Hazard Assessment and PPE Recommendations for Biochemistry Labs (CHEM 313, 371, 474, 475)

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Background: Per the department safety committee guideline adopted in March 2017, this document describes the hazard assessment performed for all laboratory experiments and procedures in 200-level and above undergraduate laboratories in the biochemistry division. The purpose of this hazard assessment is to provide justification that the default laboratory personal protective equipment (PPE) suggested for upper-division laboratories is unnecessary in most biochemistry labs except as described below. Note that the University-wide minimum requirement of splashproof goggles and closed-toed shoes remains in effect for *all* labs listed below. This assessment is not meant to replace University or Cal-OSHA requirements and in the event of a conflict, those guidelines supersede the recommendations set forth in this document.

CHEM 313: Survey of Biochemistry and Biotechnology

Lab 1: Micropipetting

Hazards identified: Eye/skin contact with basic solution (carbonate buffer, pH 11) and irritant (para-nitrophenol; harmful if swallowed, eye/skin irritant)
Recommended PPE: University minimum (goggles/closed-toed shoes)
Justification: Due to the small volumes involved (< 50 mL of all solutions, manipulated in portions of ≤ 2 mL at a time), risk of ingestion or large-scale skin contact is considered minimal.

Lab 2: Buffers and Buffer Calculations

Hazards identified: Eye/skin contact with 0.05 M sodium hydroxide (corrosive liquid) and 0.010 M histidine (eye/skin irritant).
Recommended PPE: Gloves (when handling sodium hydroxide solutions); university minimum
Justification: Sodium hydroxide is highly corrosive and can cause burns and permanent eye damage. The use of splashproof goggles should minimize the risk of eye contact. The dilute solutions used in the lab should not generate a risk of immediate burns in case of skin contact (provided that affected skin is thoroughly washed with water); therefore the use of lab coats or other full-body PPE is deemed unnecessary.

Lab 3: Protein Modeling

Hazards identified: None (computer simulation)
Recommended PPE: None (if performed in department computer lab); university minimum (if performed in 180-368)
Justification: Since this lab is entirely computer-based, PPE is unnecessary.

Lab 4: Determination of Protein Concentration Using the Biuret Method

Hazards identified: Eye/skin contact with Biuret reagent (2.0 M sodium potassium tartrate, 1.5 M sodium hydroxide, 0.6 M copper(II) sulfate; corrosive/toxic liquid).
Recommended PPE: Gloves (when handling Biuret reagent); university minimum
Justification: While the Biuret reagent is highly corrosive, the modest volumes used in the lab (≤ 25 mL per group) make the risk of large-scale exposure slight. The greatest risk of exposure

occurs during transfer to and from test tubes, and during mixing. Spills in these cases tend to be confined to the benchtops, where they can be easily cleaned up. Use of gloves is recommended during manipulations involving the reagent. Due to the small volumes involved, however, whole-body PPE is not required.

Lab 5: Enzyme Kinetic Study of Glucose Oxidase
Hazards identified: Eye/skin contact with acidic (pH 5) or basic (pH 9.5) glucose oxidase assay mixtures
Recommended PPE: University minimum
Justification: Two of the glucose oxidase assay mixtures have pH values acidic or basic enough to be potentially corrosive; however, the small volumes used (2.0 mL each) and only modestly acidic/basic pH values make the risk of injury minimal. University minimum PPE is deemed sufficient to prevent exposure.

Lab 6: Fish Protein SDS-PAGE Gel Electrophoresis
Hazards identified: Exposure to residual unpolymerized acrylamide solution (toxic/carcinogenic liquid); exposure to Coomassie stain/destain solutions (30% methanol/10% acetic acid; toxic/corrosive liquid); exposure to β-mercaptoethanol in sample preparation buffer (toxic liquid); exposure to glycine-SDS running buffer (irritant); use of electrophoresis power supplies (risk of electrical shock; fire/explosion hazard if gel apparatus allowed to overheat)
Recommended PPE: Gloves (when handling polyacrylamide gels or applying stain/destain solutions); university minimum at other times
Justification: Since gels are cast by the staff prior to lab, the risk of acrylamide exposure is considered minimal; nonetheless students are instructed to wear gloves when handling gels. Similarly, the risk of exposure to the Coomassie stain and destain solutions is limited to the transfer of gels to and from stain solution; the volumes involved (< 20 mL) are sufficiently small that large-scale exposure is not considered a significant risk. Significant β-mercaptoethanol exposure is considered very unlikely due to the small volumes (microliters) encountered in the lab. The risk of SDS running buffer exposure is limited to eye contact, which is prevented by the University-mandated splashproof goggles. PPE above and beyond the university minimum is unlikely to prevent injury in the event of an electrical shock or fire/explosion; moreover, these risks are considered very small because students are instructed in the proper use and connection of the power supplies, and the gels are continuously monitored by the teaching staff to prevent drying/overheating.

Lab 7: ELISA Immunoassay to Determine Saliva IgA Concentration
Hazards identified: Contact with tris-buffered Triton detergent wash (eye/skin irritant); o-phenylenediamine/hydrogen peroxide solution (irritant; oxidizer); human saliva (potential biohazard)
Recommended PPE: University minimum
Justification: While several of the reagents in this lab are potential irritants, the low concentrations and small volumes present make the risk of exposure small. The risk of exposure to biohazardous agents (bacteria, viruses) is considered insignificant because students are instructed to handle only their own saliva. University minimum PPE is sufficient for the risk level involved.

Lab 8: DNA Forensics
Hazards identified: Exposure to tris-acetate-EDTA (TAE) buffer (irritant); exposure to hot agarose solution (burn hazard)
Recommended PPE: University minimum
Justification: University minimum PPE is sufficient to minimize the small risk of exposure to TAE buffer. The Gel Red stain used in this lab (as a safer replacement for ethidium bromide) is nontoxic at the concentrations used, and is dispensed by the teaching staff so exposure is unlikely. Hot agarose solution presents a burn risk (especially if it boils and overflows the top of its container); however, students receive very specific instruction on how to avoid sudden boiling, and the solution remains dangerously hot only for a short period (minutes). Thus, university minimum PPE is deemed sufficient.

Lab 9: Determination of Antioxidant Levels in Consumer Beverages
Hazards identified: Exposure to 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid solution (harmful, irritant)
Recommended PPE: University minimum
Justification: The volumes of hazardous material involved in this lab are small enough (mL) that large-scale exposure is unlikely, so university minimum PPE is sufficient.

CHEM 371: Biochemical Principles

Lab 1: Buffers and UV-Visible Spectrophotometry
Hazards identified: Exposure to 0.10 M sodium phosphate solutions (irritant); exposure to 0.10 M hydrochloric acid (corrosive liquid); exposure to bovine serum albumin (BSA) solution (potential eye irritant)
Recommended PPE: University minimum
Justification: University minimum eye protection is sufficient to protect against the small risk of eye exposure of the irritants in this lab. The most dangerous compound in this lab, hydrochloric acid, is at a low enough concentration that burns/irritation are unlikely in the case of skin contact so long as exposed skin is washed. Additional PPE is therefore deemed unnecessary.

Lab 2: Determination of Concentration of a Specific Protein Using ELISA Assay
Hazards identified: Contact with tris-buffered Triton detergent wash (eye/skin irritant); o-phenylenediamine/hydrogen peroxide solution (irritant; oxidizer); human saliva (potential biohazard)
Recommended PPE: University minimum
Justification: While several of the reagents in this lab are potential irritants, the low concentrations and small volumes present make the risk of exposure small. The risk of exposure to biohazardous agents (bacteria, viruses) is considered insignificant because students are instructed to handle only their own saliva. University minimum PPE is sufficient for the risk level involved.

Lab 3: Lysozyme Purification by Ion-Exchange Chromatography
Hazards identified: Exposure to wash buffer (100 mM glycine pH 9; irritant), exposure to elution buffer (100 mM glycine, 0.50 M sodium chloride, pH 9.0; irritant); exposure to sodium

phosphate buffer solution (irritant), exposure to *Bacillus* bacterial suspension (biohazard); contact with egg white (potential allergen).
Recommended PPE: Gloves (when handling *Bacillus* suspension); university minimum at other times.
Justification: University minimum eye protection is sufficient to guard against eye exposure to the irritants in this lab. The use of resuspended *Bacillus* in the lysozyme assay creates a potential for infectivity (particularly in the case of eye contact or contact with open wounds/sores), but students are instructed to wear gloves and eye protection when handling the bacteria, and only small volumes (1-2 mL) are used, so the infectivity risk is slight. Students with egg allergies are informed ahead of time to avoid contact with the egg white solution or to wear gloves. Additional PPE beyond the university minimum is unlikely to lead to any further reduction in risk.

Lab 4: Analysis of Protein Concentration and Absorptivity (Biuret Assay)
Hazards identified: Eye/skin contact with Biuret reagent (2.0 M sodium potassium tartrate, 1.5 M sodium hydroxide, 0.6 M copper(II) sulfate; corrosive/toxic liquid).
Recommended PPE: Gloves (when handling Biuret reagent); university minimum
Justification: While the Biuret reagent is highly corrosive, the modest volumes used in the lab ($\leq$ 25 mL per group) make the risk of large-scale exposure slight. The greatest risk of exposure occurs during transfer to and from test tubes, and during mixing. Spills in these cases tend to be confined to the benchtops, where they can be easily cleaned up. Use of gloves is recommended during manipulations involving the reagent. Due to the small volumes involved, however, whole-body PPE is not required.

Lab 5: Purification of Glutamate-Oxaloacetate Transaminase (GOT)
Hazards identified: Exposure to maleate-EDTA buffer (harmful, irritant); exposure to acetate and sodium phosphate buffers (irritant); exposure to ammonium sulfate (irritant)
Recommended PPE: University minimum
Justification: Considerable volumes of buffer are used in this experiment; however, the associated hazard is largely that of eye exposure, which is prevented by university minimum PPE. Thus, additional PPE is deemed unnecessary.

Lab 6: Analysis of Purified Fractions of GOT for Activity
Hazards identified: Exposure to maleate-EDTA buffer (harmful, irritant); exposure to acetate and sodium phosphate buffers (irritant); exposure to ammonium sulfate (irritant); exposure to malate dehydrogenase/NADH assay mixture (irritant)
Recommended PPE: University minimum
Justification: The hazards listed are largely limited to eye exposure, which is mitigated by university minimum PPE. This, combined with the small volumes of solution used, makes additional PPE unnecessary.

Labs 7 and 8: Determination of K_M Values for GOT
Hazards identified: Exposure to maleate-EDTA buffer (harmful, irritant); exposure to acetate and sodium phosphate buffers (irritant); exposure to ammonium sulfate (irritant); exposure to malate dehydrogenase/NADH assay mixture (irritant)
Recommended PPE: University minimum

Justification: The hazards listed are largely limited to eye exposure, which is mitigated by university minimum PPE. This, combined with the small volumes of solution used, makes additional PPE unnecessary.

Lab 9: SDS-PAGE Electrophoresis of GOT Fractions

Hazards identified: Exposure to residual unpolymerized acrylamide solution (toxic/carcinogenic liquid); exposure to Coomassie stain/destain solutions (30% methanol/10% acetic acid; toxic/corrosive liquid); exposure to β-mercaptoethanol in sample preparation buffer (toxic liquid); exposure to glycine-SDS running buffer (irritant); exposure to 30% trichloroacetic acid solution (corrosive); use of electrophoresis power supplies (risk of electrical shock; fire/explosion hazard if gel apparatus allowed to overheat)

Recommended PPE: Gloves when working with trichloroacetic acid solution; university minimum at other times.

Justification: Trichloroacetic acid is highly corrosive and is an acute burn risk in case of skin exposure. Gloves are therefore recommended when working with this reagent. However, this substance is confined to the fume hood and students are clearly instructed to transfer only the volume needed to precipitate protein in each fraction (<1 mL per fraction, <4 mL total), so large-scale exposure is unlikely and further PPE is not warranted. Since gels are cast by the staff prior to lab, the risk of acrylamide exposure is considered minimal; nonetheless students are instructed to wear gloves when handling gels. Similarly, the risk of exposure to the Coomassie stain and destain solutions is limited to the transfer of gels to and from stain solution; the volumes involved (< 20 mL) are sufficiently small that large-scale exposure is not considered a significant risk. Significant β-mercaptoethanol exposure is considered very unlikely due to the small volumes (microliters) encountered in the lab. The risk of SDS running buffer exposure is limited to eye contact, which is prevented by the University-mandated splashproof goggles. PPE above and beyond the university minimum is unlikely to prevent injury in the event of an electrical shock or fire/explosion; moreover, these risks are considered very small because students are instructed in the proper use and connection of the power supplies, and the gels are continuously monitored by the teaching staff to prevent drying/overheating.

CHEM 474: Protein Techniques Laboratory

Project 1: Purification and Activity Assays of Bacterial Alkaline Phosphatase

Hazards identified: Exposure to *E. coli* suspension culture (biohazard), exposure to Tris/EDTA/sodium chloride/magnesium chloride/zinc chloride buffer solutions (potential irritants), exposure to SDS running buffer (irritant, harmful), exposure to para-nitrophenyl phosphate (harmful, irritant), exposure to Coomassie stain/destain solution (methanol-acetic acid, flammable and toxic liquid), exposure to acrylamide solution (toxic/carcinogenic liquid), use of electrophoresis power supplies (risk of electric shock and fire/explosion hazard)

Recommended PPE: Gloves and lab coat or apron when using acrylamide solutions to pour gels; gloves when handling bacterial cultures; university minimum at other times

Justification: Acrylamide is toxic and carcinogenic at low doses. The fact that acrylamide/bis-acrylamide solutions (rather than powder) are used in this lab helps to mitigate the exposure risk by eliminating dust. However, the high likelihood of spills while pouring gels and the non-volatile nature of acrylamide produce a considerable risk of contamination of surfaces and clothing. Therefore, it is recommended that all students wear either full-length buttoned lab

coats (preferably) or full-length aprons, with gloves, while working with acrylamide solutions and while working in the lab until all surfaces have been cleaned by staff. This greatly reduces the chance of accidental exposure through contaminated clothing or through contact with a contaminated surface (e.g., benchtop). Acrylamide is not an acute hazard by skin contact, so in cases of larger-scale exposure washing of skin can remove acrylamide. For other procedures in this project, minimum laboratory PPE (goggles and closed shoes) are sufficient to minimize the risk of exposure, due to the relatively non-hazardous nature of the materials involved. Additionally, gloves are recommended when handling *E. coli* suspensions, but the small volumes involved (< 10 mL) and the fact that viable bacteria are only encountered once in the project makes the exposure/infectivity risk minimal. The electrophoresis apparatus will be monitored by staff to prevent overheating and evaporation; additional PPE is considered unhelpful in the event of an electric shock or fire/explosion during gel electrophoresis.

Project 2: Purification and Analysis of Recombinant Green Fluorescent Protein
Hazards identified: Exposure to *E. coli* suspension culture (biohazard), exposure to Tris and sodium phosphate buffers (potential irritants), contact with imidazole powder and solution (harmful, irritant, potentially corrosive), exposure to β-mercaptoethanol (toxic liquid), exposure to SDS running buffer (irritant, harmful), exposure to acrylamide solution (toxic/carcinogenic liquid), use of electrophoresis power supplies (risk of electric shock and fire/explosion hazard)
Recommended PPE: Gloves and lab coat or apron when using acrylamide solutions to pour gels; gloves when handling bacterial cultures; university minimum at other times
Justification: See Justification for Project 1, above. The only additional hazard present in this project is the exposure to neat β-mercaptoethanol; however, use of this reagent is confined to the fume hood and students are instructed to only pipet the minimal volume needed for preparation of protease digestion buffer (≤ 1 mL). Thus, exposure to this substance is considered only a slight risk.

CHEM 475: Molecular Biology Laboratory

Lab 0: Micropipetting
Hazards identified: Eye/skin contact with para-nitrophenol (harmful, irritant)
Recommended PPE: University minimum
Justification: The small volumes of PNP involved in this experiment make the risk of exposure small; the university minimum PPE of goggles and closed shoes are thus sufficient to minimize exposure.

Lab 1: Human SNP Analysis
Hazards identified: Exposure to tris-acetate-EDTA (TAE) buffer (irritant); exposure to hot agarose solution (burn hazard); use of electrophoresis power supplies (risk of electrical shock; fire/explosion hazard if gel apparatus allowed to overheat)
Recommended PPE: University minimum
Justification: University minimum PPE is sufficient to minimize the small risk of exposure to TAE buffer. The Gel Red stain used in this lab (as a safer replacement for ethidium bromide) is nontoxic at the concentrations used, and is dispensed by the teaching staff so exposure is unlikely. Hot agarose solution presents a burn risk (especially if it boils and overflows the top of its container); however, students receive very specific instruction on how to avoid sudden

boiling, and the solution remains dangerously hot only for a short period (minutes). Thus, university minimum PPE is deemed sufficient.

Lab 2: Yeast RNA Isolation

Hazards identified: Eye/skin exposure to phosphate-buffered saline (PBS) solution containing $MgCl_2$ and sorbitol (irritant); exposure to guanidinium thiocyanate (harmful); exposure to diethyl pyrocarbonate (DEPC, toxic); contact with 50% v/v phenol in chloroform (toxic, corrosive liquid); exposure to hot agarose solution (burn hazard); exposure to ethidium bromide (carcinogen/mutagen); use of electrophoresis power supplies (risk of electrical shock; fire/explosion hazard if gel apparatus allowed to overheat).

Recommended PPE: Gloves and lab coat/apron when working with phenol/chloroform; gloves when working at ethidium bromide-contaminated workstations (e.g. gel imager) or when handling ethidium bromide-stained gels; university minimum at other times.

Justification: Phenol can produce deep skin burns on contact with even small amounts of liquid; therefore, it is advised that students working with phenol/chloroform solutions be covered from the neck down to minimize the risk of contact. Ethidium bromide is a potent carcinogen active at low concentrations; thus, workstations where ethidium bromide has been used will be marked as such and gloves will be required when working at these sites or when handling ethidium bromide-stained gels in order to minimize skin contact (Gel Red stain is ineffective at staining RNA, so ethidium bromide remains the stain of choice for this experiment). At other times, university minimum PPE is sufficient to prevent exposure to potentially harmful or irritating substances due to the relatively small volumes concerned. For the risk due to agarose solutions, see Lab 1.

Lab 3: Amplifying a Yeast Gene Using RT-PCR

Hazards identified: Exposure to PCR buffer mix (dNTP mix, buffer, enzyme, $MgCl_2$, yeast DNA; potential irritants)

Recommended PPE: University minimum

Justification: The small volumes of reagents involved in this experiment make exposure a very small concern; thus, university minimum PPE is likely sufficient to prevent exposure.

Lab 4: Cloning an RT-PCR Fragment Into a Vector

Hazards identified: Contact with *E. coli* cultures (biohazard); exposure to PCR buffer mix (dNTP mix, buffer, enzyme, $MgCl_2$, yeast DNA; potential irritants)

Recommended PPE: Gloves when handling *E. coli* cultures; university minimum at other times.

Justification: Gloves are recommended when handling *E. coli* suspensions, but the small volumes involved (< 10 mL) make exposure/infectivity risk minimal. Other procedures in this lab involve small volumes of potentially irritating or harmful chemicals for which minimal PPE will provide adequate protection.

Lab 5: Plasmid Isolation and Verification of Insert in Plasmid Clones

Hazards identified: Contact with *E. coli* cultures (biohazard);; Exposure to tris-acetate-EDTA (TAE) buffer (irritant); exposure to hot agarose solution (burn hazard); use of electrophoresis power supplies (risk of electrical shock; fire/explosion hazard if gel apparatus allowed to overheat)

Recommended PPE: Gloves when handling *E. coli* cultures; university minimum at other times.

Justification: Gloves are recommended when handling *E. coli* suspensions, but the small volumes involved (< 10 mL) make exposure/infectivity risk minimal. Other procedures in this lab involve small volumes of potentially irritating or harmful chemicals for which minimal PPE will provide adequate protection.

Lab 6: *In Silico* Analysis of Plasmid Clones
Hazards identified: None (computational only)
Recommended PPE: None
Justification: Since this lab consists entirely of data analysis and planning of experiments, PPE is not necessary.

Lab 7: Restriction Mapping of Recombinant Plasmid Clones
Hazards identified: Use of electrophoresis power supplies (risk of electrical shock; fire/explosion hazard if gel apparatus allowed to overheat); exposure to tris-acetate-EDTA (TAE) buffer (irritant); exposure to hot agarose solution (burn hazard).
Recommended PPE: University minimum
Justification: See CHEM 475 Lab 1, for which the hazards are nearly identical to those in this lab. Note that the use of GelRed DNA stain in place of ethidium bromide greatly reduces hazard.

Lab 8: DNA Sequence Analysis
Hazards identified: None (DNA sequencing performed externally)
Recommended PPE: None
Justification: Since this lab will involve only analysis of sequences determined off-site, there is no need for PPE.