Development of Analytical Methods for Agricultural Chemicals

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MR. MYERS: Our last speaker this morning will be Dr. Eugene Kennedy. He is with the Division of Physical Sciences and Engineering at NIOSH in Cincinnati, Ohio. He will be giving a presentation on the Development of Analytical Methods for Agricultural Chemicals.

DR. KENNEDY: I would like to acknowledge my co-workers in this. They are Martin Abell of NIOSH and John Reynolds and Don Wickman of Datachem Laboratories out in Salt Lake City. They were all very helpful in pulling this all together. Actually, John and Don did most of the laboratory work and we collaborated back and forth by phone and E-mail.

Well, you cannot see it on this slide, but I am from the Methods Research Branch. One of our functions is developing sampling and analytical methods for chemicals in the work place air. In response to the NIOSH initiative in the area of agriculture we initiated research to develop a model multianalyte method for agricultural chemicals. This was based on the need for a unified sampling and analytical approach for these compounds, that is the use of one single sampler for a whole series of compounds. This is one of the things that had been requested by some of our field people. We thought it was a good way to go.

We started this research effort by grouping compounds in ways that we could use a single analytical technique for the whole class. The method I will be talking about uses gas chromatography for the analysis. I will mention a few other things that we are also looking at as well.

We selected some of the chemicals that we included in our study on the basis of their recommended exposure limit or permissible exposure limit or threshold limit value. Also, often times these values were not available, so we also included annual production figures for where we could find them and the toxicity of the compounds.

Since some of the compounds that we were going to be studying were the organophosphorus pesticides, which could exist in both the aerosol and vapor phase we needed a sampler that would be able to collect both phases. In order to do that, we looked to the literature and found that researchers at the Occupational Safety and Health Administration (OSHA) Laboratories in Salt Lake City had developed a sampler (OVS) for a series of pesticides individually. This was viewed as an excellent device for method development and evaluation. Essentially it consists of a filter in front of an absorbent bed and a back up sorbent bed.

Although this looks like it is a pretty huge device it is only about two inches long so it will make personal sampling quite convenient.

This slide provides a summary of the method that we developed for the or-

ganophosphorus compounds. Basically we used the OVS sampler at a sampling rate of one liter per minute. We can use it for up to 8 hours so we can take a 480 liter sample. The analytes are desorbed with organic solvent. As I mentioned before, we use capillary gas chromatography analysis and can determine up to 19 compounds simultaneously.

We did look at other columns for analysis, so we do know we can use those columns for confirmation of the pesticide identity when we find it on the primary analysis column. We also found that the samples were fairly stable for up to 30 days at either ambient or refrigerated conditions. I will be talking about this a little bit later in my presentation.

For those of you not familiar with gas chromatography these are basically what the results look like. This slide shows all 19 compounds, base line resolved with an internal standard added. That is the type of chromatography that we always like to see when we are doing analysis. It makes life a lot easier for the chemist.

The key element in most of the NIOSH methods or in the NIOSH Manual of Analytical Methods is that the method provide a result that is plus or minus 25 percent of the true value 95 times out of 100 or essentially 95 percent of the time. This element is often referred as a NIOSH Accuracy Criteria.

In order to ensure that the method would meet this criterion we established a protocol for the evaluation of the method. This protocol was based on a previous protocol which was used back in the 1970s during the Standards Completion Program for method evaluation.

One departure that we did make from the original protocol was we did not prepare generated samples. As you can imagine, trying to generate pesticide aerosols in the laboratory and being sure what concentration you have, could be a nightmare. To simulate generation, we took some slightly different steps and fortified the samplers with the analytes contained in a solution. We pulled humid air through these samplers in order to simulate sampling.

One of the things that we did look at was the limit of detection, since we wanted to verify that we would be able to detect the analyte at levels that would be much lower than the exposure limit. We also wanted to verify that the recovery was greater than 75 percent. We wanted to make sure we had adequate sampler capacity so that we could sample at known concentrations for an extended period of time. As I mentioned, we wanted to verify what the stability of the analyte on the sampler was and also to look at the precision and bias in order to verify its performance in meeting the NIOSH accuracy criterion.

In summary of the results that we obtained from our limit of detection work, we saw a typical limits of detection of around 0.04 to 0.6 micrograms per sample per analyte. That essentially was 50 to 100 times lower than any of the recommended exposure limits for these compounds. This meant that we had plenty of sensitivity in the method to be able to detect very low levels of pesticides.

We also evaluated the recovery without pulling air through the sampler, just to see what kind of recovery factor we would have to apply when we were doing sample analysis. In most cases it was greater than 90 percent for all 19 compounds.

We looked at the capacity of the sampler. Essentially we spiked the samplers with between 50 and 400 micrograms of each pesticide. That was essentially equivalent to sampling at 2 times the exposure limit for up to 12 hours.

We did a screening study where we looked at the effect of temperature and humidity on sampler performance. We sampled from these humid atmospheres at one liter per minute and tried to observe sampler breakthrough; that is, if we found five percent of the analyte on the back up section of the sampler, then that would indicate that the sampler had broken through and we would have to look at different sampling times to see where the break through point did occur.

In order to incorporate a safety factor into the maximum recommended sampling times, we would multiply this break through point times two-thirds. It gives us increased confidence that we had not over sampled.

This was basically a summary of the results of our capacity study. Essentially we saw no effect of temperature or humidity on the capacity. We also saw no break through even after sampling for 12 hours at 1 liter per minute. That gave us a maximum recommended sampling time for all 19 analytes of 8 hours which corresponds to a 480 liter sample.

Next we wanted to look at the stability of the samples, so we fortified 24 samplers at a level which was equivalent to sampling at one-half of the exposure limit and then we weathered these for 4 hours at 30 degrees centigrade and 15 percent relative humidity. Then we analyzed this series of samplers over a period of 30 days.

The data I have here is for the pesticide methamidophos. We analyzed 6 samplers on day 1, 6 samplers on day 7, 3 samplers on day 10, 14, 21, and 30. The criterion that we were applying here was that we wanted to make sure that we could get 90 percent of the analyte back from the sampler. The 90% recovery is indicated by this straight line on the slide. As you can see here when we get out to around 28, days we start dropping below the 90 percent line.
This storage was done at room temperature. We also have repeated the storage study under refrigerated conditions and found that all the materials, including methamidophos did give us good recovery and was above 90 percent even at 30 days.

This slide summarizes the data that we had. The samples were stable for 30 days at 25 degrees centigrade and 0 degrees centigrade. You can see that the recovery tended to improve a little bit when we used refrigerated storage, so that is what we will be recommending in the method when it comes out in the Fourth Edition of the NIOSH Manual of Analytical Methods.

In order to determine whether the method met the NIOSH accuracy criterion preci-

sion and bias of the sample results were determined. This curve represents a combination of estimated bias and precision values which will meet the NIOSH accuracy criterion. We have set arbitrary limits of plus or minus 10 percent on the bias. As you can see here any set of values which falls under this curve will meet the NIOSH accuracy criterion. We do have some other limits here like the upper curve is if we have got 4 sets of samples, 6 samples per level, and here we just have 1 level with 12 samples. We use this for our short-term exposure limit data.

Based on this, we prepared 6 samples at each of 4 levels, that is 0.1, 0.5, 1:0 and 2 times the exposure limit for the amount that would be collected for an 8 hour period. These samples were weathered under the humid conditions that we have previously defined in our capacity study.

For compounds which had short-term exposure limits we prepared 12 samples based on the amount of material that would be collected during a 15 minute sampling period. We then determined the estimated average bias of all of these results, calculated the precision of the pooled sampler results and included a five percent pump error into the estimates. The short-term samples and long-term samples were treated separately in the data analysis.

This slide gives a summary of the results that we had. Basically for all 19 compounds, the biases range from between minus 2 and minus 7 percent and the precisions range between 6 and 7 percent. In all cases the methods did meet the NIOSH accuracy criterion for all 19 compounds.

Since the ability of the sorbent to collect pesticide vapor has not been fully verified in our experiments we did one additional experiment to look at this fact. We took a sampler and fitted it with a dropping pipette tip, placed and sealed that into the front end of the sampler with wraps of Teflon tape. Then we placed a piece of quartz wool into the front and then fortified this quartz wool with solutions of the pesticides. We then placed this into our humidity generator system and pulled air through the sampler.

As you can see on the next slide, the volatility of the pesticides varies. The red portion here indicates that we found the majority of that pesticide on the quartz wool. Down in the lower area of the graph, we found the majority of the pesticide on the sampler. As you can see here for azinphosmethyl, fenamiphos, and monocrotophos, they really did not volatilize at all. When we get down to mevinphos and phorate, those pesticides almost exclusively evaporated from the quartz wool and were collected onto the sampler.

I would like to mention a few other things that we are doing right now. We are taking this sampler and trying to expand its use. We are currently looking at 13 carbamate pesticides which we will be determining by high performance liquid chromatography. In a somewhat related project we are looking at the application of enzyme linked immunosorbent assay kits for pesticides. We hope to be able to apply those to filter samples or air collected in the

field. These offer several advantages: they are fairly low cost, I think $10 to $15 per analysis; they have the potential to be field analyzable; and also we want to compare them with our GC/LC methods and see how they do compare in terms of precision and bias. It may be a more cost effective way of looking at these analyses.

Finally, I would like to acknowledge a couple people who helped with a lot of the administrative aspects of getting this work done. They include Dr. Jim Perkins with Datachem Laboratories and Mr. John Holtz in our Division of Physical Sciences and Engineering. ■

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