

GENERAL REFEREE REPORTS

Methods Committee on Natural Toxins and Allergens

Mycotoxins

GORDON S. SHEPHARD

PROME C Unit, Medical Research Council, PO Box 19070, Tygerberg 75075, South Africa, Tel: +27-21-938-0279, Fax: +27-21-938-0260, E-mail: gordon.shephard@mrc.ac.za

Topic Advisors: Joe W. Dorner, Rudolf Krska, Gary A. Lombaert, Bruce Malone, Chris Maragos, Myrna Sabino, Gordon S. Shephard, Michele Solfrizzo, Mary Trucksess, Hans P. van Egmond, George M. Ware, Thomas B. Whitaker

Summary

AOAC INTERNATIONAL is currently involved in the reorganization of its Methods Committees and of the manner in which analytical methods will be assessed and approved in the future. General Referee reports are no longer published in the *J. AOAC Int.*; they are now available on the AOAC Website. As a consequence, the Topic Advisors have felt that the mycotoxin review section which has traditionally formed part of the General Referee report should be published as a separate review in a leading mycotoxin journal. Hence this report is considerably shorter than previous years.

The collaborative study of the method for determination of aflatoxins and ochratoxin A in ginseng and ginger by multitoxin immunoaffinity column cleanup and liquid chromatographic quantitation, Study Director Mary Trucksess, has been successfully completed and granted First Action status (2008.02). Two First Action methods have been moved to Final Action status and are listed below.

The papers presented at the MYCOGLOBE Conference in Bari, Italy, in September 2006, have been published in *Food Additives and Contaminants*, Vol. 24 (October 2007). The title of the conference, "Advances in Genomics, Biodiversity and Rapid Systems for Detection of Toxicogenic Fungi and Mycotoxins," illustrates the diversity of papers presented in the above publication. The Proceedings of the XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins held in Istanbul, Turkey, in May 2007, have also been published in *Food Additives and Contaminants* (Vol. 25, February 2008). The lectures presented at a workshop entitled "Mycotoxins from the Field to the Table," which was held in Omaha, NE, in November 2006, have been published in a special issue of *The International Journal of Food Microbiology* (Vol. 119, 2007).

Review articles that have come to the attention of the General Referee in the previous year include those on mycotoxin analysis (1), stable isotope dilution assays (2), mycotoxicoses associated with the major mycotoxins (3), the management and prevention of mycotoxins in peanuts (4), the

occurrence of mycotoxins in botanicals and dried fruits (5), the mycoflora and mycotoxins in various commercially important agricultural commodities (6), the stability of mycotoxins during food processing (7), the economic impacts of *Fusarium* toxins in animal feeds (8), and perspectives on mycotoxin regulations in a global and European context (9).

A recent paper has described a subject not normally associated with this report, namely the use of a public information campaign on aflatoxin contamination of maize to raise awareness of aflatoxin and reduce exposure in the west African countries of Benin, Ghana, and Togo (10).

Codex Activities

The second session of the Codex Committee on Contaminants in Food (CCCF) was held in The Hague, The Netherlands, March 31 to April 4, 2008. The draft maximum level for ochratoxin A in raw wheat, barley, and rye (5 µg/kg) and the draft maximum levels for total aflatoxins in almonds, hazelnuts, and pistachios "for further processing" (15 µg/kg) and "ready-to-eat" (10 µg/kg) are all at step 8 of the procedure. Proposed draft aflatoxin sampling plans for aflatoxin contamination in ready-to-eat treenuts (almonds, hazelnuts, and pistachios) and treenuts destined for further processing and a proposed draft code of practice for the prevention and reduction of aflatoxin contamination in dried figs are all held at step 5/8 of the procedure. The Codex Alimentarius Commission accepted the maximum limits and draft sampling plan at its meeting in Rome in June 2008. The committee has proposed new work on maximum levels for total aflatoxins in Brazil nuts, a code of practice for the prevention and reduction of ochratoxin A in coffee, and discussion papers on fumonisin and mycotoxins in sorghum (11).

Recommendations

General Referee Recommendations

- (1) Await current changes in AOAC structure before appointing a new Topic Advisor for citrinin.
- (2) Continue to investigate and develop methods for mycotoxins in all commodities relevant to human health.
- (3) Support the initiative to develop a Mycotoxins Community in AOAC INTERNATIONAL.

Topic Advisors' and Study Directors' Recommendations

(1) *Sampling and subsampling for mycotoxins.*—Topic Advisor Thomas B. Whitaker, U.S. Department of Agriculture, Agricultural Research Service, PO Box 7625, North Carolina State University, Raleigh, NC 27695-7625, Tel: 919-515-6731, Fax: 919-515-7760, E-mail: tom_whitaker@ncsu.edu. Continue study.

(2) *Aflatoxin M₁.*—Topic Advisor Hans P. van Egmond, National Institute for Public Health and the Environment,

Laboratory for Food and Residue Analysis (ARO), PO Box 1, 3720 BA Bilthoven, The Netherlands, Tel: 31-30-2742440, Fax: 31-30-2744403, E-mail: Hans.van.Egmond@rivm.nl. Continue study.

(3) *Aflatoxin methods*.—Topic Advisor Gordon S. Shephard, Program on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council, PO Box 19070, Tygerberg 7505, South Africa, Tel: 27-21-938-0279, Fax: 27-21-938-0260, E-mail: gordon.shephard@mrc.ac.za. Continue study.

(4) *Alternaria toxins*.—Topic Advisor Michele Solfrizzo, Institute of Sciences of Food Production, National Research Council, Via Amendola 122/o, Bari 70126, Italy, Tel: +39-080-5929367, Fax: 39-080-5929374, E-mail: michele.solfrizzo@ispa.cnr.it. Continue study.

(5) *Citrinin*.—Topic Advisor David Abramson has retired. Appoint new advisor and continue study.

(6) *Cyclopiazonic acid*.—Topic Advisor Joe W. Dorner, U.S. Department of Agriculture, ARS, National Peanut Research Laboratory, PO Box 509, 1011 Forrester Dr SE, Dawson, GA 31742, Tel: 912-995-7408, Fax: 912-995-7416, E-mail: jdorner@nprl.usda.gov. Continue study.

(7) *Ergot alkaloids*.—Topic Advisor George M. Ware, U.S. Food and Drug Administration, 60 8th St, Atlanta, GA 30309, Tel: 404-347-2131, ext. 5215, Fax: 404-347-4225, E-mail: gware@fda.hhs.gov. Continue study.

(8) *Fumonisin*s.—Topic Advisor Chris Maragos, U.S. Department of Agriculture, ARS, National Center for Agricultural Utilization Research, 1815 N. University St, Peoria, IL 61604, Tel: 309-681-6266, Fax: 309-681-6689, E-mail: chris.maragos@ars.usda.gov. Continue study.

(9) *Mycotoxins in botanicals*.—Topic Advisor Bruce Malone, Trilogy Analytical Laboratory, 111 West Fourth St, Washington, MO 63090, Tel: 636-239-1521, Fax: 636-239-1531, E-mail: bruce@trilogylab.com. Continue study.

(10) *Ochratoxins*.—Topic Advisor Mary Trucksess, U.S. Food and Drug Administration, 5100 Paint Branch Pkwy, College Park, MD 20740, Tel: 301-436-1957, Fax: 301-436-2665, E-mail: mary.trucksess@fda.hhs.gov. Continue study.

(11) *Patulin*.—Topic Advisor Myrna Sabino, Instituto Adolfo Lutz, Av. Dr Arnaldo 355-01246-902, São Paulo, Brazil, Tel: 55-11-3068-2921, Fax: 55-11-3085-3505, E-mail: mysabino@ial.sp.gov.br. Continue study.

(12) *Trichothecenes*.—Topic Advisor Gary A. Lombaert, Health Canada, 510 Lagimodiere Blvd, Winnipeg, MB, R2J 3Y1, Canada, Tel: 204-984-2088, Fax: 204-983-5547, E-mail: gary_lombaert@hc-sc.gc.ca. Continue study.

(13) *Zearalenone*.—Topic Advisor Rudolf Krska, University of Natural Resources and Applied Life Sciences, Department of Agrobiotechnology-IFA-Tulln, Austria; Center for Analytical Chemistry, Christian Doppler Laboratory for Mycotoxin Research, Konrad Lorenz Str. 20, A-3430 Tulln, Austria, Tel: 43-2272-66280-401, Fax: 43-2272-66280-403, E-mail: rudolf.krska@boku.ac.at. Continue study.

(14) **990.32** *Aflatoxin B₁ in Corn and Roasted Peanuts*.—Awaiting information on possible kit formulation changes. Held at First Action status.

(15) **998.03** *Aflatoxins in Peanuts (Alternative BF Method)*.—Held at First Action status. Has not been published in *J. AOAC Int.*

(16) **999.07** *Aflatoxin B₁ and Total Aflatoxins in Peanut Butter, Pistachio Paste, Fig Paste and Paprika Powder*.—Final Action status.

(17) **2001.04** *Determination of Fumonisin B₁ and B₂ in Maize Flour and Cornflakes by LC*.—Study Director Angelo Visconti, National Research Council, Institute of Toxins and Mycotoxins, Viale Einaudi 51, Bari 70125, Italy, Tel: 39-080-548-6013, Fax: 39-080-548-6063, E-mail: visconti@area.ba.cnr.it. Remove cornflake from matrix and submit for Final Action.

(18) **2004.10** *Determination of Ochratoxin A in Green Coffee by Immunoaffinity Column Cleanup and LC*.—Study Director Eugenia Vargas, Laboratory for Mycotoxin Analysis, Avenida Raja Gabaglia, 245 Cidade Jardim, Belo Horizonte 30380-090, Brazil, Tel: 55-31-250-0398, Fax: 55-31-250-0399, E-mail: gena@cdlnet.com.br. Final Action status.

(19) **2005.08** *Liquid Chromatographic Analysis of Aflatoxin Using Post-Column Derivatization (Modification of 991.31, 999.07, and 970.45)*.—Study Director Arthur Waltking, Waltking Associates, 482 Rock Rd, Glen Rock, NJ 07452. Held at First Action status pending editorial changes to encompass a wider range of post-column photochemical devices.

(20) **2008.02** *Determination of Aflatoxins and Ochratoxin A in Ginseng and Ginger by Multitoxin Immunoaffinity Column Cleanup and Liquid Chromatographic Quantitation*.—Study Director Mary Trucksess, U.S. Food and Drug Administration, 5100 Paint Branch Pkwy, College Park, MD 20740, Tel: 301-436-1957, Fax: 301-436-2665, E-mail: mary.trucksess@fda.hhs.gov. First Action status.

References

- (1) Krska, R., Schubert-Ullrich, P., Molinelli, A., Sulyok, M., MacDonald, S., & Crews, C. (2008) *Food Addit. Contam.* **25**, 152–163
- (2) Rychlik, M., & Asam, S. (2008) *Anal. Bioanal. Chem.* **390**, 617–628
- (3) Richard, J.L. (2007) *Int. J. Food Microbiol.* **119**, 3–10
- (4) Dorner, J.W. (2008) *Food Addit. Contam.* **25**, 203–208
- (5) Trucksess, M.W., & Scott, P.M. (2008) *Food Addit. Contam.* **25**, 181–192
- (6) Kumar, V., Basu, M.S., & Rajendran, T.P. (2008) *Crop Protection* **27**, 891–905
- (7) Bullerman, L.B., & Bianchini, A. (2007) *Int. J. Food Microbiol.* **119**, 140–146
- (8) Wu, F. (2007) *Animal Feed Sci. Technol.* **137**, 363–374
- (9) van Egmond, H.P., Schothorst, R.C., & Jonker, M.A. (2007) *Anal. Bioanal. Chem.* **389**, 147–157
- (10) James, B., Adda, C., Cardwell, K., Annang, D., Hell, K., Korie, S., Etorh, M., Gbeassor, F., Nagatey, K., & Houenou, G. (2007) *Food Addit. Contam.* **24**, 1283–1291
- (11) Codex Alimentarius Commission, ALINORM 08/31/41, <http://www.codexalimentarius.net>, Accessed May 5, 2008

Marine and Freshwater Toxins

JAMES M. HUNGERFORD

U.S. Food and Drug Administration, PRL-NW, 22201 23rd Dr SE, Bothell, WA 98021, Tel: 425-483-4894, Fax: 425-483-4996, E-mail: James.Hungerford.fda.hhs.gov

Topic Advisors and Study Directors: Lyn Briggs, Ambrose Furey, Jean-Marc Fremy, Ana Gago-Martinez, Philipp Hess, Patrick Holland, Hans Kleivdal, Richard Lewis, Ronald Manger, Jerome Naar, Michael Quilliam, Don Richard, Benjamin A. Suárez-Isla, Aurelia Tubaro, Fran van Dolah, Mari Yotsu-Yamashita

Summary

AOAC INTERNATIONAL is currently reorganizing its Methods Committees and the manner in which analytical methods will be assessed and approved in the future. General Referee reports are no longer published in the *J. AOAC Int.*; they are now available on the AOAC Website. As a consequence, the General Referee, Topic Advisors, and Study Directors have felt that the Marine and Freshwater Toxins reviews, traditionally forming part of the General Referee report, should be published as separate reviews in *J. AOAC Int.* or in another leading journal. The following summarizes developments in the last few years.

The proceedings of an international symposium, "Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs," have been published in a monograph of the same title edited by H. Kenneth Hudnell and published by Springer (2008). This includes an overall workgroup report (Chapter 20) and other topics of relevance to methodology (Chapters 21–24 and poster abstracts) by the Analytical Methods Workgroup, which also included members of the Marine and Freshwater Toxins Task Force.

Codex Alimentarius and Other International Groups

In April 2006, an international working group met in Ottawa, Canada, to assess the advice from the *Joint FAO/WHO/IOC Ad Hoc Expert Consultation on Biotoxins in Bivalve Molluscs*. The expert consultation group and the report they generated following a meeting held in late 2004 in Oslo, Norway, were described previously (1). In their response to the consultation report, the Ottawa working group addressed multiple regulatory issues relating to marine biotoxins found in molluscan shellfish. From the standpoint of international regulation and trade, both the consultation report (2) and the working group recommendations (3) are useful for illustrating the nature of risk assessments, as well as a proposed role played by successful monitoring programs in addressing proposed changes. Two distinctive trends were observed at the Ottawa meeting and in the resulting recommendations. The first of these was the working group agreement to include, in their recommendations on quarantine levels, the performance records of existing monitoring programs (and associated quarantine levels) as part of a

broader data set which already included risk assessments based on human illness and animal models. The second trend was to recommend that, for toxins for which no record of human illnesses exist, quarantine levels should not be established. Overall this results in working group advice to deregulate certain marine toxins and, among the remaining toxins classes of human health concern, to advise against stricter quarantine levels for cases where existing limits have been effective in protecting human health. These recommendations were carried forward to the Codex meeting on fisheries products subsequently held in Beijing, China, September 2006 (3). Most recently, a 2008 online report (4) shows that most of the Ottawa group's recommendations were retained, including suggested action levels.

Progress in Methods Implementation, PSP Toxins

Another important aspect of the Codex recommendations (4) was that OMA **2005.06** replace the mouse bioassay as a type II, or reference method. Prior to this, in June 2006, the Marine and Freshwater Toxins Task Force held a training course in Seattle, WA, developed by the Health Canada authors of the method (5). The General Referee also notes that at the time of writing, all monitoring of mussels within the United Kingdom was done using a slightly modified version of this method, and for the past year the method has also been written into European Union (EU) directives. Analyte extension work is also in preparation by the EU's Community Reference Laboratory on Marine Biotoxins, which also held their own version of a training course for the method. Most recently, a matrix extension from shellfish to human urine is in preparation via a single-laboratory validation (SLV) in work jointly by FDA and U.S. Army Medical Research Institute for Infectious Diseases. (New appointments may be necessary to address this extension effort.)

Manuscript D15.—After careful consideration, the immunobead assay for ciguatoxins (6) addressed in collaborative study manuscript D15 has been rejected by the General Referee for consideration as an Official Method of Analysis. An informal meeting of the Task Force Subgroup on Ciguatoxins was held in Honolulu, Hawaii, in December 2005, during Pacificchem 2005. The meeting was attended by 8 members of the subgroup and among them was the subgroup Chair, the author of the method, and a representative of the firm marketing the kit, and the General Referee. The conclusion reached at this meeting was that available evidence shows that the kit in its current form should not be approved by AOAC. This was based on external studies applying the kit to fish samples in both the Pacific (7) and on previous FDA investigations of its application to Caribbean fish. In these studies, both false positives and false negatives were obtained, and some of the false-negative results were obtained for fish implicated in cases of ciguatera fish poisoning. The data presented to the ciguatoxins subgroup and subsequently published (8) reportedly use the same antibody; however, the end-point is enzymatic rather than the commercialized immunobead format, and thus does not relate to the commercialized kit.

In the present systems of review, performance requirements imposed by both the AOAC requirements for prior SLVs, as well as by the voting group of the Marine and Freshwater Toxins Task Force and Analytical Community, are designed to ensure that collaborative study protocols are based on sound methods and test kits. It is in the best interests of those conducting interlaboratory studies to first thoroughly study accuracy, sensitivity, ruggedness, and other performance issues.

Recommendations

General Referee Recommendations

(1) In the General Referee report, divide reports by Topic Advisors and Study Directors into sections on Topics and also Validation efforts to better clarify which areas have active validation efforts. Submit review articles to *J. AOAC Int.* where appropriate with participating Topic Advisors and Study Directors as co-authors. Validation efforts should have more specific titles and scope than general topics.

(2) Add a topic "Scombrottoxins" to pursue validations of methods for detecting histamine (and/or scombroid poisoning-implicated bioactives) in fish. Appoint Topic Advisor(s) for specific techniques to be validated when available. Histamine as a contaminant is often considered in seafood commodities as a decomposition indicator, but the prevalence of scombroid poisoning points to a need to give the problem higher priority.

(3) Create the following new topic areas for new task force validation efforts with the following recommended Topic Advisors.

Saxitoxins by Post-Column Oxidation LC.—Appoint as Topic Advisor Jeff van de Riet, 484 Brierwood Ave, Ottawa, Ontario, K2A 2H3, Canada, Tel: 613-729-2205, E-mail: jeffrey.vanderiet@inspection.gc.ca An SLV study is in preparation and an interlaboratory/OMA study is planned, and protocol will be prepared and submitted in 2008/2009.

Brevetoxins and Their Metabolites in Shellfish by LC/MS/MS.—Appoint as Topic Advisor Steven Plakas, FDA/CFSAN, Dauphin Island, Alabama. Publication of completed SLV study is in preparation, an interlaboratory/OMA study is planned, and protocol will be prepared and submitted in 2009.

Microcystins by Recombinant PP2A Inhibition Assay.

Okadaiates by Recombinant PP2A Inhibition Assay.

(4) Revise appointment area title of (now Study Director) Frances van Dolah to the more specific title *Receptor Binding Assay for Saxitoxins in Shellfish*. (Her former area title of Receptor Assays for Phycotoxins can be addressed in review articles).

(5) Appoint Andrew Turner of CEFAS, UK as Study Director of the topic "*Saxitoxins in Shellfish by Pre-Column Oxidation LC*". The appointment reflects two changes, including revision of appointment area title formerly held by Study Director James Lawrence (from former "*Saxitoxins by LC Methods*") and also Lawrence's resignation as study director. Turner is very active in refinement, validation, and implementation of the associated topic area, OMA **2005.06**. The new topic title also clarifies appointment of Turner vs the

different (post column) approach of new appointee Jeff van de Riet.

(6) Continue to investigate and develop methods for marine and freshwater toxins in all commodities relevant to human health, using the Marine and Freshwater Toxins Task Force and Analytical Community for guidance (note that recommendations 7–10 below relate to this general task force recommendation).

(7) Establish formal ties with other Analytical Communities such as Dietary Supplements to address algae-based products.

(8) Use workshops and symposia to encourage new collaborations with biomedical, materials science, synthetic organic chemistry, and other areas to advance the state-of-the-art of toxin detection and validation of emerging technologies.

(9) To promote earlier detection of potential threats to human health, also include in task force activities those involved in detection of toxin-producing organisms such as microalgae and bacteria. Recent "FlowCytobot" detection of *Dinophysis* toxic algae (prior to detection of okadaic acid contamination of shellfish) is an example of benefit to stakeholders.

(10) Continue to support stakeholders and their implementation efforts by pursuing training courses and workshops in partnership with AOAC Sections and stakeholder laboratories, and where appropriate other AOAC Analytical Communities. Use feedback from these events to improve methodology efforts and stakeholder connections, and to recruit new task force members.

Topic Advisor and Study Director Recommendations

Topics below will be addressed in co-authored review papers which will also include the other topics—validation, implementation, and training progress—as appropriate:

(1) *Anatoxins*.—Topic Advisor Ambrose Furey, PROTEOBIO, Mass Spectrometry Centre for Proteomics and Biotoxin Research, and All-Ireland *safeFood* Biotoxin Research Network Coordinator (Food Safety Promotion Board), Cork Institute of Technology, Department of Chemistry, Bishopstown, Cork, Ireland, Tel: 00353-21-4326701, Fax: 00353-21-4345191, E-mail: afurey@cit.ie. Continue study.

(2) *Animal bioassays for marine toxins*.—Topic Advisor Donald J.A. Richard, New Brunswick, E1C 4L8, Canada, Tel: 506-855-4824, E-mail: donr@nb.sympatico.ca, Alternate: richardd@dfo-mpo.gc.ca. Continue study.

(3) *Azaspiracids*.—Topic Advisor Ambrose Furey, PROTEOBIO, Mass Spectrometry Centre for Proteomics and Biotoxin Research, and All-Ireland *SafeFood* Biotoxin Research Network Coordinator (Food Safety Promotion Board), Cork Institute of Technology, Department of Chemistry, Bishopstown, Cork, Ireland, Tel: 00353-21-4326701, Fax: 00353-21-4345191, E-mail: afurey@cit.ie. Continue study.

(4) *Capillary electrophoretic methods for marine toxins*.—Topic Advisor Ana Gago-Martinez, Universidad de Vigo, Dpto. Química Analítica y Alimentaria, Facultad de

Ciencias, Campus Universitario de Vigo 36200-Vigo-E, Spain, Tel: 34-986-812284, Fax: 34-986-812382, E-mail: anagago@uvigo.es. Continue study.

(5) *Cell bioassays for phycotoxins*.—Topic Advisor Ronald Manger, Fred Hutchinson Cancer Research Center, PO Box 19024, Seattle, WA 98109-1024, Tel: 206-667-5838, Fax: 206-667-4182, E-mail: rmanger@fhcrc.org. Continue study.

(6) *Domoic acids*.—Study Director Michael A. Quilliam, National Research Council of Canada, Institute for Marine Biosciences, 1411 Oxford St, Halifax, Nova Scotia, B3H3Z1, Canada, Tel: 902-426-9736, Fax: 902-426-9413, E-mail: michael.quilliam@nrc.ca. Continue study.

(7) *Immunochemical methods for phycotoxins*.—Topic Advisor Lyn Briggs, AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand. Tel: 64-7-838-5137, Fax: 64-7-838-5189, E-mail: lyn.briggs@agresearch.co.nz. Continue study. General Referee comments: Briggs led the laboratory portion of an AOAC Workshop on Rapid Tests which addressed phycotoxins and included histamine, antibiotics, and mycotoxins. Task force members Mark Poli and Stacey Etheridge also participated as laboratory instructors.

(8) *LC/MS/MS detection of marine toxins*.—Topic Advisor Patrick Holland, Cawthron Institute, Private Bag 2, Nelson, New Zealand, Tel: 64-3-548-2319, Fax: 64-3-546-9464, E-mail: patrick.holland@cawthron.org.nz. Continue study.

(9) *Microcystins and nodularins*.—Topic Advisor Geoffrey A. Codd, University of Dundee, School of Life Sciences, Dundee DD1 4HN, Scotland, UK, Tel: 44-1382-344272, Fax: 44-1382-344275, E-mail: g.a.codd@dundee.ac.uk. Continue study.

(10) *Okadaiates, assay methods*.—Topic Advisor J. Marc Fremy, Agence Française de Sécurité Sanitaire des Aliments, Unité d'Évaluation des Risques Physico-Chimiques, Maisons Alfort, F-94701, France, Tel: 33-1-4977-2794, Fax: 33-1-4977-1352, E-mail: j.fremy@paris.afssa.fr. Continue study.

(11) *Receptor assays for phycotoxins*.—Topic Advisor Frances van Dolah, NOAA National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, 219 Fort Johnson Rd, Charleston, SC 29412, Tel: 843-762-8529, E-mail: fran.vandolah@noaa.gov. General Referee recommends van Dolah change appointment from “*Receptor Assays for Phycotoxins*” to “*Receptor Binding Assays for Saxitoxins*” as part of being made Study Director in the saxitoxins application.

(12) *Sample pretreatment methods for marine toxin analysis*.—Topic Advisor Ana Gago-Martinez. Continue study.

(13) *Saxitoxins, electrophysiological methods*.—Topic Advisor Benjamin A. Suárez-Isla, Laboratory of Marine Toxins, Program of Physiology and Biophysics, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Av. Independencia 1027, PO Box 70005, Santiago 8380453 Chile, Tel: 562-978-6308, Fax: 562-732-9668, E-mail: bsuarez@med.uchile.cl. Continue study.

(14) *Tetrodotoxins*.—Topic Advisor Mari Yotsu-Yamashita, Tohoku University, Sendai, 981-8555,

Japan, Tel/Fax: 81-22-717-8922, E-mail: myama@biochem.tohoku.ac.jp. Continue study.

(15) *Yessotoxins*.—Topic Advisor Aurelia Tubaro, University of Trieste, Department Materials and Natural Resources, Via Valerio 6, 34127, Trieste, Italy, Tel: 39-040-558 7910, E-mail: tubaro@units.it. Continue study.

(16) *Domoic acids by ELISA*.—Study Director Hans Kleivdal, Biosense Laboratories AS, HIB-Thormhøllensgate 55, N-5008 Bergen, Norway, Tel: 47-5554-3967, Fax: 47-5554-3771, E-mail: hans.kleivdal@biosense.com. Continue study. First Action status for OMA **2006.02**.

(17) **2005.06** *Quantitative Determination of Paralytic Shellfish Poisoning Toxins in Shellfish Using Prechromatographic Oxidation and Liquid Chromatography with Fluorescence Detection*.—Study Director James Lawrence, Bureau of Chemical Safety, Food Directorate, Food Research Division, Research Center 2203D, Ottawa, Ontario K1A-OL2, Canada, Tel: 613-957-0973, Fax: 613-954-4674, E-mail: jimlawrence@sympatico.ca. Adopted as a First Action Official Method.

(18) **2006.02** *Determination of Domoic Acid Toxins in Shellfish by Biosense ASP ELISA: A Direct Competitive Enzyme-Linked Immunosorbent Assay*.—Study Director Hans Kleivdal, Biosense Laboratories AS, HIB-Thormhøllensgt. 55 N-5008 Bergen, Norway, Tel: +47-5554-3967, Fax: +47-5554-3771, E-mail: hans.kleivdal@biosense.com. Adopted as a First Action Official Method.

Validation efforts below are in development, in-process, or approved as Official First Action. These topics will also be addressed in co-authored review papers. (*Note: Other validation efforts referred to in General Referee recommendations above require appointment of Topic Advisors.*)

Brevetoxins, Immunological Methods.—Topic Advisor Jerome Naar, University of North Carolina at Wilmington, Center for Marine Science, 5600 Marvin K. Moss Ln, Wilmington, NC 28409, Tel: 910-962-2367, Fax: 910-962-2410, E-mail: naarj@uncw.edu. Continue study. An SLV study has been completed and has been submitted to *J. AOAC Int.* for publication. Commercialization of the procedure in a complete kit format is now being undertaken by MARBIONC, a biotechnology consortium of the state of North Carolina. The procedure differs only from the SLV-studied version in that it does not require presensitization of the plates. When the kit is formally commercialized, rename this topic to include the word ELISA and appoint Jerome Naar as Study Director.

Okadaiates by LC-Based Methods.—Topic Advisor Philipp Hess, Marine Institute, Galway Technology Park, Parkmore, Galway, Ireland, Tel: 353-91-730400, Fax: 353-91-730470, E-mail: philipp.hess@marine.ie. Continue study. *Note: Hess is the principal in a (non-AOAC) interlaboratory study in Europe applying LC/MS to these and other lipophilic toxins in shellfish. He interacts with AOAC to promote approaches beneficial to both approval processes.*

Receptor Binding Assay for Saxitoxins.—Study Director Frances van Dolah. Continue study. An SLV and a protocol

for an interlaboratory study has been revised and resubmitted based on initial review, as part of an OMA submission contracted between AOAC and International Atomic Energy Agency. The SLV has also been submitted for publication in *J. AOAC Int.*

Saxitoxins by Pre-Column Oxidation LC.—Study Director Andrew Turner, CEFAS, UK is new Study Director appointment. James Lawrence was former Study Director. OMA **2005.06** is at First Action status. See General Referee recommendations above on this topic.

References

- (1) Hungerford, J.M. (2006) *J. AOAC Int.* **89**, 248–269
- (2) Report of the Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs (Short Summary), <http://unesdoc.unesco.org/images/0013/001394/139421e.pdf>, Accessed July 20, 2008
- (3) Codex Alimentarius Commission, <ftp://ftp.fao.org/Codex/ccffp28/fp2806ae.pdf>, Accessed July 20, 2008
- (4) Codex Alimentarius Commission, ALINORM 08/31/18, http://codex.mohw.go.kr/files/al31_18e.pdf, Accessed 20 July 2008
- (5) *Inside Laboratory Management* (March/April 2008) 28–32
- (6) Hokama, Y., Ebesu, J.Y.M., Takenaka, W.E., Bourke, R.E., & Sullivan, P.K. (August 3, 2004) U.S. Patent No. 6770490
- (7) Wong, C.K., Hung, P., Lee, K.L., & Kam, K.M. (2005) *Toxicon* **46**, 563–571
- (8) Campora, C.E., Hokama, Y., & Ebesu, J.S.M. (2006) *J. Clin. Lab. Anal.* **20**, 121–125

Allergens

BERT POEPPING

Eurofins Scientific Group
69a Kilnwick Road
Yorkshire, Pocklington, YO42 2JY United Kingdom

Determination of Peanut Material in Food by a Sandwich Enzyme-Linked Immunosorbent Assay.—Study Director Mark Mozola, Neogen Corp., 620 Leshar Pl, Lansing, MI 48912, Tel: 517-372-9200, Fax: 517-372-0108, E-mail: mmozola@neogen.com. Study on hold.

In light of the regulations for labelling of allergens in food products in USA, Europe, Japan, and Australia/New Zealand the AOAC Presidential Taskforce on Food Allergens has been very active over the past year compiling the guidance document for validation of protein-based ELISA kits, jointly with members of the European 6th Framework Project “MoniQA”.

The document has matured over the past years with many lively debates on what is feasible and achievable as well as discussion on the statistical evaluation of data from ringtrial. Here, statisticians from Health Canada and AOAC, namely Paul Wehling contributed to a better understanding of the complicated situation when assessing the data generated in ringtrials. This document is now in its final stage and will be submitted to *J. AOAC Int.* for peer-review.

Therefore, no new method validation exercise has taken place or will take place until the draft guidelines have been accepted. However, r-biopharm was granted PTM status (Certificate 120601) on their Ridascreen Gliadin ELISA kit with effect of January 1st 2008.

In Europe, several draft standards have been generated for the standardisation of molecular biological, immunological and chromatographic methods. This work is part of the European committee CEN TC 275 WG 12 [Committee European de Normalisation Technical Committee 275 Working Group 12 (Food Allergen Methods)]. Here, the drafts prEN 15633 and prEN 15634 have been submitted for comments in 2008. These have now been accepted as standards with effect of January 2009 (EN 15633-1:2009 Immunological Methods and EN 15634-1:2009 Molecular Biological Methods). The draft standard containing general information prEN 15842 has been submitted for review and generally accepted by all memberstates with some technical and editorial comments which will be discussed at the forthcoming CEN meeting in Madrid in May 2009.

Also, 2 immunological methods have been submitted for consideration as standards, name the hazelnut ELISA methods

by r-biopharm and ELISASYSTEMS which have been successfully ringtried according to AOAC/IUPAC harmonised guidelines by the German Ministry of Consumer Protection and Food Safety (BVL – Bundesministerium fuer Verbraucherschutz und Lebensmittelsicherheit). These methods will be subject to discussion as the forthcoming CEN meeting in Madrid.

Subsequently to the the Codex Alimentarius meeting in April 2008 in Budapest where labelling thresholds for gluten-free products have been agreed on, the European Commission introduced a new regulation in January 2009, the 41/2009/EC which regulates the labelling of products with reduced gluten content. This regulation sets levels for products with reduced gluten levels. At 100mg/kg these products shall be labelled as “very low gluten”, at 20 mg/kg these products may be labelled as gluten free. The regulation does not permit products naturally gluten free to be labelled as such. Since this is the first regulation which states labelling thresholds, it is of utmost importance that further studies on validation of gluten determining ELISA kits takes places as soon as possible.

While Japan has already taken a pragmatic approach to require labelling of allergen-containing products at a level of 10 mg/kg, other countries are still discussing threshold for labels as this will benefit industry, consumers and (enforcement-)laboratories alike. Here, the VITAL GRID is used as basis for discussion (http://www.allergenbureau.net/downloads/allergen-guide/VITAL/VITAL_Grid_02_July_2007.pdf).

In Europe, two European Commission funded project also deal with Allergens: Europrevall (www.europrevall.org), led by Clare Mills, Inst Food Res., UK and MoniQA (www.moniqa.org), led by Roland Poms, ICC, Austria. These project have joined forces and mutually made research information available to each other. This has led to a first publication of a position paper (status quo of allergens) in Quality Assurance and Safety of Foods and Crops: ‘Managing food allergens in the food supply chain viewed from different stakeholder perspectives.

One of the major problems in this field is the lack of reference materials. So far, only egg (NIST RM8445 – spray dried whole egg) and milk (NIST RM 1549 non-fat milk powder) are available as RMP (Reference Material Pure). Some approaches have been made to also produce RMI (Reference Materials Incurred). These also form part of the MoniQA project. Here, cookies containing different levels of egg and milk have been produced that will be used for validation studies later in 2009.