Appendix I—Guidelines for Work with Toxins of Biological Origin

Biological toxins encompass a vast range of peptides, small molecules, and macromolecular proteins that cause disease by interfering with biological processes. As their name suggests, biological toxins reside between traditional definitions of biological and chemical agents. They are produced by living organisms, are unable to replicate, and do not result in communicable diseases. The production of novel or existing toxins by synthetic means is becoming increasingly accessible.[1,2] Many biological toxins have been evolutionarily optimized to rapidly disrupt critical biological functions at low concentrations. Their extraordinary, highly specific toxicity is mediated through a diverse set of mechanisms, including enzymatic activity against critical cellular targets, blockade of membrane ion channels and receptors, and perturbation of essential cellular functions. The remarkable combination of specificity and potency has resulted in the widespread use of diverse biological toxins for clinical and research purposes, including botulinum neurotoxins, tetrodotoxin, conotoxins, scorpion toxins, snake venom toxins, and immunotoxins. Because laboratory workers in a wide range of medical and scientific disciplines are likely to encounter biological toxins at some point during their career, it is critically important that laboratory workers understand and are able to assess the risks associated with their use.

Laboratory workers can be exposed to biological toxins through a variety of routes, including inhalation of powders, aerosols, or volatile substances; ingestion; injection; and absorption through dermal, mucosal or ocular tissues. Many biological toxins are highly potent, and internalization of even relatively low doses may result in death or severe incapacitation. Consequently, it is critically important for those working with biological toxins to understand and implement appropriate laboratory safety principles. A number of principles for the safe use of many toxins commonly encountered in the clinical or research environment are summarized below, including for those biological toxins regulated by the Federal Select Agent Program as Select Toxins (see below).

General Considerations for Toxin Use

The primary risks during laboratory use of biological toxins result from accidental injection, absorption through skin or mucous membranes, inhalation, and ingestion. Laboratory work with most toxins in amounts routinely employed in the biomedical sciences can be performed safely with minimal risk to the worker and negligible risk to the surrounding community. Under most circumstances, toxins can be handled using established general guidelines for toxic or highly-toxic chemicals with the incorporation of additional safety and security measures

based upon a risk assessment for each specific toxin and laboratory operation.[3,4] Additionally, the mixed hazard nature of toxins and their associated organisms should be considered in the risk assessment when determining appropriate facilities, practices, and equipment use for situations where both biological and chemical hazards are present. Standard use of engineering controls (e.g., Class II or Class III biosafety cabinets or open-front chemical fume hoods) and personnel protective equipment (e.g., safety glasses or goggles, mask, gloves, and lab coat) are generally sufficient to avoid accidental inhalation or topical exposure.

Training and Laboratory Planning

Each laboratory worker must be trained in the theory and practice of the toxins to be used, with special emphasis on the nature of the practical hazards associated with laboratory operations. These include risks associated with transfer of solubilized toxins; manipulation of waste solutions, contamination of materials and equipment; and decontamination after routine operations and spills. Workers must be well-trained and sufficiently adept at all laboratory procedures and safety practices before participating in toxin operations.

A risk assessment should be conducted to identify potential hazards and develop safe operating procedures before undertaking toxin operations. For example, the use of pre-operational checklists is highly recommended.[4] For complex operations, newly approved toxin workers should undergo supervised practice runs in which the exact laboratory procedures to be undertaken are rehearsed using nontoxic simulants. Technical rehearsals are particularly important to mitigate the psychological stress of working with highly dangerous agents.

The inclusion of toxins can significantly complicate otherwise routine laboratory procedures. For example, equipment with potential to produce aerosols may need to be placed in primary containment, such as a biosafety cabinet (BSC) or fume hood, and decontaminated after each use. The use of personal protective equipment (PPE) can reduce dexterity, and operations may be more difficult when conducted in crowded hoods or BSCs. If toxins and infectious agents are used together, then both must be considered in the risk assessment when selecting containment equipment, developing safety procedures, and choosing decontamination and disposal methods. Early endpoints need to be designed to balance experimental objectives with safe and ethical application of toxins to animals. The medical consequences of an accidental needlestick during animal operations may be significantly increased when toxin is involved. Team leaders should be prepared to carefully review study procedures to identify how toxin use may interfere with experimental execution and develop effective mitigation strategies.

Each laboratory that uses toxins must develop toxin-specific chemical hygiene plans. The National Research Council has provided a review entitled "Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards" with guidance on development of chemical hygiene plans and compliance with regulations governing occupational safety and health, hazard communication, and environmental protection. The 2011 edition of this review can be downloaded for free from https://www.nap.edu/catalog/12654/prudent-practices-in-the-laboratory-handling-and-management-of-chemical. These procedures are also summarized in the Occupational Safety and Health Administration's Laboratory Standard (29 CFR Section 1910.1450, Appendix A).

A number of engineering and human controls are available to decrease the risk of accidental misuse of biological toxins. An inventory control system should be established and audited on a regular basis (e.g., monthly or quarterly) to account for toxin quantity, use, and disposition. While an inventory control system is required for users of non-exempt quantities of Select Toxins (see below for exempt quantity limits), it is also useful for ensuring that exempt quantity users do not accidentally exceed permissible toxin limits. For additional information select toxin exemption requirements, see the Federal Select Agent Program website (www.selectagents.gov). Toxins should be stored in storage containers with labels that clearly list the toxin contents, points of contact for trained, responsible laboratory staff, and emergency contact information. The use of locks on storage containers offers an additional level of oversight and control over toxin access. Laboratory work with toxins should only be done in designated rooms with controlled access and at pre-determined bench areas. When toxins are in use, the room should have clearly posted signage stating, for example, "Toxins in Use—Authorized Personnel Only." Signage should provide a knowledgeable point of contact and delineate minimum requirements for PPE. Whenever possible, unrelated and nonessential work should be avoided in laboratory or clinical areas where concentrated solutions of toxins or of toxin-producing organisms are maintained. Laboratory visitors must be briefed and monitored to prevent them from inadvertently handling contaminated laboratory equipment or touching exposed surfaces without protection. Finally, treatment plans for accidental exposures should be prepared and available to emergency responders and, when possible, coordinated with primary care facilities. While there is no way to completely eliminate the dangers of biological toxin use, implementation of these controls can significantly reduce the risks associated with toxin storage and use.

Safety Equipment and Containment

Routine operations with dilute toxin solutions are conducted under BSL-2 conditions with the aid of PPE and a well-maintained BSC, chemical fume

hood, or comparable engineering controls.[5] Engineering controls should be selected according to the risk assessment for each specific toxin operation. A certified BSC or chemical fume hood will suffice for routine operations with most solubilized protein toxins. Work involving toxin powders, volatile chemicals, or radionuclides combined with toxin solutions may require additional safeguards or barriers based on the risks associated with each toxin preparation.

Handling of solubilized toxins should be conducted within the operationally effective zone of a BSC or chemical fume hood. Before initiating work, each user should verify the hood or BSC is properly working according to manufacturer guidelines. When using a BSC or hood, workers should wear suitable laboratory PPE to protect the hands, arms, and eyes, such as laboratory coats with knit or elastic cuffs, smocks or coveralls, disposable gloves, and safety glasses. When working with toxins that pose direct percutaneous hazards, special care must be taken to select gloves that are impervious to the toxin and the diluents or solvents employed. When conducting large volume liquid transfers and other operations that pose a potential splash or droplet hazard in an open-front hood or BSC, workers should wear a disposable facemask or face shield.

Toxin(s) should be removed from the hood or BSC only after the exterior of the closed primary container has been decontaminated and placed in a clean secondary container. Toxin solutions, especially concentrated stock solutions, should be transported in leak/spill-proof secondary containers. The interior of the hood or BSC should be decontaminated periodically; for example, at the end of the day or after a spill. Until thoroughly decontaminated, the hood or BSC should remain posted to indicate that toxins are present, and access should be restricted to staff trained in toxin use and decontamination.

Selected operations with toxins may require modified BSL-3 practices and procedures. The determination to use BSL-3 is made in consultation with available biosafety staff and is based upon a risk assessment that considers the variables of each specific laboratory operation, especially the toxin under study, the physical state of the toxin (solution or dry form), the total quantity of toxin used relative to the estimated human median lethal dose, the volume of the material manipulated, the methodology, and any human or equipment performance limitations.

Inadvertent Toxin Aerosols

Many biological toxins are highly potent, and emphasis must be placed on evaluating and modifying experimental procedures to avoid inadvertent generation of toxin aerosols. Tubes containing solubilized toxin under pressure should be only be opened in a BSC, chemical fume hood, or other ventilated enclosure.

Operations that expose toxin solutions to vacuum or pressure should always be handled in this manner, and the operator should also use appropriate respiratory protection to minimize the accidental inhalation of aerosolized toxins or toxin powder. If vacuum lines are used with toxin, they should be protected with a HEPA filter to prevent entry of toxins into the line and include a vacuum flask with decontamination solution between the vacuum source and vacuum line. HEPA filters should be considered to be contaminated with toxin particles and disposed of as described below.

Centrifugation of cultures or materials potentially containing toxins should only be performed using sealed, thick-walled tubes in safety centrifuge cups or sealed rotors. The outside surfaces of containers, safety cups (if applicable), and rotors should be routinely cleaned before and after each use to prevent contamination that may generate an aerosol. The sealed centrifuge safety cups or sealed rotor should be taken from the centrifuge to a BSC prior to opening or it should be taken to other suitable containment prior to breaking the seal and removing centrifugation tubes.

Mechanical Injuries

Accidental needlesticks or mechanical injury from sharps (i.e., glass or metal implements) pose a well-known risk to laboratory workers. When these accidents occur during operations using biological toxins in amounts that approach a human lethal dose, the consequences may be catastrophic. Consequently, additional care must be taken prior to and during toxin operations to reduce the risks of exposure through mechanical injury.

Only workers trained, competent, and experienced in handling animals and toxin operations should be permitted to conduct operations involving animals, especially injection of toxin solutions using hollow-bore needles. Discarded needles/syringes and other sharps should never be recapped; instead, they should be placed directly into properly labeled, puncture-resistant sharps containers and decontaminated. Glassware should be replaced with plastic for handling toxin solutions to minimize the risk of cuts or abrasions from contaminated surfaces. Thin-walled glass equipment should be completely avoided. Glass Pasteur pipettes are particularly dangerous for transferring toxin solutions and should be replaced with disposable plastic pipettes. Glass chromatography columns under pressure must be enclosed within a plastic water jacket or other secondary container.

Additional Precautions

Experiments should be planned to eliminate or minimize work with dry toxin or toxin- containing formulations (e.g., lyophilized material or freeze-dried preparations). Unavoidable operations with dry toxin should only be undertaken with appropriate respiratory protection and engineering controls. Dry toxin can be manipulated using a Class II BSC or with the use of secondary containment such as a disposable glove bag or glove box within a hood. *Static-free disposable gloves* should be worn when working with dry forms of toxins that are subject to spread by electrostatic dispersal. If a Class II BSC is used, HEPA filters should be considered to be contaminated with toxin particles and disposed of as described below. Workers should wear respiratory protection suitable to prevent accidental inhalation of toxin particles.

In specialized laboratories, the intentional, controlled generation of aerosols from toxin solutions may be required to test antidotes or vaccines in experimental animals. These are extremely hazardous operations that should only be conducted after extensive validation of equipment and personnel using non-toxic simulants. Aerosol exposure of animals should be done in a certified Class III BSC or similar containment device. Workers should take additional precautions to avoid accidental exposure to biological toxins when removing exposed animals from the exposure area and for the subsequent 24 hours after exposure; additional precautions include wearing protective clothing (e.g., disposable Tyvek suit) and appropriate respiratory protection. To minimize the risk of dry toxin generating a secondary aerosol, areas of animal skin or fur exposed to aerosols should be gently wiped with a damp cloth containing water or buffered cleaning solution before the animals are returned to holding areas. Injections of toxin solutions into animals can be conducted outside of a BSC, but attention must be paid to avoiding needlesticks and ensuring that used syringes are stored and disposed of properly to avoid accidental contamination or loss of toxin.

For high-risk operations involving dry forms of toxins, intentional aerosol formation, or the use of hollow-bore needles in conjunction with amounts of toxin estimated to be lethal for humans, consideration should be given to requiring the presence of at least two knowledgeable individuals at all times in the laboratory.[6] This is particularly important when using toxins that have acute effects. While the physicochemical properties of most toxins render interpersonal transmission highly unlikely, emergency care providers should be aware of the possibility of contamination in the environment or through direct transfer of bodily fluids (e.g., during mouth-to-mouth resuscitation). Laboratories using toxins that have acute effects on cardiopulmonary function should have emergency resuscitation training provided and equipment located in the near vicinity to sustain casualties

until the toxic effect passes and emergency caregivers are on-scene. Resuscitation equipment should include mask-bag or oxygen delivery systems to reduce the risk of exposure to emergency caregivers.

Vaccinations against some biological toxins are available and may be appropriate for laboratory workers, depending on the amount of toxin used, frequency of use, and risk of toxin exposure.

Decontamination and Spills

Decontamination of a biological toxin(s) means the toxin is rendered inactive and is no longer capable of exerting its toxic effect. Toxin stability varies considerably outside of physiological conditions depending upon the temperature, pH, ionic strength, presence of co-factors, and other characteristics of the surrounding matrix. Literature values for dry heat inactivation of toxins can be misleading due to variations in experimental conditions, matrix composition, and experimental criteria for assessing toxin activity. Inactivation is not always a linear function of heating time; some protein toxins possess a capacity to re-fold and partially reverse inactivation caused by heating. In addition, the conditions for denaturing toxins in aqueous solutions are not necessarily applicable for inactivating dry, powdered toxin preparations.

General guidelines for laboratory decontamination of selected toxins are summarized in Tables 1 and 2, but inactivation procedures should not be assumed to be 100% effective without validation using specific toxin bioassays. Most toxins are susceptible to steam inactivation (121°C for one hour) or to chemical inactivation with dilute sodium hydroxide (NaOH) at concentrations of 0.1–0.25N, and/or sodium hypochlorite (NaOCl) solutions at concentrations of 0.1–2.5% (w/v). Commercially available bleach solutions typically contain 3–6% (w/v) NaOCl. Bleach decontamination solutions should always be prepared *fresh* (i.e., <24 h).

Contaminated materials and toxin waste solutions can be inactivated by incineration, extensive autoclaving, or by soaking in a suitable decontamination solution, depending on the toxin (Table 2). Once decontaminated, liquid inactivated toxins can be absorbed onto a solid matrix (i.e., absorbent pad, filter paper, or paper towel) for incineration as hazardous waste. Alternatively, liquid inactivated toxins can be disposed of in the sink, depending on local regulations and policies. All disposable contaminated solid material should be placed in secondary containers and then autoclaved and/or disposed of as hazardous waste for incineration. Contaminated or potentially contaminated protective clothing and equipment (e.g., PPE) that is to be re-used should be decontaminated using suitable chemical methods or should be autoclaved after use, if the toxin is heat-labile, and before it is re-used or removed from the laboratory for cleaning or repair.

In the event of a liquid spill, avoid splashes or generating aerosols during clean-up by covering the spill with dry paper towels or other disposable, absorbent material. Ensure that appropriate PPE (at a minimum to include mask, gloves, safety glasses or goggles, and laboratory coat) is worn during the clean-up. Apply an appropriate decontamination solution to the spill, beginning at the perimeter and working towards the center. Allow sufficient contact time for the decontamination solution to completely inactivate the toxin (Table 2). Restrict access to the contaminated area until the decontamination is complete. Absorb the decontaminated toxin onto a solid matrix and discard as hazardous waste for incineration.

Spills involving toxin powder have an increased risk of inhalational exposure. PPE should include respiratory protection, gloves, safety glasses or goggles, and lab coat. If the spill occurs within the BSC, gently cover the powder spill with damp absorbent paper towels to avoid raising dust. Apply the appropriate chemical inactivating agent starting at the perimeter and working toward the center, allowing for sufficient contact time as specified in Table 2. Wipe the area with a paper towel soaked in bleach solution or a decontamination solution specific to the biological toxin; then, wash with soap and water. Dispose of the decontaminated physical waste by autoclaving or as hazardous waste for incineration. A powder spill outside the BSC should trigger the immediate evacuation of the area. The spill should be managed and decontaminated as above; however, access to the contaminated area should be carefully controlled in order to minimize the possibility of disturbing the powder and causing an inhalational exposure. Decontamination personnel should be equipped with respirators. Depending on the size of the spill, the area may have to be quarantined and the HVAC system turned off until the entire spill is contained and the area decontaminated. Filters in the HVAC system may need to be removed and discarded by trained personnel.

Decontamination of large areas, buildings, or offices containing sensitive equipment or documents poses special challenges. Large-scale decontamination is not covered explicitly here, but careful extrapolation from the basic principles may inform more extensive clean-up efforts.

Low molecular weight biological toxins tend to be highly stable and resistant to decontamination. Chemical decontamination with NaOCl is currently the most reliable method for inactivation.[7] Alternative methods have not proven very effective. For example, 1 N sulfuric or hydrochloric acid does not inactivate T-2 mycotoxin and only partially inactivates microcystin-LR, saxitoxin, and brevetoxin (PbTx-2). Tetrodotoxin and palytoxin are inactivated by hydrochloric acid, but only at relatively high molar concentrations. T-2 is not inactivated by exposure to 18% formaldehyde plus methanol (16 hours), 90% freon-113 + 10% acetic

acid, calcium hypochlorite, sodium bisulfate, or mild oxidizing agents. Hydrogen peroxide is ineffective in inactivating T-2 mycotoxin. Hydrogen peroxide does cause some inactivation of saxitoxin and tetrodotoxin but requires a 16-hour contact time in the presence of ultraviolet light. The addition of 3% acetone after bleach treatment has been suggested to prevent reformation of mycotoxins after bleach treatment when decontaminating spills or glassware.[8]

Select Toxins

HHS and the USDA have identified a group of toxins that pose a severe threat to human, animal, and/or plant health as Select Toxins. The Federal Select Agent Program oversees the possession, use, and transfer of these toxins, to include botulinum neurotoxins (all serotypes and subtypes), abrin, paralytic alpha conotoxins, diacetoxyscirpenol, ricin, saxitoxin, staphylococcal enterotoxins (subtypes A–E), T-2 toxin, and tetrodotoxin. A current list of Select Toxins and exempt quantities can be found at https://www.selectagents.gov/SelectAgentsandToxins.html. Registration with the CDC or USDA is required for possession, use, modification, production, storage, and/or transfer of non-exempt quantities of Select Toxins, while exempt quantities should be carefully managed by the responsible organization to prevent loss or misuse. Most Select Toxins are highly potent, and corresponding antidotes are not clinically available; thus, extreme care must be taken when using these agents for clinical or research purposes. Risk assessments and emergency treatment plans should be formulated that are specific to the dangers of each Select Toxin, and responsible parties should undertake regular reviews of laboratory procedures to ensure that laboratory procedures are understood and carefully followed by technical personnel.

Table 1. Physical Inactivation of Toxins

Toxin	Steam Autoclave	Dry Heat (10 min)	Freeze-Thaw	Gamma Irradiation
Botulinum neurotoxin A–G	Yes [a]	≥ 100° C [b]	No [c]	Incomplete [d]
Staphylococcal enterotoxin	Yes [e]	≥ 100° [C]; refold [f]	No [g]	Incomplete [h]
Ricin	Yes [i]	≥ 100° C [i]	No [j]	Incomplete [k]
Microcystin	No [l]	≥ 260° C m	No [n]	ND
Saxitoxin	No [l]	≥ 260° C [m]	No [n]	ND
Palytoxin	No [l]	≥ 260° C [m]	No [n]	ND

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Toxin	Steam Autoclave	Dry Heat (10 min)	Freeze-Thaw	Gamma Irradiation
Tetrodotoxin	No [l]	≥ 260° C [m]	No [n]	ND
T-2 mycotoxin	No [l]	≥ 815° C [m]	No [n]	ND
Brevetoxin (PbTx-2)	No [l]	≥ 815° C [m]	No [n]	ND
Abrin	Yes [o]	ND	ND	ND
Shiga toxin	Yes [p]	ND	ND	ND

ND indicates "not determined" from available literature.

a. Steam autoclaving should be at ≥121° C for 1 h For volumes larger than 1 liter, especially those containing *Clostridium botulinum* spores, autoclave at ≥121° C for 2 h to ensure that sufficient heat has penetrated to kill all spores.[9,10]

b. Exposure to 100° C for 10 min inactivates BoNT. Heat denaturation of BoNT as a function of time is biphasic with most of the activity destroyed relatively rapidly, but with some residual toxin (e.g., 1–5%) inactivated much more slowly.[11]

c. Measured using BoNT serotype A at -20° C in food matrices at pH 4.1–6.2 over a period of 180 days.[12]

d. Measured using BoNT serotypes A and B with gamma irradiation from a ^{60}Co source.[13,14]

e. Protracted steam autoclaving, similar to that described for BoNT, followed by incineration is recommended for disposal of SE-contaminated materials.

f. Inactivation may not be complete depending upon the extent of toxin re-folding after denaturation. Biological activity of SE can be retained despite heat and pressure treatment routinely used in canned food product processing.[15]

g. SE toxins are resistant to degradation from freezing, chilling or storage at ambient temperature. Active SEB in the freeze-dried state can be stored for years.[16]

h. References[16,17]

i. Dry heat of >100º C for 60 min in an ashing oven or steam autoclave treatment at >121º C for 1 h reduced the activity of pure ricin by >99%.[7] Heat inactivation of impure toxin preparations (e.g., crude ricin plant extracts) may vary. Heat-denatured ricin can undergo limited refolding (<1%) to yield active toxin.

j. Ricin holotoxin is not inactivated significantly by freezing, chilling, or storage at ambient temperature. In the liquid state with a preservative (sodium azide), ricin can be stored at 4º C for years with little loss in potency.

k. Irradiation causes a dose-dependent loss of activity for aqueous solutions of ricin, but complete inactivation is difficult to achieve; 75 MRad reduced activity 90%, but complete inactivation was not achieved even at 100 MRad.[18] Gamma irradiation from a laboratory ^{60}Co source can be used to partially inactivate aqueous solutions of ricin, but dried ricin powders are significantly resistant to inactivation by this method.

l. Autoclaving with 17 lb pressure (123º C) for 30 min failed to inactivate LMW toxins.[7,19] All burnable waste from LMW toxins should be incinerated at temperatures in excess of 815º C (1,500º F).

m. Toxin solutions were dried at 150º C in a crucible, placed in an ashing oven at various temperatures for either 10 or 30 min, reconstituted, and tested for concentration and/or activity; tabulated values are temperatures exceeding those required to achieve 99% toxin inactivation.[7]

n. LMW toxins are generally very resistant to temperature fluctuations and can be stored in the freeze-dried state for years and retain toxicity.

o. Reference[20]

p. Reference[21,22]

Table 2. Chemical Inactivation of Toxins

Toxin	NaOCl (30 min)	NaOH	Freeze-Thaw	Gamma Irradiation
Botulinum neurotoxin A–G	≥ 0.1% [a]	≥ 0.25 N	ND	Yes [b]
Staphylococcal enterotoxin	≥ 0.5% [c]	≥ 0.25 N	ND	ND
Ricin	≥ 1.0% [d]	ND	> 0.1% + 0.25 N [e]	ND
Saxitoxin	≥ 0.1% [e]	ND	0.25% + 0.25 N [e]	ND
Palytoxin	≥ 0.1% [e]	ND	0.25% + 0.25 N [e]	ND
Microcystin	≥ 0.5% [e]	ND	0.25% + 0.25 N [e]	ND
Tetrodotoxin	≥ 0.5% [e]	ND	0.25% + 0.25 N [e]	ND
T-2 mycotoxin	≥ 2.5% [e,f]	ND	0.25% + 0.25 N [e]	ND
Brevetoxin (PbTx-2)	≥ 2.5% [e,f]	ND	0.25% + 0.25 N [e]	ND
Alpha conotoxins	≥ 0.5% [g]	10 N [g]	ND	No [g]
Abrin	≥ 0.7% [h]	ND	ND	ND
Shiga toxin	≥ 0.5%	ND	0.25% + 0.25 N [e]	ND

ND indicates "not determined" from available literature.

a. Solutions of NaOCl (≥ 0.1% final concentration; typically a 1:50 dilution of commercial bleach into distilled water) or NaOH (> 0.25 N) for 30 min inactivate BoNT and are recommended for decontaminating work surfaces and spills of *C. botulinum* or BoNT. Chlorine at a concentration of 0.3–0.5 mg/L as a solution of hypochlorite rapidly inactivates BoNT (serotypes B or E tested) in water.[23] Chlorine dioxide inactivates BoNT, but chloramine is less effective.[23,24] After decontamination, the solution is safe to discard in the sink as long as local ordinances are obeyed. Alternatively, BoNT can be absorbed onto a disposable napkin, dried, and disposed of in hazardous waste for incineration.

b. Ozone (> 2 mg/L) or powdered activated charcoal treatment also completely inactivate BoNT (serotypes A, B tested) in water under defined conditions.[24,25]

c. SEB is inactivated with 0.5% hypochlorite for 10–15 min.[26]

d. Ricin is inactivated by a 30-min exposure to concentrations of NaOCl ranging from 0.1–2.5%, or by a mixture of 0.25% NaOCl plus 0.25 N NaOH.[7] In general, solutions of 1.0% NaOCl are effective for decontamination of ricin from laboratory surfaces, equipment, animal cages, or small spills.

e. The minimal effective concentration of NaOCl was dependent on toxin and contact time; all LMW toxins tested were inactivated at least 99% by treatment with 2.5% NaOCl, or with a combination of 0.25% NaOCl and 0.25 N NaOH.[7]

f. For T-2 mycotoxin and brevetoxin, liquid samples, accidental spills, and nonburnable waste should be soaked in 2.5% NaOCl with 0.25 N NaOH for 4 h. Cages and bedding from animals exposed to T-2 mycotoxin or brevetoxin should be treated with 2.5% NaOCl and 0.25 N NaOH for 4 h. Exposure for 30 min to 1.0% NaOCl is an effective procedure for the laboratory (working solutions, equipment, animal cages, working area and spills) for the inactivation of saxitoxin or tetrodotoxin. Decontamination of equipment and waste contaminated with select brevetoxins has been reviewed.[19]

g. Conotoxins can also be inactivated using reducing agents such as dithiothreitol β- mercaptoethanol, or tris (2-carboxyethyl) phosphine (100 mM) at 65–100° C for 15 min, followed by alkylation with 100 mM maleimide in isopropanol at 65° C for 15 min. Alternatively, alpha conotoxins can be inactivated by hydrolysis in 10 N NaOH or HCl at 100° C for 30 min.[27]

h. Exposure of crude abrin solution and dried abrin to 0.67% NaOCl eliminated over 90% of cytotoxicity within 5 min.[28]

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