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1 interpretation of bioassays? Can we start looking
2 at susceptible subpopulations?
3 [Slide]
4 Really what we are interested in here is
5 how much acrylamide gets in the body, how much is
6 metabolized, what is our area under the curve in
7 blood and the tissues? Do we have adducts in
8 proteins, and do we have adducts in DNA? And, the
9 same thing for glycidamide. And, what is the
10 balance between the various processes?
11 [Slide]
12 To go back to a little history, one of the
13 more comprehensive studies on pharmacokinetics was
14 conducted by Miller et al., in 1982. They
15 administered radioactive acrylamide to rats. I
16 just draw your attention to the very last bullet.
17 The half-life of acrylamide, they found, was about
18 two hours and about 12 percent of the radioactivity
19 remained associated with red blood cells. That is
20 something that is actually specific to the rat.
21 They have a very high level of binding in red blood
22 cells and it is because of the presence of a highly

1 reactive cysteine in rat hemoglobin that does not
2 occur in mouse or in human red cells.
3 [Slide]
4 I have already alluded to this study that
5 was conducted in 1992. This was the first study
6 that I was involved in with acrylamide, looking at
7 urinary metabolites following administration of
8 this 1,2,3 ^{14}C acrylamide in rats and mice. We used
9 a dose of 50 mg/kg in male rats and mice and we
10 were able to characterize five urinary metabolites
11 including four derived from glycidamide.
12 Interestingly, we actually found glycidamide itself
13 in the urine. We find that there was a big species
14 difference, with rats metabolizing most of the
15 acrylamide directly by glutathione conjugation and
16 about 33 percent by glycidamide, whereas with mice
17 it was the opposite, much more was oxidized by
18 glycidamide than was conjugated directly with
19 glutathione.
20 [Slide]
21 On this slide I just have a summary of the
22 various metabolic pathways. The acrylamide

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1 undergoes direct conjugation with glutathione to
2 form this mercapturic acid that we see in urine.
3 This is the epoxide glycidamide and this is
4 actually detected in urine and we see it is ring
5 opened form, the dihydroxy form, and we also see
6 two glutathione conjugates at each of the epoxide
7 carbons to form also mercapturic acids.
8 [Slide]
9 Now I am going to switch to hemoglobin
10 adducts. These are one of the great ways of
11 detecting exposure long after the exposure may have
12 happened. So, what do we know about hemoglobin
13 adducts and how do they behave? Well, they are
14 proportional to area under the curve for reactive
15 chemicals or metabolites. You can get a lot of
16 hemoglobin rapidly from a blood sample so it is
17 easy to get and easy to measure. It has a number
18 of reactive amino acid residues that can react with
19 chemicals and their metabolites, cysteine,
20 histidine, the internal valine and carboxyl groups.
21 The internal valine is particularly useful in
22 performing adducts because it is the same in both

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1 the alpha and beta chain of hemoglobin and it is
2 the same in rats, mice and humans. So, that gives
3 you a lot of simplification. You don't need to
4 have different assays for different species.
5 The red cell has a long lifetime in
6 circulation of about 120 days, and it is removed
7 with zero order kinetics. That also is a big
8 advantage. It is not a first order process so it
9 is very predictable and we can really nicely model
10 what is going on with adduct accumulation. What
11 happens is if you have continuous exposure over the
12 life span of the red cell, in humans, if you have
13 an adduct formed per day of exposure of X, you
14 would expect that the life span of the red cell, if
15 you had continuous exposure, is that your adduct
16 level would reach 60 times X. So, that is very
17 useful for long lived exposures and long
18 experiments but it makes it very difficult to
19 actually do a short-term study because you need to
20 have a fairly high level of exposure if you are
21 trying to do a calibration study.
22 With repeated exposures the adduct

1 accumulate and reach a steady state when the
2 duration of exposure exceeds the red cell life
3 span. So, it is good for things like dietary
4 exposure, smoking, exposures in the workplace,
5 those kind of endpoints.
6 [Slide]
7 This is just a very crude sketch of what
8 happens if you have a reactive chemical in the
9 blood. This is the concentration with time and, as
10 your chemical would go up and down maybe in one or
11 two exposures on consecutive days the adduct
12 concentration would increase and would reach
13 plateau when your chemical has disappeared from
14 circulation, and then would increase again on the
15 second exposure. So, that is sort of an
16 illustration of the integration. You are looking
17 at accumulation over time and the dose or the area
18 under the curve equals the adduct concentration
19 that you measure divided by the reaction rate
20 constant for basically a second order reaction, a
21 rate constant that you can determine chemically _in
22 vitro_. So, that is one way that we can actually go

1 from an adduct measurement to an indication of what
2 the area under the curve was and can give us a way
3 of tying back into pharmacokinetics and
4 pharmacokinetic modeling.
5 [Slide]
6 Again to review just a little bit of the
7 history, the first measurements were done with
8 cysteine adducts in the rat. There has been some
9 indication of non-linearity, first in a study by
10 Bailey and then in two studies by Calleman. These
11 are probably the more authoritative ones. They
12 measured adducts from acrylamide and glycidamide
13 produced by reaction with cysteine. They detected
14 the adduct from glycidamide as well as acrylamide.
15 Then they did a very elaborate compartmental
16 pharmacokinetic model and it ended up with a
17 description of a saturable metabolic process,
18 oxidation of acrylamide to glycidamide. what it
19 implies is that there is a greater risk per unit
20 exposure of low doses if glycidamide is the
21 metabolite that generates adverse effects.
22 [Slide]

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1 This just shows acrylamide internal dose
2 that was calculated by Calleman. This is the
3 administered dose in milligrams per kilogram body
4 weight. You can see this is a straight line
5 extrapolated from these points, and then this is
6 the actual curve of the model with individual data
7 points of each dose. So, that is for acrylamide.
8 [Slide]
9 For glycidamide it shows this evidence of
10 saturation where, as you increase the dose up to
11 100 mg/kg, the amount of glycidamide produced is
12 falling off.
13 [Slide]
14 Then, the last slide from this paper shows
15 that they calculated the percent of acrylamide that
16 would be metabolized through glycidamide at 15
17 mg/kg, which was actually the concentration we used
18 or the dose that we used in our studies, and find
19 about 30 percent or so metabolized through
20 glycidamide in the rat. We got pretty good
21 agreement. As you go down to lower doses the
22 percentage that gets metabolized through

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1 glycidamide appears to go up.

2 [Slide]

3 Now I would like to switch gears to valine

4 adducts. I am sure this is a name that is familiar

5 to you all. Before Margarita was working on

6 acrylamide in food she did a lot of work on

7 hemoglobin adducts and actually developed one of

8 the major methods that we use for acrylamide adduct

9 analysis. That is when valine is involved in the

10 adduct formation. It is the internal residue in

11 both the alpha and beta chains, as I indicated

12 before.

13 She developed a modified Edman degradation

14 that selectively cleaves the N-terminus with its

15 adduct, using a variant of the classic Edman

16 degradation for peptide sequencing. This has been

17 used widely with gas chromatography with negative

18 chemical ionization mass spectrometry, and more

19 recently with GS-MS. It is, however, a real pain

20 to do this assay. It is tremendously labor

21 intensive, although as assays go it is pretty good.

22 [Slide]

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1 This method was applied in a number of
2 studies. One was by Bergman et al. They looked at
3 analysis of acrylamide and glycidamide adducts in
4 China. Just to go through these really quickly,
5 they looked at acrylamide and glycidamide adducts
6 in 41 workers exposed to acrylamide. They only
7 looked at glycidamide adducts in six samples and
8 they find that there were about 30-100 percent of
9 the acrylamide adduct levels. This methodology
10 though left something to be desired. It was a
11 completely different method for the AAVal or
12 acrylamide valine versus the glycidamide valine.
13 There were other measurements reported by
14 Emma Bergmark on acrylamide valine in smokers and
15 non-smokers in laboratory workers. Then Perez et
16 al. developed a method for acrylamide valine and
17 glycidamide valine that was applied to workers in a
18 production plant, and they recorded glycidamide
19 valine levels that were about 3-12 percent of the
20 acrylamide valine levels. These are probably a
21 reasonably good measurement.
22 Then we have the Hagmar study, in 2001,

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1 where acrylamide valine was measured in 210 tunnel
2 workers. This is the one that precipitated a lot
3 of the measurements in food. In the unexposed
4 workers it measured from 0.02 to 0.07
5 nanomoles/gram for acrylamide valine and in the
6 exposed workers it was found in considerably
7 elevated levels.
8 [Slide]
9 One of the things that I have been doing
10 over the last few years has been to work on a
11 number of different aspects. These are metabolic
12 data on acrylamide and adduct data. One of these
13 has been how is the internal dose related to
14 exposure with different routes of exposure and
15 different species. Is the GSH conjugation to
16 oxidation ratio altered by exposure route and by
17 dose?
18 We have looked at comparing route of
19 exposure, dermal and inhalation and IP, and we have
20 looked at comparing inhalation exposure in rats and
21 mice, and looked at Cyp 2E1 metabolism and its role
22 in acrylamide metabolism.

1 [Slide]

2 This just shows sort of a summary plot of
3 glutathione conjugation versus oxidation. The
4 first bar is the glutathione conjugation. The
5 second bar is all of the metabolites of
6 glycidamide. The last one is glycidamide itself in
7 urine. When we look at dermal in the rat, it
8 actually has the lowest amount of glutathione
9 conjugation, the highest amount of oxidation, and
10 compare that with inhalation IP and gavage and we
11 see about 70 percent here at the peak. Whereas, in
12 mice when we look at inhalation and gavage, we have
13 a much greater percent of oxidation in the mouse
14 compared with the rat.

15 [Slide]

16 We looked at metabolism of acrylamide via
17 glutathione conjugation, oxidation to glycidamide
18 and further metabolites of glycidamide in wild type
19 mice and Cyp 2E1 null mice. We administered
20 acrylamide at a dose of 50 mg/kg. In the wild type
21 mice we find basically what we have seen before.
22 About 50 percent or so was metabolized by

1 glutathione conjugation, the remainder by
2 glycidamide and glycidamide further metabolites.
3 Whereas, in the 2E1 null we saw absolutely nothing
4 derived from glycidamide or glycidamide itself.
5 So, it argues that Cyp 2E1, at least in the mice,
6 is the major or the only form of p450 that
7 catalyzes the reaction and there doesn't appear to
8 be any other oxidation pathway.
9 [Slide]
10 In my lab recently we have been working on
11 a new method for adduct analysis, basically using
12 the Edman degradation. We have adapted this for
13 using LC/MS/MS for analysis. We get a higher
14 throughput, a greater sensitivity with a smaller
15 amount of globin and we can distinguish adducts
16 from natural abundance acrylamide and ^{13}C-labeled
17 acrylamide. We have just recently published this,
18 actually this month.
19 [Slide]
20 The method essentially works on taking our
21 acrylamide valine hemoglobin, adding Edmund reagent
22 and we selectively cleave our adduct and we form

1 this kind of an adduct derivative, where this part,
2 here, is derived from the acrylamide and this part,
3 here, is derived from the valine, and this part,
4 here, is derived from our derivatizing agent. We
5 add an internal standard and we monitor by
6 LC/MS/MS. We have three different ion reactions
7 that we monitor. One is for our analyte; the
8 second is for our ^{13}C-labeled analyte where we have
9 acrylamide administered to animals. The third one
10 is our internal standard which is derived from
11 labeling the valine with ^{13}C.
12 [Slide]
13 We have a similar kind of procedure for
14 analyzing glycidamide valine. This basically is
15 the adduct. I won't bore you with the details
16 here.
17 [Slide]
18 Our chromatograms look something like
19 this. This is acrylamide valine. I think it is in
20 the mouse. Here is our natural abundance channel,
21 our ^{13}C channel where we have administered
22 ^{13}C-labeled acrylamide, and this is our internal

1 standard. From this we can understand our curves,
2 we can quantitate and we can make measurements.
3 [Slide]
4 This is what we get with glycidamide
5 valine. We actually have two isomers of our
6 analyte and here is our internal standard.
7 [Slide]
8 We have basically looked at the same kind
9 of studies as I mentioned with our metabolism
10 studies so I won't run through all this.
11 [Slide]
12 We have looked at acrylamide valine and
13 glycidamide valine in rats administered acrylamide.
14 We have used a number of different routes and
15 exposure scenarios. What I wanted to draw your
16 attention to, since we are talking about food,
17 primarily gavage as a route of exposure. We have
18 done two studies here, at 50 mg/kg and at 3 mg/kg.
19 These are actually exposed to ^{13}C so the channel to
20 really focus on is this one, AAVal and GAVal, and
21 we see high levels at 50 mg/kg and much lower
22 levels at 3 mg/kg.

1 The important thing is that the ratio of
2 glycidamide valine to acrylamide valine changes in
3 going from high dose to low dose. So, we do seem
4 to have a dose-response difference in the percent
5 going to acrylamide valine versus glycidamide
6 valine, and that fits with the Calleman data.
7 [Slide]
8 I am just going to bypass this.
9 [Slide]
10 To look at inhalation exposure we used
11 rats and mice actually during the same exposure
12 event. Again, I am going to call your attention to
13 these two sets of data and just look at the
14 numbers. These got the same exposure and we have
15 similar amounts of acrylamide valine from the
16 acrylamide in blood, whereas in the glycidamide
17 valine we have very considerably higher levels in
18 the mouse than in the rat. Our ratio here is
19 completely different. It is about 1.0 for the rat
20 and about 3.5 for the mouse. So, we have big
21 species differences and mice seem to make a whole
22 lot more glycidamide valine than do rats.

1 [Slide]

2 So, what kind of range of adduct
3 concentrations have we seen and what kind of ranges
4 have been reported? There have been a number of
5 studies by Bergmark, by Hagmar and by Perez. The
6 background is sort of in general agreement in
7 humans from these studies. Some are in the 20-70
8 range. In smokers that may be elevated. In
9 acrylamide exposed workers we can have fairly high
10 levels that have been reported and that vary from
11 300-34,000 in the Bergmark study and in the 3-12
12 percent range in the Perez study for glycidamide
13 valine compared to acrylamide valine.

14 In rats, what is in the literature, apart
15 from what I summarized on cysteine adducts, we have
16 a background and in rats administered a fried diet
17 there is an increase from about 20 up to
18 about--well, an increase of the order of I guess
19 somewhere around seven-fold. So, there has been
20 reported an increase in rats fed a fried diet.

21 [Slide]

22 So I would like to conclude from some of

1 our published work that we have seen route
2 differences in internal dose in metabolism to
3 glycidamide. We see that dermal administration
4 results in a lower percentage of absorption. I
5 didn't really go into that but I think dermal is
6 one of the significant worker place exposure rates.
7 We do see a species difference in metabolism to
8 glycidamide.
9 [Slide]
10 We can readily measure acrylamide valine
11 and glycidamide valine by LC/MS/MS. We do see a
12 background for both of our adducts. The ratio of
13 acrylamide valine to glycidamide valine depends on
14 dose, rate of exposure and species. We do see a
15 higher amount of glycidamide valine in mice
16 compared to rats. Although I didn't really go into
17 it, we do see a good correlation between metabolism
18 data and hemoglobin adduct data.
19 [Slide]
20 I think one of the things we are all
21 concerned about is where are the data gaps. I have
22 my opinions on where these are. One of the big

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1 ones is when are we concerned about glycidamide and
2 when are we concerned about acrylamide, and how
3 metabolism fits into mode of action when you are
4 dealing with risk assessment. How is acrylamide
5 taken up and metabolized in people and what is the
6 relationship between exposure and hemoglobin adduct
7 levels in humans. Then, a final point is how good
8 are the data on DNA adducts.
9 [Slide]
10 We have a number of studies in progress
11 and I just wanted to summarize those very quickly.
12 [Slide]
13 The first one is a human study that has
14 been funded by SNF. We are actually just doing the
15 measurement of the adduct concentrations. That is
16 to evaluate uptake of acrylamide, metabolism to
17 glycidamide and to calibrate hemoglobin adducts
18 with the known exposure and to compare uptake on
19 dermal and oral administration. This was an
20 exposure of sterile male volunteers to three
21 different dose levels of ^{13}C-labeled acrylamide, a
22 dermal exposure to three times three doses of

1 acrylamide, and then collection of urine for
2 analysis of metabolites by ^{13}C NMR, collection of
3 blood for analysis of hemoglobin adducts before and
4 after administration. We are almost at the
5 conclusion of this study and we hope to have this
6 finished probably by the end of next month.
7 [Slide]
8 There has been one paper published on
9 acrylamide DNA adducts, and that is this paper by
10 Segerback. They had synthesized an adduct,
11 characterized an adduct standard from the reaction
12 of glycidamide with guanine and then administered
13 ^{14}C-labeled acrylamide to rats and mice. They had
14 identified DNA adduct based on comigration of ^{14}C
15 with adduct standard on HPLC. They quantitated the
16 adducts.
17 These kind of approaches are limited by
18 the specific activity of the acrylamide you can
19 prepare and we do have more sophisticated methods
20 for this. We have been working on one. I know
21 that at NCTR there has been work going on, on this
22 also and I think they are further ahead than we

1 are. But I think it is something that needs to be
2 done and we need to have a better understanding of
3 is this, for example, the only DNA adduct and how
4 is it repaired. There are quite a number of issues
5 of how one deals with an adduct if it is formed.
6 [Slide]
7 I just wanted to finish up with this slide
8 which is basically a summary of some of the points
9 from the FAO/WHO consultation recommendations. At
10 the time when these came out we were actually
11 actively involved in pursuing some of these. One
12 is evaluating and calibrating biomarkers of
13 exposure. We need data on absorption, metabolism
14 and distribution and excretion in humans by the
15 oral route. We need information on glycidamide and
16 binding to the DNA as a marker of toxicity and
17 carcinogenicity. We need dose-response
18 characteristics of acrylamide and glycidamide and
19 the relationship between adducts with hemoglobin
20 and adducts with DNA in different organs.
21 With that, I would like to stop and thank
22 you for your attention.

1 **Questions of Clarification**

2 DR. MILLER: Comments or questions? Yes,
3 Frank?
4 DR. BUSTA: I your human study that you
5 are just concluding, the dose of 0.5--
6 DR. FENNELL: That was the lowest dose.
7 DR. BUSTA: Was it given orally?
8 DR. FENNELL: Yes.
9 DR. BUSTA: I am just guessing, I tried to
10 calculate that, that is about 100 and 200 times
11 above what you might get with a high concentration
12 in food?
13 DR. FENNELL: Yes, I think we are talking
14 about ball park. There are a couple of things that
15 we were interested in, in this study. One is that
16 when we initially developed our exposure protocol
17 the driver was not acrylamide in food. The driver
18 was acrylamide in the workplace. These are not,
19 you know, outrageous levels that could be achieved
20 in the workplace. I think that is issue number
21 one.
22 The other is that when you come to

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1 measuring DNA adducts or hemoglobin adducts, or any
2 of these things that have a background, and you are
3 accumulating a background from the dietary exposure
4 it is difficult to actually see a significant
5 increment above that background without going to a
6 higher dose than you would see ordinarily for
7 example in food. So, in order to see, for example,
8 a two-fold or just a doubling of a background in
9 hemoglobin adducts you would probably need to
10 administer something like 60 times the daily dose
11 to see an increase statistically, and the levels
12 that we operate for determining background that is
13 quite a challenge. So, one needs to actually get
14 to much higher levels in order to make that happen.
15 One thing that would did was to use
16 ^{13}C-labeled acrylamide so we are actually looking at
17 a different species of acrylamide adduct and
18 glycidamide adduct than we see in our indigenous
19 background but even then we were concerned that it
20 was going to be too big a challenge for us to go
21 much lower than 0.5.
22 DR. MILLER: How do you deal with that

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1 when you have a substance that appears to be
2 metabolized differently at high concentrations than
3 low concentrations?
4 DR. FENNELL: I think the range that we
5 are in--well, one of the things that we did want to
6 find out was over that small concentration range
7 did we see a difference. Certainly, what has been
8 shown in the rat has been 0.5 to 100 mg/kg. So, in
9 the range of 0.5 to 3.0 there is probably not going
10 to be as wide a difference. You know, that big
11 difference has been in the rat for the two dose
12 points that showed the maximum or that showed the
13 best evidence of saturation were at 50 mg/kg and
14 100 mg/kg. So, it is quite possible we won't be
15 anywhere close to that at this point.
16 DR. MILLER: You said you calculated it
17 was about 60 times?
18 DR. BUSTA: Well, if you have 1000 ppb in
19 a food, I calculated 1 mg/kg of food and feeding at
20 0.5, for an average person that is 35 mg that you
21 have to feed. So, you would have to have 35 kg of
22 French fries to get that average load. Maybe my

1 numbers are a little off.

2 DR. FENNELL: No, I think your numbers are
3 about right. I think this is one of the issues.
4 You know, this study is not going to be the answer
5 to what happens to acrylamide in food but I think
6 it is going to go a long way to providing a
7 calibration for hemoglobin adducts which in the end
8 will give us some idea of what is happening in
9 food.

10 DR. BUSTA: Where is the background coming
11 from?

12 DR. FENNELL: The background is coming
13 presumably from food. One of the things that we
14 have had difficulty with is as you get down to the
15 low end of the calibration curve your matrix blank
16 always has a measurement, there is always something
17 in that, so how do you deal with that if you are
18 going to use the appropriate matrix? We are still
19 wrestling with some of those issues on our assay at
20 the moment for dealing with our pre-exposure
21 measurements. We clearly do see adducts. You
22 know, it is quite easy to find them. I guess the

1 question becomes when you do a calibration curve
2 how you deal with them, and we are wrestling with
3 some of those issues at the moment.
4 DR. BUSTA: I would think that there are
5 groups of people that are consuming very, very low
6 levels of acrylamide in the kinds of foods they are
7 eating. So, you should be able to run an assay on
8 their blood and find the minimum amount of adducts
9 as a result of their consumption of that food. But
10 you say you still get a baseline.
11 DR. FENNELL: We haven't done a huge
12 number but we get a baseline even when we set up
13 with rat hemoglobin where we are dealing with rats
14 on defined diets, none of which we believe are
15 fried but at least may be heated, and we do see a
16 background. At the time we first observed it, it
17 was more of an extreme annoyance and we thought,
18 you know, when are we ever going to get a decent
19 batch with a low background but now we know how to
20 interpret this and it is more of an interesting
21 observation.
22 DR. MILLER: Other comments?

1 DR. RUSSELL: With regard to the
2 consultation recommendations that are up here now
3 on the data on absorption, distribution and
4 excretion in humans by the oral route, were those
5 recommendations both for acrylamide in the food
6 matrix as well as pure acrylamide? I guess another
7 way of asking is not so much about the
8 recommendations but is the work envisioned to go on
9 in both realms, both looking at the pure acrylamide
10 and in a food matrix?
11 DR. FENNELL: I wasn't at the consultation
12 and I know there is at least one person in the room
13 that was so.
14 DR. MILLER: Go ahead.
15 DR. CANADY: Yes, our intention was just
16 for food but information from other routes would be
17 informative to interpreting existing tox. studies.
18 DR. MILLER: Just one question, was there
19 any suggestion made about the matrix itself, what
20 the matrix should consist of? If everyone uses a
21 different diet you may end up with different
22 results.

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1 DR. CANADY: Right. No, that level of
2 detail was not gone into. It was more just the
3 observation that we don't have information for
4 absorption of acrylamide through the food matrix
5 and so we need information that can help us
6 interpret how we receive acrylamide through food
7 versus what the animal studies tell us. That was a
8 simple observation.
9 DR. MILLER: I have to admit that I am
10 just uneasy about the apparent lack sometimes of
11 agreement on fundamental standards. For example,
12 if you are going to argue that there is a
13 possibility that absorption or metabolism is going
14 to have some impact on the outcome of the
15 experiment, it would also be probably clear that
16 different matrices are going to cause different
17 effects, or at least it is possible. So, it seems
18 to me there has to be some venue where people can
19 agree on what that matrix is going to be. That
20 equally applies to the analysis too. I mean, if
21 everybody is using different analyses you have to
22 show it doesn't make any difference, the

1 differences between the different analytical
2 methods, or you are going to have to agree on which
3 method you are going to use.
4 DR. DWYER: I am just curious. It seems
5 to me there are a lot of feeding studies around the
6 country where people are fed defined diets or some
7 kind of diet, kept samples of what they are fed or
8 at least records of what they were fed, where they
9 have blood samples. Have people looked at the
10 similarities or has there been any work done to get
11 an estimated dose and then looking at the
12 hemoglobin adducts and so forth? Rather than
13 starting from scratch and doing new studies, it
14 would seem like this would be far less expensive
15 and might give some first approximations.
16 DR. FENNELL: That is a good point. I
17 think we are at a level of complexity in analysis
18 that is much, much higher than acrylamide analysis
19 in food when we are dealing with hemoglobin
20 adducts, and the cost reflects that difference.
21 The way that we have collected samples in
22 general has involved a fairly extensive samp...

1 preparation right at the point where the samples
2 are collected. We wash the red blood cells is
3 isotonic saline and then we store them frozen. We
4 do that in order to decrease the amount of albumin
5 binding or serum protein contamination. Once we
6 have the red blood cell frozen it is pretty stable
7 and we can do a lot of things with it at a later
8 time.

9 So, I think if there are banked samples
10 that are stored as red blood cells we think that we
11 can analyze them. We are not quite there yet in
12 having a definitive answer. You know, we can take
13 an unwashed red cell where somebody has spun off
14 the plasma or serum and see what we can get out of
15 that. I think that is one important question. It
16 has been raised to us. We have actually done one
17 study and we think it was successful but we would
18 like to do it a few more times before we make any
19 definitive statements on it.

20 DR. MILLER: If you are going to use
21 NHANES samples you are going to have to determine
22 whether the storage conditions allow you to develop

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1 any worthwhile conclusions.

2 DR. FENNELL: To address just one point

3 about NHANES, the procedure that is planned by CDC

4 for NHANES is different from the one that I

5 presented. I attended a meeting at CDC to discuss

6 that and I think CDC's plan is that we will have

7 some standards that can be passed around the labs

8 to make sure that we are all in agreement as to

9 what the measurement is, but they are planning to

10 set up a new method which will involve HPLC mass

11 spec. with tryptic digests and whether they can do

12 that on banked samples or not I don't know.

13 DR. MILLER: If what you say is true, the

14 sample storage might make a difference in the

15 results.

16 DR. FENNELL: I think the way the sample

17 is stored can make a difference depending on

18 whether you want to store it to go prospectively or

19 whether you are looking at retrospectively stored

20 bank samples and I think they are looking at it

21 from the standpoint of going forward from here on.

22 DR. MILLER: I see.

sgg

1 DR. DWYER: Would you say that again? Why
2 are they developing another method if you already
3 have a method?
4 DR. FENNELL: I am not sure I can give you
5 an answer for that.
6 DR. DWYER: Aren't you all being funded by
7 the Department of Health and Human Services?
8 DR. FENNELL: I am not. I am funded
9 entirely by either by grant or contract and I am
10 not funded by CDC at all.
11 DR. CANADY: One of the reasons the CDC
12 has given for developing a new methodology is that
13 the same methodology, if used for acrylamide and
14 glycidamide adducts, could also then be used for
15 other adducts to other chemicals. So, they are
16 trying to develop a more generalized approach that
17 they can use for other chemicals that they would
18 asses through NHANES.
19 Having said that, they are in the process
20 of determining what the method will be, whether it
21 works and how well it correlates with other
22 methods. They haven't decided on the method they

sgg

1 will use, rather, they are developing a method that
2 is appropriate. So, it is still in a method
3 development stage although they are taking another
4 approach, as Dr. Fennell indicated. They are
5 trying to take another approach.
6 DR. DWYER: What are they doing with the
7 blood they are collecting each day while they are
8 waiting since the survey is in the field right now?
9 DR. CANADY: Right. The red blood cells
10 are being stored for this purpose. There are
11 samples being set aside of red blood cells for
12 future analysis including acrylamide adducts and
13 glycidamide adducts.
14 DR. MILLER: That is bothersome, frankly.
15 I can understand why they would want to develop a
16 more multi-purpose analysis given the things they
17 have to deal with, but this is an issue that needs
18 some resolution, and why wait until they have a
19 multi-purpose analysis when it might be easier just
20 to produce a more specific assay to begin with?
21 Any other comments or questions? Since
22 there is no one registered for public comment,

sgg

1 which is required by the rules of the committee,

2 then we have come to the end of our first day's

3 activities and, unless there is some further

4 question or comment from the committee, I am going

5 to adjourn the committee until tomorrow morning at

6 8:30.

7 [Whereupon, at 4:00 p.m., the proceedings

8 were adjourned, to resume on Tuesday, February 25

9 2003 at 8:30 a.m.]

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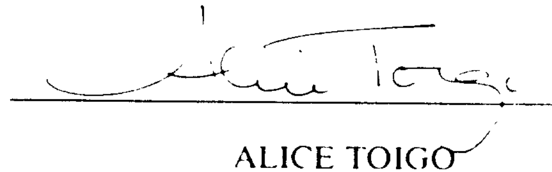
C E R T I F I C A T E

I, **ALICE TOIGO,** the Official Court Reporter for Miller Reporting Company, Inc., hereby certify that I recorded the foregoing proceedings; that the proceedings have been reduced to typewriting by me, or under my direction and that the foregoing transcript is a correct and accurate record of the proceedings to the best of my knowledge, ability and belief.



ALICE TOIGO