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**Safety and security guidelines for working with biological toxins**

**Background**: Biological toxins are hazardous substances produced by microorganisms, animals, insects, fungi and plants that can be harmful when inhaled, ingested, injected or absorbed. This document applies to the use of biological toxins (either native or synthetically /recombinantly produced) that present a life-threatening or severe irreversible health risk in an incident of a single exposure. This document provides guidance for researchers in Dublin City University and stipulates that research activities involving toxins be subject to review and approval by the Biological Safety Committee (BSC). A list of these toxins covered by this document is summarised in Table 1 and this list is subject to updating if deemed necessary by the Biological Safety Advisor (BSA). The risk of laboratory exposure is primarily from accidental injection, absorption through skin or mucous membranes, inhalation, and ingestion. Some toxins are also on a list of biological agents and toxins for “dual use research” because of the potential risk for the benevolent or harmful use of these toxins and therefore additional controls may be implemented if deemed necessary1. In Ireland dual use research is subject to EU Regulation 2018/1922 which implements recommendations of the Australia Group of nations on import / export controls in relation to the Chemicals Weapons Convention and the Biological Agents and Toxin Weapons Convention2. The UK and USA have additionally developed stringent controls on the use and storage of certain specified biological agents and toxins, which are termed ‘Select Agents’, under their own national security and anti-terrorism regulations. Current Select Agent Toxins are highlighted in bold in Table 1; note that not all of the highlighted toxins are considered Select Agents in both jurisdictions. DCU will adopt best practices internationally. In the UK the National Counter Terrorism Security Office (NaCTSO) carries out inspections and audits on sites registered to work with Select Agents under the Anti-Terrorism, Crime and Security Act 2001. Their security requirements expect a full inventory of stocks to be held and that all movement of Select Agents is tracked and recorded. Their expectation is for a site to carry out an annual audit, which they then review. NaCTSO do not give permission for sites to continue working with material until they can be assured that all samples are accounted for and recorded3. In the USA, in addition to standard Select Agent regulations there are further requirements for Botulinum neurotoxin because it has been designated as a tier 1 Select Agent presenting the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects to the economy, critical infrastructure, or public confidence. In addition to Botulinum neurotoxins (BoNT), the nucleic acids that encode for the functional forms of Botulinum neurotoxins that are expressed recombinantly or synthetically made are also subject to Select Agent regulations and (in the USA) Tier 1 requirements1. All work in DCU involving toxins from the list in Table 1 will require registration with, and approval by the Biological Safety Committee.

1. **Review and approval for possession and use of toxins:**
	1. Prior to working with the toxin the Principal Investigator (PIs) must complete and submit the following documents to the BSC:
		1. A copy of the biological toxin(s) registration form (Appendix 1)
		2. A copy of the risk assessment
		3. Manufacturer’s safety data sheet and or protocols for culture of biological agents or isolation or production of toxins (synthetically or recombinantly produced).
		4. A clear and user-friendly inventory for each toxin so the end user and audit team can quickly determine the amount of toxin in storage including undiluted vials, diluted stocks and aliquots.
		5. Standard operating procedures with relevant safety information.
		6. Occupational health advice must be sought on the requirement for vaccination.
		7. **PI must submit an SOP on how to deal with exposure and show evidence that they have liaised with a local accident and emergency department to make them aware of any potential hazards or risks in the event that an individual is exposed.**
	2. Prior to the final BSC approval the DCU BSA will meet with laboratory personnel who plan to use toxins in order to inspect the premises, review the proposed procedures and provide guidance on procedural refinements.
	3. Post BSC approval the following procedures must be followed:
		1. Quarterly audits will be performed to monitor stocks and PIs must agree to participate in occasional unplanned inspections by the BSA.
		2. HSA and EPA approval must be sought post-BSC approval if relevant.
		3. Up-to-date training records for all personnel authorised to handle the toxins must be maintained and available for inspection by BSC.
	4. All work performed in the Bioresource unit (BRU) involving toxins in animals (including modified toxins) is subject to approval by HPRA only following BSC approval. All adverse events for all toxins (including modified native, synthetic or recombinant toxins) involving animals must be reported to the BSA immediately. Quarterly updates of projects involving toxin must be submitted in writing to the BSC by BRU manager. These reports include a description of the nature of work, amount of toxin administered, the disposal of any toxin and a list of adverse events. All animal fatalities or severe adverse events must be reported to the BSA immediately.
	5. The BSA has the authority to discontinue any work with toxins if there is failure to record and account for any toxins or if there are failures to adhere to agreed protocols and processes.
2. **Registration of Toxins** Possession and use of toxin(s) must be registered with the BSC using the toxin registration form along with a Risk Assessment and standard operating procedures (SOPs) for the activity. The BSC must also approve projects involving the deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules prior to the initiation of the project. Work cannot begin until project has been authorised by the BSC and other relevant agencies such as the EPA. The toxins that require registration are selected based upon known LD50 values (Table 1). Please note that this list is not exhaustive, is subject to regular revision with additional biological toxins being registered as appropriate. Please contact the BSA prior to working with any toxin.

Modified toxins that are less toxic than the original toxin may be subject to less stringent controls. The BSC will review a request by the PI based upon LD50 experiments using OECD approved methods4. The BSC may consider advice from consultations with approved experts, evidence from relevant published studies and evidence from novel data generated in DCU. A case must be presented to the BSC in order to reduce any controls. Use of the toxin will continue to be monitored and additional controls reintroduced if there is a reintroduction of factor(s) associated with virulence, toxic activity, or other manipulations that modify the attenuation such that virulence or toxic activity is restored or enhanced. Please note modified toxins are not exempt from other regulatory, safety and good laboratory practice requirements. All work performed in the Bioresource unit (BRU) involving modified toxins in animals will continue to adhere to the same reporting requirements.

1. **Exposure Hazards associated with the toxins:** When working with the toxin avoid high risk procedures (see examples below) and if this is necessary, put in processes to mitigate risk. Exposure to dry toxin product can be a significant exposure risk through accidental inhalation and through dispersal of toxin residues to the surrounding environment. The BSC recommends that researchers work with toxin suspended in a diluent. If a manufacturer supplies toxin as a dried powder, use a premeasured product that will permit the addition of the diluent without the need to open the container and manipulate the toxin. If you MUST manipulate a dry toxin in an open vessel, you MUST develop specific safety procedures addressing that exposure risk.

Suitable containment measures and personal protective equipment must be used when working with toxins, especially if large amounts are used or high risk procedures are being performed (see below). Consider that toxins may not be retained fully by filters in Microbiological Safety Cabinets (MSCs) and that it might be necessary to use cabinets that are ducted to the external environment, or a fume hood. The most appropriate equipment to use will depend on the properties of the toxin in use and the types of activity being performed, with determination by risk assessment. Recommendations on physical containment measures to use when working with biological toxins can be found in the USA NIH/CDC guidebook ‘Biosafety in Microbiological and Biomedical Laboratories’5

#### The other significant exposure risk scenario is an accidental injection or cut while transferring or administering the toxin with a sharp device. Minimize as much as possible the use of sharps and those handling these devices need to follow sound safety practices to protect themselves and others who share the lab space from exposure to the toxin. Sharps handling procedures should be reviewed by the BSC and for toxin use additional steps may be required such as the use of pen needles.

#### It is advisable that additional safe work practices are implemented such as a “Buddy System” when working with concentrations or amounts near or exceeding the LD50 dose for a human or when conducting higher risk procedures. The researcher should limit glassware (no glass Pasteur pipettes), work over disposable, absorbent paper, use disposable containers and have spill kits or decontamination materials at the ready.

####  **Higher risk procedures:**

1. Use of aerosol or splatter-generating procedures (e.g. vortexing, grinding, centrifuging, intra-nasal inoculation of animals).
2. Utilization of concentrated stocks or large quantities of toxins (vial could contain more than a LD50 for an average-sized person).
3. Work with powdered or lyophilised/dried toxins: potential for inhalation and a tendency for electrostatic attachment to gloves, weighing spatulas, etc.
4. Use of needles or sharps in experimental procedures.
5. Sealed vials that are difficult to open (glass breakage, sharp metal band).
6. Removal of septa may result in dispersal of concentrated powder.
7. Alternatively, puncturing the septum with a needle and syringe provides potential for sharps injuries.
8. **Storage of and restricted access to toxins:** Biological toxins can only be stored and used under containment level 2 in limited amounts by a Principal Investigator (Table 2). They must be secured and accounted for at all times. If larger quantities are required an additional containment process will be required by the BSC (such as BSL3). Do not transfer the toxin to another Principal Investigator or another facility within DCU without notification of, and approval by the BSA. In all circumstances, personnel who work with the toxins that pose an acute high-risk exposure need to be on record with Occupational Health and other relevant parties to ensure that plans are put into place for a timely and effective medical response in the event of an exposure and that vaccination / health monitoring programs are implemented, if necessary. Maintain toxins in a secure storage device (i.e., locked box in a locked storage unit or locked box in a storage unit inside a locked lab) with access restricted to personnel authorized to work with the toxin. When toxins are in use, ensure adequate signage to prevent unauthorised person from entering the area where the work is being conducted.

 Toxins should be stored in a minimum of two layers of sturdy, leak-proof packaging with a biosafety hazard displayed on the secondary container. Display biohazard-warning labels in the designated areas or on items where toxins may be present. Only use minimal amounts of toxin when performing research procedures and track toxin usage from receipt to destruction in a secure inventory log. Report any suspicious activities or security concerns to the Biological Safety Advisor and to the Biological Safety Committee. To prevent stockpiling the amount permitted to be stored will be limited for selected toxins (Table 2).

1. **Transport of Toxins:** No transport of toxin is permitted without consultation with the BSA who liaises with a Dangerous Goods Safety Advisor to ensure that transportation is compliant with the requirement of ADR6 and or IATA7 guidelines. Transfer of toxins between laboratories must have BSA approval and must be done when human traffic is reduced. Technical officers or reception staff must be aware of the estimated delivery date and time.

### Exposure Management: Each toxin will present its own unique exposure risk and an SOP dealing with aerosol, skin, wound or parenteral exposure should be put in place including emergency planning. The PI must show evidence of liaison with the local accident and emergency department to make them aware of any potential hazards or risks in the event that an individual is exposed. In the event of exposure to the toxin you may need to isolate the individual or move to (or move person to) an uncontaminated area. The BSA may strongly recommend [vaccination](https://www.cdc.gov/vaccines/hcp/acip-recs/vacc-specific/tdap-td.html) for individuals working directly with certain toxins. This decision will be based on best practice and following the advice of the Occupational Health Nurse.

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1. **Training:** All persons with potential exposure to toxins must be familiar with the Safety Data Sheet (SDS) supplied by the manufacturer and must also read lab-specific toxin SOPs with signed training records indicating that they understand them. SOPs will cover both experimental procedures with toxins as well as emergency procedures. If procedures need to be updated, for example, in view of new information becoming available, SOPs must be updated and issued. Staff must read the new SOP as soon as possible and update their training records. Irrespective of whether SOPs have been updated or not, staff must re-read all SOPs at least once per year and record this in their training records. Work with toxins of biological origin should be carried out in accordance with BSL-2 or BSL-3 containment procedures. Training records for all personnel authorised to handle the toxins must be maintained and reviewed annually.

**Table 1: Non-exhaustive list of toxins of biological origin (with LD50 data) for registration with the BSC.**

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| **Toxin Name** | **Administration Route** | **LD50 (**g/kg) | **Ref** |
| **Abrin** | oral (humans) | 0.7 | 8 |
| Aflatoxin B1 | oral (rat) | 7200 | 9 |
| Aflatoxin B2 | oral(duckling) | 1680  | 9 |
| Aflatoxin M1 | oral (mouse) | 9000  | 9 |
| Aflatoxin G1 | oral (duckling) | 790  | 9 |
| Aflatoxin G2 | oral (duckling) | 2500  | 9 |
| Azaspiracid |  humans | LOAEL 0.4-2 g/kg | 10 |
| Aerolysin | IV | 7 | 11 |
| **Botulinum Toxin A** | IP |  0.0012 | 11 |
| **Botulinum Toxin B** | IP |  0.0012 | 11 |
| **Botulinum Toxin C1** | IV | 0.0011  | 11 |
| **Botulinum Toxin C2** | IP | 0.0012 | 11 |
| **Botulinum Toxin D** | IP | 0.0004  | 11 |
| **Botulinum Toxin E** |   | 0.0011 | 11 |
| **Botulinum Toxin F** | IV | 0.0025  | 11 |
| Β-Bungarotoxin | IP/IV | 7.8/10 | 11 |
| Caeruleotoxin | IV | 40 | 11 |
| Cereolysin | IV | 40**–**80 | 11 |
| Ciguatoxin 2 | IP | 1 | 11 |
| Ciguatoxin 3 | IP | 0.9 | 11 |
| *Clostridium difficile* enterotoxin A | IP | 0.5 | 11 |
| *Clostridium perfringens* alpha toxin, lecithinase | IV | 3 | 11 |
| *Clostridium perfringens* theta toxin, perfringolysin O | IV | 13-16 | 11 |
| ***Clostridium perfringens* enterotoxin** | IV | 81 | 11 |
| *Clostridium perfringens* beta-toxin | IP/IV | 4.5/0.31 | 11 |
| *Clostridium perfringens* delta-toxin | IV | 5 | 11 |
| ***Clostridium perfringens* epsilon-toxin** | IV  | 0.1 | 11 |
| **Conotoxins** | IV  | 10-100  | 11 |
| Crotoxin | IV | 82 | 11 |
| Deoxynivalenol | Oral (mouse) | 46000-78000 | 12 |
| **Diacetoxyscirpenol (DAS)** | IV | 1100 | 11 |
| Diphtheria toxin | IV/IP | 0.01/6.5 | 11 |
| Fumonisin B1 | Ingestion (Cultured Tilapia) | 600 | 12 |
| Listeriolysin | IV | 3-12 | 11 |
| Maitotoxin | IP | 0.6 | 11 |
| Microcystin LR | Oral (mouse); IP (mouse) | 5000; 32.5 | 12 |
| **Modeccin** | IP | 1.3 | 11 |
| Nematocyst toxins | IV | 33-70 | 11 |
| Notexin | IV | 5 | 11 |
| Ochratoxin A | Oral (rat) | 20000 | 12 |
| Palytoxin | IV/IP | 0.089/0.05 | 11 |
| Patulin | Oral (rat) | 27790 | 12 |
| Pertussis toxin | IP | 18 | 11 |
| Pneumolysin | IV | 1.5 | 11 |
| *Pseudomonas aeruginosa* exotoxin A | IP/IV | 14/3 | 11 |
| **Ricin** | IV/IP | 2.2/1.5 | 11 |
| **Saxitoxin** | IV | 3-9 | 11 |
| Scaritoxin | IP | 50 | 11 |
| **Shiga toxin** | IP/IV | 0.25/0.0022  | 11 |
| *Shigella dysenteriae* neurotoxin | IP/IV | 1.3/0.45 | 11 |
| Streptolysin O | IV | 8 | 11 |
| Streptolysin S | IV | 25 | 11 |
| *Staphylococcus aureus* alpha toxin | IV | 0.04-0.06 | 11 |
| ***Staphylococcus aureus* enterotoxin A,B,C,D,E** | IV | 25-1333 | 13 |
| **T-2 toxin** | IV | 1000 | 11 |
| Taipoxin | IV | 2 | 11 |
| Tetanus toxin | SC | 0.003 | 11 |
| **Tetrodotoxin** | IV/IP | 8 | 11 |
| **Viscumin** | IV | 2.4 | 11 |
| **Volkensin** | IP | 1.38 | 11 |
| *Yersinia pestis* murine toxin | IV | 10 | 11 |
| -Zearalenone | oral (rat) | 4000000 | 12 |

IV = intravenous; IP = intraperitoneal; SC = subcutaneous
Toxins in bold are designated Select Agent toxins in Schedule 5 of the (UK) Anti-Terrorism, Crime and Security Act 2001 or by the US Department of Health and Human Services (HHS) and Department of Agriculture (USDA). <http://www.healthandsafety.manchester.ac.uk/toolkits/returns/schedule5pathogensandtoxins/schedule5list/>
LD50 = median lethal dose; the smallest amount that can kill 50 % of a test group of subjects

LOAEL=Lowest observed adverse effect level

**Table 2. Limits of Select Agent toxins permitted in Containment level 2**

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| **Toxin Name** | **LD50****(µg/kg)** | **Recommended Limit per PI** | **Maximum Limit****in storage at DCU** |
| Abrin | 0.7 | 1000 mg | 5000 mg |
| Botulinum neurotoxins | 0.001 | 1 mg | 5 mg |
| Short, paralytic alpha conotoxins | 12-30 | 100 mg | 500 mg |
| Diacetoxyscirpenol (DAS) | 750 | 10,000 mg | 50,000 mg |
| Ricin | 2.7 | 1000 mg | 5000 mg |
| Saxitoxin | 8 | 500 mg | 2500 mg |
| Staphylococcal Enterotoxins (Subtypes A, B, C, D & E) | 25 | 100 mg | 500 mg |
| T-2 toxin | 2700 | 10,000 mg | 50,000 mg |
| Tetrodotoxin | 8 | 500 mg | 2500 mg |
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