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M E M O R A N D U M

TO: Laboratory Quality Assurance Committee Members

FROM: Ken B. Moore, Executive Director Ken B. Moore

DATE: July 23, 2004

RE: 2004-2005 Committee Charges

This memorandum is to confirm your appointment by the Conference Executive Board Chairman to the Laboratory Quality Assurance Committee. Joel Hansel will serve as Committee Chairman.

The Laboratory Quality Assurance Committee is assigned the following tasks for 2004-2005:

- Proposal 03-107: Neurotoxic Shellfish Toxins (Mouse Bioassay) Laboratory Evaluation Checklist (in conjunction with the Biotoxin Committee)
- Proposal 03-109: Education Requirements for Program Supporting Laboratories

If you are unable to participate in the activities of this subcommittee, please contact us at 803-788-7559 or issc@issc.org. Thank you for your interest and support of the ISSC and we look forward to working with you. Your Committee Chairperson will be contacting you soon.

/nsd
Attachments

2004-2005 Laboratory Quality Assurance Committee Roster		
Chair:	Hansel, Joel EPA	hansel.joel@epa.gov
Members:		
	Burrow, Richard	burrowr@agriquality.com
	Chandler, Linda FDA	linda.chandler@cfsan.fda.gov
	Cumbo, Mercuria	mercuria.cumbo@maine.gov
	Dorsey, Carol	caroldorsey@adph.state.al.us
	Fowler, Patti	patti.fowler@ncmail.net
	Karolus, John	jjkarolus@snet.net
	LaValley, Ken	kjlavalley@spinneycreek.com
	Porter, Leonora	lxporter@gw.dec.state.ny.us
	Ruple, Angela NOAA	angela.ruple@noaa.gov
	Suavé, Gilbert	sauveg@inspection.gc.ca
	Thompson, Eric	eric.thompson@doh.wa.gov
	Vargas, Arturo	aprandiz@salud.gob.mx
	Wickman, Kathleen	kwickman@oda.state.or.us

PROPOSAL NO. 03-107:

Specific Reference:	Chapter III Laboratory @ .01 Quality Assurance D. (1) Page 38
Text of Proposal/ Requested Action:	Chapter III@.01.D. (1) add the following to the end of the existing sentence (Laboratory evaluation criteria listed in Section IV Guidance Documents). The suggested NSP checklist is provided in the attached file.
Public Health Significance:	An NSSP standardized NSP laboratory evaluation checklist will allow objective evaluation of laboratory conformance with NSSP requirements.
Cost Information (if available):	N/A
ACTION BY 2003 TASK FORCE I	Recommended that Proposal 03-107 be referred the appropriate committee as determined by the Conference Chairman.
ACTION BY 2003 GENERAL ASSEMBLY	Adopted recommendation of 2003 Task Force I.
ACTION BY USFDA	Concurred with Conference action.

PROPOSAL NO. 03-107:

DRAFT

Analysis for Neurotoxic Shellfish Toxins – Mouse Bioassay

*** Indicates that this is not in the *Recommended Procedures*, 4th Edition**

Weighted code		Item Description
		Quality Assurance (QA) Plan
C		1. Written Plan adequately covers the following (check those that apply): a. _____ Organization of the laboratory. b. _____ Staff training requirements. c. _____ Standard operating procedures. d. _____ Internal quality control measures for equipment, calibration, maintenance, repair and performance. e. _____ Laboratory safety. f. _____ External FDA proficiency testing.
C*		2. QA Plan is implemented.
		Work Area
O		1. Adequate for workload and storage.
O*		2. Clean and well lighted.
O*		3. All work surfaces are nonporous and easily cleaned.
K*		4. A separate, quiet area with adequate temperature control is maintained for acclimation and injection of mice.
C*		5. Following CIS guidelines, a closed system, e.g., room with adequate ventilation With explosion- proof electrical equipment and lighting has to be used for diethyl ether extractions. All electrical outlets and switches have to be on the outside of the room to avoid sparks and the fume hood should be without electrical service in the hood.
		Laboratory Equipment
K		1. The differing sensitivities in weight measurements required by various steps in the extraction procedure as well as the bioassay are met by the balances being used. a. _____ To determine sample weight, a sensitivity of at least 0.1 g at load of 100 g is required. b. _____ To determine the weight of the lipid extract and its subsequent volume adjustment, a sensitivity of at least 10 mg at loads of 1 and 10 g is required. c. _____ To determine the weight of the mice used in the bioassay, a sensitivity of 0.1 g at a load of 20 g is required.
O*		2. The calibrations of the balances are checked monthly using NIST Class S or ASTM Class 1 or 2 weights or equivalent. Records are maintained.
K*		3. The temperature maintained by the refrigerator is between 0 and 5°C.
O*		4. Refrigerator temperature is monitored at least once daily. Temperatures are recorded and records are maintained.

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		Reagents
K		1. Concentrated (12N) HCl is used to acidify the homogenate.
O		2. Reagent grade NaCl is used in the extraction procedure.
C		3. Diethyl ether purified for lipid extraction is used for extracting lipids from the shellfish homogenates.
C*		4. Cottonseed oil (0.917 g/ml) or a solvent with a similar density is used as the toxin delivery system. Name of the solvent if substituted for cottonseed oil. Density _____
		Collection and Transportation of Samples
O*		1. Shellstock are collected in clean, waterproof, puncture resistant containers.
K*		2. Samples are appropriately labeled with the collector's name, the harvest area and the time and date of collection.
K*		3. Immediately after collection, shellstock samples are placed in dry storage between 0 and 10°C until analyzed.
K*		4. Shellstock samples are analyzed within 24 hours of collection or refrigerated unshucked until analyzed.
K*		5. Refrigerated storage of shellstock does not exceed 48 hours.
K*		6. If shellstock is refrigerated, only live animals are used in the analysis.
K*		7. If shellfish are shucked in a location other than the laboratory, they must be prepared according to steps 1- 9 in "Preparation of Sample" section below. Samples are then double bagged.
		Preparation of Sample
C*		1. At least 12 animals are used per sample and a minimum of 100 grams of meat.
O		2. The outside of the shell is thoroughly cleaned with fresh water.
K*		3. Shellstock is opened by cutting the adductor muscles.
C		4. Shell liquor is discarded.
O*		5. The inside of the shells is rinsed with fresh water to remove sand or other foreign material.
K*		6. Shellfish meats are removed from the shell by separating the adductor muscles and tissue connecting at the hinge.
K*		7. Damage to the body of the mollusk is minimized in the process of opening.
O		8. Shucked shellfish are drained on a #10 mesh sieve or equivalent without layering for 5 minutes.
K*		9. Pieces of shell and drainings are discarded.
C		10. Drained meats are blended at high speed until homogenous (60-120 seconds).
K*		11. Shellfish homogenates are digested the same day they were blended.
		Digestion of Sample
K*		1. All glassware used is clean and properly washed with a succession of at least three fresh water rinses, 1.2 N HCl, and a final distilled/deionized rinse to remove residual detergent.
K		2. 100 grams (or entire sample amount if less than 100 grams is available) homogenized sample is weighted into a beaker.

C*		3. 1 ml of HCl and 5 g NaCl is added to the 100 gram homogenate and thoroughly mixed. (For samples <100 g, add reagents to obtain final concentrations of 0.12N HCl and 5% NaCl.)
C*		4. The homogenate is brought to a rolling boil and once 100 + 1°C (sea level) is reached, gently boil for a minimum of 5 minutes and until frothing ceases.
O*		5. The beaker is covered with a watch glass or equivalent during boiling to prevent excessive evaporation.
O*		6. The homogenate is boiled under adequate ventilation (fume hood).
O*		7. The boiled, acidified homogenate is cooled to room temperature or below in a refrigerator or in an ice bath.
		Extraction
C*		1. All steps in the extraction procedure which involve any manipulation of diethyl ether are carried out under adequate ventilation in a closed system that has explosion-proof electrical equipment and lighting following CIS guidelines. NO sparks.
C		2. 100 ml of diethyl ether is added to the cooled, acidified homogenate in a stoppered centrifuge tube and shaken vigorously for 5 minutes.
O		3. Centrifuge tubes are vented frequently while being shaken and before being centrifuged to avoid accidents.
C		4. The content of the centrifuge tubes are centrifuged at 2000 rpm for 10 to 15minutes.
C*		5. The clear upper ether phase is transferred to a large separatory funnel or pre-weighed beaker. Any emulsion in the centrifuge bottle is excluded.
C*		6. The contents of the centrifuge tube are extracted three additional times for a total of four times, each time with 100 ml of diethyl ether. The upper phases are combined together in either the separatory funnel or the pre-weighed beaker (as in step 5).
C		7. If a separatory funnel is used, the ether extract is transferred to a large, clean, dry pre-weighed beaker (first discarding any emulsion or tissue that may have settled in the funnel.)
C		8. Ether is evaporated to dryness.
C		9. The final lipid residue is weighted and the weight is recorded.
		Bioassay
C		1. The volume of the lipid residue is adjusted by weight to 10 ml (9.17 g) per 100 g shellfish extracted using cottonseed oil. If a solvent with a density similar to cottonseed oil is used, the volume is adjusted to a weight 10 times the density of the solvent. Specify the weight to which the volume is adjusted to. _____
K*		2. A 25 gauge hypodermic needle is used for injection.
C		3. Healthy male mice in the weight range of 17 to 23 grams from a stock colony are used for routine assays. Stock strain used _____. Source of the mice _____.
C*		4. Mice are allowed to acclimate for at least 24 hours prior to injection. In some cases up to 48 hours may be required. Typical length of the period of acclimation is _____.
O*		5. Mice are weighed to the nearest 0.1 gram.
C		6. The extract is completely mixed before it is injected.
C		7. Mice are injected intraperitoneally with 1 ml of the lipid extract.
C*		8. A total of 5 mice are injected with undiluted extract.

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C		9. The time of completed injection is recorded.
C*		10. Mice are continuously observed for at least 6 hours (360 minutes).
C		11. If death occurs within the period of continuous observation, the time of death to the nearest minute is noted by the last gasping breath.
K		12. If mice survive the test, the time of death is recorded as ">" the period of continuous observation.
		Calculation of Toxicity
C		1. The death time of each mouse is converted to mouse units (MU) using Table 8 in *Recommended Procedures*, 4th Edition.
O		2. Table 8 is interpolated for death times between 110 and 360 minutes that are not listed in the Table.
K		3. A weight correction in MU is made for each mouse injected using Table 8 in *Recommended Procedures*, 4th Edition.
O		4. Table 8 is interpolated to accommodate weights which are not listed.
C		5. The death time for each mouse in MU is multiplied by a weight correction in MU to give the corrected mouse unit (CMU) for each mouse.
C		6. The mean corrected mouse unit of the array of corrected mouse units (CMU) is used when all the mice injected with diluted or undiluted extract die during the period of continuous observation.
C		7. The median corrected mouse unit of the array of corrected mouse units (CMU) is used when at least one mouse either survives the test or dies.
C		8. The concentration of toxin is determined by the formula: Mean or median CMU x Dilution Factor x 10.
C		9. When the time of death is known for certain for all mice injected, toxicity is determinate and the toxin concentration is reported as the number of mouse units per 100 grams of sample.

PROPOSAL NO. 03-109:

Specific Reference: Guidance Documents, Chapter II Growing Areas, 11. Evaluation of Laboratories…including Checklists, Laboratory Evaluation Checklist, Microbiology 2, Part 1 Quality Assurance, NSSP Form LAB-100 rev. 2001-11-27, Page 318

Text of Proposal / Requested Action: Add new items to Laboratory Checklist as 2, 3, 4, and 5 to the Quality Assurance Plan Section. Making old 2 and 3, new 6 and 7. Add new references to Checklist References as follows:

Educational - Personnel Qualifications

Add new items as 2, 3, 4 and 5 to the Quality Assurance Plan section.
Make old 2 & 3, new 6 & 7.
Add new references to checklist References.

Code	Reference	Quality Assurance Plan
K	8, 11	1. Written Plan
		a. through g.
C	State's Human Resources Department	2. In state laboratories, the supervisor meets the state educational and experience requirements for managing a public health laboratory.
K	State's Human Resources Department	3. In state laboratories, the analyst(s) meets the state educational and experience requirements for processing samples in a public health laboratory.
C	USDA Microbiology & EELAP	4. In private laboratories, the supervisor must have at least a **bachelor's degree** in microbiology, biology, or equivalent discipline with **at least two years of laboratory experience**.
K	USDA Microbiology & EELAP	5. In private laboratories, the analyst(s) must have at least a **high school diploma and** shall have **at least two years of laboratory experience** with the testing concerned.
C	8	6. QA Plan Implemented.
K	11	7. Participates in a proficiency-testing program annually. Specify Program(s) _____

USDA Microbiology - U.S. DEPARTMENT OF AGRICULTURE – Microbiology Branch

EELAP – Ecology Environmental Laboratory Accreditation Program – Washington State

State's Human Resources Department

Supporting documentation is our chart.

PROPOSAL NO. 03-109:

RATIONALE AND SUPPORTING DOCUMENTATION:

Part I: Supervisory minimum requirements for Federal Agencies

	EPA	USDA Microbiology	U.S. Army Veterinary	EELAP
Supervisor	The supervisor of the microbiology laboratory should have a **bachelor's degree** in microbiology, biology, or equivalent. Supervisors who have a degree in a subject other than microbiology should have had at least one college-level microbiology laboratory course in which environmental microbiology was covered. In addition, the supervisor should have a **minimum of two weeks training** at a Federal agency, State agency, or academic institution in microbiological analysis of drinking water or, 80 hours of **on-the-job training** in water microbiology at a certified laboratory, or other training acceptable to the State or EPA. If a supervisor is not available, a **consultant having the same qualifications may be substituted**, as long as the laboratory can document that the consultant is acceptable to the State and is present on-site frequently enough to satisfactorily perform a supervisor's duties.	In full service laboratories (where most organisms – if not all – of significance to foods are tested) there shall be a trained, competent supervisory microbiologist on staff having at least **a Bachelors Degree** in microbiology, food science or a related discipline with **at least two years of laboratory experience**. The person filling this position shall have successfully completed **at least 20 credit hours** in microbiology, public health, food safety or other related topics. ELAP – Dept of Ecology *** Bachelors Degree** *** One-year experience.**	This is a full service laboratory that tests for a wide variety of pathogens. We have a competent supervisory microbiologist on staff with **at least a Bachelors Degree** in microbiology, food science or a related discipline and **at least two years of laboratory experience.** The person filling this position has successfully completed **at least 20 credit hours** in microbiology, public health, food safety or other related topics. Such information is found in the personnel file that is kept in the administrative office. ELAP The Technical Manager must have an earned science degree, minimally at **the baccalaureate level** with **at least twenty (20) semester hours** in microbiology and **a minimum of two (2) years** of documented environmental microbiological work experience (bacteriology and/or mycology).	**Minimum of a bachelor's degree** in chemistry or a biology science, or, if bachelor's degree is in a field other than chemistry or a biology science, the individual should have college-level credit hours sufficient to qualify for a minor in chemistry or biology. • Experience: **Minimum of two years experience** in an environmental lab. NELAC *** Bachelors Degree** *** At least 16 credit hours** *** Minimum of two years experience**

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RATIONALE AND SUPPORTING DOCUMENTATION (continued):

Part II: Technical support personnel minimum requirements for Federal Agencies

	EPA	USDA Microbiology	U.S. Army Veterinary	EELAP
Analyst	The analyst should perform microbiological tests with minimal supervision, and have **at least a high school education**. In addition, the analyst should have a minimum of **at least three months of bench experience** in water, milk, or food microbiology. The analyst should also have training acceptable to the State (or EPA for non-primacy States), in microbiological analysis of drinking water **and a minimum of 30 days of on-the-job training** under an experienced analyst. Analysts should take advantage of workshops and training programs that may be available from State regulatory agencies and professional societies. Before analyzing compliance samples, the analyst must demonstrate acceptable results for precision, specificity and satisfactory analysis on unknown samples.	The person filling this position shall have **at least two years of laboratory experience** with the testing concerned. ELAP – Dept of Ecology *** High school diploma.**	In small, limited service laboratories (usually of no more than 5 people; where 1-3 tests are performed) the technician performing the tests or supervising the tests shall be trained with demonstrated competence in the limited number of tests performed by the laboratory. The person filling this position shall have **at least two years of laboratory experience** with the testing concerned.	Other analysts (e.g., chemistry, biology, or microbiology technicians) should meet the following minimum requirements: • Academic Training: **High school diploma.**

Public Health Significance: To ensure overall laboratory conformity and integrity with respect to educational background and laboratory experience.

Cost Information (if available): Not available.

ACTION BY 2003 TASK FORCE I Recommended referral of Proposal 03-109 to the appropriate committee as determined by the Conference Chairman.

ACTION BY 2003 GENERAL ASSEMBLY Adopted recommendation of 2003 Task Force I.

ACTION BY USFDA Concurred with Conference action.