Report of the Analytical Methods Working Group

On October 29 and 30, 2002, experts in the analysis of foods for acrylamide and experts in general food analysis convened to discuss any needs in the analysis of foods for acrylamide. For the first half day, the Working Group heard reports from seven laboratories on the methods that they were employing for acrylamide analyses. Each of the presenters provided information on sample preparation procedures, chromatographic and determinative steps, and method performance. The presenters represented a spectrum of commercial laboratories offering acrylamide analyses for a fee, government and regulatory control laboratories, private company laboratories and laboratories within academic institutions. Following the presentations, the Working Group discussed the methods and method performance characteristics and reached the conclusions presented below on the status of current methods and the reliability of results obtained using those methods.

Current Status of Analytical Methods

The table presented below is a summary of analytical methods that are currently known to be employed in this analysis. The methods are grouped by determinative procedure. It should be noted that laboratories have used and are using a variety of procedures to extract acrylamide from food samples and to clean up the extracts prior to analysis. These individual variations do not appear to affect the analytical results. Dr. Castle showed the Working Group information from the FAPAS round of testing involving over thirty laboratories, using variations of at least five of the methods in the table, that demonstrated no significant difference between results from any of the methods. It should be noted that this round involved only one sample of crisp bread at a level of over 1200 ppb. Thus, as will be discussed in a later section, a range of matrices with varying levels of acrylamide should be developed for further proficiency testing. Data was shown that demonstrates, for a simple cripsbread matrix at least, that water, polar organic solvents, or water-solvent mixtures, give the same extraction efficiency. Also, that the temperature and duration of extraction is not crucial since acrylamide seems to be quickly and efficiently extracted at ambient temperatures or at a higher temperature. The major effect of extraction liquid and condition seemed to be on subsequent need to clean up the extract of materials that would interfere with the analysis. Presenters using solid phase extraction columns mentioned the need to qualify such materials both for efficiency and for the presence of interfering substances. One presenter reported that development is underway for a method employing SPME fibres (Solid Phase Microextraction) either in the headspace or in direct contact with the liquid extract.

The Working Group arrived at the following conclusions regarding the methods in the table.

- Evidence available to date suggests that there is no apparent bias in the results between the first five methods in the table.

- Laboratories have also demonstrated good recoveries of acrylamide with the first five methods.
- The working group has limited experience with the LC/UV method and further method performance evaluation is needed before it can be compared with the others.
- Known problem matrices in common to all methods are: coffee (roasted and ground), chocolate, cocoa, some soy sauces, high salt products, molasses.

Method	No. Labs	Validation Data	LOQ, ug/kg	CV, %	Rec, %	Selectivity*	Est. Cost/Sample, USD	Assays/Day/Person
GC-ECD (bromination)	1+	1,3	10	12	75-112	Low-Med	100-200	6-8
GC-MS (no derivatization)	3+	2,4,5	50	10	80-90	High	160	10
GC-MS (bromination)	20+	1,2,3,4,5	10	2-9	95	High	150-200	5-15
LC/MS	2+	2,4,5	20	5-8	90-98	Med-High	200-300	20
LC/MS/MS	20+	1,2,3,4,5	20	5-10	90+	High	200-300	20-25
LC/UV	1+	2	Unk	Unk	Unk	Low**	100-200	unk

1- Peer reviewed, published
2- Unpublished
3- Satisfactory results obtained in FAPAS series 30 round 1
4- Interlaboratory comparisons
5- Internal lab validation

* Level of confidence in correct identification
**All positive results should be confirmed

Status of Method Validation

Previous groups that have met and discussed acrylamide in foods have pointed to the need for validated methods. The Working Group developed the following statements regarding the status of method validation.

- A number of the methods have been validated internally within the individual laboratories that are performing the analysis.
- These laboratories have been taking part in proficiency testing programs such as FAPAS and have obtained satisfactory results.
- In Europe, DG SANCO (Health Protection and Consumers Directorate) has commissioned JRC (Joint Research Centre, Geel, Belgium) to develop and validate a method and prepare a standard reference material. JRC also intends to do proficiency testing and establish a database of analytical methods and test results, in cooperation with CIAA (Confederation of European Food Industries).
- USFDA is planning a peer verified round of testing of its LC/MS/MS method.
- At least one other method will be undergoing a round of peer verified testing.
- There was no consensus that, other than the effort being commissioned DG SANCO, the development of a reference method was needed. The timetable would, anyway, be rather long.

Recommendations for Proficiency Samples

The Working Group recognized that while we have confidence in the results from the methods presented above, confidence could be greatly improved with more proficiency testing and a greater variety of samples. Accordingly, the Working Group recommended that proficiency samples based on the following matrices be developed. Based upon previous analytical data, these matrices are expected to represent samples with acrylamide contents ranging from approximately 10 ug/kg to perhaps several hundred ug/kg.

- Breadcrumbs
- Cereal
- Cocoa Powder
- Cookie
- Instant Coffee
- Peanut Butter
- Potato chip

Who can provide the samples?

With respect to possible providers for these proficiency samples, the Working Group suggested that the following organizations should be approached.

- FAPAS (Food Analysis Performance Assessment Scheme)
- AACC/NFPA/AIB (American Association of Cereal Chemists/National Food Processors Association/American Institute of Baking)
- JRC (Joint Research Center)
- AOCS (American Oil Chemists Society)
- NIST (National Institute of Standards and Technology)

Recognizing the need to have samples as soon as possible the Working Group recommended that samples be available before Spring 2003 or as soon as reasonably possible. In the event that it is not possible to prepare all matrices within that time frame the Exposure Assessment Working Group is asked to help prioritize the samples.

Proficiency test materials should be made in sufficient quantity so that they can be available as in-house Working Reference Materials.

Method Performance

The Working Group made the following statement regarding the acceptance of analytical data.

- Reported data should include performance characteristics which would include; measurement uncertainty, range of linearity, precision and accuracy information, recovery data, limit of quantitation, and other quality control characteristics as defined in established guidelines.

Rapid Methods

While there is an interest in simple, deployable, lower cost methods of analysis, the consensus of the Working Group was that some of the listed methods are suitable for routine testing and that the development of rapid ELISA type assays for this analyte is unlikely to occur anytime in the near future.

Workshop Questions

The Analytical Methods Working Group was also asked to address some questions that were common to the all of the Workshop Groups.

- **What are the primary areas concerning the occurrence of acrylamide in food in which research is needed?**
  1. Is any acrylamide physically 'bound but still potentially bioactive'? If so do current methods extract all acrylamide (including "bound" acrylamide)?
  2. Is there a need to establish methods of analysis for asparagine, and other possible precursors in food?
  3. Establish proficiency testing program and materials
  4. Need data for more foods

- **Are methods currently available to accomplish this research?**
  1. To be advised by Toxicology group
  2. To be advised by Mechanism group
  3. Yes, there are organizations capable of operating a proficiency testing program and developing test materials
  4. Yes, methods are validated to approx. 30 ppb for a limited range of matrices, lower levels will require additional validation

- **What is the time frame for getting results for the research identified?**
  1. Contingent upon need
  2. Contingent upon need
  3. ASAP
  4. ASAP

- **What missing information is needed to enable the proposed research to be even initiated or accomplished?**
  1. Contingent upon need
  2. Contingent upon need or further discoveries about precursors
  3. Development of prioritized list of desired test materials
  4. Resources

- **What questions will be answered by each research area proposed?**
  1. Whether we're missing any sources of exposure
  2. Help identify other high exposure potential products. Provide data to support the formulation of mitigation strategies
  3. Improve overall confidence in analytical results and better understanding of method capability
  4. Better exposure estimates

- **Rank the research areas/projects identified in order of priority for accomplishment**
  1. Proficiency testing program
  2. More data
  3. Asparagine and precursors
  4. Bioactive "bound" acrylamide

- **Where is your Working Group linked to others, i.e., from what other Working Groups do you need assistance?**
  1. Need input from mechanism group about analytical needs for precursors
  2. Need input from Exposure group on detection limits and help with prioritizing the development of proficiency samples
  3. Need input from Toxicology group about need to analyze for "bound" acrylamide