

METHODS COMMITTEE REPORTS

Committee on Feeds, Fertilizers, and Related Agricultural Topics

LUANN WETZLER, CHAIR

Nebraska Department of Agriculture, 3703 S. 14th St,
Lincoln, NE 68502

NANCY THIEX, SECRETARY

South Dakota State University, Olson Biochemistry
Laboratories, Box 2170 ASC 151, Brookings, SD 57007

VACANT, SAFETY ADVISOR

VACANT, STATISTICAL ADVISOR

MICHAEL P. CARLSON

University Nebraska-Lincoln, 145 Veterinary Diagnostic
Center, Fair St and E. Campus Loop, Lincoln, NE 68583

MARY G. LEADBETTER

U.S. Food and Drug Administration, Center for Veterinary
Medicine, HFV-143, Rm 337, 7500 Standish Pl, Rockville,
MD 20855

JOHN DENNIS MCCURDY

U.S. Food and Drug Administration, 3949 Sugarloaf Dr,
Monrovia, MD 21770

JAMES MACNEIL

Canadian Food Inspection Agency, 116 Veterinary Rd,
Saskatoon, SK S7N-2R3, Canada

DAVE MERTENS

U.S. Department of Agriculture, Fiber Utilization
Laboratory, Room 261, 1925 Linden Dr West, Madison,
WI 53706

RODNEY J. NOEL

Office of the Indiana State Chemist, Purdue University,
1154 Biochemistry Bldg, West Lafayette, IN 47907-1154

ROBERT L. ROSER

Scotts Co., 1411 Scottslawn Rd, Marysville, OH 43041

Committee Actions

One collaborative study has been successfully completed and 3 new protocols have been approved. OMA-2004-Jul-012 *Monensin, Narasin, and Salinomycin, LC Method* is pending approval and currently under final review by the Statistician. Protocols for OMA-2004-Oct-017 *Oxytetracycline, LC Method*, OMA-2004-Nov-023 *Arsenic, Cadmium, Cobalt, Chromium, Lead, Molybdenum, Nickel, and Selenium in Fertilizer by Microwave Digestion and ICP-OES Detection*;

and OMA-2005-Jan-002 *Lasalocid* were approved and studies are underway. Three topics have been recommended to be discontinued. Seven antibiotic methods are recommended to be archived as some are no longer manufactured and others are no longer added to animal feeds.

The driving force for new methods entering the collaborative study process in the past year has been the Agricultural Materials Community and its subgroups. The Fertilizer and Feed Additive Contaminants Subgroups have been meeting regularly, both by conference call and in-person meetings. A summary of the activities of the community is available on the AOAC website at: http://www.aoac.org/Ag_Materials/community.htm. Information on the Fertilizer Subgroup is available at http://www.aoac.org/Ag_Materials/fertilizer/mission.htm and information on the Feed Additives and Contaminants Subgroup is available at http://www.aoac.org/Ag_Materials/additives/main.htm. The Fertilizer Subgroup has emerged from and draws strength from the Fertilizer Forum, which has been meeting in conjunction with the AAPFCO Laboratory Committee in past years. The Feed Additives and Contaminant Subgroup has roots in the AAFCO Laboratory Methods and Services Committee, which has also been active in method development. These groups have provided the Committee and volunteer infrastructure upon which to build and expand into a larger community.

Antibiotics in Feeds, Hussein Ragheb

Most current methods are performing satisfactorily. The General Referee recommends the following Official Methods be archived: erythromycin (971.48); hygromycin (960.67); novobiocin (962.25); nystatin (974.48); oleandomycin (974.49); spectinomycin (973.81); and streptomycin (971.49). Some of these antibiotics are no longer manufactured and others are no longer added to animal feeds. It should be mentioned however that certain antibiotics still are in use for some veterinary applications. Microbiological methods of analysis are essentially the same as feed methods.

(1) **993.29 Bacitracin in Feeds:** Topic Advisor Elizabeth Luigs for Zinc and MD Bacitracin (Alpharma Animal Health Division, 400 State St, Chicago Heights, IL 60411, Tel: 708-758-0111, Fax: 708-757-2510). Continue topic.

(2) **Neomycin in Feeds:** Topic Advisor Scott Panagiotis (Pennfield Animal Health, 14040 Industrial Rd, Omaha, NE 68144, Tel: 402-330-6000, Fax: 402-330-6004, E-mail: Panagiotis@noria.net). Continue topic.

(3) **Low Level Chlortetracycline in Feeds:** Topic Advisor Scott Panagiotis. Continue topic.

(4) **Tylosin in Premix and Animal Feeds:** Topic Advisor Mark R. Coleman (Eli Lilly and Co., 2001 W. Main St, GL 36, Greenfield, IN 46140-0108, Tel: 317-277-463, Fax:

317-277-4993, E-mail: coleman_mark_r@lilly.com). Continue topic.

(5) *Narasin*: Topic Advisor Mark R. Coleman (for Microbiological Method). Continue topic.

(6) *Penicillin*: Topic Advisor Steven Holmes (Alpharma, 400 State St, Chicago Heights, IL 60411, Tel: 708-757-2505, Fax: 708-757-3510). Continue topic.

(7) *Chlortetracycline*: Topic Advisor Steven Holmes. Continue topic.

(8) *Bambermycin*: Topic Advisor Denise Riley Moore (Woodson Tenent Laboratories, 345 Arbor Ave, Memphis, TN 38103, Tel: 901-521-4500, Fax: 901-521-4510, E-mail: Dmoore@wtlabs.com). Topic Advisor retired. Discontinue topic.

(9) *Direct Fed Microbiological Products and Silage Inoculants*: Vacant.

Drugs in Feeds, Harold Campbell

(1) *Amprolium, LC Method*: Study Director Fred Armstrong, Canadian Food Inspection Agency, Ottawa Laboratory (Carling), Bldg 22, Central Experimental Farm, 960 Carling Ave, Ottawa, ON K1A 0C6, Canada, Tel: 613-759-1299, Fax: 613-759-1260, E-mail: farmstrong@inspection.gc.ca. The Study Director reports that LC separation on a silica column using a mobile phase of acetonitrile and water, buffered with ammonium acetate, is being investigated for use with both UV and LC/MS detection.

There is one paper of note in the literature for 2004. Fink studied the response of amprolium's primary degradation products to the colorimetric derivatization reaction used in the official AOAC spectrophotometric method for amprolium in feeds (961.24), demonstrating that the method may be used to determine amprolium in stability studies [Fink, D.W., deFontenay, G., Bonnefille, P., Camarade, M., & Monier, C. (2004) *J. AOAC Int.* **87**, 677–680]. Continue study.

(2) *Carbadox*: Topic Advisor Alexander MacDonald, Pharma Science, Inc., 16 Cypress Ave, North Caldwell, NJ 07006, Tel: 973-228-2392, Fax: 973-228-3498, E-mail: BeeMac201@aol.com. No further activity was reported regarding the study of LC method based on the method of Aerts and Werdmuller (1988) *J. Assoc. Off. Anal. Chem.* **71**, 484–489. Continue topic.

(3) *Chlortetracycline, LC Method*: Study Director Richard Larson, South Dakota State University, ASC 133, PO Box 2170, Brookings, SD 57007-1217, Tel: 605-688-6706, Fax: 605-688-6295, E-mail: richard_larson@sdstate.edu. Work continues on optimizing the method of Houghlum and Larson (1997) *J. AOAC Int.* **80**, 961–965. Mobile phase pressure problems are being experienced by some laboratories, apparently related to solubility of the EDTA as the mobile phase gradient approaches 60% methanol. Flushing the column with 15% methanol for 5 min before equilibrating at 35% methanol seems to help eliminate the buildup of buffer salts in the system. The flow rate has also been reduced from 1.5 to 1.3 mL/min. These changes have added 5 min to the run time but do not negatively affect the

chromatography. Another laboratory has suggested reducing the EDTA concentration of the mobile phase buffer from 20 to 5 mM, which also affects (reduces) the relative detector response. Some comparative analyses and validation of the 5 mM versus 20 mM EDTA are being conducted. Continue study.

(4) *Ethopabate*: Topic Advisor Joseph Hillebrandt, Cornell University Diagnostic Laboratory, Nutritional and Environmental Analytical Services, 777 Warren Rd, Ithaca, NY 14850, Tel: 607-257-2345, Fax: 607-257-5041, E-mail: jh119@cornell.edu. Continue topic.

(5) *Ivermectin*: Topic Advisor James A. Whitehead, Merial Ltd, 3360 Maury Ave, St. Louis, MO 63116, Tel: 314-752-8053, Fax: 314-752-8061. The current Peer-Verified Method and interlaboratory study information is published, *J. AOAC Int.* (1994) **77**, 1353–1358. Continue topic.

(6) *Lasalocid*: Study Director Charles L. Focht, Nebraska Department of Agriculture, 3703 S. 14th St, Lincoln, NE 68502-5399, Tel: 402-471-2176, Fax: 402-471-0091, E-mail: cfocht@agr.ne.gov. A collaborative study protocol was submitted for Lasalocid in Feeds and Premixes by Reversed-Phase HPLC with Fluorescence Detection to AOAC in January 2005. The protocol was circulated among expert reviewers selected from AOAC Committee I members. Reviewers' comments and suggestions were returned to the Study Director in late March 2005. Key criticisms of the protocol were that the document did not meet all the method applicability criteria as described in the "Method Needs and Fitness for Purpose Statement". The Study Director was advised that a residue level component must be added to the procedure so laboratories could apply the method to determine the presence of drug contamination in nonlasalocid medicated feeds.

Further work is being done at the Study Director's laboratory and it is expected that the inclusion of the residue level applicability to the procedure will be successful. The protocol will be resubmitted, and once it is approved the collaborative study will follow shortly thereafter. Continue study.

(7) *Monensin, Narasin, and Salinomycin, LC Method*: Study Director Harold Campbell, Canadian Food Inspection Agency, Ottawa Laboratory (Carling), Bldg 22, Central Experimental Farm, 960 Carling Ave, Ottawa, ON K1A 0C6, Canada, Tel: 613-759-1227, Fax: 613-759-1260, E-mail: hcampbell@inspection.gc.ca. The Study Director reports that the manuscript for the joint ISO/AOAC collaborative study was resubmitted to AOAC INTERNATIONAL following a revision of the statistical analysis. The study was successful for all feeds (trace levels and medicated feeds and premixes), but it failed for drug premixes. Continue study.

(8) *Monensin, LC Method*: Topic Advisor Mark Coleman. Method 997.04 "Monensin in Premix and Animal Feeds" has Final Action status. Continue topic.

(9) *Morantel Tartrate*: A Topic Advisor is needed. An LC method is desired; method published by Goras and Gauthier, (1985) *J. Assoc. Off. Anal. Chem.* **68**, 598–601, is a good possibility.

(10) *Narasin, LC Method*. Topic Advisor Mark Coleman. Continue topic.

(11) *Nicarbazin*: The Topic Advisor position is vacant. An interlaboratory study of an LC method was reported by de Jong et al. (2004) *J. AOAC Int.* **87**, 1269–1277. Nineteen laboratories participated in the study of 4 medicated broiler feeds, 1 blank swine feed, and 1 broiler premixture. The RSD_r of the feeds (20 to 240 mg/kg) varied between 2.6 and 10.2%, the HorRat ranged between 0.70 and 1.22, and recoveries were 91–108%. For the premixture, acceptable results were obtained after the test portion and extraction volumes were doubled, and the range of the calibration curve was doubled. The RSD_r was 5.7%, and the HorRat was 1.95 (10 laboratories). An LC method reported by Krael et al. (2000) *J. AOAC Int.* **83**, 1027–1037, is also a viable method. Continue topic.

(12) *Oxytetracycline, LC Method*: Study Director Richard Larson. A collaborative study of the method of Houglum, Larson, Mutchler, and Wetzler, (1998) *J. AOAC Int.* **81**, 919–922, is being conducted. Completion of the study is expected by the end of 2005. Continue study.

(13) *Pyrantel Tartrate*: A Topic Advisor is required. An LC method is desired.

(14) *Roxarsone*: Topic Advisor Margaret Pomeroy, Alparma Inc., Animal Health Division, 400 State St, Chicago Heights, IL 60411-1242, Tel: 708-758-0111, Fax: 708-757-2510, E-mail: peggy.pomeroy@alparma.com. AOAC Official Method **971.47** is the most commonly used procedure for roxarsone (3-nitro-4-hydroxyphenyl arsonic acid) in feeds. There was no activity on the development of an LC method. Continue topic.

(15) *Sulfamethazine, LC Post-Column Method*: Topic Advisor Kendrick Albert, Office of the Indiana State Chemist, Purdue University, 1154 Biochemistry Bldg, West Lafayette, IN 47907-1154, Tel: 765-496-3079, Fax: 765-494-4331, E-mail: albertk@purdue.edu. Noting that other approaches to analysis of sulfamethazine (residues in dairy products and in muscle and organ tissue) with and without use of an internal standard and derivatization have been reported in 2004–2005, there are nevertheless no new developments to report with regard to Official Method **999.16** for determination of sulfamethazine in feeds by HPLC with post-column derivatization. The following articles appearing in 2004–2005, all of which treat analysis of multiple sulfonamides residues, may be of interest: Pecorelli, I., Bibi, R., Fioroni, L., & Galarini, R. (2004) *J. Chromatogr.* **1032**, 23–29. A liquid chromatographic method with diode-array detection (270 nm) is applied to determination of 10 sulfonamides residues in swine, bovine, and poultry muscle samples. The analytes are extracted by homogenization of tissue in ethyl acetate followed by cleanup on a cation-exchange SPE column; chromatographic separation follows by gradient elution (30 min) on a C8 analytical column in acetonitrile–acetate buffer mobile phase. The authors seek “a possible compromise between the requirement of [European Union regulatory] decision 2002/657/EC and the resources of an official laboratory with

high samples throughput.” Furusawa, N., & Kishida, K. (2004) *LCGC North America* **22**, 1092–1096. The authors describe an HPLC method for determination of sulfamethazine residue in pork using 100% water as the only solvent. Sample preparation makes use of extraction by homogenization in water, separation on a reversed-phase C1 column in a water mobile phase, and detection at 261 nm by a photodiode-array detector. The authors aim to avoid generating solvent and other hazardous reagent waste and also to put forth a method they consider useful for “an international standardized analytical method for routine residue monitoring of sulfamethazine in pork.” Maudens, K.E., Guo, F.Z., & Lambert, W.E. (2004) *J. Chromatogr.* **1047**, 85–92. The authors report a quantitative HPLC-fluorescence method with post-column derivatization for analysis of 12 sulfonamides in honey. A review of 4 other methods employing an initial acidic hydrolysis step is included (acidic hydrolysis of the sample to eliminate the problem of sugar-bound sulfonamide compounds and underestimation of contamination). Hydrolysis is followed by liquid–liquid extraction (aqueous acetonitrile) with sulfamethazole added as internal standard, evaporation, and reconstitution, and finally by solid-phase extraction on a cation-exchange SPE column. The method employs gradient elution on an RP-18 endcapped column in sodium acetate buffer–acetonitrile, followed by post-column derivatization with fluorescamine. Fluorescence excitation and emission wavelengths are 420 and 485 nm, respectively. Sulfamethazine and sulfamethoxy pyridazine have a “minimal overlap” in this procedure. Continue topic.

(16) *Tilmicosin*: The Topic Advisor position is vacant. The current method and interlaboratory study information is published, (1997) *J. AOAC Int.* **80**, 1156–1170. Continue topic.

(17) *Tylosin, LC Method*: Topic Advisor Mark Coleman. There is no activity to report. Continue topic.

(18) *New Topics*: Study Directors are solicited for other topics such as clopidol, decoquinat, diclazuril, fenbendazole, halofuginone, levamisole, maduramicin, ormetoprim/sulfadimethoxine, ractopamine, robenidine, semduramicin, and tiamulin. Interested scientists or organizations are encouraged to contact the General Referee or AOAC INTERNATIONAL for more information.

(19) *OMA Chapter 5, Section 5.1.02*: As part of the revision of this chapter, the General Referee recommends a revision of AOAC Official Method **950.02**, Preparation of Test Sample, to provide additional and current information and to make it consistent with the same method in Section 4.1.02 of Chapter 4. The proposed wording is: Reduce particle size of laboratory sample selecting a method that minimizes loss of moisture and maintains integrity of constituents of analytical interest. For feeds containing heat-sensitive analytes, grind to pass 2.0 mm sieve; for other feeds grind to pass 1.0 mm sieve. Store analytical sample under conditions that minimize degradation of components and exposure to the atmosphere.

Feeds, Charles Focht

(1) **2003.05** *Crude Fat, Diethyl Ether Extraction, in Feeds, Cereal Grains, and Forages by Randall/Soxtec/Submersion Method*: Study Directors Nancy Thiex and Bryan Gildemeister, South Dakota State University, Brookings, SD, Tel: 605-688-5466, Fax: 605-688-6295, E-mail: nancy.thiex@sdstate.edu and bryan.gildemeister@sdstate.edu, Shirley Anderson, Foss North America, 7682 Executive Dr, Eden Prairie, MN 55344, Tel: 952-974-9892, ext. 161, Fax: 952-974-9823, E-mail: sanderson@fossnorthamerica.com. Approved as First Action. Method recommended for Final Action status. Continue topic.

(2) **2003.06** *Crude Fat, Hexanes Extraction, in Feeds, Cereal Grains, and Forages by Randall/Soxtec/Submersion Method*: Study Directors Nancy Thiex, & Bryan Gildemeister, and Shirley Anderson. Approved as First Action. Method recommended for Final Action status. Continue topic.

(3) *Inorganic Elemental Constituents of Plant Samples, Microwave Digestion*: Topic Advisors Robert Miller, Colorado State University, Fort Collins, CO, Tel: 970-493-4382, Fax: 970-416-5820, E-mail: rmiller@lamar.colostate.edu and Nancy Thiex. No activity during the past year. Investigate applicability to feed sample matrixes. Continue topic.

(4) *Microscopy*: Topic Advisor Mike Buckner, Division of Consolidated Lab Services (DCLS), 1 N. 14th St, Rm 127, Richmond, VA 23219, Tel: 804-225-4070, Fax: 804-796-7795, E-mail: mbuckner@dgs.state.va.us. No activity during the past year. Continue topic.

(5) *Neutral Detergent Fiber, Acid Detergent Fiber, and Lignin Using Filter Bag Technology*: Topic Advisor Andrew Komarek, ANKOM Technology Corp., Fairport, NY, 14450, Tel: 716-425-3940, Fax: 716-425-3941, E-mail: akomarek@ankom.com. Continue topic.

(6) **2000.12** *Phytase Activity in Feeds*. Topic Advisor Peter Randsdorp, SDM Food Specialties, PO Box 1, Delft, 2613-MA, The Netherlands, Tel: 31-15-2793543, Fax: 31-15-2792357, E-mail: Peter.Randsdorp@dsm-group.com. Method has Official First Action Status. Discontinue topic.

(7) *Vitamin A, LC Method*. Continue topic.

Fertilizer and Agricultural Liming Materials, Peter Kane

(1) **929.01** *Sampling*: Study Director Peter Kane. Kane has been appointed Study Director in order to complete the work of former Study Director Douglas Caine, a method modification to the bulk sampling procedure, to address the need of sampling from 1 ton mini-bulk fertilizer bags. Continue study.

(2) **983.02** *Potassium, Flame Photometric Detection*: Study Director Natalie Newlon, Office of the Indiana State Chemist, Purdue University, Biochemistry Bldg, 175 S. University St, West Lafayette, IN 47907-2063, Tel: 765-494-1563, Fax: 765-494-4331, E-mail: newlonn@purdue.edu. Newlon is currently working on a method for K₂O using ICP-OES. After the traditional

EDTA-citrate extraction, ICP-OES and **983.02** Flame Photometry are run in parallel. Preliminary statistical comparison looks encouraging. The Study Director recommends continued study.

(3) *Controlled Release Nutrient Extraction*: Study Director William Hall, The Mosaic Co., PO Box 2000, 3095 County Rd, 640 W, Mulberry, FL 33860-1100, Tel: 863-428-7161, Fax: 863-428-7398, E-mail: Bill.Hall@mosaicco.com. Precollaborative study data was received; a review of the data indicated minor modifications in the procedure. All suggested method modifications are complete. In-laboratory evaluations show better accuracy, precision, and ease of use. The procedure has been revised and the study is planned for fall 2005. Additional collaborators are still being sought.

Preliminary data was submitted to the AAPFCO Slow Release Committee in 2004. Additionally, a reference incubation method for testing products in a biologically active soil system has been tested and is complete. This allows correlation of the laboratory method to data generated by an agronomic (reference) method. Correlations of the 2 methods is high (>90). The study manuscript and method will be submitted to JAOAC and presented at the 2006 AOAC Annual Meeting. The Study Director recommends continued study.

(4) *Trace Elements in Fertilizers*: Study Director David W. Averitt, The Mosaic Co., PO Box 2000, Mulberry, FL 33860, Tel: 863-428-2500 ext. 4436, Fax: 863-428-7374, E-mail: david.averitt@mosaic.com. No report. The General Referee recommends continued study.

(5) *Determination of Urea in Fertilizers by LC*, **2003.14**: Study Director Michael Hojjatie, Tessengerlo Kerley Inc., 2480 Twin Buttes, Sahuarita, AZ 85629, Tel: 520-791-2940, Fax: 520-625-8091, E-mail: mhhojjatie@tkinet.com. No report. Continue study.

(6) **982.01** *Boron in Fertilizers*: Study Director Wesley Hsu, U.S. Borax Inc., 26877 Tournay Rd, Valencia, CA 91355-1847, Tel: 661-287-5400, Fax: 661-287-5495, E-mail: Wesley.hsu@borax.com. No report. Continue study.

Nutrients in Soils, Charles Focht

The Soil Science Society of America and its subcommittees (S889 and S890) are trying to regenerate a soil methods validation program, and they want to remain in contact with AOAC, so the Nutrient in Soils topic will remain until a decision is reached about further involvement with AOAC.

Tobacco, Harold Burton

Historically, the Analytical Methods Committee (AMC) has been associated with the Tobacco Science Research Conference (formerly Tobacco Chemists' Research Conference) for over 50 years. In the early years, participants were from academic, government (primarily USDA), private, and industry. Due to changes, participants now are primarily from industry. The AMC provided a means for initiating and completing collaborative studies for analyses in tobacco. Until 1998, the AMC met yearly but did not have any collaborative studies. Because of the increasing emphasis on regulating the

tobacco industry, there is a need to continue the GR for tobacco. In the past 5 years, the AMC have conducted collaborative studies for a GC method for nicotine in tobacco, a GC method for water in tobacco, tobacco-specific nitrosamines using GC-TEA, and a study for determining the minor alkaloids. This is the most active the AMC has been in 30 years, so it is providing a need for the industry. The leader for the minor alkaloid study is Serban Moldoveanu,

RJ Reynolds Tobacco, PO Box 1487, Winston-Salem, NC 27102.

Veterinary Analytical Toxicology, Vacant

No report. The General Referee position is vacant and will be appointed by AAVLD Committee of Veterinary Analytical Toxicology at their November 2005 meeting.