| **Proposal for Task Force Consideration at the ISSC 2015 Biennial Meeting** | ☒ Growing Area<br>☐ Harvesting/Handling/Distribution<br>☐ Administrative |
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| Proposal Subject | PCOX Method Status |
| Specific NSSP Guide Reference | Section IV. Guidance Documents<br>Chapter II. Growing Areas<br>.11 Approved Laboratory Tests |
| Text of Proposal/ Requested Action | This request is for a change in the status of the PCOX method for determining paralytic shellfish poisoning (PSP) toxins from "Approved Limited Use" to "Approved". This change would be reflected by:<br>1. Adding the PCOX method to NSSP Section IV Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests, Table 2. Approved Methods for Marine Biotoxin Testing with Biotoxin Type: Paralytic Shellfish Poisoning (PSP), Application: Growing Area Survey & Classification, Sample Type: Shellfish, and Application: Controlled Relaying Sample Type: Shellfish; and<br>2. Deleting the PCOX method from NSSP Section IV Guidance Documents Chapter II. Growing Areas .11 Approved NSSP Laboratory Tests, Table 4. Approved Limited Use Methods for Marine Biotoxin Testing.<br><br>The PCOX method for paralytic shellfish poisoning toxins (PSTs) was developed by the Canadian Food Inspection Agency (CFIA) and National Research Council Canada (NRCC) using post-column oxidation and fluorescence detection (PCOX). This method was optimized, tested, and used extensively in the authors' laboratory before the formal validation process was initiated to ensure that it could perform in the "real-life" setting of a regulatory monitoring laboratory. The method performed well, and was subjected to a single-laboratory validation (SLV) study [1]. The data generated in the SLV study was used to support proposal 09-104 to the Interstate Shellfish Sanitation Conference (ISSC) to approve the PCOX method for official use; the result of this proposal was that the method was approved as a Type IV method. The PCOX method was implemented for screening PST levels in shellfish at the Canadian Food Inspection Agency Dartmouth Laboratory in November, 2009, following ISSC approval; all samples were analysed using the PCOX method, and results leading to regulatory action were confirmed by mouse bioassay (MBA), AOAC OMA 959.08[2]. The method was next subjected to an international collaborative inter-laboratory study [3]. This collaborative study was successful, and the results were used to support the approval of the PCOX method as an AOAC official method of analysis (OMA), First Action status – OMA 2011.02 [4]. All MBA analyses for PSTs were eliminated in CFIA laboratories when the PCOX method was granted OMA, First Action status in April, 2011, and the PCOX method was considered a quantitative, regulatory method, without the need for MBA confirmation of results. The PCOX method was promoted to AOAC OMA, Final Action status in 2013 in response to positive method performance feedback from users. |

The PCOX method has been used to analyse almost 50,000 shellfish samples since it was implemented in Canada in November, 2009, with the Canadian Food Inspection Agency (CFIA) Dartmouth Laboratory completing almost 19,000 of those tests. This large dataset from CFIA laboratories provides an opportunity to verify performance characteristics with routine use over an extended period of time. A summary of QC performance at the CFIA Dartmouth Laboratory is shown in Table 1 below. These data demonstrate excellent precision (CV of <10% for average total PSTs) and accuracy (102 ± 17% for total PSTs) in method performance examined over a span of five and a half years, including multiple instruments, multiple analysts, and numerous batches of reagents. Additional data from other CFIA laboratories reveal similar results for >1500 additional QC points. The performance characteristics of the method were also evaluated and confirmed as part of a ring study on PSTs in oyster tissue organized by a laboratory in the United Kingdom [5]. Accuracy has also been evaluated through successful participation in CFIA and international proficiency testing programs by all three CFIA laboratories using the PCOX method. These performance characteristics exceed those specified by Codex [6] for quantitative chemical methods; recovery guidelines at these concentration are 80-110% with ≤44% RSD and repeatability guidelines for these concentration are <15% RSD.

Table 1: CFIA Dartmouth Laboratory summary of QC performance from November, 2009 – June, 2015

| | | GTX1 | GTX3 | STX | TOTAL PST |
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| In-house reference material 1 | n | 520 | | | |
| | Average | 24[b] | 29[b] | 139[b] | 264[b] |
| | Standard Deviation | 3.3 | 2.3 | 11.9 | 17.0 |
| | % RSD | 13% | 8% | 9% | 6% |
| In-house reference material 2 | n | 504 | | | |
| | Average | 45[b] | 50[b] | 62[b] | 244[b] |
| | Standard Deviation | 3.4 | 2.3 | 6.2 | 12.8 |
| | % RSD | 8% | 5% | 10% | 5% |
| SPIKE RECOVERY (%) | n | 1024 | | | |
| | Average | 100%[a] | 100% | 98% | 102% |
| | Standard Deviation | 38%[a] | 10% | 15% | 17% |
| | Concentration Range [b,c] | 3-11[a] | 7-10 | 28-61 | 57-92[d] |

[a] higher variability is observed because spiking levels are below the method LOD
[b] µg STXdiHCl eq/100g
[c] multiple spiking solutions were used over time; range reflects minimum and maximum spiking levels
[d] including only individual toxins that were above the method LOD

The method is also being used outside of Canada. The Norwegian School of Veterinary Science (NSVS) completed a validation study before implementing the PCOX method for all samples in January, 2013. Again, the performance of the method in the Norwegian laboratory was consistent with results from the collaborative study. It is also worth noting that all CFIA laboratories and the NSVS are accredited to ISO 17025 and maintain the PCOX method on their scope of accreditation. Within the United States, Maine has completed validation studies and been approved to use the PCOX method for regulatory samples since April, 2014, and

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| | Alaska has completed validation studies [7] and is currently awaiting final FDA approval to implement the method for regulatory testing (but currently uses the method for non-regulatory samples). Oregon has recently expressed interest in the method as well. Chilean laboratories at the University of Chile plan to validate the PCOX method and transition from MBA to the PCOX method in the near future. The method is also being used for non-routine or research purposes in New Zealand (Cawthron Laboratory), the United Kingdom (CEFAS laboratory), Ireland (Marine Institute), Chile (University of Chile), the United States (e.g., Alaska Environmental Health Laboratory, US FDA), and Canada (NRCC).<br><br>Training has been requested and delivered to groups in the United States (2010) and Europe (2012), and scientists from the Maine Department of Marine Resources and Bigelow Laboratory for Ocean Sciences were hosted for training at the CFIA Dartmouth Laboratory (2012). There was also interest in a training course organized by the China Section of AOAC International, but logistical difficulties have prevented the course from taking place thus far.<br><br>Feedback from participants in the collaborative study was very positive, and most laboratories experienced no problems with the method; however, like all methods, there are limitations and weaknesses. One weakness of the method is that it cannot resolve neosaxitoxin (NEO) from decarbamoylneosaxitoxin (dcNEO), or gonyautoxin-6 (GTX6) from gonyautoxin-4 (GTX4). The inability to resolve these toxins is an issue for samples contaminated by *Gymnodinium catenatum*, in which dcNEO and GTX6 are often present. This challenge is being examined, and the European Union Reference Laboratory for Marine Biotoxins has expressed interest in collaborating to overcome it. Another weakness of the method is the LC column, which suffers from a short lifespan. An alternative column has been proposed, but research continues to find a more suitable replacement. A weakness of all chemical PST methods is the unavailability of analytical standards for some toxins (such as GTX6, and C3/C4). The unavailable toxins are uncommon in North American toxin profiles (these toxins are common in samples contaminated by *Gymnodinium catenatum*) and have very low toxicity. These challenges are included here to provide a complete description of the method, and also to highlight that these issues are not serious enough to prevent implementation of the method. Research will continue to improve the robustness and flexibility of the method to make it easier to implement in different laboratories.<br><br>The PCOX method is more sensitive than the MBA, and can be used to provide earlier warning of rising PST levels in shellfish. This earlier warning capacity can be used to focus additional sampling and increase the probability of detecting toxin levels before they exceed the regulatory limit [8], resulting in increased food safety, and fewer product recalls for industry.<br><br>The ISSC terminology describing method status has been updated since the PCOX method was approved in 2009, and the PCOX method status is currently "Approved Limited Use"; however, there are currently no clear statements of what "limited use" means for this method. The method has been successfully implemented for regulatory samples in multiple accredited laboratories for several years, and performance data from these laboratories agree with those generated during the original inter-laboratory study. The status of this method should be changed to "Approved" to reflect the fact that this method is no longer in limited use, and no critical limitations to the method have been identified. This change would also be consistent with the changes resulting from adoption of Proposal 13-309, which recognizes AOAC OMA status when considering proposed methods that are |

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| | demonstrated as fit-for-purpose.<br><br>1. Van de Riet, J.M., et al., *Liquid Chromatographic Post-Column Oxidation Method for Analysis of Paralytic Shellfish Toxins in Mussels, Clams, Scallops,and Oysters: Single-Laboratory Validation.* Journal of AOAC International, 2009. **92**(6): p. 1690-1704.<br>2. INTERNATIONAL, A., *Method **959.08***, in *Official Methods of Analysis, 19th Ed.* 2012, AOAC INTERNATIONAL: Gaithersburg, MD.<br>3. Van de Riet, J., et al., *Liquid Chromatography Post-Column Oxidation (PCOX) Method for the Determination of Paralytic Shellfish Toxins in Mussels, Clams, Oysters, and Scallops Collaborative Study.* Journal of AOAC International, 2011. **94**(4): p. 1154-1176.<br>4. INTERNATIONAL, A., *Method **2011.02***, in *Official Methods of Analysis, 19th Ed.* 2012, AOAC INTERNATIONAL: Gaithersburg, MD.<br>5. Turner, A.D., et al., *Interlaboratory Comparison of Two AOAC Liquid Chromatographic Fluorescence Detection Methods for Paralytic Shellfish Toxin Analysis through Characterization of an Oyster Reference Material.* Journal of AOAC International, 2014. **97**(2): p. 380-390.<br>6. Commission, C.A., *Procedural Manual, 23rd edition.* 2015.<br>7. Hignutt, J.E., *Suitability of Postcolumn Oxidation Liquid Chromatography Method AOAC 2011.02 for Monitoring Paralytic Shellfish Toxins in Alaskan Shellfish—Initial Pilot Study versus Mouse Bioassay and In-House Validation.* Journal of AOAC International, 2014. **97**(2): p. 293-298.<br>8. Rourke, W.A. and C.J. Murphy, *Animal-Free Paralytic Shellfish Toxin Testing—The Canadian Perspective to Improved Health Protection.* Journal of AOAC International, 2014. **97**(2): p. 334-338. |
| Public Health Significance | The detection limit for PSTs by the MBA method is 40 µg STX diHCl eq/100g, while that of the sum of individual PSTs are significantly lower using the PCOX method - <10 µg STX diHCl eq/100g. This lower detection limit improves food safety and minimizes closures in southwestern New Brunswick, Canada, where PST levels in the Bay of Fundy are chronically high and can change very rapidly. Since the PCOX method has been implemented, the local CFIA office has determined that harvest sites with PST levels >35 µg STX diHCl eq/100g should be sampled a second time in the same week instead of waiting to sample the site the following week; by contrast, those same samples would show no toxin by the MBA method and sampling would be delayed until the regularly scheduled sample the following week. This delay potentially leaves harvest areas with increasing PST levels open over the weekend and beginning of the following week; this could lead to illnesses, food safety investigations, and product recalls that are now prevented because of the lower detection limits of the PCOX method. This information has been used to maintain harvest areas in an open status longer – an advantage for the shellfish harvesting industry - and simultaneously close the harvest areas before toxin levels exceed the regulatory limits. This change in sampling frequency has resulted in fewer food safety investigations and product recalls and was not possible before the PCOX method was implemented because the MBA method does not have enough sensitivity to detect low levels of PSTs. |
| Cost Information | There should be no direct cost implications to this change. It may make the transition from the MBA to the PCOX method slightly easier for laboratories not currently using the latter, or for those gearing up for PST testing for the first time. The PCOX method is less expensive than the MBA if capital purchases (LC systems) are averaged over the life of the equipment. |