

National Shellfish Sanitation Program (NSSP) Guide for the Control of Molluscan Shellfish: 2015 Revision

Laboratory Evaluation Checklist – Analysis for NSP (Mouse Bioassay)

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SHELLFISH LABORATORY EVALUATION CHECKLIST			
LABORATORY:			
ADDRESS:			
TELEPHONE:		FAX:	EMAIL:
DATE OF EVALUATION:	DATE OF REPORT:		LAST EVALUATION:
LABORATORY REPRESENTED BY:		TITLE:	
LABORATORY EVALUATION OFFICER:		SHELLFISH SPECIALIST:	
		REGION:	
OTHER OFFICIALS PRESENT:		TITLE:	
Items which do not conform are noted by:			
C- Critical K - Key O - Other NA - Not Applicable Conformity is noted by a "√"			

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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. Internal performance assessment.
		g. External performance assessment.
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.
C		4. A separate, quiet area with adequate temperature control is maintained for acclimation and injection of mice.
		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.
		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 (0.915 to 0.927 g/ml) is used as the toxin delivery system. Name of the solvent if substituted for cottonseed oil. Specify 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.

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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 1-12 in "Preparation of Sample" section below.
		Preparation of Sample	
C		1.	At least 12 animals are used per sample.
O		2.	The outside of the shell is thoroughly cleaned with fresh water.
K		3.	Shellstock are 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.
K		8.	100 – 150 grams of meat are collected or all the available sample if there is less than 100 grams.
O		9.	Shucked shellfish are drained on a #10 mesh sieve or equivalent without layering for 5 minutes.
K		10.	Pieces of shell and drainings are discarded.
C		11.	Drained meats are blended at high speed until homogenous (60-120 seconds).
C		12.	Shellfish homogenates are digested within 2 hours of blending.
		Digestion of Sample	
K		1.	All glassware used is clean and properly washed with a succession of at least three fresh water rinses, 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) of homogenized sample is weighed into a beaker.
C		3.	1 ml of concentrated 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 boil and once 100 ± 1°C (sea level) is reached, gently boil for 5 minutes.
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.
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 15 minutes.
C		5.	The clear upper ether phase is transferred to a large separatory funnel.
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 the separatory funnel (as in step 5).
C		7.	The ether extract is transferred to a large, clean, dry pre-weighed beaker (discard 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 weighed and the weight is recorded.

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		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 or diluted extract as appropriate per sample in routine assays. <ul style="list-style-type: none"> a. The extract is not diluted when all test/assay mice survive beyond 110 minutes of injection. b. The extract is diluted when 2 of 2 test mice or 3 of 5 assay mice survive for fewer than 110 minutes after injection c. When dilution is required, only dilutions which produce mean/median death times within 110 to 360 minutes of injection are used in the analysis.
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 <i>Recommended Procedures</i> , 4 th 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 <i>Recommended Procedures</i> , 4 th 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.

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LABORATORY STATUS	
LABORATORY	DATE
LABORATORY REPRESENTATIVE:	
NEUROTOXIC SHELLFISH POISON COMPONENT:	
<p>A. Results</p> <p>Total # of Critical (C) Nonconformities _____</p> <p>Total # of Key (K) Nonconformities _____</p> <p>Total # of Critical, Key and Other (O) nonconformities _____</p>	
<p>B. Criteria for Determining Laboratory Status of the NSP Component</p> <p>1. Does Not Conform Status The NSP component of this laboratory is not in conformity with NSSP requirements if:</p> <p>a. The total # of Critical nonconformities is ≥ 3 or</p> <p>b. The total # of Key nonconformities is ≥ 6 or</p> <p>c. The total # of Critical, Key and Other is ≥ 10</p> <p>2. Provisionally Conforms Status: The NSP component of this laboratory is determined to be provisionally conforming to NSSP requirements if the number of critical nonconformities is ≥ 1 but < 3</p>	
<p>C. Laboratory Status (<i>circle appropriate</i>)</p> <p>Does Not Conform Provisionally Conforms Conforms</p>	