Considerations at Time of Confiscation (Time Zero)-DRAFT (12/14/21)
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Introduction: Confiscated turtles are often severely compromised, need supportive care, and have an unknown history of exposure to potential pathogens of concern. Given this, immediate actions taken at time of confiscation can help reduce animal suffering, improve welfare, and maintain appropriate biosecurity and chain of custody to ensure animals may be releasable. The duration that turtles have been held in captivity prior to confiscation is often unknown. Illegally collected turtles are often held in crowded, unsanitary conditions at inappropriate temperatures while awaiting distribution. In the immediate post-confiscation period, we seek to hold the turtles in a safe, secure location, provide them with appropriate environmental conditions, and ensure that they are hydrated while waiting for transport to longer term holding facilities. Ideally this immediate care is provided by biologists, rehabbers, or an animal care facility to assist Law Enforcement.

Objectives: With these guidelines, we seek to optimize actions taken at time of confiscation (time zero) for the long-term care and releasability of confiscated turtles and eggs (if present). A checklist (figure 1) is also provided at the end of the document that outlines the key actions taken at the time of confiscation.

Audience: These guidelines are intended for law enforcement, wildlife inspectors at ports, and biologists who may be on-the ground at time of confiscation.

Immediate considerations:

1. Species – is this a native or exotic species? Is it federally or state listed as endangered or threatened, invasive, nuisance? If it is listed as an injurious species under Lacey Act, it

can be humanely euthanized. The status of the species may dictate where the animal is sent after confiscation.

2. Health – it is imperative that an initial, very general health screening, or triage (see below), be conducted to ensure the overall status of the confiscated turtles.
3. Housing – at time of initial confiscation, turtles need to be housed in appropriate short-term housing that addresses basic life and welfare needs. Housing may be simple (e.g., totes, buckets), but must be easily disinfected and of appropriate size for individuals or groups of turtles.
4. Conditions – at time of initial confiscation the conditions from which the turtles are confiscated must be considered and recorded (see example datasheet below). For example, were the turtles confiscated from an airport in suboptimal conditions (heat, cold)? Or were they confiscated from a collection and in appropriate housing? How many species are present? Were they mixed?
5. Quarantine – strict quarantine should be implemented immediately upon confiscation. This includes separation of different species within the same confiscated container, appropriate disinfection of equipment, documentation of groups by container, and enhanced biosecurity measures. It is necessary to ensure, and document, quarantine procedures in the immediate time of confiscation and through time for chain of custody measures, and to help future decisions be made pertaining to long-term outcome(s) of the turtles, such as being deemed releasable.
6. Contact of appropriate authorities – State(s) and Federal partners (if it is a federally listed species). A directory of state and federal contacts for SGCN species and federally listed species will be developed.
7. Other? (see example datasheet below, Figure 1)

Biosecurity Considerations:
Biosecurity at time of confiscation is of utmost importance, especially if confiscated turtles were held together and/or with other species. More detailed information on biosecurity measures are provided at the end of the document (Figure 2).

- Be sure to wear gloves and change gloves between handling individuals. If not feasible, wash hands with soap and water with gloves on before moving to the next individual.
- Quarantine sick turtles from healthy turtles.
- Quarantine different species, if multiple species are confiscated at one time.
- If confiscating multiple groups or containers of turtles at one time quarantine each group of turtles separately.
- All temporary containers, equipment, or contact surfaces used to hold animals should be disinfected between uses. Warm water and liquid dish soap may be used to wash, followed by disinfection with a dilute bleach solution (32 parts water to one part household bleach). All equipment and containers should then be thoroughly rinsed.
- If turtles are clearly sick be sure to clean (with soap and water) or disinfect outside of the totes before transport of turtles in a vehicle (to reduce risk of transmission of potential pathogens).
- If sick turtles are transported in a vehicle, disinfecting the inside of the vehicle using appropriate disinfectant is recommended. Using disinfectant wipes for hard surfaces and spray Lysol for soft surfaces, coupled with UV and heat can reduce pathogen risk inside vehicles.

Guidelines for Immediate Confiscation (see figure 3 for checklist):
Be sure to keep turtles out of direct sunlight, wind, or other environmental conditions that may impact their ability to thermoregulate. Provide adequate shade, even if temperatures are cool. Turtles can rapidly overheat in closed containers in direct sunlight. Relocate the turtles to an environment with ambient temperature of 60-80 degrees F. Their body temperature is dependent on the environment, and proper body temperature is important for all of their vital functions. Most native species can tolerate colder temperatures if needed, but it is not ideal for confiscated specimens (AZA Turtle SAFE program Health and Welfare Working Group protocols).

Identify the species if not already done. If multiple species are present, if possible, separate them from each other.

Initial Health Screening:

- Remove any dead individuals.

Note that turtles may sometimes appear dead even if not. Given this, keep apparently dead animals separate, but in similar conditions to the others, until they can be examined by a veterinarian. It is not always easy to tell if a turtle is dead. If in doubt, assume that it may be alive and maintain in same temporary, but separate, housing conditions as other turtles confiscated at same time. Dead turtles often have very sunken eyes, and rigor mortis may be noted. Look for swollen eyes, nasal discharge, skin and shell lesions, bleeding, etc. Look for turtles that feel too light (when picked up, healthy turtles should feel solid, roughly like a rock of similar size). If clearly dead, remove dead turtles and place them into a refrigerator or cooler containing ice. Do not freeze or discard them unless specifically told to do so by Law Enforcement (AZA Turtle SAFE program Health and Welfare Working Group protocols).

Separate clearly unhealthy turtles from those that appear healthy, but be sure to keep track of those that were held together on site of confiscation (see information on marking below). Signs of ill health include, but are not limited to: eye, oral, or nasal discharge, swellings around ear (tympanum), cloacal discharge or diarrhea, oral plaques, skin or shell lesions, lethargic or moribund, other noticeable differences in individuals compared to their counterparts

  - Once unhealthy turtles are separated from healthy ones be sure to adhere to strict quarantine between the groups. Do not handle apparently unhealthy animals and then healthy animals. Keep unhealthy animals as far as possible from those that are healthy.
  - Wear gloves and change between individuals, if feasible, or wash hands with soap and water with gloves on before moving to the next individual.
  - If feasible, have a veterinarian to do a more thorough health exam and collect any warranted samples.

- Check temperature of turtles. Put your hand on the shell. The shell should feel like room temperature.
- Collect any relevant information/data on the health of turtles. Log what was done (temp changes, soaks, etc).
- Turtles will **not** need to eat in timeframe around the immediate confiscation. Turtles are often ok going without food for weeks or even months.
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Temporary Housing:

Housing for turtles immediately after confiscation can be simple. Buckets, totes, or other containers that are easily disinfected (plastic) and have a secure lid will work. **If solid lid(s) are used, be sure air holes are drilled in the lids and/or sides of the containers**. Reduce crowding and reduce stocking density as much as possible by separating turtles into smaller groups, or if possible, distributing them into individual containers. Individuals do not need a large amount of space in the short-term. Plastic containers offer ease of cleaning and ease of water provision compared to wooden or cardboard containers (see below). For small turtles, small individual totes (size of shoe box) can be used to house them individually.

- Keep totes or buckets housing turtles out of direct sunlight. Turtles will easily overheat if they cannot access water or shade for thermoregulation.
- Clearly label each container with appropriate information (date of collection, turtle species, number of turtles, IDs if appropriate, etc).
    - Mark individual turtles as well with unique ID. A paint pen or fingernail polish can be used to mark the carapace (top of shell). DO NOT NOTCH (or other permanent mark). If turtles need to be marked quickly and tracked upon first confiscation, turtles can be "batch marked" where turtles that were packaged together can be tracked, and each group or container receives the same initial marking.
- Clean housing container (tote, bucket, etc) at least daily. If groups of turtles are kept in a single container, more frequent cleaning may be necessary.
    - Remove feces as quickly as possible. Have separate cleaning supplies (gloves, nets, scoops, etc) for each tote or group of turtles. This is especially important between the sick and healthy turtles, or different species/groups confiscated from separate locations.
- Keep a daily health record. Information recorded can include overall health observations, behavior, feces (presence/absence), etc.
    - If possible, obtain a weight at time of confiscation or within 24 hours.
- Keep a clear log of where turtles are moved after time of confiscation and before being placed in longer term housing.
- Place turtles in more appropriate, long-term housing as soon as possible. This should be specific and appropriate based on species and if the turtles are aquatic or terrestrial.

Supportive Care at Time of Confiscation:

- Turtles should be provided with a fresh-water soak within 24 hours of being confiscated. This will help prevent and/or rectify dehydration. Be sure to soak turtles in only an inch or two of fresh, clean water; do not make this too deep (even if an aquatic species). The turtle(s) should be able to easily pick head up out of the water.
    - Ensure water is about the same temperature as the environment, ideally room temperature.
    - Soak turtles for 1-2 hours under direct supervision; watch for defecation and remove if observed. Watch for turtles to drink. Turtles can be removed from water once they have had an opportunity to drink.
    - If direct supervision is not feasible, soak for 5-10 minutes.
    - Soak turtles, or groups, separately. Do not soak unhealthy with healthy turtles.

- Disinfect soak water after turtles are done. This can be done using bleach (10%) or another disinfectant prior to disposal of the water.
- Buckets or totes can be used to soak turtles. Do not use the same buckets or totes for healthy and unhealthy turtles.
- Disinfect totes/buckets after turtles have soaked. (Bleach or Virkon are good disinfectants.)

Supportive Care for Subsequent Days (AZA Turtle SAFE program Health and Welfare Working Group protocols):

- Most species can continue to be kept in shallow water, with water changed daily. Box turtles can be kept on paper substrate or bark mulch, and provided with a shallow water bowl.

- If time permits, provide each turtle with an opportunity for visual security. They often choose to be covered and hidden. Cereal boxes, clean milk cartons, large plastic bottles, halved flower pots, etc. can be used as shelters. A few inches of dry leaves may serve the same purpose.

- If resources permit, the substrate and water should be discarded and the containers should be washed and disinfected daily. Warm water and liquid dish soap may be used to wash the enclosure, followed by disinfection with a dilute bleach solution (32 parts water to one part household bleach). Enclosures should then be thoroughly rinsed.

- Provide a light cycle of 12-16 hours per day. Lights should be left on continuously (i.e. do not leave lights on overnight). Failure to provide at least partial darkness at night may lead to physiological stress that exacerbates other medical problems.

- It is not essential to provide food during the first few days, but it can be offered if resources permit. Earthworms are a favorite food for most species. Brightly colored, fragrant fruits such as strawberries, raspberries, and melon may also be accepted.

Transport (from AZA Turtle SAFE Program Health and Welfare Group protocols):
Transport the turtles from the confiscation site to the temporary holding location. Turtles can be packed in plastic totes, bins, buckets, kiddie pools, concrete mixing tubs, cardboard boxes, etc. Boxes used for shipping fruits and vegetables are often available from grocery stores and are useful for transport. If possible, line the containers with absorbent material such as paper towels, newspaper, towels, etc. During transport turtles often urinate and defecate, and the absorbent material helps to reduce contamination of other turtles and transport vehicles. Turtles are good climbers, so ideally secure a lid on the containers. If containers are air tight, drill some air holes in the sides or top. Try to minimize crowding if possible. A single "layer" of turtles is ideal. Turtles that are packed two or more layers deep often compress, traumatize, urinate, and defecate on each other, causing eye, skin, muscle, and shell injuries. Studies have shown that transport is a physiologic stressor for turtles, so develop a plan to minimize the duration of transport as much as possible. Avoid direct sunlight as this can cause turtles to rapidly overheat.

Figure 1. Information to be collected at time of confiscation.

- Law Enforcement Case Number
- Species of turtle present
- Location of confiscation
- Location of origin (if known)
- Number of individuals
- Number of species present, if >1
    - Other species present (amphibians, other turtles or herps)
- Name and contact information of confiscating individual
- General conditions of confiscation (take photos if possible)
    - For example, at a port from a box with # of individuals in the container
    - From a home collection, general conditions, species present
- Temperature at time of collection
    - Other general weather conditions
- First impression of overall health of confiscated turtles
    - Can be general (good, moderate, poor)
- ID of each turtle/life stage (hatchling, juvenile, adult)
- Specific health info for each turtle
    - Weight
    - General condition
    - Other info (did it eat, drink, etc)
- Other??

Figure 2. "How to" for biosecurity measures implemented at time of confiscation.

1. Wear disposable gloves and/or wash hands with soap and water for 20 seconds between handling individual animals or any substrate/biological waste material to avoid contamination and transfer of pathogens.
   https://www.cdc.gov/handwashing/when-how-handwashing.html

2. Use unique tools/containers/brooms, etc. for each individual and its enclosure, or clean and disinfect them between enclosures.

3. Cleaning is done before disinfecting with the goal of removing debris. Cleaning can be done using a scrub brush and water.

4. Disinfection of totes, tubs, or buckets can be accomplished with bleach solution or Virkon™. Disinfection protocols can be found at: http://www.northeastparc.org/products/pdfs/NEPARC_Pub_2014-2_Disinfection_Protocol.pdf (table provided below).

5. Disinfecting soak water can be done with a 10% bleach solution.

Disinfection Options for Ranavirus (RV) and *Batrachochytrium dendrobatidis* (*Bd*)

Although these chemicals were not developed specifically for RV or Bd, these recommendations represent the minimum concentration and contact time demonstrated as effective

	Clorox Bleach®	Nolvasan®	Virkon S®	Ethanol
Active Ingredient (AI)	Sodium hypochlorite	Chlorhexidine	Potassium peroxymonosulfate	Ethyl alcohol
Concentration of AI	6.0%	2.0%	20.4%	70.0%
Relative cost	$4.99/gal	$65.95/gal	$76.50/10 lb or $1.60/gal	$23.45/L or $88.83/gal
Min. Contact Time RV[9]/Bd[10]	1 min / 30 sec	1 min / not determined	1 min / 20 sec	1 min[11] / 20 sec
Min. Concentration RV[9]/Bd[10]	3.0% / 1.0%	0.75% / not determined	1.0% / 1.0%	70% / 70%
Effective dilution ratio for both RV and Bd	1:32 dilution (bleach:water) for 3% solution using 6% concentration of household bleach.	1:127 (Nolvasan®: water) for 0.75% solution (RV only)	1 scoop (1.3 oz) or 1 tablet per gal of water	Effective when applied undiluted (70%)
Toxicity to Humans	• Vapor may cause severe irritation or damage to eyes and skin • Harmful if swallowed	• May be fatal if inhaled • Avoid breathing spray mist • Causes irreversible eye damage • Harmful if swallowed	• Harmful if swallowed • Irritating to respiratory system and skin • May cause serious eye damage	• May be fatal if swallowed or inhaled • Can damage liver, kidneys and nervous system by repeated or prolonged exposure • May be absorbed through skin. Repeated or prolonged contact can cause eye irritation or dermatitis[12]
Toxicity to Amphibians	• Fatal at high concentrations	• Safe for short durations [13]	• Non-toxic [14]	• May destroy mucus and wax resulting in dehydration and microbial infection[11]
Effects on Equipment	• Corrodes metals • Will fade colors and break down cloth fibers	• None reported	• Safe on fabric • May cause pitting on galvanized or soft metal if not rinsed with water	• May damage rubber and plastics • May cause deterioration of glues[12]

Special Instructions:

• Remove debris from equipment prior to treatment.[15] • Wear safety glasses and gloves when handling chemicals. • Water pH can affect chemicals; all information in this table assumes the use of tap or municipal water. • Keep out of lakes, streams, or ponds; stand at least 50 m from any natural water source. • Do not clean equipment or dispose of waste solutions at field sites. • For disposal, follow local, state, and federal guidelines.

Bleach: Inactivated by organic material. • Inactivated by sunlight. • If in an opaque container, diluted bleach will last 1 month[16]. If exposed to sunlight or air, it will only last 5 days.

Nolvasan: Can be inactivated by organic material.[15] • Store at room temperature in sealed container.[17] • Dilute concentrate with water of pH 5-7.[18] • Remains stable for 1 week if dilute with tap water, and for up to 6 weeks if diluted with deionized water.[17] • Use concentrate within 36 months.[17] • Toxic to fish.[18]

Virkon-S: Store at room temperature.[19] • Keep solution away from extreme cold or heat. • Shelf life for tablets is 2 years and for powder is 3 years. • Remains stable for 1 week if diluted with tap water.

Ethanol: Highly flammable. • Use and store in a well ventilated area. • Evaporation may diminish effective concentration.[12,18]

Agent	Concentration	Contact Time	Application	Efficacy	Human Health and Environmental Risks	Mitigation of Negative Effects
Virkon S™ **Virkon Aquatic™** **(potassium peroxymonosulfate)**[1,2,3,4,8,10,11]	1% solution (1 scoop (1.3 oz or 36.86 g) or 1 tablet per 1 gal or 3.8 L water)[9,11]	1 min[1,2,3,4] & 5 min[1] 2 min & 10 min[11]	Safe for all field equipment	Ranavirus[1,2,3,4] Bd[2,3,8] SFD[11]	Powder poses risks if inhaled or contacts mucous membranes; must be mixed in a well-ventilated area, preferably outdoors or fume hood Appropriate PPE should be used (splash apron, gloves and safety goggles) Tablets more stable and less hazardous to personnel for transporting and reconstitution Test strips available to check viability of solution	Once in solution non-toxic to amphibians, environment, humans
	0.02%[4]	1 min[4]		Bd[4]		
Phenols (Lysol™)[6]	2-5%	10 min	Hard surfaces Must rinse with water	SFD[6]	Not labeled for use on field gear Irritating to skin	
Ethanol[3,4,6]	70%	10-30 min 1 min[3,4] 2 hr[3]	Surgical equipment; hands; countertops	Ranavirus Bd SFD[6] Bd[3,4] Ranavirus[3,4]	Inactivated by sunlight; evaporates quickly and may not get proper contact time; drying to skin; expensive; not good for field equipment; fixes organics to hard surfaces.	
Benzalkonium Chloride[3,4,6]	0.1% 0.2%	1 min	Surgical equipment	Bd[3,4] SFD[6]		

Figure 3. Checklist for actions taken at the time of confiscation.

Conditions at time of confiscation

- ☐ How were the turtles being held? (ex. crowded in box and covered with sock/duct tape or in appropriate housing)
- ☐ Are they in extreme temperatures (hot or cold)?
- ☐ Number of individuals and species present, and how they're packaged (number of packages, number of animals per package, are multiple species mixed?)

Immediate Handling

- ☐ Keep turtles out of direct sunlight, wind or other conditions that can impact thermoregulation, and keep an eye on changing conditions..
- ☐ Relocate to an environment with ambient temperature of 60-80°F.
- ☐ **Wear gloves and change gloves between handling different individuals and groups. Wash hands with gloves with soap and water if changing gloves for every turtle is not feasible.**
- ☐ ID and separate out different species within groups held together.
- ☐ For sorting, turtles can be placed in plastic totes or buckets that are appropriately sized for individual turtles or groups of turtles and can be disinfected.

Health Screening

- ☐ Remove dead individuals and place in a plastic bag in a refrigerator or in a cooler with ice. Do not freeze or discard without approval from law enforcement. If unsure whether an individual is dead, keep separate from other individuals until they can be examined by a veterinarian.
- ☐ Separate sick turtles from healthy turtles. Ensure that different groups found at the time of confiscation remain separated (i.e., do not combine turtles found in different shipments/containers even if they appear healthy). Signs of ill health include, but are not limited to: eye, oral, or nasal discharge, swellings around ear (tympanum), cloacal discharge or diarrhea, oral plaques, skin or shell lesions, lethargic or moribund, other noticeable differences in individuals compared to their counterparts.

Temporary housing

- ☐ Buckets, totes, or other containers that are easily disinfected (plastic) and have a secure lid will work. If solid lid(s) are used, be sure air holes are drilled in the lids and/or sides of the containers.
- ☐ Clearly label each container with appropriate information (date of confiscation, turtle species, number of turtles, IDs if appropriate, etc.).
    - o Mark individual turtles as well with unique ID. A paint pen or fingernail polish can be used to mark the carapace. DO NOT NOTCH (or other permanent mark).
- ☐ Clean housing container (tote, bucket, etc) at least daily (see Biosecurity below). If groups of turtles are kept in a single container, more frequent cleaning may be necessary.
    - o Remove feces as quickly as possible. Have separate cleaning supplies (nets, scoops, etc) for each tote or group of turtles. This is especially important between the sick and healthy turtles, or different species/groups confiscated.

- ☐ If feasible, keep a daily health record. Information recorded can include overall health observations, behavior, feces (presence/absence), etc.
    - o If possible, obtain a weight at time of confiscation or within 24 hours.

Biosecurity

- ☐ Be sure to wear gloves and change gloves between handling individuals. If not feasible, wash hands with soap and water with gloves on before moving to the next individual.
- ☐ Quarantine sick turtles from healthy turtlesIdeally all individuals should be housed separately, if feasible.
- ☐ Quarantine different species, and if confiscating multiple groups or containers, quarantine each group separately.
- ☐ All temporary containers, equipment, or contact surfaces used to hold animals should be disinfected between uses. A 1:10 dilution of household bleach solution can be used by mixing 1 part new, regular household bleach (sodium hypochlorite) to 9 parts water, AVOID no-splash varieties; AVOID opened or old bleach). Thoroughly (triple) rinse afterwards.
- ☐ If turtles are clearly sick be sure to clean (with soap and water) or disinfect outside of the totes before transport of turtles in a vehicle (to reduce risk of transmission of potential pathogens).
- ☐ If sick turtles are transported in a vehicle, disinfecting the inside of the vehicle using appropriate disinfectant is recommended. Using disinfectant wipes for hard surfaces and spray Lysol for soft surfaces, coupled with UV and heat can reduce pathogen risk inside vehicles.

Supportive Care

- ☐ Turtles should be provided with a fresh-water soak within 24 hours of being confiscated. Be sure to soak turtles in only an inch or two of fresh, clean water. The turtle(s) should be able to easily pick head up out of the water.
    - o Ensure water is about the same temperature as the environment, ideally room temperature.
    - o Soak turtles for 1-2 hours under direct supervision; watch for defecation and remove if observed. Watch for turtles to drink. Turtles can be removed from water once they have had an opportunity to drink.
    - o If direct supervision is not feasible, soak for 5-10 minutes.
    - o Soak turtles, or groups, separately. Do not soak unhealthy with healthy turtles.
    - o Disinfect soak water after turtles are done. This can be done using household bleach (1/10 dilution) or another disinfectant prior to disposal of the water.
- ☐ Disinfect totes/buckets after turtles have soaked. Rinse equipment well.
- ☐ If turtles need to be held for several days, most species can continue to be kept in shallow water, with water changed daily. Box turtles can be kept on paper substrate or bark mulch, and provided with a shallow water bowl.
- ☐ If resources permit, the substrate and water should be discarded and the containers should be washed and disinfected daily.

Figure 4. Examples of temporary housing.