artificial burrows will be constructed and tortoises initially blocked into these burrows. This pen will be monitored during construction in Winter 2004 to insure its integrity and tortoise safety; it is assumed that ongoing site construction will deter vandalism. Alternatively, very small transmitters may be obtained for use on these juveniles and monitoring will proceed as for larger animals.

While not all monitors will be permitted to handle or transmitter tortoises, all will be adequately trained to ensure thorough monitoring. A sufficient number of biologists permitted to handle and/or transmitter tortoises will be onsite during fence construction and clearance surveys to move animals efficiently. A lead biologist will always be present to ensure that monitoring runs smoothly and to solve problems.

Tortoises will be handled smoothly, quickly, and with clean techniques, following techniques outlined by the Desert Tortoise Council (1994), and incorporating newer research (e.g., Brown 2003).

Clearance and Translocation - Year 1

The fenced construction areas will have beencleared of tortoises during Winter 2004. Although tortoises will have been moved only very short distances, probably still within their home ranges, some tortoises may be observed pacing a long fence (e.g., the test track) excessively, as the weather warms. These tortoises may be moved, as appropriate, to a site immediately outside the opposite fence and monitored.

During the last week in March (or slightly earlier if ambient temperatures are sufficiently warm) and first week in April, control site tortoises will be found and transmittered. Host tortoises will be located and transmittered on the translocation site during the first and second weeks in April.

Fencing of all phases of the project site (construction and traffic zones, northwest disease control area, and perimeter) and the translocation site will have been completed by the time clearance surveys begin on the Hyundai site and tortoises are translocated to the translocation site. Remaining searches for tortoises on the Hyundai site will start approximately April 1, beginning with clearance of the disease control area. Clearance will include aboveground searches as well as

burrow searches and will require at least two more clearance passes[6]. At least two consecutive passes with no tortoises will be required for a segment of the site to be considered cleared (see final EA/ HCP). All burrows that could potentially host a tortoise will either be (a) excavated with hand tools in the method prescribed by the Desert Tortoise Council (1994), if in an area where the burrow would ultimately be destroyed by construction activities, or (b) visually examined with fiberoptics tools for tortoise presence.

Until translocation at the end of approximately the first week in April, new tortoises will be transmittered as they are found, including tortoises that are in excess of the study cohort size (for ease of re-locating). Smaller tortoises will be sequestered as explained above. All tortoises found and transmittered will remain on the site to forage, protected from construction activities by tortoise-proof fencing and/or monitoring until translocation. Translocation in early April will permit translocatees to locate new burrows on the translocation site prior to ambient temperatures becoming lethal. Concurrently, most or all of the host tortoises and, hopefully, all of the control study tortoises will have been found by this time. All non-clinically ill tortoises, including juvenile tortoises that are too small for transmitters (see above), will be translocated.

Clinically ill tortoises that are seropositive for *M. agassizii* will be translocated to the disease control area after all non-clinically ill tortoises there have been translocated to the translocation site. Clinically ill tortoises that are not seropositive will be transported to the translocation site, rather than moving them to the disease control area and potentially exposing them to *M. agassizii*.

Tortoises will be transported to the translocation site in individual, sterilized tubs with taped plywood lids. Transportation will occur inside a vehicle offering shade (e.g., camper, sports utility vehicle or van). The tubs will be cushioned, shaded, cool, and not placed over the catalytic converter; vehicle speeds will be minimized on dirt roads to prevent jarring. Tortoises will be released at several locations throughout the translocation site that have been identified prior to tortoise transport. Because releases will only proceed under suitable ambient temperatures, no artificial burrows will be constructed. All tortoises will be offered free water prior to transport to aid in minimizing stresses associated with translocation.

Juvenile tortoises are not only more subject to depredation than are adults, but a juvenile tortoise's large surface area to volume ratio results in its heating faster than its larger counterparts. Because unfamiliarity with the translocation site could exacerbate these factors, juvenile tortoises will be provided with extended artificial protection from canid and avian predators and ambient conditions by use of a predator-proof enclosure erected at the translocation site. The size of the enclosure will depend on the number of tortoises found, but will start at 20 feet in diameter and will be extended to approximately 50 feet if more than three

[6] The October 2003 survey will be considered the first pass.

tortoises are contained. [Morafka *et al*. 1997 successfully penned juvenile tortoises at the rate of 152-305 animals per hectare (62-123 tortoises per acre).] The current plan for subsequently releasing juveniles includes opening escape holes in the lower edge of the enclosure after tortoises have become familiar with the site's odors and landmarks for at least one-two weeks (Morafka *et al* . 1997). This plan may be mitigated depending on predator interest in the enclosure and/or juvenile tortoise behavior in the enclosure.

Nests found during burrow excavation or during other searches after April 15 will be moved to as identical a microsite as possible (e.g., cover, plant species, soil type, substrate, aspect) on the translocation site, using standard techniques (Desert Tortoise Council 1994). Translocated nests will be fenced with open-mesh fencing (e.g. 2-inch) and have open-mesh fencing or avian netting on the roof to prevent depredation by canids that might be attracted to the new nests by human scent, while still permitting allow passage by hatchlings. Nests will be monitored from a 30-foot distance once a month until November, at which time they will be excavated for examination. (Hatching should be complete by October.) If possible, hatchlings will be weighed, measured, photographed, described and marked.

Blood and nasal samples will be taken on host and control tortoises during the first and second weeks of April. [Since translocatees do not have definitive clinical signs, they will be unlikely to infect host tortoises immediately upon translocation. Clinical signs in host tortoises, following infection, would not occur until at least two weeks post infection and it can take up to eight weeks for antibody levels to build sufficiently for reliable detection (Brown *et al.* 2003).] Translocated tortoises, including tortoises in the study as well as those of sufficient size to extract blood, will be re-sampled at this time for temporal comparability to control and host tortoises. Transmitters on adult tortoises that are in excess of the study cohort size will be removed.

All tortoises will be located twice weekly in April following the initial large release, to help insure their safety, identify potential problems, and monitor behavior. They will be located approximately weekly in May, and once a month thereafter, until hibernation. No locations will occur during winter. During each location, tortoises will be examined for clinical disease signs to assess whether the stress of translocation or direct contact with a diseased host tortoise has resulted in illness.

It may take most of April or even more time to find all of the tortoises on the Hyundai site. As such, tortoises may be found after aboveground, ambient temperatures regularly exceed lethal thresholds most days (generally late April). The temperature limits for translocation will follow those that have been set by USFWS for handling - 35°C (95° F) at 5 cm above the ground surface (http://ventura.fws.gov/SurveyProt/de_tortoise_prtstatement.htm) - or

43° C (109° F) ground surface temperature, whichever is lower, to ensure tortoise safety. (Karl 1992 and Zimmerman *et al.* 1994 observed that 43° C was the approximate surface temperature at which tortoises go underground to escape heat.) During Year 1, tortoises found on the Hyundai project site after ambient temperatures exceed this threshold will be processed, transmittered, released at their capture sites, and monitored until October, when temperatures cool sufficiently to permit further translocation. The exception to this procedure will be for juvenile tortoises, which will be sequestered on the Hyundai site in a predator-proof pen (see description above). All tortoises will have blood and nasal samples analyzed prior to translocation and seropositive, clinically ill tortoises will be handled as explained above.

Study tortoises will be weighed and measured to determine condition indices, identify clinical signs, and monitor transmitter condition in July (following the nesting period) and mid-October. Blood and nasal samples will also be taken in mid-October. In general, handling will be minimized to minimize stress to the animals.

Primary productivity of annual plants will be measured on biomass plots on the translocation and control sites during the third week (approximately) of April, when maximum biomass of current-year production has been reached. Precipitation will be monitored using rain gauges on the translocation and control sites; daily temperature maxima and minima and relative humidity will be collected with data logger stations on each site and/or from stations at Edward Air Force Base, Mojave Airport, or another nearby weather station.

Translocation Study - Years 2-4 (March 15, 2005 to April 1, 2008)

Translocated tortoises will be located on a sufficiently intense schedule to collect the necessary health data, download data loggers, change transmitters, and identify faulty transmitters and other equipment. Unless circumstances arise, this will probably be limited to weighing and measuring for monitoring condition indices at exiting from hibernation (late March), following the spring activity period and after nesting (July) and immediately prior to hibernation (late October). Transmitters will be changed and data loggers downloaded as necessary. Blood and nasal samples will be taken and analyzed annually, in October. Sampling frequency and techniques for disease analysis will be updated as necessary during the study, based on the newest disease information from this and other studies. This may include tests for other pathogens (e.g. *Mycoplasma.* spp., herpesvirus, iridovirus) as their importance and evaluation techniques become known. Any time a tortoise is handled, it will be examined for clinical signs of disease.

Primary productivity will be measured on biomass plots at the same phenological stage each spring. Precipitation will be monitored as for Year 1 (see above).

The tortoise-proof portion of the translocation site fence will be removed during approximately Year 2, between October and March, to assess site repatriation. This point in the study has been chosen based on data from earlier studies that suggests that translocatees settle into a new area within two years of translocation (Stewart 1993, Phil Medica and S. Corn, pers. comm. to A. Karl). This "settling" will be determined based on behavior and movements of translocatees compared to control tortoises. The timing within the year is in consideration of activity patterns and ambient temperatures. Locating tortoises will need to be increased slightly (e.g., additional locations once a month during the activity seasons) to monitor movements and examine whether tortoises remain on the translocation site.

Transmitters and data loggers will be removed at the conclusion of the study, in March-April 2008.

In the event that a tortoise appears on the Hyundai project site after clearance surveys have been completed but prior to the removal of interior tortoise-proof fences in Year 5, a protocol has been established to remove the tortoise from the site (see Appendix 1-Chart 5). The protocol requires that there be an onsite, Hyundai Environmental Compliance Officer (ECO), educated in basic tortoise handling procedures, who would contact the translocation study principal investigator or other team member (during the translocation study period) or one of several designated biologists (after the translocation study is completed) who can come to the site within 24 hours to attend to the tortoise. In the meantime, the ECO would safely hold the tortoise, either by fencing or blocking it into its burrow if it's inactive (winter) or putting it in a sterilized tub with a taped, plywood lid which would then be stored in a cool, dark site overnight at air temperatures between 25° and 33° C (77° and 91° F) (spring through fall). (These temperatures simulate preferred burrow temperatures in late spring and summer [Karl 1992].) Disposition of the tortoise the next day (e.g., translocated, adopted, or used for headstarting or research) would depend upon timing (i.e., ambient temperatures), clinical signs and if the tortoise can temporarily be left on the Hyundai site safely. Translocation of non-clinically ill tortoises to the translocation site would occur if the tortoise were found between October 1 and approximately April 25. The tortoise would be processed and numbered, and blood and nasal samples would be taken, but the tortoise would not be transmittered. If the tortoise were found on the Hyundai site between April 25 and October 1, and it could temporarily remain on the site, it would be transmittered and left at the release site or moved immediately off any high-traffic area until either (a) it could be translocated to the translocation site at the appropriate time of the year for translocation (i.e., October 1 to April 25) or (b) if the tortoise had clinical signs of disease, ELISA test results had returned. Any clinically ill, seropositive tortoise would be would be transferred to the disease control area at the appropriate time of year. (Protocols for juvenile tortoises would follow those outlined for the 2004 construction year.) Clinically ill,

seronegative tortoises would be translocated to translocation site at the appropriate time of the year for translocation. Any tortoise translocated to the translocation site during this period would have their transmitters removed, with the exception of clinically ill tortoises in the disease control area that cleared of disease signs (see *Health Considerations*, above). As with other aspects of the project, approaches may be changed based upon new information from this and other studies.

By the end of the translocation study, all clinically ill tortoises still in the disease control area that had not cleared of clinical signs would be adopted or used for research.

In the event that a tortoise appears on the Hyundai project site after interior tortoise-proof fences are removed (Year 5), it would be assumed that the tortoise must be immediately removed from the site. The protocol would be similar to that above, with the following exceptions:

- Between October 1 and approximately April 25, the tortoise would only be processed and numbered prior to translocation

- Any clinically ill tortoise would be adopted or used for research.

Injuries and Mortalities for Tortoises Associated with the Translocation Study or Hyundai Mojave Test Track Project

All study tortoises that are injured as a direct result of the study or Hyundai Mojave Test Track Project construction or operation will be transported to a qualified veterinarian for medical care.

All study tortoises that die during the study or as a result of project construction or operation will be submitted for necropsy to the Mycoplasma research laboratory at the University of Florida to assist in ongoing evaluations of western Mojave tortoise health conditions, if the tissues are in condition to be analyzed by the lab.

Unforeseen Circumstances

If unforeseen circumstances arise, they will be addressed through discussions with experts, CDFG, and USFWS.

Reporting

An annual report will be prepared for federal and state permit requirements. This report will include an analysis of data collected that year, annual and cumulative results and conclusions, and recommendations. Following the final year, a comprehensive report will be written to encompass the entire study.

Study results will be submitted to a peer-reviewed journal for publication.

FUNDING

Adequate funds will be available to complete the study as described. This will include all field work, data analysis, publication of results, and transmitter removal at the conclusion of the study. Hyundai will be financially responsible for the translocation program. The funds will be separate from compensation habitat enhancement and endowment fees.

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APPENDIX 1. Decision Matrix for Implementation of the Hyundai Desert Tortoise Translocation Program

Chart 1. 2004 Construction Year: Tortoise found on Hyundai Site

Chart 2. 2004 Construction Year: Tortoise < 428 g Found on Hyundai Site

Chart 3. 2004 Construction Year Through End of Translocation Study (April 2008): Tortoises with Clinical Signs

Chart 4. Post Construction, Through Year 5 (January 2009): Tortoise found on Hyundai Site

Chart 5. Project Operation, Following Year 5 (January 2009): Tortoise Found on Hyundai Site

CHART 1

PROJECT PERIOD – **2004 CONSTRUCTION YEAR**
SITUATION - **UNTRANSMITTERED TORTOISE FOUND ON HYUNDAI SITE**

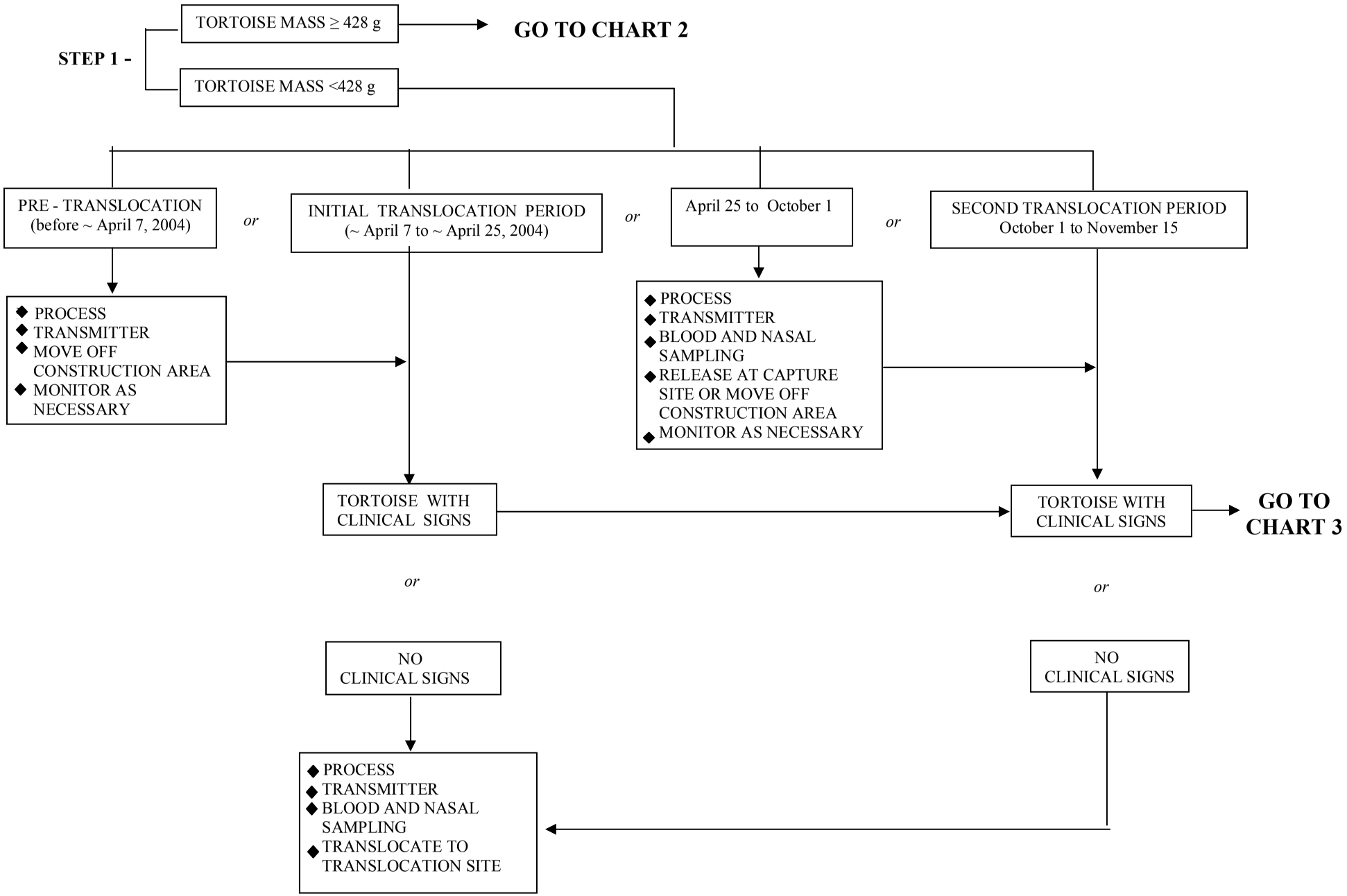

CHART 2

PROJECT PERIOD – **2004 CONSTRUCTION YEAR**
SITUATION - **TORTOISE <428 G FOUND ON HYUNDAI SITE**

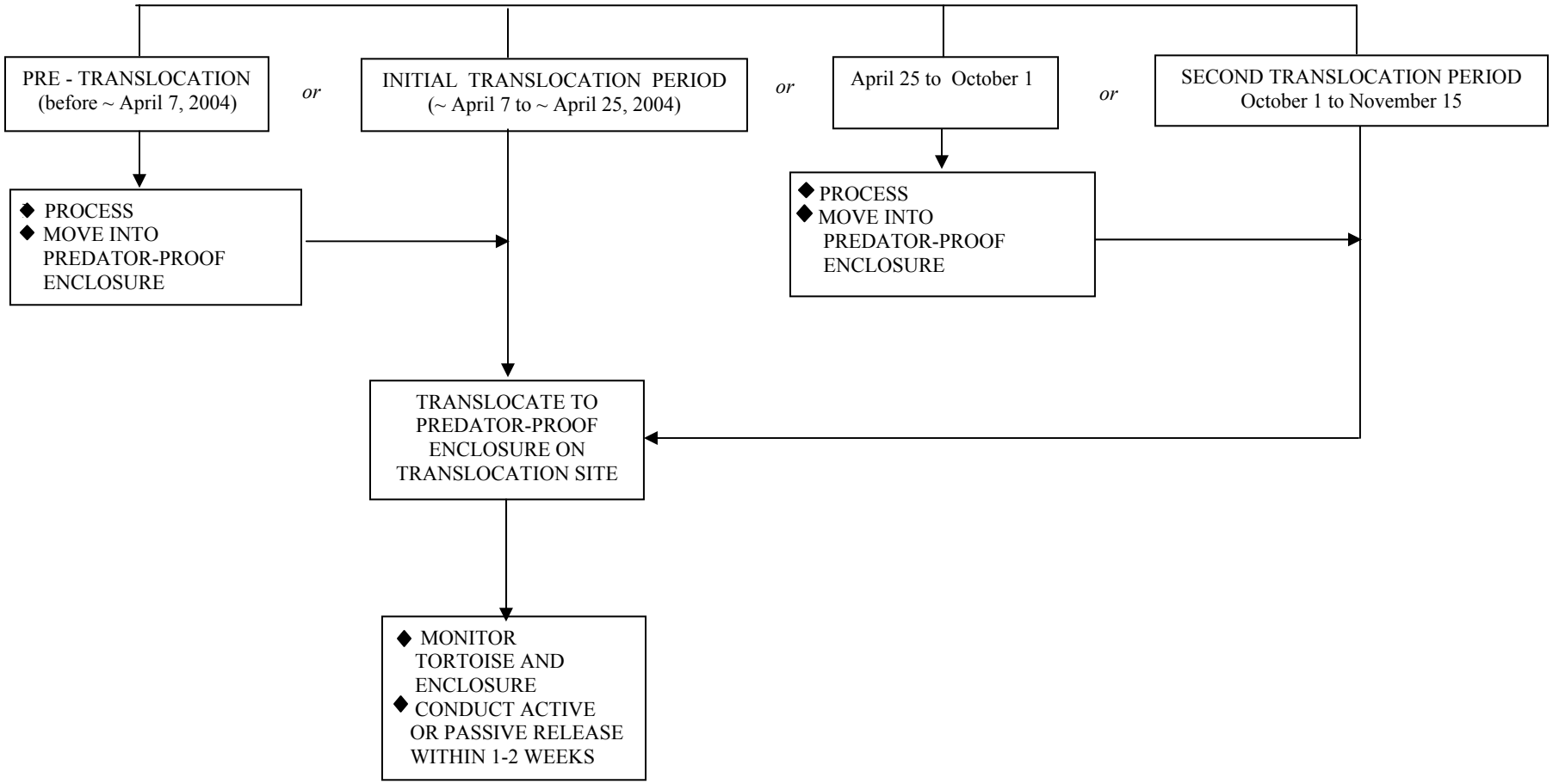

CHART 3

PROJECT PERIOD – **2004 CONSTRUCTION YEAR THROUGH END OF TRANSLOCATION STUDY (APRIL 2008)**
SITUATION - **TORTOISE WITH CLINICAL SIGNS**

STEP 1 -

- PROCESS
- TRANSMITTER
- BLOOD AND NASAL SAMPLES
- RELEASE AT CAPTURE SITE

STEP 2 -

TORTOISE SERONEGATIVE

TRANSLOCATE TO TRANSLOCATION SITE AT APPROPRIATE TRANSLOCATION PERIOD (SEE CHART 1)

or

TORTOISE SEROPOSITIVE

MOVE TO DISEASE CONTROL AREA AT APPROPRIATE TRANSLOCATION PERIOD (SEE CHART 1)

WEIGH AND MEASURE AT INTERVALS IN TRANSLOCATION PROGRAM

and

RADIOGRAPH ADULT FEMALES MAY 1 TO JULY 15

TRANSLOCATE NESTS TO TRANSLOCATION SITE

CLEAR OF CLINICAL SIGNS FOR TWO CONSECUTIVE WEIGH/MEASURE SAMPLINGS

DON'T CLEAR OF CLINICAL SIGNS FOR TWO CONSECUTIVE WEIGH/MEASURE SAMPLINGS

KEEP IN DISEASE CONTROL AREA

CHART 4

PROJECT PERIOD – **POST CONSTRUCTION, THROUGH YEAR 5 (JANUARY 2009)**
SITUATION - **UNTRANSMITTERED TORTOISE FOUND ON HYUNDAI SITE**

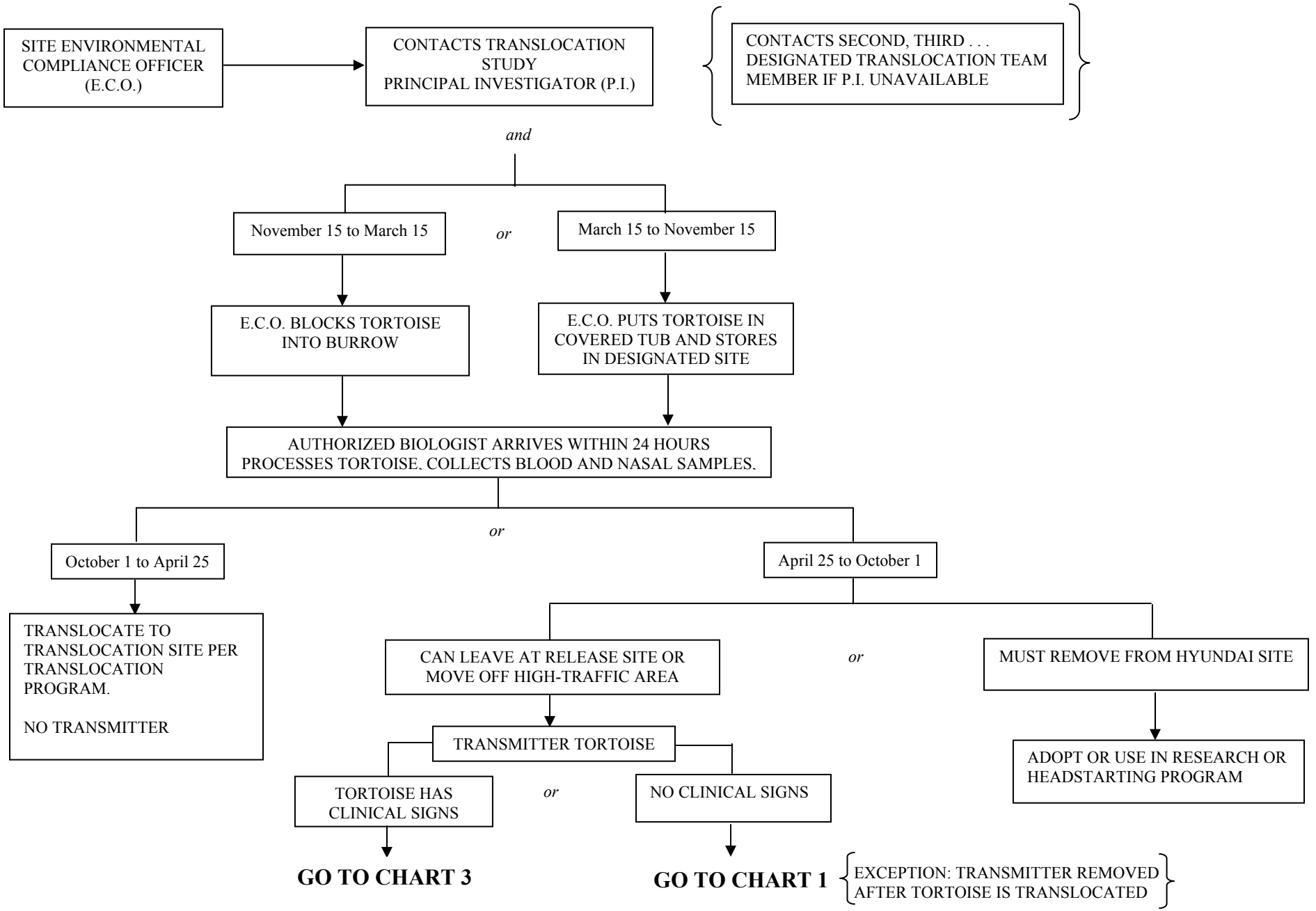

CHART 5

PROJECT PERIOD – **PROJECT OPERATION, FOLLOWING YEAR 5 (JANUARY 2009)**
SITUATION - **TORTOISE FOUND ON HYUNDAI SITE**