#### TRANSLOCATION OF MOJAVE DESERT TORTOISES FROM PROJECT SITES: PLAN DEVELOPMENT GUIDANCE U.S. Fish and Wildlife Service November 2011

Recent studies of Mojave desert tortoise translocation have shown promise. However, these studies have occurred over durations of less than five years, various risks have not been fully evaluated, and long-term success has not been demonstrated. We do not fully understand the long-term impacts of translocation, including for example, altered disease dynamics or changes to effective population size (relative to reproduction of translocated tortoises within the resident population). Likewise, impacts on population fragmentation and gene flow as a result of utility-scale development within occupied desert tortoise habitat are unclear. The following guidance is designed to address risks and minimize impacts based on the best scientific information currently available and will be updated as new information and data are obtained. It is important that new translocation projects be designed in a research or effectiveness-monitoring framework to address outstanding questions related to the success or impacts of translocation of Mojave desert tortoise populations.

This guidance is complementary to existing protocols for the Mojave desert tortoise (*Gopherus agassizii*; hereafter, "desert tortoise" or simply "tortoise") that should be referenced when planning and implementing surveys, translocation plans, and other activities involving this species. To ensure that you are referring to the most current guidance and protocols, contact your local U.S. Fish and Wildlife Service (USFWS) field office or see <a href="http://www.fws.gov/ventura/speciesinfo/protocols\_guidelines/">http://www.fws.gov/ventura/speciesinfo/protocols\_guidelines/</a>, where the following can be accessed: Pre-project Survey Protocol, Desert Tortoise Field Manual (includes Pre-project Survey Guidelines, Handling Guidelines, and Exclusion Fence Specifications), Qualifications and Requirements for Authorized Biologists, and Desert Tortoise Exclusion Fence Specifications.

The purpose of this document is to provide guidance for the development of project-specific translocation plans for activities that may impact desert tortoises when avoidance of these impacts is not feasible and adverse effects of the incidental take of desert tortoises associated with the proposed action need to be minimized. Prior to drafting a translocation plan, however, project proponents should identify, review, and consider all potential measures to avoid adverse effects to desert tortoises at the project site. If translocation can be justified as the most appropriate course of action, this document should be used as an outline that, when combined with project-specific input from the USFWS and other permitting agencies, will facilitate the completion of a translocation plan. This guidance does not constitute a translocation plan "template." Rather, the guidance describes considerations and recommendations that must be taken into account when developing a translocation plan and preparing to move desert tortoises across the landscape. Deviations from the guidance may be appropriate and acceptable if addressed by a project-specific effectiveness-monitoring program within, or otherwise justified by, the translocation plan.

The implementation of any translocation activity will necessitate take of desert tortoises in some form. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct. Section 9 of the Endangered Species Act of 1973, as

amended, and Federal regulation pursuant to section 4(d) of the Act prohibit the take of endangered and threatened species (fish and wildlife), respectively, without special exemption. Consequently, activities described by this guidance may be undertaken only when authorized by the USFWS through the issuance of a recovery permit pursuant to section 10(a)(1)(A) of the Act, an incidental take permit pursuant to section 10(a)(1)(B) of the Act, or if an exemption against the prohibitions against take is granted through the issuance of a biological opinion that contains an incidental take statement under the authorities of section 7(a)(2) of the Act and the action agency issues their decision document. The translocation plan, after approval by the USFWS, would be incorporated into the project design or included in the terms and conditions of the USFWS's biological opinion or incidental take permit. Without a recovery permit, activities outlined in Step 6 through Step 10 of this guidance can only be conducted in accordance with an incidental take permit or biological opinion.

In addition to this guidance, *project proponents should confer with the respective State wildlife agencies within the range of the Mojave population of the desert tortoise where compliance with State laws is mandated or different survey and translocation protocols exist.* Collection or take permits may also be required by other Federal agencies or by State laws and regulations.

We have summarized the actions associated with translocation in chronological order. Table 1 (at the end of the document) provides a quick reference of the various recommendations based on number of desert tortoises expected to be moved, as translocation options and monitoring are different for projects affecting five or fewer desert tortoises. Because any given project may have unique circumstances, we recommend project proponents and the lead action agency work closely with the appropriate USFWS field office and State wildlife agencies as early in the planning process as possible to determine which of the components and to what degree each of the following should be included in project-specific translocation plans.

**1. Determine need for translocation of desert tortoises.** Is the project site or project footprint designed to avoid and/or minimize impacts to existing desert tortoise populations to the maximum extent possible, including impacts to connectivity between populations?

**2.** Estimate the number of desert tortoises that will be affected at the project site. Conduct desert tortoise surveys according to the most recent USFWS Pre-project Survey Protocol (accessed at the website above) and include data on all desert tortoise sign observed during surveys. A list of minimum data to be collected is given in Table 2. Surveys should be conducted during the desert tortoise's most active periods (*i.e.*, typically April 1 through May 31 or September 1 through October 15) when air temperatures are below 40°C ( $104^{\circ}F$ ), which should be verified by activity in the field. Temperature should be measured in the shade and protected from the wind at a height of 5 centimeters above the ground. Survey data will be used to estimate the number of desert tortoises expected to be impacted by the project; assist in identifying potential recipient sites<sup>1</sup> based on the density estimates; and, if applicable, determine the minimum number of resident<sup>2</sup> and control<sup>3</sup> desert tortoises needed for monitoring purposes. If

<sup>&</sup>lt;sup>1</sup> The *recipient site* is the location to which desert tortoises removed from the project site will be translocated. <sup>2</sup> *Resident tortoises* are those desert tortoises living within the recipient site prior to the release of tortoises from the project site. Once identified, they continue to be designated as resident tortoises after the translocation.

<sup>&</sup>lt;sup>3</sup> *Control tortoises* are at a location separate from and unaffected by the project or recipient site and tortoises. This location is selected for comparative monitoring purposes relative to the translocated and resident tortoises.

out-of-season surveys, probabilistic sampling, or non-protocol surveys are proposed for the project site, approval from the USFWS and State wildlife agencies should be obtained prior to conducting any surveys; this increases the likelihood that survey results will be accepted.

*If the USFWS has issued a recovery permit for this project, health assessments*<sup>4</sup> *conducted in accordance with the most recent protocols (USFWS 2011) may be performed concurrent with these surveys (instead of waiting until Step 8).* Health assessments will include a physical inspection (including body condition, measurements, and notation of clinical signs of disease) and collection of biological samples to determine disease status of the individuals. Because physiological activity of the desert tortoise immune system is related to behavioral activity and is important for the types of tests that will be run, blood samples should be drawn beginning May 15 or, upon specific approval from USFWS, four weeks after the date that the individual of interest left its hibernaculum or was first found active and above ground (not in association with a shelter site). The last date for blood sampling is October 31. Results from the biological samples will be valid for 1 year from the date that the samples were collected.

*Five or fewer desert tortoises.* If 5 or fewer desert tortoises need to be removed from the project site and project fence line, a mitigation fee may be assessed in lieu of translocating these tortoises and implementing an effectiveness-monitoring program. Disposition of the affected tortoises may be determined as follows. These guidelines apply to the cumulative number of tortoises across the entire project site (*i.e.*, projects cannot be subdivided into smaller phases to avoid requirements otherwise contained in this guidance).

- If a desert tortoise is within 500 meters (m) of the project fence, that individual may be moved over the nearest fence (if it is a protected area with suitable habitat) after a health assessment is conducted. Subsequent monitoring of this desert tortoise via telemetry will only be required until the project-site clearance is completed and final numbers are confirmed.
- Desert tortoises may be moved to a depleted area (as defined in Step 3), if an appropriate site has been identified (see recipient-site criteria in Step 3), after a health assessment has been conducted. To determine size of the area needed, all release points should be encompassed by a 6.5-kilometer (km) radius (see Step 3). Subsequent monitoring will not be required. If the tortoises are moved to a population that is not depleted as defined in Step 3, then the remaining translocation guidance applies, including density surveys and health assessments within the recipient population, with the exception of the post-translocation monitoring requirements and the need for a control site.
- Desert tortoises may be removed from the wild and placed within a USFWS- and Stateapproved program.

**3.** Identify potential recipient and control sites<sup>5</sup> for projects. Lands that may be considered as potential recipient sites may include designated critical habitat or lands identified as Desert

<sup>&</sup>lt;sup>4</sup> Health assessments include disease surveillance testing as described in USFWS (2011).

<sup>&</sup>lt;sup>5</sup> See Table 1 for circumstances when identification of recipient and control sites is necessary.

Tortoise Conservation Areas (TCAs) in the revised recovery plan for the species; lands outside TCAs that are important for maintaining habitat and population connectivity, that are not subject to future impacts, or are a minimum of 10 km from areas expected to be developed; or lands where management actions are currently being tested. Planning should be done in coordination with Federal and State wildlife and land management agencies, and approval from the landowner/manager for use of the sites must be obtained.

<u>Determination of recipient-site size</u>. Recipient sites include the expected area of dispersal of translocated tortoises. Desert tortoises moved up to 500 m from their capture location are expected to settle within 1.5 km of their release point. Most desert tortoises moved greater than 500 m are expected to settle within 6.5 km of their release point.

- For tortoises moved up to 500 m: the recipient site encompasses the area within a 1.5-km radius of the set of potential release points.
- For tortoises moved greater than 500 m: the recipient site encompasses the area within a 6.5-km radius of the set of potential release points.

<u>Recipient-site selection criteria</u>. The following criteria should be considered when selecting prospective recipient sites. Each of the criteria is important, but failure to meet criteria 1-3 automatically results in disqualification of the recipient site.

- 1. \*The site supports desert tortoise habitat suitable for all life stages.
- 2. \*Disease prevalence within the resident desert tortoise population is less than 20 percent (see Step 6).
- 3. \*The site is at least 10 km from major unfenced roads or highways. Distances from roads may be reduced if the proposed action includes provisions to install and maintain desert tortoise exclusion fencing as a minimization measure.
- 4. The site is within 40 km of the project site, with no natural barriers to movement between them, to ensure that the desert tortoises at the two sites were likely part of a larger mixing population and similar genetically.
- 5. The site occurs on lands where desert tortoise populations have been depleted or extirpated yet still support suitable habitat. Depleted areas<sup>6</sup> may include lands adjacent to highways.
- 6. The site has no detrimental rights-of-way (ROWs) or other encumbrances.
- 7. The site will be managed for conservation so that potential threats from future impacts are precluded.

Little information exists on what constitutes carrying capacity for desert tortoise habitat or what specific variables relate to quantifiable measures of habitat quality. However, project proponents should provide a qualitative assessment of habitat conditions at prospective recipient sites relative to the project site, including perennial cover and density, annual forage, plant species diversity, and soil conditions for burrowing. Prospective recipient sites should be evaluated relative to potential threats that may affect desert tortoises translocated to the area, including

<sup>&</sup>lt;sup>6</sup> For the purposes of this guidance, an area is considered *depleted* if the density is below the 95-percent confidence limit of the density of the respective desert tortoise recovery unit (Table 3), ecologically appropriate habitat is present, and there is either a lack of desert tortoise sign or a preponderance of desert tortoise shells in the area.

those that may originate nearby (*e.g.*, dogs or elevated coyote or raven populations associated with human development, proximity to major highways, and proximity to existing and future utility infrastructure). Sites with identified threats may be used if the translocation plan incorporates management actions to ameliorate those threats, an associated effectiveness-monitoring program is designed, and contingency measures are identified in the event that management actions prove unsuccessful.

Some recipient sites may need to be temporarily or permanently fenced (partially or completely) if adjacent areas (*e.g.*, adjacent to highways) are not protected or desert tortoise movements need to be restricted. We recommend that more than one potential recipient site be identified during planning in the event that disease status, desert tortoise densities, or other factors prevent the use of a potential site(s). As stated above, the selection of the recipient sites should be coordinated with and approved by Federal and State wildlife and land management agencies.

Control-site selection considerations. Potential control sites should:

- be similar in habitat type/quality, desert tortoise population size/structure, and disease status to the recipient sites;
- not have been previously used as a recipient site for other projects; and
- be a minimum distance of 10 km away from an unfenced recipient site that has no substantial anthropogenic or natural barriers to prevent the interaction of control, resident, and translocated desert tortoises.

# **4. Estimate desert tortoise abundance at agreed-upon potential recipient and control sites**<sup>5</sup>**.** Conduct desert tortoise surveys according to the most recent USFWS Pre-project Survey

Conduct desert fortoise surveys according to the most recent USFWS Pre-project Survey Protocol and include data on all desert tortoise sign, including carcasses, observed during surveys. Surveys should be conducted during the desert tortoise's most active periods (*i.e.*, typically April 1 through May 31 or September 1 through October 15 when air temperatures are below 40°C [104°F], which should be verified by tortoise activity in the field). *If the USFWS has issued a recovery permit for this project, health assessments conducted in accordance with the most recent protocols (USFWS 2011) may be performed concurrent with these surveys (instead of waiting until Step 6)*. Health assessments will include a physical inspection and collection of biological samples to determine disease status of the individuals. Because physiological activity of the desert tortoise immune system is related to behavioral activity and is important for the types of tests that will be run, blood samples may be drawn beginning May 15 or, upon specific approval from USFWS, four weeks after the date that the individual of interest left its hibernaculum or was first found active and above ground (not in association with a shelter site). The last date for blood sampling is October 31. Results from the biological samples will be valid for 1 year from the date that the samples were collected. *Without a recovery permit, surveyors should closely observe, but not handle, desert tortoises at this time.* 

Translocation will increase desert tortoise densities in recipient sites, but for a successful translocation, the number of tortoises in any area should not exceed the capacity of the surrounding desert. Densities described by a single standard deviation of the mean tortoise density for a recovery unit are not unusually high. Therefore, assuming appropriate habitat and management exist, as described above, projected density after translocation at the recipient sites (residents plus translocated adult individuals [at least 180 millimeters carapace length]) should

not exceed the 68% confidence interval of the mean density (this is an asymmetrical interval based on one standard deviation to each side of the mean) in the respective desert tortoise recovery unit (Table 3). In some circumstances it may be most appropriate to use site-specific density information, thus close coordination with Federal and State wildlife and land management agencies is recommended. Contact the USFWS for most current data on desert tortoise densities within each recovery unit.

Selection and approval of recipient sites also depends on the disease status of the tortoises at the potential recipient site compared to those at the project site. If handling tortoises for health assessments has not been approved at this stage, it may be useful to document clinical signs of disease that can be observed without handling. This information might alert you to disease issues at the site that could preclude its approval. See Appendices B and F in USFWS (2011) for a datasheet with the clinical signs of interest and an algorithm that identifies the most serious and risky of the clinical signs.

**5.** Develop the translocation plan and associated effectiveness-monitoring program. Project proponents should work in close coordination with USFWS, State wildlife agencies, and land management agencies. Note that the translocation recommendations vary according to the number of desert tortoises expected to be moved, and many of the details may be project specific (Table 1). Unless a recovery permit has been obtained to conduct the activities described in steps 6-8, we recommend that the translocation plan and proposed project be approved and permits secured prior to beginning Step 6. *As previously stated, activities requiring the handling of desert tortoises may be conducted only under the authorities of a recovery permit, an incidental take permit or biological opinion, and applicable State permits.* 

**6.** Confirm desert tortoise abundance at the potential recipient and control sites<sup>5</sup> as *in situ* health-assessment sampling is conducted and transmitters are attached. Methods used to confirm desert tortoise densities at recipient and control sites should be consistent with the USFWS Pre-project Survey Protocol. Minimum sample sizes are determined by those needed to detect 10-percent disease prevalence<sup>7</sup> at the 95-percent confidence level (Figure 1)<sup>8</sup>. These sample sizes will likely be greater than the minimum number of observations described by the USFWS Pre-project Survey Protocol needed to estimate abundance. Project proponents should coordinate with the appropriate USFWS office to determine sample size requirements based on recipient-site abundance estimates collected at Step 4.

Health assessments will include a physical inspection and collection of biological samples and as previously stated, it is important that the desert tortoise's immune system is actively responsive when blood samples are drawn. Blood may be drawn beginning May 15 or, upon specific approval from USFWS, four weeks after the date that the individual of interest left its hibernaculum or was first found active and above ground (not in association with a shelter site). The last date for blood sampling is October 31. Health assessments should be performed on all

<sup>&</sup>lt;sup>7</sup> *Disease prevalence* is defined as the cumulative proportion of tortoises within the population of interest that are seropositive to *Mycoplasma agassizii* antibodies, those that are seropositive to *Mycoplasma testudineum* antibodies, and those that have other clinical signs that disqualify an individual from being translocated (USFWS 2011).

<sup>&</sup>lt;sup>8</sup> This targeted level of sampling (*i.e.*, to detect 10-percent prevalence instead of 20 percent) is necessary to more precisely document baseline conditions relative to monitoring potential changes in disease prevalence throughout the required effectiveness-monitoring period (see Step 10).

desert tortoises encountered during surveys at the recipient and control sites according to the most recent protocols (USFWS 2011). Results from the biological samples will be valid for 1 year from the date that they were collected. Additional health assessments of the recipient and control desert tortoises may be required if desert tortoises are not translocated to the recipient site within 1 year of the original assessment dates. Handling of resident and control desert tortoises in order to perform health assessments and attach transmitters should be done in accordance with the most recent protocols contained in the Desert Tortoise Field Manual; particular attention should be paid to temperature thresholds and measures to prevent spread of disease.



Figure 1. Minimum sample sizes needed to detect 10-percent disease prevalence at the 95-percent confidence level if fewer than 2 positive samples (dashed blue lines) are obtained <u>or</u> if no positive samples (solid red lines) are detected. This analysis was conducted using the Survey Toolbox of Cameron (1999) and is based on the serological test for *Mycoplasma agassizii*, for which documented sensitivity and specificity estimates exist. Other health observations must also be considered in making decisions about translocation.

All desert tortoises should be assigned a unique identifier (provided by USFWS), and individuals that will be monitored as part of the translocation plan should be fitted with a transmitter. Again, projected density of resident and translocated desert tortoises after translocation at the recipient sites should not exceed the asymmetrical confidence interval described using 1 standard deviation of the mean density detected in the respective desert tortoise recovery unit (Table 3) unless otherwise determined by the Federal and State wildlife and land management agencies. When greater than 5 desert tortoises will be translocated from the project site and project fence line, a long-term monitoring plan is needed, and an equal number of desert tortoises within the recipient site and an equal number of control desert tortoises should be assigned a unique identifier (provided by USFWS) and be fitted with a transmitter by qualified personnel.

### 7. Determine if desert tortoises on the project site will be held in- or ex situ. The

translocation plan should identify which of the following interim holding/monitoring arrangements will be used for the desert tortoises on the project site. This step can be conducted

concurrently with Step 6 and construction of fencing at the project site under Step 8. Regardless of the option selected, desert tortoises should be translocated within 18 months of collection.

Regardless of which holding/monitoring option (*in-* or *ex situ*) is selected, all desert tortoises too small to be transmittered (*i.e.*, transmitter packages weighing no more than 10 percent of the desert tortoise's body mass are unavailable; project proponents should procure transmitters with a range of sizes to accommodate sub-adult and juvenile desert tortoises) should be placed into individual quarantine pens (individuals located in the same burrow may be housed together) so they can be relocated at the time of translocation.

<u>Option 1:</u> Ex situ monitoring – construction of individual quarantine facilities. Desert tortoises located during protocol clearance surveys (see Desert Tortoise Field Manual at website above) would be transferred to a quarantine facility. The quarantine facility may be located off-site or within an area on-site not scheduled for development activities until following the translocation. Attachment 1 provides an example for facility design, animal husbandry, and operating protocols, but the quarantine facility and operating plan must be approved by USFWS and State wildlife agencies for each project. Facilities should be constructed and managed to prevent desert tortoises from coming into contact with one another, exclude predators, provide ability for appropriate thermoregulation, and allow for necessary husbandry activities by a caretaker that is certified to conduct health assessments and administer care. If this option is selected, quarantine facilities should be constructed to avoid inadvertently capturing any resident desert tortoises within the facility. If suitable USFWS and State wildlife agency-approved facilities exist in the area, the project proponent may inquire with facility managers about temporary use; however, these opportunities are extremely limited.

<u>Option 2: In situ monitoring – monitoring desert tortoises on the project site via telemetry</u>. As protocol clearance surveys are conducted, health assessments, assignment of unique identifiers (provided by USFWS), and affixing transmitters should be performed on each desert tortoise as it is located. Telemetry monitoring would then be conducted a minimum of once per month with more frequent monitoring under certain circumstances. A minimum dataset to be collected will be standardized for all projects to include the fields listed in Table 2.

# 8. Construct project fencing, conduct protocol clearance surveys of the project site, and perform health assessments.

<u>General clearance protocols</u>. Any desert tortoises encountered during clearance of the project site or the perimeter fence should be given a health assessment (USFWS 2011) prior to being moved. During the health assessment, desert tortoises will be assigned a unique identifier (provided by USFWS), and a transmitter will be attached for monitoring purposes. If the desert tortoise is being moved to a quarantine facility, it will not be fitted with a transmitter until it leaves the quarantine facility.

Data collected during clearance surveys will be standardized for all projects and should include detailed information about the exact point of collection, as described in Table 2. For those desert tortoises that will be monitored *in situ*, these data should be collected again on the day of translocation from the project site. The unique identifiers will allow us to link each individual desert tortoise with data obtained during clearance surveys and subsequent health assessments.

The placement of desert tortoises will depend on the health-assessment results (USFWS 2011) and translocation plan (*i.e.*, holding/monitoring option) approved under the incidental take permit or biological opinion. With the exceptions for projects with 5 or fewer desert tortoises described in Step 2, tortoises from project-site populations in which disease prevalence<sup>7</sup> is 20 percent or greater must be moved to unoccupied, but ecologically appropriate, habitat if they are approved for translocation. Unoccupied sites must meet the recipient-site criteria described in Step 3, and the final density must fall under the prescribed density limits (Table 3). Only those tortoises meeting the translocation requirements will be moved to the unoccupied site. Individuals from project-site populations in which disease prevalence is less than 20 percent. Individual tortoises eligible for translocation in either scenario are those that exhibit normal attitude/ activity, acceptable body condition, no-to-mild mucoid nasal discharge, no oral lesions, and no other condition that may impact its survival (USFWS 2011).

As individuals are located during clearance surveys of the project site, once at least 5 individuals have been located, there are three potential outcomes for each desert tortoise. See Step 2 for options in which 5 or fewer tortoises must be moved.

- a. Transfer of desert tortoises to quarantine facility for holding (*ex situ*). Final translocation decisions depend on concurrence with results of health assessments and disposition plan (see USFWS 2011, Appendix H), but desert tortoises should not be held in the quarantine facilities for greater than 18 months.
- b. Remain on-site for *in situ* monitoring until translocation, pending concurrence with results of health assessments and disposition plan (USFWS 2011, Appendix H).
- c. Transfer to Desert Tortoise Conservation Center in Las Vegas, Nevada, or another agency-approved facility. Transferring desert tortoises to an approved facility is only appropriate for individuals deemed inappropriate for translocation. Project proponents will be invoiced by the San Diego Zoo in for costs associated with the provision of housing, care, treatment, and other services in one-month increments, not to exceed one year and \$14,800 (\$1600 for the first month and \$1200 for each month, thereafter).

<u>Health Assessments</u>: Health assessments will include a physical inspection and collection of biological samples.<sup>9</sup> Also, immediately prior to (ideally within a day of) the scheduled translocation, additional health assessments (without additional biological sampling) need to be conducted. This will serve as a baseline condition with which to compare post-translocation assessments and as a final check against the algorithm (USFWS 2011, Appendix G) that the tortoises are suitable for translocation. Any tortoises that were previously approved for translocation, but now show clinical signs that disqualify it according to the algorithm, should not be translocated and their disposition discussed with USFWS. Biological samples should be submitted to the USFWS (see USFWS 2011, Appendix F.8) or delivered directly to the DTCC for banking/analysis on a monthly basis. Upon completion of the health assessments for a given

<sup>&</sup>lt;sup>9</sup> Biological samples are used for monitoring purposes, assessing disease prevalence on the project site, and assessing the suitability of a recipient site, not for making individual translocation decisions (see USFWS 2011).

season (*i.e.*, spring or fall), a copy of all health data sheets should be submitted to the USFWS. Additionally, data sheets should be available when disposition plans are being reviewed by USFWS.

Health assessments must be conducted by individuals approved and permitted by the USFWS and State wildlife agencies to conduct such assessments. Individuals should inquire with USFWS about opportunities to receive certification. Because of new health assessment standards and the need for standardized data, certification will not be granted solely on past experience. Training for performing health assessments and collecting biological samples is conducted at the DTCC, subject to scheduling availability. Due to the newness of the training program and to further minimize risks associated with translocating desert tortoises, all projects should have a qualified veterinarian verify a sample of the health assessment results to ensure proper placement of the desert tortoises.

#### Component-specific protocols.

<u>Perimeter Fence</u>: Fence construction may be done during any season. Individuals located within the fence-clearance area, should be moved into adjacent habitat outside the fence line in accordance with clearance and handling procedures outlined in the current USFWS Desert Tortoise Field Manual. These individuals should be given a unique identifier, a health assessment, and be fitted with a transmitter (in the event the individual moves back into the project site before the fence is completed).

If a desert tortoise that was moved out of the fence alignment moves back into the project site prior to the completion of the fence, the individual will be translocated as identified in the translocation plan and considered a translocatee. If the individual remains on the outside of the fence, it will be considered a resident of the area, and if resident individuals in this area do not need to be monitored as part of the translocation plan, the transmitter would be removed. If there is no suitable habitat adjacent to the fence line and the recipient site is not yet available to receive desert tortoises from the project site, the individuals should be fitted with transmitters and placed inside the perimeter fence or in quarantine pens for *in-situ* monitoring.

If clearance of the perimeter fence is conducted outside of the desert tortoise active season, then any desert tortoises located along the fence alignment should be moved as described above, fitted with a transmitter, blocked into an artificial or empty natural burrow, and monitored as described below.

<u>Project Site (*i.e.*, power plant, substation, etc.)</u>: Clearance surveys, health assessments, and subsequent translocation should be conducted during the active season. Pursuant to the USFWS Desert Tortoise Field Manual, the maximum transect width for clearance surveys is 5 m. All desert tortoise scat should be collected during each pass of the clearance surveys to facilitate locating desert tortoises that may have been missed on previous passes. Juvenile desert tortoises are most likely to be observed during the early morning hours; therefore, clearance surveys should be conducted to maximize the likelihood of finding juveniles.

<u>Linear Facilities (*e.g.*, transmission and buried lines)</u>: Clearance surveys may be conducted during any season. Any desert tortoises found during clearance of linear facilities should be

moved out of harm's way following clearance and handling procedures outlined in the current USFWS Desert Tortoise Field Manual.

**9. Translocation of desert tortoises following acceptance of translocation-review package.** Once health assessments are complete, a translocation-review package should be submitted to the USFWS and the State wildlife agencies for concurrence of the proposed disposition of each tortoise to be translocated. The translocation-review package should include the following:

- Disposition plan (see USFWS 2011, Appendix H) for the project-site tortoises;
- Summary information for sampled recipient-site tortoises (*i.e.*, follow the disposition plan template);
- Photographs of individual tortoises as specified on the health assessment data sheet;
- Health assessment data sheets, if not submitted previously;
- Recipient-site density information, including Table 3 from the USFWS Pre-project Survey Protocol and calculation of post-translocation density;
- Maps of the recipient site, including all relevant digital GIS layers, illustrating distribution and health status of resident tortoises and proposed release sites of project-site tortoises;
- Maps of the project site (including all project phases and all relevant digital GIS layers), illustrating distribution and health status of project-site tortoises and proposed release sites of tortoises to be moved <500 m (if applicable); and
- Any other project-specific information that supports or clarifies translocation decisions.

Disposition plans describe the proposed fate of each desert tortoise (*i.e.*, translocated to recipient site or removed from population due to suspected disease) from the project site and must be completed within the spring or fall season in which translocation occurs. Project delays that result in translocation occurring in a subsequent desert tortoise activity season than that in which the disposition plan was developed also result in the need to complete updated health assessments (with the exception that results from biological samples are valid for 1 year). A minimum of two weeks should be provided for evaluation of the translocation-review package. Desert tortoises should not be moved prior to acceptance by the USFWS of the translocation-review package.

Translocation proceeds to the USFWS-approved, final recipient site(s) in a manner consistent with existing protocols, this guidance, and the project-specific translocation and monitoring plan. Some flexibility may exist for individual projects based on the time of year, local/regional weather patterns, actual weather conditions during the proposed release event, and condition of the project sites and final selected recipient sites (*e.g.*, degraded or recently burned). Translocations should occur in spring (April 1 through May 31). Translocations may occur between September 1 and September 30 if weather conditions are favorable and approved by the USFWS and appropriate State wildlife agency. Desert tortoises may be removed from project sites between October 1 and 15; this date may be extended under favorable weather conditions. Any desert tortoise removed from a project site after September 30 must be placed in a quarantine facility. Desert tortoises should not be removed from project sites at any other time of year. In addition, the following conditions should be met for translocation to proceed:

- Releases should occur when temperatures range from 18-30°C (65-85°F) and are not forecasted to exceed 32°C (90°F) within 3 hours of release or 35° (95°F) within 1 week of release. Additionally, forecasted daily low temperatures should not be cooler than 10° C (50°F) for one week post-release.<sup>10</sup>
- Release points for desert tortoises should be pre-selected during visits to the recipient site and specified in the disposition plan (configuration of release points is project-specific).
- Desert tortoises should be transported to their release sites in clean, ventilated protective containers. If re-used, these containers must be disinfected using a solution and methods approved by USFWS and the State wildlife agency before being used for another desert tortoise (see USFWS 2011, Appendix A, for discussion on disinfection).
- Depending on environmental conditions and hydration state, desert tortoises to be translocated may need to be hydrated within 12 hours before release. All tortoises that void will need to be hydrated according to existing protocols (USFWS 2011, Appendix F.6).
- Desert tortoises should be released at unoccupied shelter sites. Shelters include unoccupied soil burrows, spaces within rock outcrops, caliche caves, and the shade of shrubs.

Desert tortoises determined to be infectious or unhealthy should be sent to the Desert Tortoise Conservation Center or other agency-approved facility (hereafter, approved facility) where they will undergo further assessment, treatment, and/or necropsy; some desert tortoises may be rehabilitated and potentially be eligible for subsequent release. Coordination with the USFWS, State wildlife agencies, and the approved facility should be initiated when clearance surveys commence to facilitate prompt transport of unhealthy desert tortoises, as necessary. The agencies and approved facility should be notified of the number of desert tortoises estimated to be removed from the project site to allow for advanced preparation.

# 10. Implement post-translocation monitoring (30-yr minimum<sup>11</sup>) and adaptive management to evaluate effectiveness of translocation as a take-minimization measure. For

<sup>&</sup>lt;sup>10</sup> Temperature thresholds for translocation differ from those for handling resident and control desert tortoises because translocated desert tortoises spend more time above ground subsequent to release as they habituate to unfamiliar surroundings, increasing their susceptibility to stress factors such as temperature extremes.

<sup>&</sup>lt;sup>11</sup> Effectiveness monitoring has been extended to 30 years from prior guidance on the basis of several factors. Research results reported subsequent to the prior guidance, indicate that translocated desert tortoises may not "equilibrate" to "resident" status, relative to movements and reproductive output, as quickly as indicated in previous studies. More importantly, a recent review of reptile and amphibian translocations considered translocations to be successful when a) substantial new recruits were added to the adult population due to successful reproduction at the translocation site, and b) the site was monitored for at least as long as the time to reach maturity (13-20 years for desert tortoises). Success of prior translocations is often uncertain due to inadequate monitoring time (Germano and Bishop 2009). Given the time to reach maturity in desert tortoises and low and variable survival rates of juveniles, it may require 30 or more years to detect substantial recruitment to the adult population. Thirty years is commensurate to the duration of development permits for which this guidance was developed and is less than the duration of direct project impacts, which are essentially permanent. Therefore, we have determined that at least 30 years is a reasonable timeframe over which to evaluate effects of projects of this magnitude.

projects that require translocation of more than 5 desert tortoises, an effectiveness-monitoring program should be designed to evaluate the translocation relative to project-specific conditions (*e.g.*, considering site-specific environmental conditions or land-management actions, health conditions within the translocated/recipient populations). Unless otherwise justified by the effectiveness-monitoring program and approved by USFWS and the State wildlife agency, monitoring will include an equal number of translocated desert tortoises, desert tortoises that are resident at recipient sites, and desert tortoises at control sites. For example, if six desert tortoises are to be translocated, six resident, and six control desert tortoises should also be monitored at even sex ratios (regardless of whether or not the group of translocatees has an even sex ratio), if possible. In situations where fewer resident desert tortoises exist at the recipient site than translocatees being added (likely in targeted depleted areas), as many residents as possible should be monitored.

*Frequency of Monitoring*. Monitoring refers to pinpointing the exact location of the desert tortoise and attempting to view it without disturbance unless entrapment, a scheduled health assessment, or equipment maintenance requires handling. Unless otherwise approved by the USFWS in the effectiveness-monitoring program, frequency of monitoring should occur as follows:

Desert tortoises confined to an artificial or empty burrow during perimeter fence construction:

- Once a day during first week;
- once a week for the following three weeks; then
- twice per month until the clearance survey is conducted.

Translocated desert tortoises:

- Once within 24 hours of release; and
- a minimum of twice weekly for the first two weeks after release;
- a minimum of once a week from March through early November for the 30-year monitoring period; and
- once every other week from November through February starting after the third week of release and for the duration of the 30-year monitoring period.

Resident and control desert tortoises, for the 30-year monitoring period:

- A minimum of once a week from March through early November; and
- A minimum of once every other week from November through February.

<u>Health assessments during the monitoring period</u>. Health assessments should be conducted during each year of monitoring; one assessment prior to and one assessment subsequent to overwintering. Any health problems observed (*e.g.*, rapid declines in body condition, perceived outbreaks of disease, mortality events) should be reported to the USFWS and State wildlife agency such that appropriate actions can be taken in a timely manner. Mortalities should be investigated as thoroughly as possible. Information on health concerns and mortalities, including desert tortoise unique identifier, location, and cause of death (if determined) should be provided

to USFWS and State wildlife agency upon discovery (verbally within 48 hours or via email within 5 business days). Fresh carcasses should be submitted for necropsy (details to be provided during project planning and coordination with USFWS) and the cost covered by the proponent.

If the total number of monitored desert tortoises (translocatees and residents, combined) within the recipient area is less than that needed to detect disease prevalence within that population at the 10-percent level with 95-percent confidence (see Figure 1), additional surveys to locate additional tortoises for health assessments will periodically be required throughout the monitoring period to track any shifts within the population. In such cases, surveys and health assessments within the population, including additional, non-telemetered residents, should be conducted every 5 years (*e.g.*, years 1, 5, 10, 15, 20, 25, and 30) in order to detect changes in disease prevalence within the overall population over time. For example, if only 10 desert tortoises are being monitored at the recipient site (5 translocated and 5 resident), but a sample of 40 is required to detect 10-percent disease prevalence with 95-percent confidence, 30 additional tortoises in the resident population should be sampled at the appropriate interval. Health assessment surveys should be completed within the control population at the same time.

<u>Other effectiveness-monitoring considerations</u>. In addition to monitoring the desert tortoises, we recommend that the effectiveness-monitoring program include activities such as annual vegetation sampling to capture potential changes in habitat characteristics. At a minimum, monitoring of the annual species component is recommended to identify changes in forage diversity and availability. Additional monitoring variables may be necessary to evaluate site-specific conditions (*e.g.*, potential toxicants adjacent to roadways). The USFWS will provide additional guidance to project proponents on appropriate methods of vegetation monitoring and sampling during the planning process.

Explicit triggers for implementation of adaptive management will be project specific and developed through coordination with USFWS and State wildlife agencies, as appropriate. Such triggers may be based on statistical comparison between translocated, resident, or control populations (*i.e.*, survival, movements, reproductive output, or disease incidence between groups relative to site-specific environmental or anthropogenic conditions such as annual vegetation production or toxicant levels potentially associated with roadways), thereby emphasizing the need for a rigorous effectiveness-monitoring program.

Projects occurring within important habitat linkages between desert tortoise conservation areas (see your local USFWS office) should also include a monitoring component that evaluates large-scale population connectivity and gene flow through the linkage occupied by the development project. This element of monitoring is particularly important to evaluate whether project impacts are truly minimized.

#### 11. Compile and synthesize data throughout duration of translocation and monitoring.

Findings, data, and recommendations will be submitted to USFWS and appropriate wildlife and/or permitting agencies annually at minimum or more frequently for some data as previously described (projects translocating 5 desert tortoises or fewer, and not conducting subsequent monitoring, need only report the health assessment and release data, as appropriate). Minimum data requirements include the fields listed in Table 2 and, for health assessments, those listed in USFWS (2011, Appendix B). Additional data should be incorporated into the database according to the project-specific effectiveness-monitoring program. Reporting requirements will be determined during the planning process with the appropriate land management and permitting agencies and incorporated into associated permits and/or biological opinions.

Upon conclusion of the 30-year monitoring period, transmitters should remain attached until the USFWS and State wildlife agencies have determined whether or not further action is warranted at the site.

#### Literature Cited

Cameron, A.R. 1999. Survey Toolbox for Livestock Diseases - A Practical Manual and Software Package for Active Surveillance in Developing Countries. ACIAR Monograph #54, vii + 330 pp.

Germano, J.M., and P.J. Bishop. 2009. Suitability of amphibians and reptiles for translocation. Conservation Biology 23:7-15.

U.S. Fish and Wildlife Service. 2011. Health Assessment Procedures for the Desert Tortoise (*Gopherus agassizii*): A Handbook Pertinent to Translocation.

Table 1. Desert tortoise translocation components at a glance based on the number		
of desert tortoises expected to be translocated.		
	# of dt ≤5	# of dt > 5
Translocatees: health assessment	yes	yes
Translocatees: disposition plan	yes	yes
Recipient site required	project specific <sup>1</sup>	yes <sup>4</sup>
Recipient site density surveys required	project specific <sup>2</sup>	yes
Recipient desert tortoises: health assessment	project specific <sup>3</sup>	yes
Control site required	No	yes
Monitoring of translocatees	No	yes
Monitoring of residents	No	yes

Monitoring of controls

<sup>1</sup>Yes, for tortoises moved >500 m (see Step 2, page 3, 2<sup>nd</sup> bullet). <sup>2</sup>Yes, when identifying a depleted area for translocation (see Step 2, page 3, 2<sup>nd</sup> bullet). <sup>3</sup>Yes, when translocating >500 m into a non-depleted population (see Step 2, page 3, 2<sup>nd</sup> bullet). <sup>4</sup>Projects with tortoise populations with disease prevalence of 20 percent or greater must move all individuals to suitable unoccupied habitat.

No

yes

Table 2. Minimum list of standardized data to be collected and reported during all phases of a project. For health assessments, also refer to USFWS (2011, Appendix B).

Date	Coverage #	Transmitter manufacturer
Time	Field_crew_vendor	Transmitter_serial_#
Temperature (°C)	Surveyor (First Last)	Transmitter_frequency
		Transmitter_install_date
Project_Name	ID#	Battery_life_months
Site_type (project/recipient/control)	MCL (mm)	Status (alive/dead/lost)
Landowner (BLM, NPS, etc.)	Sex	
Permit/BO #	UTM_Easting	
	UTM_Northing	
	Location (burrow, etc.)	

Table 3. Density thresholds for identifying "depleted areas" and maximum post-translocation			
densities within each recovery unit (RU; as delineated in the 2011 revised recovery plan).			
	Mean RU Density Maximum depleted- Maximum recipient +		
<b>Recovery Unit</b>	(tortoises/km <sup>2</sup> ) <sup>1</sup>	area density	translocatee density
Western Mojave	4.0	2.15	5.55
Eastern Mojave	4.2	2.19	5.77
Colorado Deserts	5.3	2.79	7.41
Northeastern Mojave	2.8	1.63	3.62
Upper Virgin River	14.3	10.79	16.41

<sup>1</sup> Mean density from 2007-2010 surveys of designated critical habitat and other conservation areas.

ATTACHMENT 1



Temporary Captive Care of Wild Mojave Desert Tortoises: Examples of Protocols Used at the Desert Tortoise Conservation Center

Updated Oct 12, 2010

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**Disclaimer:** This document is provided as a courtesy to companies that are authorized to place and maintain wild desert tortoises in captivity. While the protocols described within the document are standard at the Desert Tortoise Conservation Center, the San Diego Zoo makes no claims that the protocols, techniques, and structures described herein will work properly for every wild desert tortoise in every captive situation. It is expected that projects involving the captive care of wild desert tortoises will consult with US Fish and Wildlife Service to ensure that the protocols, techniques, and structures that they choose to use are in compliance with project permits and all other requirements. The San Diego Zoo is not responsible for illness, injury, or death to desert tortoises on projects that choose to use these protocols as guidance.



# I. Housing

## A. Individual Quarantine Enclosures

Desert tortoises (DTs) at the DTCC are housed outdoors in escape-proof pens, and if under 100mm are maintained in escape-proof, predator-proof pens. Each DT is housed individually to prevent potential disease transmission. The sites where pens are constructed have ample vegetation that is minimally disturbed during construction, and the soil is appropriate for tortoises to dig their own burrows. Pens are constructed with tortoise fencing with at least 60cm of fencing above the ground and at least 30cm buried below ground to ensure that they cannot escape (note that the fencing at the DTCC differs slightly from FWS-approved desert tortoise exclusion fencing). The fencing flares out 25cm at the bottom to ensure that if tortoises try to dig out from the inside or if predators try to dig in from the outside, they will hit fencing. Ideally, each pen has ample vegetation such as creosote bush, yuccas, ephedra, and bursage to provide shade, and other plants like globemallow to serve as food sources. In order to access each pen without the potential for contamination (see the section on Aseptic Techniques), walkways are constructed between individual pens and between each row of pens. If we need to walk inside any pen for any reason, we always disinfect our shoes while moving out of a pen and into a walkway.

Pen 1	Pen 2	Pen 3	Pen 4
Pen 5	Pen 6	Pen 7	Pen 8
Pen 9	Pen 10	Pen 11	Pen 12

Figure 1. Pen Design, Including Walkways

Wild DTs are housed individually to prevent potential disease transmission. Larger DTs (MCL > 124mm) that are less likely to be predated are ideally housed in pens that are  $6m \times 6m$ , while smaller DTs (MCL < 125mm) that are more likely to be predated are ideally housed in pens that are  $2m \times 2m$ . It is particularly important to us to make sure there are ample native food sources growing in the pens for smaller tortoises. In addition, all pens for smaller tortoises have a lid or cover, and all parts of the enclosure are predator proof, not just against large animals, but against smaller animals such as ground squirrels, which are able to squeeze through  $\frac{1}{2}$  inch x  $\frac{1}{2}$  inch fencing. We use  $\frac{1}{2}$  inch x  $\frac{1}{4}$  inch fencing,



but not anything smaller, because smaller fencing can result in small animals, like lizards and snakes, getting stuck in the fence and attracting potential predators. The most critical part of constructing a predator-proof enclosure is making sure that the entry points (the places where we open and close the fencing or lid) are completely predator-proof when they are in the closed position. As an example, here at the DTCC our predator proof enclosures are constructed of cement block and mortar with a steel diamond-pattern lid painted with reflective paint (picture A). We place shade cloth in the middle of the lid during summer to provide extra shade. Another type we use is a large walk-in predator-proof enclosure with 2m x 2m pens inside it (picture B). Other walk-in enclosures have been constructed at FWS approved head-starting and research facilities, and those facilities may be able to provide example designs and protocols.

#### Picture A: Hatchling pens with lids



#### Picture B: Hatchling pens, walk-in structure



#### B. Burrows

At least one artificial burrow is provided in each pen to fit the particular tortoise it will house. The burrow width is 5-8cm larger than the total length of the tortoise to ensure that the tortoise does not get stuck inside the burrow when it turns around. We measure from the longest anterior and posterior points on the tortoise's shells to get the correct measurement. Similarly, the height of the burrow is 3-5cm taller the height of the tortoise's shell to accommodate movement through the burrow. We do not provide burrows that are more than 10cm larger than the tortoise because tortoises naturally create burrows to fit their size - we are concerned that extra space in a burrow could be detrimental over the winter because it could allow more air flow when temperatures are too low for tortoises to tolerate. Also note that tortoises with special needs, such as those with missing limbs and other conditions that impair movement, are provided with a bigger burrow than they need because they are more likely to become stuck in what would normally be considered a proper size burrow for their body size.

At the DTCC we use PVC pipes to create artificial burrows. We cut them in half lengthwise and use the half pipe as the top of the burrow. The length of an artificial burrow is about 2 meters. To construct a burrow, we dig a trench at a 30-45 degree angle, and 2 meters long. The burrow is approximately 60cm deep at the chamber end. We lay the PVC half pipe in the trench to form the burrow with the front end of the pipe resting 10-15cm higher than the ground to serve as the mouth of the burrow. We sometimes add dirt in the front to make this possible because it's very important to raise the mouth of the burrow to prevent possible flooding. The outer sides of the PVC are lined with large rocks and the entire length of the PVC is covered with rocks and dirt – the rocks help prevent the dirt from blowing away in the wind. We pack at least 30cm of dirt and rocks on top of every part of the PVC. Dirt is moistened daily for 3 to 4 days to



promote compaction. It is important that the PVC remains completely covered all the time because without the added layers of dirt and rock, it provides no protection and can actually become an oven in summer and an ice box in winter – tortoises that try to use a burrow that is not properly covered are at a high risk for mortality. Once the burrow is complete, we look inside with a flashlight or mirror to make sure the entire path down the burrow is clear of debris and ready for a tortoise to enter. We check the temperature at the mouth and chamber of the burrow during the coolest and hottest parts of the day twice each week to ensure that temperatures inside the burrow are within a normal tolerable range for tortoises (15-30C).

#### Picture C: Artificial burrow



If you will be housing tortoises for an undetermined amount of time, another option for constructing more permanent artificial burrows is offered on the Tortoise Group website (<u>http://www.tortoisegroup.org/pamphlet.php</u>) on pages 10-14 of their Tortoise Adoption and Care Handbook. Please note that the San Diego Zoo is not affiliated with Tortoise Group, but offers this as a viable option for the construction of long term tortoise burrows because the method has been used extensively and is shown to be effective. However, the rest of the handbook specifically addresses long term care of captive pet desert tortoises, not wild tortoises, so we do not endorse any of their other material for use with wild tortoises.

We inspect and maintain all burrows, both natural and artificial, nearly every day. Table 1 shows our burrow maintenance schedule.

Season	Maintenance
March-April	After most tortoises emerge from hibernation, remove remaining berms from burrows where DTs have not emerged. Check burrows daily and add dirt and rocks when necessary
May-October	Check burrows daily and add dirt and rocks when necessary
November-March	After most tortoises have bermed themselves in, berm in the remaining tortoises. Check burrows weekly and add dirt and rocks when necessary

#### Table 1. Burrow Maintenance Schedule



After heavy rains, we check every burrow by looking inside with a mirror or flashlight. If burrows collapse, tortoises can become entombed so the burrow must be dug out immediately. For more information regarding what we do when we find a tortoise entombed after rains, see the Surveillance section.

In order to prevent disease transmission, tortoises are not moved among the pens, or newly placed in a pen where another tortoise was living without first disinfecting the burrow. For more information on how to disinfect burrows and pens, see the Aseptic Techniques section.

## C. Irrigation

Every individual pen at the DTCC receives water on a regular basis. For most pens we use a drip system from a central well that leads to a drip head in every pen. We point the drip head toward the downward slope of each pen to avoid flooding the burrow if something goes wrong with the irrigation system. We dig out the earth below the drip head to make a catch basin or we use plant trays to catch the water so the tortoises will have plenty of time to drink before it evaporates.

A second option is to use a row of sprinklers connected to water tanks that are operated with generators and pumps. If you allow a 360 degree spray pattern, then many pens can be watered at once and it promotes the growth of native vegetation.

A final option that we use to irrigate some of our pens is to carry it to the pens manually. We put a large shallow terra cotta or plastic dish in each pen, and sink it into the ground so it is level. We only use shallow dishes so smaller tortoises won't drown if they flip over into it. We carry water to the pens and pour it directly into the dishes.

## D. Site Security

It is important that the site where the tortoises are kept is secure from both predators and humans. The DTCC is surrounded with a chain link fence buried at least 30cm into the ground with the bottom flared outward to prevent digging in from the outside. Our fence it topped with 2 to 3 rows of razor wire. It is not recommended that lights be installed, even if they are on motion sensors, because they will likely come on frequently during the night, which can be disturbing to wildlife. If you believe you may have an issue with security at night, you could consider installing motion sensor cameras with night vision in key locations.

### Picture D: Perimeter fence





# II. Aseptic Techniques

The primary disinfectant we use for surfaces, totes, equipment, instruments, PVC burrows, footbaths, and potentially contaminated clothing and shoes is Trifectant<sup>™</sup> (www.amazon.com). This product is a broad spectrum virucidal, bactericidal (including against *Mycoplasma spp*), and fungicidal product. It is effective on porous surfaces such as wood, in the presence of organic matter, in hard water, and at low and high temperatures. Unlike bleach, it is not inactivated by UV light. In addition, it has low toxicity and is biodegradable, and the required contact time is less than 5 minutes.

Trifectant<sup>™</sup> is stored as a powder, dissolves quickly in water, and remains stable for 7 days. As per the instructions, we use a 1% solution using warm water - 0.325 oz (1/4 scoop) per quart or 1.3 oz (1 scoop) per gallon. We spray Trifectant<sup>™</sup> on all potentially contaminated field equipment and allow it to air dry.

We always wear gloves when handling tortoises, regardless of their health condition, and we change gloves between handling different tortoises. To make sure that tortoises do not come into contact with our clothes, we wear reusable aprons that we disinfected with Trifectant<sup>™</sup> after each use and between handling different tortoises. All equipment and supplies that touched a tortoise or that we touch with a gloved hand is disinfected. To prevent contamination of data sheets and some field equipment, we use the one glove technique for handling tortoises and recording data in which one hand remains ungloved and can therefore touch writing utensils, clipboards, field equipment, etc, without potential contamination. If the second hand is needed to help hold or manipulate the tortoise, we slide on a glove one size bigger than the proper fit so we can slide into it and out of it as needed to use equipment and record data.

In order to prevent disease transmission across the site, tortoises are not moved among the pens, or newly placed in a pen where another tortoise was living without first disinfecting the burrow. To disinfect a burrow, we remove the tortoise from the pen and pull the PVC burrow out of the ground. We spray both sides of the PVC with Trifectant<sup>™</sup> and expose it to direct sunlight with no rain or moisture for a minimum of 5 days. In addition to sterilizing the burrow, we rake out the area where we pulled the burrow out of the ground and we spray the entire area with Trifectant<sup>™</sup>. We leave the pen unoccupied (with the burrow removed) and exposed to direct sunlight with no rain or moisture for a minimum of 5 days. However, if there is any moisture in the dirt when we rake it the first time, we spray the area with Trifectant<sup>™</sup> and continue to rake and spray it every day until the area appears dry. We then begin the 5 day UV exposure period.

## III. Food

Native vegetation and some produce (kale, collards, dandelion greens, etc) are the best sources of nutrition for desert tortoises. However, with large numbers of tortoises in captivity, SDZ Nutritionists and Veterinarians recommend supplementally feeding DTs Mazuri Tortoise Diet 5M21 (https://shop.mazuri.com/mazuritortoisediet.aspx). As per the product's feeding instructions, we give healthy tortoises 10 kibbles (10g)/kg of body weight at each feeding during the active season. The food comes in pellet form so we add water until it just reaches the top of the pellets and let it sit for 5 minutes before mixing it up with a gloved hand. If so much water is added that it can be poured off the top after 5 minutes, the food can lose water soluble nutrients so it is important not to overwater the kibble. For tortoises that need to gain weight, we provide additional moistened pellets at each feeding. When feeding the DTs at the DTCC, we walk outside the perimeter of each pen (not inside the pens) and place the approximate amount of food in a ball in front of or near the tortoise. If the tortoise is not out, we toss the ball of food near, but not in, the burrow. Before or during feeding, we remove all old food left behind from the last feeding, disinfecting our shoes when moving from pen to pen. It is important to note if you consistently do not see a tortoise out or if there is consistently food left behind at the next feeding.



Picture E: Mazuri Tortoise Diet soaking



Picture F: DT eating Mazuri Tortoise Diet



#### Table 2. Feeding Schedule

Season	Frequency
March/April-August	2x/week
September	1x/week
October-March/April	Do not feed

We feed the tortoises at the DTCC at the time of day when they are most active, usually as early in the morning as possible. In summer, we start feeding just before sunrise, with slightly later feeding times in spring and fall. In the spring, we begin feeding tortoises small amounts of food (3 kibbles/kg) for a week or two after they come out of hibernation, then proceed to the normal feeding schedule. In the fall, it is important that tortoises not go into brumation with food in the gastrointestinal tract so we stop feeding after the last week of September, even if temperatures are still relatively warm. It can take up to a month for DTs to digest and pass their food so it's best not to risk feeding so late in the season.

## IV. Water

We provide the tortoises with water throughout the active season until the time they enter hibernation. Table 3 shows the frequency for watering captive tortoises using a drip system



Table 3. Watering Schedule for Drip Syste	m
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Season	Frequency
March-April	3 days/week 2x/day for 15 minutes
May-August	4 days/week/ 2x/day for 15 minutes
September-October	3 days/week 1x/day for 15 minutes
October-March	Do not water*

\* Note: While water is not provided to tortoises during the winter, continued watering of the pens in the winter months using a spray type of irrigation will increase the germination of annual plants in the spring.

We begin providing water during the warmest part of the day as soon as tortoises come out of brumation. Once comfortable spring temperatures set in and tortoises are more active in the morning, we provide water early in the day and evening. During the summer, we provide water during the coolest times of day. We check the irrigation system once a week throughout the season to make sure all drippers and/or sprinklers are functioning properly. When most of the tortoises have gone into brumation in October or November, we shut off the irrigation system and blow out all the water lines to prevent breakage over winter, which can lead to flooding in the burrows.

# V. Surveillance

Captive DTs, their burrows, and their enclosures need to be examined consistently throughout the year, not just during the active season. It is particularly important to check on tortoises several times each day (morning, noon, and evening) for at least 2 weeks following their transfer to captivity because it is common for tortoises that are newly placed in captivity to pace the perimeter of the enclosure and not use the burrow provided. This can result in mortality from overheating or other complications related to exposure. To minimize the potential stress and effects of extreme temperatures, it is strongly recommended that tortoises not be removed from the wild and/or introduced to a captive environment when daily low temperatures are below 15C or daily high temperatures are above 30C on the day of and for 3 days following the move. It is also recommended that tortoises not be removed from the wild and/or introduced to a captive environment from Oct 1 to the time that brumation begins since this is a physiologically critical time for desert tortoises. Furthermore, tortoises should never be disturbed from brumation for any reason unless they are in mortal danger.

## A. Keep a Daily Record of Tortoises

From spring emergence through winter hibernation, we try to get a visual of every tortoise held in individual quarantine pens every day, and record it in a field notebook and/or on an electronic spreadsheet. It is best to do this in the early morning when tortoises are active. We try to get a good look at their faces and record if they are showing any signs of disease or if they appear lethargic. This is important information in the future if their condition deteriorates.

For tortoises that are in burrows when we locate them, we record how deep they are in the burrow, and which direction they are facing. If they have not moved in 3 days, we either coax them out or tap them gently with a stick to make sure



that they are alive and that they are not stuck. If a tortoise doesn't move from its burrow for 5 consecutive days during active season, we gently pull him from the burrow to assess his condition. We do this because we have had cases in which we saw a tortoise in its burrow facing away from us, and it appeared to be fine, but after tapping it, we realized that it was lodged in the burrow and could not escape on its own.

During the winter months, tortoises are checked weekly and are not disturbed from their brumation. We do not pull them from their burrows unless they are sick, in distress, or dead.

## i. Sick, Injured, and Dying Tortoises

We keep daily records of tortoises during the active season because it is helpful in identifying sick and injured tortoises, but our surveillance also includes tortoise checks during the hottest part of the day in warm months and during the coldest part of the day in cool months to check for tortoises that are improperly thermoregulating. If we find a tortoise that is sick, injured, or dying, we immediately remove it from the enclosure and place it in a clean tote. If it is injured or extremely ill, veterinary attention is sought immediately. If the tortoise appears mildly to moderately sick, we soak it in a tote of water with the water level just below the tortoise's chin. The water should be tepid to cool in warm months, and tepid to slightly warm in cool months. Often times a good soak to rehydrate is all a tortoise needs to feel better. Once the tortoise's condition has improved, if the temperatures are 15-30C and will remain in that range for at least 3 days following release, we place the tortoise back in its pen and monitor it 3 times each day for 7 days. If temperatures are outside that range, the tortoise is maintained indoors in a temperature controlled environment until outdoor temperatures are appropriate for release. The indoor housing consists of a very large, clean, dry bin or penned area with Timothy hay for bedding and a mercury halide light in a ceramic fixture on a timer for heat and UV light (ZooMed 160 watt PowerSun UV lamp). If tortoises are brought indoors and not allowed to hibernate due to their condition, we feed and water them weekly.

It is strongly advised that you make arrangements in advance with a veterinarian that specializes in desert tortoises in case an emergency occurs. Most veterinarians do not have desert tortoise or even reptile experience, but it is critical to find one that does. Your local reptile rescue organization may be able to direct you to the nearest desert tortoise veterinarian in your area.

### ii. Dead Tortoises

If you find a dead tortoise, take photographs before removing the carcass and follow instructions provided by US Fish and Wildlife Service. If it is a recent death and your permit allows transfer of the carcass to a research institution, place the carcass in a plastic bag in the refrigerator so the tissues will remain viable for necropsy, and contact that institution immediately (for necropsies to be conducted by the San Diego Zoo, contact Dr. Josephine Braun, Pathology Postdoctoral Fellow 760-291-5470 or Dr. Paula Kahn, Conservation Program Manager at the DTCC 702-885-7640)

## B. Check on Tortoises After Rains

Tortoises can become entombed in both artificial and natural burrows during the rainy seasons. During and/or after a rainstorm, we shine a light inside every individual burrow to make sure that the tortoises are safe. Many tortoises come out for a drink while we are inspecting the burrow, but we still look inside their burrows to make sure they have not collapsed. For those burrows that we can't see to the back, we use a burrow scope to check them.

If we find that a burrow has collapsed with a tortoise in it, we dig it out as quickly and as carefully as we can. It can be useful to put a hose, shovel handle, or other type of placeholder inside the burrow so we can find the tunnel in case of a collapse while we are digging. Once we remove the tortoise, we keep it in a safe place until we are able to re-dig the burrow. We have found that sometimes hatchlings and small juveniles will dig further back and sometimes upwards in their burrows during rainstorms, so we are particularly careful when digging in a pen with a small tortoise in it.



## C. Burrows

Every burrow must be covered completely to effectively protect tortoises from harsh weather conditions so during routine surveillance of tortoises, we also check the burrows in each pen, looking for proper coverage over the top of the burrow, as well as inspecting them for cave-ins toward the back of the burrow. We carry a shovel to put more dirt on top of marginally covered burrows to ensure there is at least 25cm of dirt on top. We use rocks to hold down the dirt and sprinkle the dirt with water to promote compaction. If we consistently see a problem with a specific burrow, we dig it up and start over, digging it deeper or placing it in a different area of the pen.

## D. Enclosures and the Perimeter Fence

We check the enclosures and perimeter fence weekly to make sure there are no holes, slides, or other evidence of breaches or escape routes. Tortoises can climb low fences to escape from their pens so if the dirt inside the pen is piled up too high near the fencing, we dig some of the dirt out and place it away from the fence. We also repair any holes or other issues immediately.

**Disclaimer:** This document is provided as a courtesy to companies that are authorized to place and maintain wild desert tortoises in captivity. While the protocols described within the document are standard at the Desert Tortoise Conservation Center, the San Diego Zoo makes no claims that the protocols, techniques, and structures described herein will work properly for every wild desert tortoise in every captive situation. It is expected that projects involving the captive care of wild desert tortoises will consult with US Fish and Wildlife Service to ensure that the protocols, techniques, and structures that they choose to use are in compliance with project permits and all other requirements. The San Diego Zoo is not responsible for illness, injury, or death to desert tortoises on projects that choose to use these protocols as guidance.

