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Proceedings of the Meeting on

Ultrasensitive Techniques for Measurement of Uranium in Biological Samples and the Nephrotoxicity of Uranium

Held at
General Services Administration
Washington, DC
December 4—5, 1985

Edited by R.L. Kathren, J.R. Weber

Sponsored by
Office of Nuclear Regulatory Research
U.S. Nuclear Regulatory Commission

Proceedings prepared by
Pacific Northwest Laboratory

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ABSTRACT

Edited transcripts are provided of two public meetings sponsored by the Division of Radiation Programs and Earth Sciences of the Nuclear Regulatory Commission, Occupational Radiation Protection Branch. The first meeting, held on December 3, 1985, included nine presentations covering ultrasensitive techniques for measurement of uranium in biological specimens. Topics included laser-spectrometric techniques for uranium bioassay, correlation of urinary uranium samples with air sampling results in industrial settings, delayed neutron counting, laser-kinetic phosphometry, isotope dilution mass spectrometry, resonance ionization spectroscopy, fission track analysis, laser-induced fluorescence, and costs of sampling and processing. The nine presentations of the second meeting, on December 4, 1985, dealt with the nephrotoxicity of uranium. Among the topics presented were the physiology of the kidney, the effects of heavy metals on the kidney, animal studies in uranium nephrotoxicity, comparisons of kidney histology in nine humans, renal effects in uranium mill workers, renal damage from different uranium isotopes, and Canadian studies on uranium toxicity. Discussions following the presentations are included in the edited transcripts.

SUMMARY

Public meetings sponsored by the Nuclear Regulatory Commission (NRC), Division of Radiation Programs and Earth Sciences (Occupational Radiation Protection Branch), were held on December 3 and 4, 1985, in Washington, D.C. Two topics were covered: the ultrasensitive techniques for measuring uranium in biological specimens, and recent research on the toxicity of uranium in the kidney.

The meeting of December 3 on ultrasensitive techniques included nine speakers. Rod Melgard (Eberline Electrical Laboratory) spoke on radioassay techniques using alphaspectrometry. The presentation of Gary Chase (Bear Creek Uranium), read by Allen Brodsky, correlated air monitoring in a uranium plant with bioassay results of workers' urine samples. Donald Gray (Inhalation Toxicology Research Institute) summarized his laboratory's experience with biological sampling and commercial analyzers. Ernest Gladney (Los Alamos National Laboratory), in a presentation read by William Moss, described his laboratory's bioassay procedures. Alfred Robinson (U.S. Testing Co.) explained laser-kinetic phosphometry in the analysis of uranium. Frank Dyer (Oak Ridge National Laboratory) discussed that laboratory's development of an isotopic dilution mass spectrometer. James Parks reported on investigations at Atom Sciences, Inc., on urinalysis using resonance ionization. Narayani Singh (University of Utah) addressed the need for uranium standards. A. R. Moorthy (Brookhaven National Laboratory) then added the technique of fission track analysis to the techniques discussed. Max Zinger of Scintrex, Inc., explained the chemistry and practicality of the technique of laser-induced fluorescence. Finally, Charles May (University of Utah) described the work of Fred Bruenger (University of Utah), who has developed a fission-track quantification of uranium in biological samples. R. B. Neel (U.S. NRC) then compared the sensitivity, cost, and availability of the techniques.

The meeting of the second day, December 4, 1985, also involved nine speakers, though now discussing the toxicity of uranium in the kidney. The first presentation, that of Jeff Sands (National Heart, Lung, and Blood Institute) discussed the anatomy and physiology of the kidney. After that background, Gary Diamond (University of Rochester) spoke on the effects of heavy metals such as uranium on kidney function. In a submitted statement presented by Gary Diamond, Paul Morrow (University of Rochester) summarized the current research on the nephrotoxicity of uranium. Robert Moore (United States Uranium Registry) then presented the results of a study comparing the pathologies of four former uranium workers' with five other control subjects who had no known work exposure to uranium. This presentation was followed by Max Scott (Louisiana State University), who presented case studies gathered over 16 years at Oak Ridge National Laboratory, and drew conclusions about standards from those cases. Michael Thun (National Institute of Occupational Safety and Health) presented the case for closer examination of the glomerulus of the kidney in workers exposed to uranium. In the first of two presentations on uranium studies with animals, Glenn Taylor (University of Utah) spoke about experiments on uranium toxicity in dogs. Bliss Taylor (Radiation Protection Bureau, Health and Welfare Canada) described uranium studies in Canada

conducted on rats and rabbits. Returning to human subjects, Narayani Singh (University of Utah) spoke on a study comparing human absorption of natural uranium in drinking water with volunteers injected with ^{237}U. The Nuclear Regulatory Commission's concerns with appropriate standards for uranium doses to the kidney were summarized by Robert Alexander (Nuclear Regulatory Commission), who opened the floor to discussion of possible new standards.

PREFACE

In December, 1985, the United States Nuclear Regulatory Commission (NRC) sponsored two days of public meetings dealing with two specific aspects of uranium of particular interest to those involved with the control of hazards from exposure to uranium. The first day was devoted to a public meeting on ultrasensitive techniques for measurement of uranium in biological samples and the second day to the nephrotoxicity of uranium. The meetings were chaired by Robert Alexander of the NRC and featured oral presentations on specific topics in the two areas under consideration by persons with known expertise. Each presentation was followed by a general discussion and question-and-answer session open to all in attendance.

In addition to providing an overview of these two important areas of concern, the oral presentations and subsequent discussions contained previously unpublished new information representative of current knowledge about uranium nephrotoxicity and ultrasensitive measurements. To make this important information available to scientists and other interested persons unable to attend these meetings, this published version of the transcripts has been prepared.

Transfer of the spoken to the written word is a task fraught with difficulties. Unedited verbatim transcripts reflect the idiosyncrasies of common speech--broken and incomplete sentences, repetition, jargon, and grammatical errors--as well as errors in the transcription, especially in the rendering of technical terms and mathematical relationships. No transcription can reproduce slides or other graphic presentation of information, particularly of the ad hoc sorts in a speaker's gestures or manners of expression. When transcribed, what was acceptable and understandable to the listener may be unacceptable and incomprehensible to the reader.

In preparing this written record of the meetings, the primary goal of the editors has been to transform the verbatim transcripts into more readable, serviceable form without losing the accuracy, tone, or sense of either presentations or discussions. However, with few exceptions, formal versions of the presentations were unavailable to the editors. For example, although graphs and slides were available for some presentations, the visual information for most of them, which contributes to their continuity and intelligibility, was unavailable. Thus, the transcripts needed to be vigorously edited in some places in an attempt to retain coherence in the written version.

By far, the larger number of editorial changes were cosmetic--eliminating side comments such as those relating to running a public meeting, references to the identity of slides or other visual materials not available for publication, and other commentary extraneous to the substance of the presentation. In other words, the editors have attempted to spare the reader the tedium and the hems-and-haws of a public meeting of this nature, while at the same time maintaining its substance and flavor. Ellipses (. . .) have been used where the absence of visuals might prove confusing or frustrating to readers.

If we have erred, we have done so in a spirit of conserving the speakers' original words and sense, despite an occasional awkwardness. In all, our basic principle has been to edit as we would wish our own presentations to be edited.

We acknowledge the contribution and assistance of Dr. Judith Foulke, formerly of the Nuclear Regulatory Commission staff, who oversaw the initial cleanup of the transcripts and whose numerous contacts with the speakers cleared up many questions. Finally, we note that Ronald L. Kathren is on loan to the Hanford Environmental Health Foundation from Pacific Northwest Laboratory.

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PUBLIC MEETING ON

ULTRASENSITIVE TECHNIQUES FOR

MEASUREMENT OF URANIUM IN BIOLOGICAL SPECIMENS

General Services
Administration
Auditorium
18th & F Streets, N.W.
Washington, D. C.

Monday, December 3, 1985
8:55 a.m.

R. ALEXANDER, U.S. Nuclear Regulatory Commission, Presiding.

MORNING SESSION

R. ALEXANDER: Rod Melgard, if you'll take the podium now and present your paper on Radioassay Techniques.

R. MELGARD: I'm Rod Melgard from the Eberline Electrical Laboratory in New Mexico. I'm standing in for Mike Ortiz, who could not be with us this morning.

I'm going to talk about spectometric techniques. The application of alpha spectrometry as a technique for the quantitative isotopic uranium measurement in urine has been in use for several decades. Not too many years ago, the attempt to achieve reliable high sensitivity analysis was considered a research project, primarily used for LT detectors and electronics. In the past decade, however, the availability of modern electronics, along with a computer-based acquisition of data, has brought spectrometry to the realm of routine laboratory analysis.

One of the differences between the ultraspectrometric techniques and other techniques of uranium analysis is the ability of the former to isolate the sample from the background trace materials normally associated with the original sample.

Since the final counting is usually performed on a disk of nickel, on which the uranium has been electro-deposited, any significant thickness of material would degrade the energies and substantially complicate the quantification.

The purification process usually involves a combination of co-precipitation extraction and ion exchange. The exact purification techniques vary from laboratory to laboratory and are not particularly critical, as long as satisfactory chemical and radiochemical purity is achieved.

Typically, on the plate which is being counted, the macrocontamination level of about 20 μg per square centimeter will not affect the ability to characterize the uranium present. However, additional amounts of macrocontamination will noticeably affect the quality of the spectrum; at about 100 μg per square centimeter, the quality of the spectrum will be seriously affected, and at 200 μg per square centimeter the spectrum will be unusable.

Typically, ^{232}U tracer is added to the beginning of the chemical process. This tracer has an ultra-energy spectrum which is unique from the point of view of the radioactivity one is looking for in this sample, so it is used to determine and to correct for losses of uranium in the ultrapurification process.

The detectors, electronics and data acquisition equipment are now commercially available from several sources. Systems can be purchased which can handle single and multiple detectors. A typical system is installed and operates 16 detectors in a single analog-to-digital converter, such that these

detection systems are served by 256 channels, by establishing a total fixed energy interval, about 3-1/2 to 7 MeV. The vast majority of samples can be recorded without requiring operator adjustments to the gain settings of the amplifier. Not only does this eliminate the possibility of operator error, but greatly simplifies the computer program required to test the stability, that is, to keep track of the instruments' backgrounds....

In this slide, you have mostly ^{238}U. I left out some of the minor lines, but basically, the crosshatches to the right are the ^{238}U energies. They are fairly close together, falling from one peak, as ^{234}U does on the right. Uranium-235 has a substantial number of fine structures. Uranium-238 and -234 are fairly uncomplicated. Uranium-235 has a more complicated structure. And we deal with that by taking the main big broad arrow heading up there, which has about 55% of the alpha energies falling in that peak.

You can't necessarily see all these things on an alpha spectrum without very sophisticated electronics. In a practical sense, we're not concerned about all these fine structures, but rather the total.

Around that main peak at 55% are several smaller peaks. We include that as a fudge in the integration process. You get around 83% of the uranium falling in that region, done by alpha spectrometry.

Since you know that all the ^{235}U is not included in that peak area, a correction factor is included in the calculational process to account for this loss, as well as to account for minor contributions to the ^{238}U region or perhaps due to a minor branch of the ^{235}U. If they're fairly sophisticated, you will see that ^{235}U does have a several percent branch that actually will be incorporated into the ^{238}U energies. So, that has to be arithmetically correct.

The actual spectrum of natural uranium is considerably different than the relative alpha intensities plotted here. This is due to a combination of different natural abundances of these isotopes, as well as differences in their half-lives....

Uranium-238 and -234 are members of the same natural decay chain. The other members have short half-lives and are essentially in equilibrium with the natural uranium. One expects the areas under their respective peaks to have all the alphas relatively equal. Uranium-235, however, belongs to a different decay chain, and its alpha activity is only a few percent of that of ^{234}U and ^{238}U. As a result of these different natural abundances and half-lives, the alpha spectrum for natural uranium appears as shown in this slide.

We have about 200 channels of spectra, with the alpha energies and MeV at the top. At the very left is ^{238}U, centering there at about channel 45. At about channel 60, we see a little bit of ^{235}U. Then comes the ^{234}U and the peak at the very right is the ^{232}U tracer, that was added to trace the sample. The number 10 over there is an indicator of the full-scale number of counts per channel in those spectra. So you can see how much we're dealing with here. This amount of uranium would be considered very large....

In this spectrum, you can see that the ^{234}U region has a very observable, readily observable peak at about 4.77 MeV. The peak over on the right at about 5.3 MeV is the ^{232}U peak. This might be typical of enriched uranium.

Counter-efficiency is about 27% so the yield in performing net QC was about 35%. When we get into the actual data calculation, the ^{238}U, ^{235}U, ^{233}U, and ^{234}U are separated in different integration regions. In actual practice, the ^{233}U and the ^{234}U in the sample are very, very close to the same energies and cannot be isolated by alpha spectometry, but it's also true that you have a measure of ^{235}U and ^{236}U in the sample.

If we have a client that wants an analysis for both ^{233}U and ^{234}U, we would have somewhat broader integrations. Channels 74 to 101 were used for the ^{233}U, which we also used for the ^{234}U, by the way. If we do only ^{234}U, we use a narrower integration region, channels 80 to 96.

In the cases where the uranium observed is not natural, a spectrum such as shown in the next slide might be obtained....

Here's a spectrum, again--^{238}U at about 4.2 MeV with little or nothing at 4.4 MeV, the ^{235}U peak. You can see the ^{234}U region has a very observable, readily observable peak at about 4.75 MeV. The higher energy 5.3 MeV peak over on the right is the ^{232}U peak. This spectrum might be typical of enriched uranium.

I might comment that normally people think of enriched uranium as having larger natural amounts of ^{235}U, but they're picking on a mass basis, when you actually get to a radioactive disintegration basis, ^{234}U will be the most prominent.

So far, I've given the outline of technology development spectrometry and talked about the interpretation process as it was taken.

I would like to point out that many detectors are involved in the process, as they normally would be at our laboratory. Since each of these detectors has to be periodically checked for efficiency and background, and the use of the computer and computer programs is highly desirable, if not absolutely mandatory.

The tests that are required for the instrumentation and the data-base update are less direct and more mathematically complicated than the actual calculation of the data for samples.

In calculating the theoretical detection capability of a procedure such as this, I often use a special form of the general counting statistics formula:

$$\text{count rate required} = 1 \pm \quad 1 + \frac{800^2 \cdot CPM(b)}{t\sigma^2}$$

where t = length of counting time
σ = fractional standard deviation
CPM(b) = background count.

This is coming backwards at the problem by asking how much sample do I need to achieve a given statistical error in a reasonable counting time, if my detector has a certain background.

In the case of alpha spectometry, if you're using a background of 0.02 CPM or 2 counts per 1000 minutes, one could improve that. That is probably a reasonably low-level, background-type counting. And you count about 1000 minutes. That allows you to count one sample per day, plus take some quality control tests and run backgrounds. If you want a fractional standard deviation of 0.5, which statistically works out to about a ±100%, with 95 as the confidence level, then the sample count rate that is required is 0.0065, or roughly 6 to 7 counts per 1000 minutes.

Under those conditions, if your instrument efficiency equals about 30% and you can achieve about a 50% yield from the sample through the purification process from one liter of urine, this count rate is going to be equivalent to about 0.043 dpm per liter of urine (~0.02 pCi/L).

Now, of course, when you are using formulas like this, your chemical yield and your instrument efficiency background will all interact strongly and redirect the sensitivity level for better or for worse. I would like to note that this type of calculation applies to each peak; i.e., one has the theoretical capability of sensing about 200 pCi/L in each of the isotopes, ^{234}U, ^{235}U, and ^{238}U. A review of the data therefore allows a decision to be made as to whether or not the source was enriched or configured.

I have nowhere used the words "theoretical capability" in this discussion because the conditions chosen for demonstration are very stringent. To achieve this theoretical capability in practice essentially means that nothing would go wrong in the laboratory. A minor relaxation of these optimum conditions--for instance, if background equals 0.005 dpm, instrument efficiency 25% and chemical yield 30%--would result in dropping capabilities to about 0.05 pCi/L.

In addition to these variables presented, there is an impact on procedural sensitivities. Both chemicals and glassware contain small amounts of uranium if you look hard enough for it with sensitive enough techniques. So, to achieve the theoretical capability presented requires that the values for the laboratory blank, determined by a variety of tests, be essentially from the instrument background only. In practice, if one randomly selects reagent grade chemicals and glassware, it is almost certain that a suitable blank will result.

Further, it is the nature of science that this blank will not be predictable. It will be random and variable enough so that you can be very hard-pressed to decide what it is and apply it to sample data.

A laboratory blank can be controlled and generally reduced to the nondetectable level with careful selection of chemicals and by purification and repurification of these chemicals at the laboratory. All glassware must be washed with strong acid solution prior to use. In some instances, such as where you have to use resins, initial bath treatment of the resin should be performed and repeated by additional uranium removal treatments prior to the use of a resin in active samples. In practice, we purchase chemicals on a lot number basis, and each time a new lot of chemical or supply is specifically tested to assure it works properly in the sequence in which it will be used.

All of these variables thus far, however, are still within the control of the laboratory, and a meaningful high-sensitivity urine analysis has been achieved by the care exercised in cleaning sample containers and keeping them clean, as well as the industrial hygiene of the site and the personal hygiene of the individuals who are sampled. In some cases collecting and removing samples from the site of operations without subjecting them to cross-contamination have proved difficult, almost to the point of impossibility. Clearly, the need to obtain uranium analysis of a high sensitivity will require the utmost cooperation of our clients' managements and supervisors.

The commercial availability of this type of procedural sensitivity is not yet established, largely due to this problem of contamination during sampling and in transit. While the fundamental methods and techniques are consistent in the laboratories, the capability for obtaining sensitivity at around 102 to 105 pCi/L probably does not exist, primarily due to lack of demand for this analysis as well as a lack of testing on a controlled basis.

As was seen above, each element of the procedure from the sampling bottles through the instrument background must be very carefully examined and continuously reexamined. For the laboratory volume-control procedures, such processes could easily consume 30% of the effort to install and maintain such a program. Commercial laboratories are unlikely to maintain such a stringent quality-control program without having demonstrated demand and a reasonably secure supply.

It is also likely that commercial laboratories must analyze samples for uranium from a wide variety of sources in a wide variety of uranium concentrations and enrichments. They may find it necessary to establish separate facilities in order to properly control the backgrounds necessary to routinely process urine samples at very high sensitivity levels. While such a task is certainly not unwelcome, the initial cost would have to be amortized over the expected life of the business and the expected replacement curves for equipment at various facilities.

The requirement to extend typical counting times conceivably into several thousands of minutes rather than the 1000 minutes used here in the examples would further increase the need for a substantial increase in equipment.

Current commercial prices for the analysis of samples of urine for various calculators range from about $65 to $150 per sample, depending on the complexity of the analysis, the number of samples available for scientific analysis, and the sensitivity required.

I am not aware of any commercial laboratory that would "guarantee" a sensitivity of 0.02 or even 0.05 pCi.L for uranium analysis or in the laboratory, retaining some responsibility for control of all of the variables involved in achieving this sensitivity.

In the future, should there be a relatively large demand for high sensitivity uranium analyses, the funding that is already indigenous could be applied. The increased demand would undoubtedly create economies of scale that would reduce the processing costs. However, this would be offset to some extent by the increased quality-control requirements and the need to increase the inventory of equipment and separate processing of bioassay samples.

In summary, alpha spectrometry can offer a reasonable alternative to other techniques as a fairly sensitive technique for analyzing uranium in urine. It allows decisions to be made as to the relative enrichment of uranium. The practical level of sensitivity is most likely to be controlled by contamination levels both inside and outside. However, this problem will be shared by all the methods of analysis presented and discussed here today.

Thank you.

Q: I have a question. How does your program take account of when the alpha spectrum is degraded and you have counts in more channels than there should be?

R. MELGARD: It doesn't. It will incorporate exactly what is in the predefined L bands. That is why we use computer programs. We have various degrees of degradation or shifting on the instrumentation, and sure enough, if you fool around with enough math long enough you can use your test samples to get the right data.

When you are actually processing real samples, though, generally the samples don't cooperate. You get a few counts here and a few counts there. If the real sample is degraded, you probably don't know. So, you have to do a physical quality inspection. You take a look at it, you count it. You do have tracer in there. You have seen the tracer beads smeared around. You do the sample over again because the few counts per thousand should be in the sample, and you will never know where they are or what happened.

Q: At what concentration do you begin to see the contamination problem becoming so severe that you just use normal reagents?

R. MELGARD: If you go out to the lab and just grab reagents, chemicals, and elastomers and count them, you will get probably 10 to 15 counts as the peak area. It would roughly triple or quadruple your overall background--that is, the instrument plus blank--which would have a tremendous effect.

Q: How much would that affect the sample?

R. MELGARD: By about a factor of 4. However, the trouble with blanks is they don't behave statistically. If you change your chemical, or change reagents or you change your resin (or somebody else in the laboratory does) and you don't know about it, the counting level of your blank could go up or down, and you would be applying the wrong blank background level to your sample, which would essentially give you data from client samples.

Q: But if the background level was, say, a factor of 10 lower than what NRC is thinking about, then do the backgrounds vary by more than a factor of 10?

R. MELGARD: If the instrument backgrounds vary statistically, you had better fix your instruments. Blanks, yes. Blanks can easily vary by a factor of 10.

Q: A lot of people are telling us that when you put uranium in containers it will stick on the glass walls. Do you or anybody else who makes this argument know how much is sticking on the walls? I have heard so much about uranium in the container sticking on the walls. Has anyone really done this work so that they know what percent is sticking on the walls?

R. MELGARD: I personally can't tell you that I have seen an instance where I have done research and found uranium sticking on the walls. Not personally. I have tested plutonium and actinium, and it is on the walls, period, no doubt about it. It goes away from solution after a while and it is on the walls.

Q: What percent do we lose every day? Do you have any data to tell us?

VOICE: Lots. With actinium it could be 100%.

R. MELGARD: I have seen them go 100% on walls or poly bottles. Just put it in 6 normal HCl with high acidic concentration. I have seen that broad a solution.

VOICE: For the last six months I have gotten water samples containing very high uranium content. Just water samples in a plastic container. No acid bases. Nothing added to that. And after six months, I haven't seen any loss. I intend to really get the data, not just talking. So I will give you data for the next 10 years, and we will see how much we lose every six months or every year for 10 years and see really what happens.

I hope anybody who looks at data like this gets some real numbers about what we are really losing for each actinide and in what amount of time.

R. MELGARD: Well, actinide loss on bottle walls is certainly a problem. I think, however, if one could select an element that would be a minimum problem in that respect, it would be uranium, just from the chemical nature of uranium. I personally have not gone into glass bottles or poly bottles and

poured them out, assayed them, and said, look, aha, it is all on the walls. I haven't done that personally on other actinides. I have letters on other actinides.

Q: I think it is worth mentioning as well that shifting to Teflon containers doesn't necessarily solve your problem. Uranium hangs up beautifully on Teflon walls.

R. MELGARD: There is no perfect container.

Q: Did you give a detection limit in μg/L? For, say, ^{238}U.

R. MELGARD: I did not. Uranium-238? In this case 0.02 dpm/L. There are about 1-1/2 dpm/μg. Total uranium of about 0.75 dpm/μg. So, you are in very, very high sensitivities. Again, the actual practical detection is not going to be detectors; it is going to be laboratory contamination, blanks, and things like this.

There was a time when some uranium mines were trying to abide by the rules of Regulatory Guide 8.11, and we went to more stringent analytical techniques with higher sensitivity. All we did, really, was find out that the customers could not control the blanks and the samples and eventually they had to go to trickle blocks, sample bottles, rewash tables at the site and giving people showers and washing them down, doing this and that at the site, putting on gloves during the sampling period, taking the samples, boxing them up, taking the equipment in plastic bags, putting it in clean boxes. Then, after a lot of money was spent, finally the blanks could be reasonably well controlled. Unfortunately, even though they were paid for all the time for being showered and cleaning themselves up, the employees felt this was an invasion of privacy. Then, they decided that Reg Guide 8.11 didn't apply to uranium mills, anyway.

Q: In regard to loss of sample on the walls of the sampling container, probably having your carrier in the bulk first isn't going to work very effectively independent of the carrier. That is probably because your alpha detection is in the lines. I would like to know for sure.

R. MELGARD: There are certainly references to that--Mike will bring them out--of carriers of any species regardless of what you have. It is unfortunate that the uranium carrier is going to be what you are analyzing for unless you have got something in mind. For example, a carrier for uranium would most likely interact with uranium, which is the problem.

Q: These levels--are these the levels close to the levels you see in your normal population? What numbers do you see in those?

R. MELGARD: Well, I don't really remember offhand. I don't remember what the normal population level of uranium is.

R. ALEXANDER: 101 μg/L is often mentioned.

Q: We do a lot of urinanalysis, and the background urines in our area in Washington State, which is a fairly low uranium area, tend to run down to around 0.017 μg/L up to 0.6 or 0.7 or 0.9. I understand it is up from that in other parts of the country.

R. MELGARD: Of course, that's the whole concept of ALARA coming back to haunt us. Statistically, is this person significantly higher than the general population?

R. ALEXANDER: I think the ALARA concept, the way it's handled in the nuclear power industry, may start working the same way in bioassaying. The way ALARA really works in this country, since we haven't found out how to do optimization analysis, is that government health physicists and the plant health physicists just keep pressing for lower and lower and lower doses at higher and higher and higher expense and more and more and more operational interference, until finally some manager steps out on the floor and says, "Now wait just a damn minute." That's ALARA.

(Laughter.)

It has a practical ring to it, and it works pretty well. And we're worried, of course, that if the costs for the more sensitive techniques get too high, that sort of argument will prevail. It's a real possibility we all have to recognize.

Gary Chase, from the Bear Creek Mill, wasn't able to be with us today, so we're fortunate to have Dr. Allen Brodsky present Gary's paper. Ever since the first uranium meeting back in the late '50s started looking for correlations between urinary uranium samples and air sampling results, none has ever been shown. But Gary appeared over at the National Academy of Sciences the other day with papers showing correlations--good correlations--at his mill between air sampling and bioassay results. I think you'll find his paper quite interesting. It will be given by Allen Brodsky of the Nuclear Regulatory Commission.

A. BRODSKY: I hope that paper by Melgard will be appearing in detail. I would like to be able to refer back to a lot of the things he said. It was an excellent paper.

Unfortunately, Gary Chase could not be here today, but he provided me with the information to be presented and briefed me by telephone last night. I cannot be as familiar, of course, with this plant as Gary is after his eight years of being a chemist there and in charge, most of that time, of radiation protection. But we have been impressed, in previous presentations of his data, by the uniqueness of an individual working on a production installation who will take the time to examine his health protection data carefully, in addition to his other duties, and to let it accumulate to scientifically evaluate what the results might be.

This paper is not an instrumental method of increasing sensitivity. However, it is an operational method of increasing sensitivity. I think we ought

to keep in mind--I think Mr. Melgard alluded to this a bit--that the law of averages can increase sensitivity, if you collect data properly, keep it properly, and have proper quality control and blank control over time. I think that's what Gary Chase really has done here. He has allowed data to accumulate over time, recorded it in a constant environment, and quality-controlled it in a constant manner for a long period of time. Therefore, correlations that do not seem to appear when perhaps you look at tens or hundreds of points begin to arise when you look at thousands of points. This is really related to the law of averages, the law of large numbers in statistics.

Bear Creek Uranium Company's mill has been in operation since September of 1977. The mill employs semi-antogenous grinding. The ore helps to grind itself. Grinding, leaching, washing, countercurrent decantation, ammonia precipitation, and drying and packaging are housed in one metal-frame building. The drying and packaging circuits are enclosed in a steel-frame, fiberglass-wall structure with negative pressure, provided with a dust-collection and scrubber system, in conjunction with a dryer off-gas scrubber system.

The solvent extraction circuit is enclosed in another metal-frame building, next to two of the main process areas by an enclosed hallway. The other building does only solvent extraction, which is a liquid ion-exchange process. It is enclosed in a separate building because the organics may be flammable....

On one end of the main building, the ore is dumped from the trucks onto the ore pad and goes up the conveyer belt. By the way, the ore when it comes out of the ground has about 13% moisture, so it's a fairly wet ore. There's not a whole lot of dust in that area, in this particular mill.

After going up the conveyor belt, it's dumped into the ball mill, which is the semi-antogenous grinder. It's crushed; then, it's put into the leaching circuit, where sulfuric acid is added. Some of that is stored in tanks. In the primary leach tank, sodium chlorate is added to convert the uranium from the tetravalent to the hexavalent state, so it will be solubilized out of the particles of ore.

This slurry is then transferred to eight tanks. The uranium is in water solution at this point, and actually, in these tanks the solids are not separated out. It's mixed thoroughly from one tank to the other and slurried, so that you get a good mixing here of the perchlorate and sulfuric acid with the ore slurry to dissolve out the uranium.

At this point, it's pretty well dissolved out: 94 to 96% of the uranium is dissolved out of the ore, but here you have to separate out the solids by the countercurrent decantation (COD) process. You separate out the sludge at the bottom of the tanks and keep countercurrent decantation going until you get a fairly clear water solution of uranium. Then, that water solution of uranium is sent over to a building, where you have a lot of organic liquid. The commercial name is Alamine 336. It's a long-chain tertiary amine, which serves as a liquid ion exchanger.

The uranium solution in the tanks is then jacked down to a pH of about 1.5 to 2 with sulfuric acid. With that pH, the uranium then attaches itself to the liquid ion exchanger. A large volume of water can then be taken off.

The uranium is now concentrated on the liquid ion exchanger. Then, the pH is raised up a little bit again with anhydrous ammonia to the pH 4. This, then, causes a reversal of the process. The liquid ion exchanger tertiary amine releases the uranium back into a water solution, but it's a smaller volume of water. So, now you have a concentrated solution of uranium and water, pH 4. The next step is to precipitate out ammonium diuranate by adding some more anhydrous ammonia. That comes out as a yellow powder. It flocculates down as this yellow powder, which we call yellowcake, as you know.

This ammonium diuranate is put into a dryer, where it's dried to 1200°F. That dryer was the source of the problem in this particular plant. They don't have a great deal of problem now. But this dryer is where, in the past, some materials have been released in the drying process. They originally had a metal enclosure around the dryer, which eventually rusted through so there were holes. Some of the dust got out of those holes and dispersed throughout the whole plant.

Actually, over the years in the grinding area here in this particular plant, they've had less than 1% of maximum permissible concentrations (MPC) of uranium in air. In the early days, it went up to maybe 13%, but in the first few years, there were instances where some of the uranium from the drying process got out into the air, and the general air concentration rose. I think you can see that otherwise most of the processing is wet processing, so you would not expect to see a whole lot of air exposure.

In a way, this may be an ideal facility for somebody like Gary Chase to see if there are correlations between air monitoring and bioassay. Most of us don't see those correlations in those plants. We usually find great variability between measurements, and we don't see a great deal of correlation. We've often discussed the problem of how much to use air monitoring and how much to use bioassay. We've tried to go to the foundations of both to assay exposure of workers.

There were 41 people employed in the mill though I think the plant population has decreased now. They're going to close down the mill in a few months altogether, but there are still about 20 involved in mill operations, 11 involved in mill maintenance. The remainder are laboratory, warehouse, clerical, and supervisory personnel. The mill operates 24 hours a day with the operators being rotated between the three existing operating positions at a routine frequency. So, the operators in this plant circulate all around the mill. All other personnel work day shifts.

Personnel monitoring equipment, respiratory protection, protective clothing, and radiation monitoring request forms are provided for all nonroutine maintenance, and any work in drying and packaging, as well as in the precipitation sections of the mill. No credit is taken for respiratory protection in calculating personal exposures for purposes of compliance with NRC regulations.

For conservatism, the personal air-sample results are added to the exposures calculated from the air concentrations. This puts a little bias in some of the data we're going to look at. We will be able to see some of that, but it's conservative on the side of safety. This is referred to as an "add-on" for projecting exposure. Calculated exposures are based on length of time at work, multiplied by the time-average exposure for particular workers. Everyone here knows what "time-weighted exposure" means.

Uranium samples are collected at each of the 23 designated sample locations, once per month, with at least one airborne uranium sample collected on each day of the month. They have dispersed air monitoring stations around the plant. They were not as closely spaced as they might have been in other types of facilities where people work more closely with smaller sources of exposure possible. Airborne samples are monitored in each of the 23 designated sample locations, once per month, with at least one airborne uranium sample collected each working day. That's only from one site.

I might say that these sample locations were picked, not by the plant people alone, but together with the NRC licensing officials. The NRC officials came to see where they wanted these sample locations and checked to see whether they were placed properly. So most of these locations over the years have been sites that were picked jointly by the plant management and the NRC Staff.

Samples are collected with high-volume samplers on Whatman 41 filter paper, at an average flow of 16 cubic feet per minute. Collection efficiency is calculated to be 98% at a 15-cubic-feet-per-minute flow with 0.3-μ-diameter particle size. The particle size has been checked to be 5 μ, activity median aerodynamic diameter (AMAD), but keeping in mind that we're talking about uranium oxides that have densities above 10, the probable actual diameters of these particles is in the tenth-micron range. Still, with the collection of 0.3 μ at 98% efficiency, it can be believed, as Gary Chase asserts, that these efficiencies are very high for collection.

The filter papers are wet ashed, digested and analyzed by fluorimetric analysis at the plant. The air samples are measured in the plant by Gary Chase himself. He runs the chemistry laboratory. Of course, you have him working carefully to control the process.

Bioassay samples are split and sent out to commercial laboratories for processing on a routine basis. Side-by-side comparison of sample collection and analytical results have been checked with NRC's Inspection and Enforcement personnel.

Urine samples have been collected from each employee on a monthly frequency since the fall of 1978. Samples are collected after the employee has been off the job for a minimum of 48 hours (two days, a weekend) and a maximum of 96 hours, averaging 70 hours.

This time is very important to Gary Chase's analysis and very important to Gary Chase's entire work because he finds that in this plant most of the material seems to act like D-class material. It leaves the body very quickly, and

he believes that he could very easily have fudged his data if he wanted to just wait a little longer and see nothing in his employees. So he has to keep the time well-controlled, and he has kept good track of the times and has calculated the averages; that is part of the data.

The samples collected before the employee entered the mill area are labeled, dated, and shipped to a commercial laboratory for analysis. Blanks, samples, and standards are included, as required by USNRC Regulatory Guide 8.22. That is a draft regulatory guide that came out in 1978, and hopefully, by God, we will finally get another one out for your comments by next year. It hasn't circulated for comment yet....

Q: Would you mind telling us where Bear Creek Mill is located?

A. BRODSKY: Bear Creek Mill is near Casper, Wyoming, on road 95. Don't depend on finding the sign, or you would turn around and go 10 miles back, as I did. It is northeast of Casper, Wyoming, I guess about 30 or 40 miles, something like that.

The same commercial laboratory has been used for these analyses since mid-1978. The name is not here, I guess because of the Privacy Act, but if some of you who have used that laboratory were to shout it out, I guess I can't stop you.

Over a period of eight years, over 4300 individuals' urine samples have been collected and analyzed for people included in the mill operation. During that period of time, over 2300 airborne uranium samples have been collected and analyzed to estimate the exposure of these people. The samples are smaller because there are more people than there are sample stations.

I might mention a fixed value of 2 μg/L was assigned to all samples reported, at less than 5 mg/L for averaging purposes. We are getting away from assigning numbers for uranium measurements below MDAs. If you measure something and you want to increase your detection capability while keeping good records, you should just report them as you see them, calculating your standard errors later.

By graphing the average annual airborne uranium concentrations and comparing this with the average annual bioassay results, it is apparent that a correlation does exist.... If you compare not just averages of the air concentrations and averages of the urine samples, but actually plotting the time-weighted average air concentrations, observing the individuals and how they spent their time in various areas (which Gary Chase also has done), then you find the correlation improves, as you would hope....

Over the years, when they controlled the dust coming out of the dryer area, the uranium concentrations came down to be in approximate equilibrium with the ^{230}Th and ^{226}Ra concentrations, sort of verifying his feeling that this material was coming from the dryer area. In the dryer area, you have about 200 times the concentration of uranium relative to ^{230}Th and ^{226}Ra because the uranium has been concentrated by the chemical processes.

Gary Chase also examined the radon levels in the areas in the plant and outside the plant to see if they may have affected the whole body counts. Whole body counts are still being analyzed by Gary. Whole body counting is done in the lung region in a portable laboratory that has sometimes been downwind from the tailings pile. So, there is a suspicion that there might be some lung and skin contamination influenced by the radon there.

Here is the whole body count that he is trying to analyze. However, if you look at the scale on the left, you will see that all these numbers that he is trying to squeeze information out of really are less than the minimum detectable amount (MDA) that can be detected with any assurance.

However, we should make Gary Chase an honorary statistician for trying to do this kind of thing because he recognizes that there is a law of large numbers in statistics. Because you have an MDA for a single measurement does not mean that if you keep your quality control and your data reliable, you cannot squeeze an additional order of magnitude of sensitivity out of your measurements. I guess this goes back to what Dr. Melgard was mentioning in his talk.

On the other hand, the background on your urines and your controls is really the limitation in squeezing the ultimate sensitivity out of the measurements. So, you need to keep the instrument detection capabilities in perspective with regard to the entire measurement process, including methods of analyzing and recording data.

Again, you can see these are averages, averages of hundreds of measurements. That is why you can start looking for correlations. You will notice all these numbers are again less than the 9 nCi that Regulatory Guide 8.22 says is an acceptable minimum detectable amount (MDA) for any lung measurement....

The annual average lung count is coming down; it does look like a trend, doesn't it? So, Gary Chase is trying to explain these trends.

Now, out of all this, in conclusion, comes the fact that you can predict average urinary concentrations from average air concentrations. Gary Chase has been working with this data intimately over the years and is very confident of that fact; and he uses these rules of thumb:

Take C, the average concentration in air at the location of the plant when a man is there, in $\mu Ci/L \times 10^{10}$, since 1×10^{-10} is the MPC. You can see these are in MPC units now in this equation; the fraction C is in MPC. You multiply by your conversion factor for mL to L per day breathed in, and the $Ci/\mu Ci$ and the $\mu g/g$. Then, the 0.25 is what he assumes by the lung model is deposited in the lung, remaining in the lung, as a rough factor.

The 0.5 has been adjusted basically to fit the relationship. To interpret the meaning of 0.5, it really means that there is an average of 2.9 days after exposure when he takes his sample (sometimes between shifts from day to evening shift, sometimes because of weekends you have variability in this time, but he gets an average of 2.9 days). The 2.9 days is the half-time for elimination of the D-type uranium from the body after deposition if you assume there is a

single time of deposition. So, this is an effective half-life of 2.9 days for the amount of uranium to come down to 0.5 of that amount originally deposited. If you assume that 0.5 has that meaning, then the rest of these are all well-known conversion factors; 1.4 liters per day is the ICRP amount of urine eliminated per day.

So, he is saying what comes into the body has to go out. What is breathed in and deposited and retained in 2.9 days has to be the same thing as what is being eliminated into the urine bottles. Common sense.

Out of this multiplication of these numbers, he gets the relationship that 126 times the average air concentration gives the expected urine concentration in μg/L, where C bar is the average air concentration in units of MPC.

An example calculation: the monthly average air concentration of 5% of MPC. You pull the number out of the air, 126. Easy, handy-dandy rules of thumb: 126 times 0.05 yields 6 μg/L expected in the urine.

When you look at this correlation for individuals, using this equation to predict urinary output, you find good predictions. Most of his concentrations in urine today are in this μg/L range. It is a pretty clean plant. Unfortunately, the plant has to close down. You almost hope that they could market more uranium when you see operations as well conducted like that.

Thank you. It has been a privilege to present Gary's material to this group.

R. ALEXANDER: Any questions?

Q: Allen, was there any data or information that you have that indicated that the 6 μg/L had caused any kidney damage?

A. BRODSKY: I don't have any information on that. I don't think there are any serious medical studies, unfortunately, on that. We are following it up, but we haven't been doing very much.

N. SINGH: In my opinion, it does not make any sense to correlate the concentration in air versus the concentration in urine. All of us know that the amount of uranium in the urine will depend upon what kind of exposure you have. But if a person is exposed to ammonium diuranate, you have a lot more uranium in the urine than for the same amount of exposure in the rock-crushing area. So, how can he really have any correlations based on urine specimens? To me it does not make any sense at all. We should always look into what kind of material the person is exposed to. Just plotting concentrations in air versus concentrations in urine does not make any sense at all. It is quite wrong.

A. BRODSKY: You've made a very good point. Let me answer that. Your point is very good. Absolutely. But it doesn't mean that it makes no sense at all. Obviously, some information came out of such a correlation, in this case. The question is: Why? What is this telling you? You see, this whole thing is

telling me something. You don't see anything if you don't look at the data. You have to look at data and also let it tell you something, not just treat data to prove or disapprove your hypothesis. This is the kind of study where the data is telling you something. You are simply looking at correlations.

The data tells you in this case that the material appears to be mostly in Class D--and there are some other things that I mentioned. We could discuss this more to verify his hypothesis. This data gives you an hypothesis applicable to the material in this particular plant to which people are being exposed. This is a particular material coming from the drying area, and it is in a particular chemical form. It is not that they are ignoring that; it is just that they can combine knowledge about plant materials and history with air and urine data to obtain correlations yielding quantitative relationships between air concentrations and average urine concentrations over long periods of time.

The point is there are many ways to approach research. Gary, I think, has prepared a foundation for an operational plant manager to take an interest in the monitoring data, to let the data tell him something, and to look at it and analyze it like any scientist.

Q: In other words, you are saying everybody in that plant is exposed in the drying area?

A. BRODSKY: No, it doesn't say that. Or, if there was uranium, if they were exposed to uranium oxide, perhaps it was not completely dry. Perhaps it was not high-fired as they say, do you see? I don't say that we should not look at the lungs. We should still look at the lungs.

Q: Is this only good for this milling operation?

A. BRODSKY: Of course.

Q: It cannot be used by any other place?

A. BRODSKY: Obviously. I mentioned that before. I said it was fortunate that he happened to look at this particular mill. I implied strongly that other mills would be different. And other locations in each mill would have to be looked at.

Q: I just want to add a comment. In the paper, it's very clearly stated the clearance is determined at an average 2.9 days. In some cases, like ammonium diuranate, there's a clear indication that it is indeed ammonium diuranate, which has only days of clearance time--two days.

A. BRODSKY: Absolutely. We should not assume we could ignore possible long-term burdens in the lung, and other materials, right.

Q: For U_3O_8, it would have taken years for such elimination. There should be a urinanalysis done on all the systems. It's a good proposal and very good work.

A. BRODSKY: And some of us think that it's very important, although, you know, you don't want to make people spend so much money on too much whole body sampling that they don't have enough to do anything else for other purposes. Occasionally, we should look at the lungs, even in this particular plant.

Q: Brian mentioned that his employees were rotated. Therefore, every employee worked every job; they were all over the plant. That would wash out.

A. BRODSKY: That's another good point, yes. I didn't mention that but that's a good point to emphasize at this time, that you do have to consider that.

VOICE: Every employee is all over the plant.

Q: I think we're getting lost in numbers here, and overlooking a good operational point. That is, if your data is the air sampling and doing a once-a-week or whatever urine sample, the air sample will suddenly say, "Hey, you've got a problem, you'd better look at people." The daily air sample will let you know that something has gone wrong.

A. BRODSKY: Right. And I don't need to emphasize that there's more than one purpose in air sampling. The primary purpose is protecting employees. At some plants, where you have the possibility of release of very highly radiotoxic material, that could really cause tremendous doses. You want several types of air samples. You want one close to the source of the leak that gives an alarm. We're not just here to study exposure to humans; we're here to prevent it.

D. GRAY: I'm Don Gray, with the Inhalation Toxicology Research Institute. I wanted to ask a question in connection with your sampling that was done in the dryer packaging area. Do you consider that that sample is a good representative sample for the individuals involved in the package processing?

A. BRODSKY: It depends on what you mean by representative. I wouldn't think it would be representative of most operations. There is the case in some organizations where you have such high variability in some representative samples that you may be off by a factor of several or more. But I think in this particular kind of a plant, your sources are large boxes and vessels and the individuals are standing back from them. There's a lot of natural air mixing convection in any room--in this room we're standing in today. So, you could expect in that situation that a sampler over there three yards away from me might be, over a long-term period, averaging out the fluctuations and be a better indication of my exposure during the time I'm in that particular area. They use the judgment factor when they place the sampler, of course.

D. GRAY: The reason I asked the question is that Dr. Frank Ibsen studied some uranium mills in Grants, New Mexico, and found that one of the significant contributors to personnel exposure was in the packaging process. I don't know how the packaging is done at Bear Creek, but it's strong at the Grants Mills.

And a lot of it is in positioning the drum: whacking it and suspending a lot of this material. So, he was concerned about personal exposures, inhalation exposures.

A. BRODSKY: This could be a factor here; I don't know. I can't pretend to be an expert on that. It looks like the Bear Creek Mill was a pretty well-closed operation, once those holes in the enclosure were fixed.

R. ALEXANDER: Thank you very much, Allen....

R. ALEXANDER: The next speaker will tell us about laser phosphorimetry. That will be Don Gray from ITRI, Lovelace Foundation.

D. GRAY: My name is Don Gray. I'm with ITRI and we're out in New Mexico.

I'm going to talk briefly about applications to biological samples in support of ITRI's research program, a little bit about the background of the method. I'm going to say something with regard to the Scintrex UA-3 uranium analyzer. This is a device routinely used now for uranium analysis at ITRI. I'm also going to say a little bit about the correlation obtained with prior analyses. In addition, I'll make some references to pellet fusion fluorometry, and I'll say why in a little bit. Further, I'm going to talk briefly about blanks, background and detection limits, just to echo what Rod Melgard has already said. Then, cost and availability. Finally, a few words about some recent work using the Scintrex UA-3 analyzer.

In the area of applications to biological samples, the mission of ITRI is to conduct studies on the deposition, retention, and excretion of uranium and a whole variety of toxic materials, following inhalation by laboratory animals. Hopefully, these studies will provide information on potential human health effects.

The samples that result from these studies have to be chemically treated prior to analysis for uranium. The solutions resulting from these kinds of treatments generally are very highly acidic. In addition to that, they contain relatively large amounts of dissolved minerals, principally various forms of calcium phosphate.

In order to draw valid conclusions relative to health effects for a large population, it is frequently necessary to study a large sample. In our case, this translates into a large number of the animals in the study, which translates into large numbers of excreta samples, fecal samples. And because of the large numbers of animals involved, it's very important that we use the most cost-effective analytical method.

Now, in connection with the information and data I'm going to give this morning, keep in mind that these are experimental samples; they are from laboratory animals. We have deliberately chosen to exclude animals that were experimentally exposed. So, the data I talk about now will be animals for which pre-exposure collections were made.

A little bit about the basic principle or method, on excitation: the uranyl ion emits a characteristic pattern having three peaks. These peaks are at 494, 516 and 540 nm. Now, in a sample which also contains both organics and uranyl ion, the organics will also luminesce in a region around 400 nm. So, an instrument designed to determine uranium can discriminate against organics by introducing a green filter with a cut-off around 450 nm between the sample and the photomultiplier tube. That's what's done in the Scintrex UA-3....

In addition to the wavelength differences that characterize the luminescence that resulted from a sample containing both uranyl ion and organics, there's a very drastic difference in the lifetime of the luminescence that you get. The laser itself has a very short pulse, on the order of 2 ns. And the scattering on that from the laser itself decays very rapidly. The next longest decay time is from the organic fluorescence; this is beginning with a lifetime of a few to a few tens of nanoseconds. The ^{238}U phosphorescence, however, decays with a much longer half-life. The phosphorescence from the uranyl ion decays with lifetimes on the order of a few tens to several tens of microseconds. That's one reason that measurement of phosphorescences is useful. The fluorescence is a nanosecond-type phenomenon and the phosphorescence is longer. This longer decay time is the principal characteristic utilized in the UA-3 analyzer to discriminate uranium.

The measuring circuit of the UA-3 is gated such that the organic fluorescence would have decayed before the measurement is actually begun. In the case of the UA-3, I believe the gate opens at about 30 μs and it remains open for 100 μs.

In practice, the phosphorescence of the uranyl ion is enhanced by the addition of a proprietary reagent called Fluran. It has the effect of complexing the uranium function to maximize the amount of luminescence from the ^{238}U and to block the others.

The heart of the UA-3 is the laser. This laser is a sealed pulsed nitrogen laser that fires 16 times a second. The sample itself is contained. What happens in operation is that the laser fires and then sends a signal to the gating circuitry. The gating circuitry waits for 30 μs after the laser fires. It opens, passes the amplified signal to the integrator, and remains open for about 100μs. The integrator then sums up four seconds' worth of signals from the gate and passes that sum on to a sample to the readout, which is just a simple dial. The sample then holds that reading every 4 sec. It has a gain control which adjusts sensitivity, and it has a balance control which is used in practice to balance out the residual organic fluorescence.

The principle of pulse laser phosphorimetry has been used prior to our applications. In 1978, Robbins used the method to measure levels of uranium in geological materials, and in surface and ground waters. And he got a 0.05-μg/L detection limit. In 1981, Harms and coworkers used the method to determine the uranium in vegetation. This involved dry-ashing and wet-ashing samples and then determining the uranium in the residue. He quoted a 15-ng detection limit in ash. These first two applications were both used in the UA-3 uranium analyzer. And in 1983, Bushaw, using the principle of pulse laser phosphori-

metry with some additional refinements, determined ^{238}U in untreated urine samples, and he quoted the detection of about 10 μg/L.

Of course, we're using the instrument. We used the standard addition method. We needed a great deal of work when we first got the instrument to see if it was possible just to use a calibration method. It was not possible to do so because one typically encounters high concentrations of calcium ions and hydrogen ions, which quench the phosphorescence of the uranyl ion. With the kinds of samples that we encounter, one gets fairly high concentrations of calcium and iron in solution, resulting from dissolution of urine samples and various kinds of tissues. Those concentrations can be very variable. That precluded the use of a sample calibration line. We settled on standard addition because it compensates for quenches as long as the standard itself does not quench, taking precautions to insure that the standards we're using for the standard additions are freshly prepared. They're prepared on the day of use. Nitric acid is what is usually used. So, it's prepared daily....

We've done an awful lot of work prior to the UA-3 analyzer by pellet-fusion fluorometry. That involved taking a 5-mL to 10-mL aliquot of sample; extracting it: pellet fusion using that sample (and, of course, a considerable amount of pellet preparation); and then measuring the fluorescence in that case by fluorometry.

By comparison, in standard-addition phosphorimetry the samples themselves are digested according to the method of Keough and Powers. This includes muffling, drying and wet ashing for varying periods of time; wet ashing with various combinations of nitric acid, hydrogen peroxide, and HF; and then repeating that process until the sample is free of carbon and you get a good water-clear solution. That residue then is dissolved in 2-M nitric acid. Now with the UA-3 uranium analyzer, we're taking 0.1-mL to 1-mL aliquots from the sample and adding Fluran. We take the first reading and add the standard addition, and then take the second. The simplicity of this method is that a technician who is skilled in the Keough and Powers method can pick up the uranium analysis in a very short period of time. That's one of the areas where we believe we have affected the cost savings and cut our original cost from that of pellet-fusion fluorometry. Calculating results, based on first and second readings, involves a very simple equation, easily handled on a programmable computer.

I'd like to talk just a minute now about what Rod Melgard said a minute ago. What is a blank? What is a background? and so forth. Our blanks, in this particular case, are 2-M nitric acid carried through the process. We haven't made any particular efforts to obtain ultrapure nitric acid. We haven't made any particular efforts to correct for leachable uranium because of the high volume of operation.

Repeated analysis of the 2-M nitric acid offers the best measure of the precision of the analytical method. And when we go beyond that and look at actual samples that result from the studies, you're talking (in a sense--I'd be uncomfortable in calling them blanks) about the measurement of the backgrounds.

So, there's really, as we see it, three levels of concern in the area of experimental samples: one can do repeated analysis of aliquots from the same sample, one can take samples from a single animal and analyze them over time, or one can take a block of samples from different animals and analyze them over time. The first of those measurements will give you a good measure of the accuracy and precision of the method. The analysis of a sample from a single animal over time is largely influenced by the individual health status, in terms of variability. The analysis of samples from several animals over time is really a true population variability. The first of these is, we feel, a fair measure of the accuracy and precision of the method.

We next turn to the results of some of the uranium determinations that we have done in various kinds of samples--urine, feces, soft tissue, and bone-- by laser phosphorimetry. As I said at the beginning, they result primarily from analyses of animals, which were either controls in the study or were collected prior to the exposures. And I think the main point to make here is to note the rather large difference in the standard deviation that results when one compares the reagent and sample measurements with the individual and population measurements (an individual being a single animal over time, and a population being a group of animals over time).

The variability increases quite drastically, based on these data and applying the factor of 4.65 times standard deviation, a reagent value of 0.37 as a detection limit, and the sample value of 0.60. Repeated analysis of the same sample results in standard deviations which give, I think, a fair measure of the method. For feces, you get about 2.3 μg/kg.

In the area of cost and availability: I'm basing the cost figures we have here on salary, overhead, operating dollars, and equipment amortization. Applying these criteria to the costs, we got $25 to $30 per sample for the first analysis. For replicate analysis for the same sample, cost decreases quite a bit, $5 to $6 per analysis for replicative analysis of the same sample.

Of course, the Scintrex UA-3 is commercially available. Since we've had it, we've sent it back for repairs once. We got it back in less than three weeks' time. So our experience would say that repairs are available with a two-to-three-week turnaround.

Recently, we have made some attempts to try to determine uranium directly in an untreated sample. We haven't had a whole lot of success. This system involves taking 1 mL of raw urine. The residual organic fluorescence in the sample really raises the apparent background to very high levels, approximately 700 μg/L. One of the things we think that might help this situation is if it were possible to delay the opening of the gate. In fact, I think that may be what Dr. Bushaw did with his instrument at Battelle--delay the opening of the gate.

In summary, then, for the three areas that we were specifically concerned with, I have a comparison of our application of standard-addition phosphorimetry with pellet-fusion fluorometry. And our projection is about 0.6 μg/L for phosphorimetry compared to about 0.2 for fluorometry. You can see the values

for the cost: $25 to $30 versus $35 to $40, and $15 to $18 for fluorometry versus $5 to $6 for phosphorimetry. And, of course, the instrumentation for both methods is commercially available.

So, in conclusion, I would just say the Scintrex UA-3 has been used at ITRI for about a year now to do routine uranium analysis, and we support a large research program with it. We have realized, we think, significant reductions in technician time, both from the standpoint of training operators and from the standpoint of daily operations. Because of that, we feel there are significant cost savings that have resulted in comparison with our previous costs for fluorometric analysis. We've seen that sensitivity and the detection limits satisfy our program requirements.

I'll be happy to entertain any questions.

Q: How many samples per day are your cost analyses based on?

D. GRAY: For original analyses, there are probably about--well, let me back up a bit and give you a little bit of background. The original sample was based on an analysis of, let's say, 80 to 100 samples. That takes a period of time stretched over about two weeks because the primary cost there is involved in obtaining the acid digest solution. If we were just going to analyze the uranium in the acid digestion solution, it would probably be more.

Q: What is the name of the manufacturer?

D. GRAY: Scintrex.

Q: What's the average lifetime of your nitrogen laser?

D. GRAY: We've been using it pretty steadily now for about a year and a half. I think the average lifetime is something on the order of a year. They don't recommend that you stockpile these things because the shelf life is not particularly good.

Q: What is the difference between phosphorimetry and fluorometry?

D. GRAY: Fluorometry is a measure of fluorescence. Fluorescence is a fairly short-lived phenomenon of the order of a few nanoseconds. Phosphorimetry is measuring a phenomenon which is much longer. The phosphorescence has a lifetime on the order of microseconds, tens of microseconds. That's one difference. The other difference has to do with excitation states.

Q: Did any of these studies correlate the Scintrex laser with alpha spectometry?

D. GRAY: No, we did not. We don't study uranium with alpha spectometry.

R. ALEXANDER: Thank you very much for an interesting paper. I notice that four of the papers deal today with laser techniques for uranium bioassay--laser phosphorimetry, laser-kinetic phosphorimetry, laser-induced fluorescence,

and then the computer-initiated resonance ionization spectroscopy, a laser technique that Dr. Parks will discuss this afternoon.

Our next paper will be presented by Bill Moss from Los Alamos, rather than Dr. Gladney.

W. MOSS: I am glad that I was asked to give this presentation. Dr. Gladney had to back out of it because of other commitments.

We published this procedure and the findings in 1979 in Health Physics, Volume 37. Also, the procedures which are referred to here were published in a manual called "Manual of Methods for Radiobioassays." The LA number is 9763M if you would like to copy that.

Let's talk about the procedure in detail and the technical aspects. I will break this talk down into three sections. First, we are going to talk about delayed neutron counting; then, we will talk a bit of our experience at Los Alamos; and, third, about the investigators.

In thermal neutron activation only fission can produce radioactive isotopes with excitation energies high enough that neutrons are emitted. The technique of counting delayed neutrons--we call it either delayed neutron counting (DNC) or delayed neutron analysis (DNA)--following thermal neutron irradiation for analytical detection of uranium has been used for many, many years, principally in water samples. Originally, the procedure we had at Los Alamos was set up as part of the DOE program for uranium research in water samples.

Delayed neutron emitters produced in the fission process undergo beta decay. The neutrons' unbound levels and daughter nuclei either gamma decay or emit a several-hundred-kilovolt neutron in the deexcitation process. The delay in neutron emission is governed entirely by the half-life of the parent of the active neutron emitter. Experimental studies have shown that these neutrons fall into six main groups, each characterized by a specific half-life....

In the present measurements made at Los Alamos with the Omega West reactor, a neutron-emitting background activity exists in ^{17}N and ^{17}O. Counting is started after a delay of 30 seconds. The ^{17}N activity is then essentially gone, but the relatively long 23- and 55-s fission-delayed neutron groups still persist.

We took a raw urine sample and placed it into a 25-mm polyethylene vial; we then sampled it and put it directly into the reactor. We had some problems with the urine leaking. It wasn't sealed up properly. So, we put the sample through a steam bath. We put it in a polyethylene envelope, placed it in a steam bath, dried it, and put it in the polyethylene vial.

The samples were then transferred to the neutron counter. The efficiency of that count was 40%. It counts for 55 s following the 30-s decay period. The total neutron flux is 10^{13} n/cm^2-s, and the average background is about 40 counts per minute.

One of the advantages of this procedure is the simplicity in analyzing samples. You can render calibration for quite a lot of samples.

So, we routinely make up our calibrations depending on what material you are looking for. You have to specify. If you are collecting urine samples for Los Alamos, we have people working with depleted uranium, normal uranium, or enriched uranium, and they have to specify. When you collect the sample, you have to run your calibration material with the same type of material the people are being exposed to. For instance, if they are working with depleted uranium, you run a calibration to depleted uranium standards. If they are working with enriched uranium, you do likewise with enriched uranium. If people are working with both, you take that into account. We use NBS standards in all cases.

We have 2500 μg of normal uranium, 140 μg (calibration measurements) of urine solution spiked with known amounts of ^{238}U which were prepared and subjected to the activation procedures. Studies used depleted uranium, 1.8% enrichment, normal uranium, 0.7, and 93%-enriched ^{235}U made at ranges from 0 to 2500 μg, from 0 to 140 μg, and 0 to 1000 pCi/L , respectively. All measures resulted in linear calibration curves over the ranges studied. In every case, correlation occurred.

A series of urine samples were spiked with known amounts of normal ^{238}U, analyzed by both the DNC and the fluorometric analysis, and compared. The uranium spike was NBS-9508 Standard Reference Material. The mean of the ratio of the known concentration divided by the analysis values was 1, and the correlation coefficient between the two sets of analyses was also 1....

The variability of the DNCC method is much less than that associated with the fluorometric analysis. That is quite important.

A series of urine samples were spiked with known amounts of enriched uranium, 93% ^{235}U, and analyzed by DNC and wet chemical isolation, followed by alpha activation measurements. The correlation between the sample results was also 1.

We had concentrations of uranium from 1 pCi/L for DNC to 52 in the radiometric measurements. Notice the correlation. The standard deviation associated with the radiometric analysis is 28, and for the DNC it is 3. We believe the DNC measurement is much superior and gives a more reliable idea.

The sensitivity of the method is, of course, of fundamental importance here. The reproducibility of the uranium calibration measurements was within the statistical error of the counts obtained. The sensitivity for the uranium detection and the accuracy of the measurement can be determined by statistical analysis of the counting data.

Because we are using this method of analysis to screen a large number of samples, our concern is to have a high level of assurance. In the event we have a sample which exceeds our concern level--and our concern level for normal

uranium is 10 μg/L and for enriched uranium it is 5 pCi/L--we then request a reanalysis by the same method. We usually expect about a 100-mL sample until the analysis is completed.

If there are positive results, we reanalyze the sample to confirm the deactivation control. In the event that we again see this level, then we do a radiometric analysis. In the experience we have had in the last six years at Los Alamos, I think, it is quite important to recognize we have had to reanalyze about half of the samples, and we have done a further analysis on only two of those samples. In each case, subsequent analysis confirmed the DNC method.

There is a possibility that there was some contamination of the sample in two cases that we analyzed. I mention this as a possibility only.

The concern levels are based on exposure to the toxic metal properties and radiation levels outlined in NRC Regulatory Guide 8.11. The average background from a series of urine samples was 40 counts.

Using the Kurie method with respect to counting statistics already referred to and a background of 40 counts yields a normal uranium detection limit of 1 μg/L. For depleted uranium, which people are exposed to at Los Alamos, it is 4 μg/L, and for enriched uranium 1.5 pCi/L.

I mentioned briefly some experiences about using samples. We routinely make up QC samples. Those are usually made up at least a month ahead of time and stored in refrigerators as a method of preserving the sample. But in no cases have we noticed any deterioration of the quality of the results from using those samples. So, I don't particularly see a problem in the purity of samples that are out there.

The other thing I have to mention, because I think it's awfully important, is what constitutes a blank. How do you know when you take a blank urine samples that that really represents the control? One of the limitations of this procedure is that, as you increase the volumes sampled, you increase the interference potential and the spectrums produced by the delayed neutron counts.

We looked at a correlation between people's potential exposure because we increased the sensitivity by obtaining a large sample. Routinely, we get 25 mm. We went to our shop people and we asked them. We did a series of experiments in which we increased the volume samples from 25 to 50, up to 100, up to 150, up to 200. We thought this was a beautiful way of looking for some possible correlation with air samples and to do sensitivity results. The limitations were that at 200 mm we got spectrum. We weren't able to resolve this at the 1.5 pCi level. But we did find that a number of people gave us samples which gave a positive response. No relationship at all to the air samples.

So, in the course of doing the study, we asked the people in our own laboratory to supply us with urine samples. And the blank urine samples showed

positive responses. So, we said, this is quite interesting. They're not working, they're giving us a positive response.

As you remember from another publication in Health Physics recently, Los Alamos water is well water and has one of the lowest concentrations of uranium of any water samples that were studied in the United States. In fact, it wasn't even detected in our drinking water. So, we broke the people down into two categories. The other class of drinking water which was found in the lab consisted of people who live outside the boundaries of Los Alamos, people who live in Santa Fe or people who live in the Valley. Los Alamos is on a plateau at about 7000 ft. People in the Valley are as low as about 5000 ft.

We found that the people who lived in the Valley gave us the positive responses, as did the well water that was found. We haven't done any followup studies, but it could use some more work. It's obvious. One of our people in the lab, for instance, was excreting in our urine 2 μg/L per day. We took that. We didn't recognize that person lived off the hill and drank 10% of her water off the hill.

So, you have to know where your people are getting their drinking water. Secondly, you have to increase the volume of samples taken.... Previously, we had two people routinely doing full-time analysis, 2000 samples a year by fluorometric analysis. Using this procedure, we have one technician that spends 26 days a year for 2000 samples. It's a critical cost of manpower and the reactor is available for our use.

We'll talk very quickly about something that Ernie Gladney has investigated, very experimentally at this time. It's not been proven but, nevertheless, it has potential. It's an analysis of uranium by inductively coupled plasma spectrometry, known as ICPMS. He hurried over to Data Control in Oklahoma and used their instrument.

The ICP mass spectrometer is a brand new tool available in this country. The detection limit is 0.02 ng of uranium per mg of sample. The inductively coupled plasma ionization source is interfaced through a quadrupole mass spectrometer, with a controlled leak so that a small fraction of the plasma is drawn into the mass spectrometer.

The mass spectrometer detection system has three principal advantages over the more common optical emission spectrometers, which for nearly a decade have been coupled to ICP sources. First, the cost is about $200,000 for the research tool available at Los Alamos to do some studies on uranium. The mass spectrum is also much simpler than the optical emission system, with some 390,000 lines versus about 300 in the mass spectrum across the periodic table. Third, since the mass spectrometer operates on an ion-counting detection, it is far more sensitive than the photon detector and capable of achieving much lower background. The ICPMS provides isotopic information with typical precisions of ± 1/2 to 1-1/2%.

The procedure, then, is to take a raw urine sample and introduce it directly into the ICP without chemical preparation or pretreatment. There

appears to be no chemical interference at the uranium mass region. A few minutes of counting time are required and 2 mL of sample. A concentration of 10 ng/mL of natural isotopic uranium urine exhibits about a 300-count peak on a 20-count background.

The high levels of totally dissolved solids in the urine matrix may physically interfere with injection of the sample into the plasma. It hasn't been proven but, I think, on the large sample volume a detection limit for direct analysis of depleted natural or highly enriched uranium would be 0.1 ng/mL, or about 7 pCi/L. The poorest detection limits are encountered in equal ratio of about 50/50 for ^{235}U and ^{238}U and would be about 3 ng/mL. For enriched uranium, of course, DNC will be much more sensitive....

We've been using this procedure since 1979. We've had about 2000 samples a year. We have taken about 50,000 samples at Los Alamos.

The thing that I think is important is that we've had very few significant exposures over the years. The experience of fluorescence analysis always leaves a question about why we're getting positive results. But I'm convinced at this time, after six years, that what we probably have were introduced from outside.

R. ALEXANDER: If you don't show any positive results, our licensees will certainly be interested in that technique.

(Laughter.)

R. ALEXANDER: No matter how much it costs.

W. MOSS: Now, seriously, Bob, we had the same problem at Los Alamos with plutonium before we approved the method by tracer technique. We also had this question with the results: Where did that fellow get his exposure? With the tracer technique, we don't see those anymore. I would say, in the last 10 years of analyzing samples with tracers, which gives us a handle on what's happening, we've had less than one exposure per year for which we don't have a documented reason, or we got a positive result. So, I think analytical techniques do resolve a lot of those questions which result when you have some questionable procedure.

R. ALEXANDER: That is a good point as far as the justification for cost of these more expensive techniques.

W. MOSS: We didn't buy the instrument for uranium analysis. We bought it for other purposes; we were simply exploring the possibility of using it.

F. DYER: Frank Dyer at Oak Ridge. The delayed neutron analysis cost is a little bit higher than we had thought it might be; if you broke it down into, say, two or three parts, what would be the largest contributing factor to the cost?

W. MOSS: In putting this talk together, I figure we'd spent about $2 on test samples. The other costs would be associated with reactor costs. Of course, the reactor is a big investment. I can't get into the details of how they come up with the value of $58, but that's what they told us.

Q: Could you just give me a ballpark idea of how many urine samples per year are analyzed throughout the whole United States?

R. ALEXANDER: We made an estimate a few years ago.... Does anybody have a guess as to what it is, to answer that question? It's fairly low now that the mills are shut down.

VOICE: H&L might do a thousand a year.

MR. BRODSKY: Bear Creek Mill is doing maybe 20 a month, but they're going to close down in a month.

VOICE: It would probably be at least 10,000 a year.

R. ALEXANDER: On the order of about 10,000? Do you have any feeling for that?

Q: What was the figure given--10,000 per what?

R. ALEXANDER: Year.

VOICE: Total for all those five mills that are operating.

VOICE: No mills.

R. ALEXANDER: No, no I don't think he's even including the mills. Other than mills. The Department of Energy still has a very large uranium operation. In fact, I hope we'll have a statement, possibly a question, from the Department of Energy right now.

VOICE: In 1982, 28 Department of Energy facilities did a total of 81,246 urine analyses.

R. ALEXANDER: There's where the business is, boys.

(Laughter)

Q: Was that all uranium?

VOICE: No, not totally.

VOICE: In the depleted-uranium industry at our manufacturing facility, we analyzed 8400 samples a week, and we have a total population of about 500 employees.

R. ALEXANDER: Where are they located?

VOICE: Concord, Massachusetts.

R. ALEXANDER: That's Air Force work?

VOICE: We do industrial, military, the whole gamut.

AFTERNOON SESSION

R. ALEXANDER: The next paper is by Al Robinson of U.S. Testing. Al has distinguished himself in this area. He's formerly from Battelle and worked for several years on a contract with us and the DOE to test a bioassay performance standard, a laboratory performance standard. That work will be finished before long and it will be in the open literature. It will result in an ANSI standard for the performance of bioassay laboratories, very similar to the one we conducted for personnel dosimetry, which also resulted in the NVLAP accreditation program. And we hope the same things happen with the bioassay program.

Laser-kinetic phosphometry is, I believe, the technique that Bushaw developed at Battelle. Al is now at U.S. Testing and he's going to bring us up-to-date on Bushaw's technique of laser-kinetic phosphometry.

Al Robinson.

A. ROBINSON: Thank you.

The Richland Division of U.S. Testing provides a number of analytical services in radiochemistry, analytical chemistry, dosimetry, and hazardous substance analysis, as specified by EPA. But one of the major components of our work is uranium analysis for both the DOE and a number of commercial customers.

Last year, we performed approximately 4000 uranium analyses, and this year it looks like the number will be up significantly, as a result of several new contracts. Some of our customers have for years required a detection limit of around 0.03 μg/L. This necessitates the use of large-volume samples, as you might realize, and certainly stretches a pellet fluorimeter to the limit. It requires a lot of extra care and essentially running an internal spike for each and every sample. It also exacerbates a lot of problems including constant burn parameters and others.

As you're all aware, for years fused-salt UV-lamp fluorimetry has been the standard for low-level uranium analysis. It takes a number of different types of samples. It has good sensitivity and low cost. But those of you who have worked with it understand that there's also some problems.

Among these, the principal problems are in efforts to reproduce the effect of the matrix. Even with something like urine, the variations from individual to individual are significant. Excitation, light scattering, emissions--again, largely unwanted emissions from the matrix--the temperature of the burn, the pH of the solution, all enter in. It takes careful control of all of these to compensate for the quenching effects of the matrix.

That's one of the reasons that you can't perform direct analysis by this method and get the kind of sensitivity that you might like.

A couple of years ago, Bruce Bushaw and Tom Whitaker of Pacific Northwest Laboratory utilized Bruce Bushaw's development of laser phosphometry and

christened it, at the time, laser-kinetic phosphorimetry. Shortly thereafter, U.S. Testing decided to build upon their early work, and Dr. Al Miller and Phil Friend of U.S. Testing subsequently designed and built a prototype commercial model of a laser kinetic phosphorimeter.

Today, my talk will describe the phosphorimeter briefly and describe some of the data that we have generated as part of an ongoing qualifications program. Essentially, it's an in-house testing program to check out the limits of the phosphorimeter, take a look at how it would fit into our commercial operation, and, in general, gain enough operating history and confidence to be able to put it into widespread use.

The basic principle of the phosphorimeter is very much like the Scintrex discussed earlier. It's simply the measurement of phosphorescence emitted after excitation of the uranyl phosphate complex. This is the excitation and emission spectrum of uranyl phosphate. The laser that we use runs around 420 nm in the middle of an excitation band. We then filter the output light and essentially look at about 520 nm as the output. I think the major important aspect of this methodology that differentiates it from some of the others is in the treatment of the data.

I'd like to briefly describe the basis for doing a calculation for this instrument. Basically, if we assume that the excitation occurs in a short pulse, and if the decay of the excited state follows first-order kinetics, then you can describe the decay of the uranium phosphorescence by a differential equation including the rate constants for the phosphorescence decay and the quenching factors that would be inherent in the matrix of the sample. Integration of this equation gives essentially a linear equation, the equation of a straight line, where the intercept is independent of quenching. The log of the intensity of the phosphorescence at time zero is proportional to uranium concentration. So, if you can get back to what you would have seen at time zero, then you would be able to bypass a lot of the problems in correcting for quenching.

R. ALEXANDER: In theory, then, Al, you have a straight line. Does that turn out to be linear in the test?

A. ROBINSON: Yes, it does, within limits.

The next slide illustrates the effect of chloride, a severe quencher of uranium phosphorescence. This was generated on the instrument, and we'll get to the details of it a little bit later, but what I wanted to show here is the effect of increasing quench. Basically, the top red line is the response with no quenching, no chloride added. Then a few millimolar is added to generate the green line, a few tenths more for the second red line, a few tenths more for the purple, a few tenths more for the blue. It shows the general effect of increasing the decay rate, and that's the general effect of quenching on the output, the light output from the uranyl complex.

Essentially, the first 50 channels will illustrate the calculational method. These are part of the same curves that we just saw. There are approximately 10 μs per channel. So, at time zero, the laser fires, and the instrument would then look at the light output in 10 μs chunks throughout a number of channels. And then plot that information. If you take a log of the counts recorded versus the channel number, for that matter, the microseconds after firing, and extrapolate that back to time T equals 0, you can see that they all meet at a given point.

Here we have a sample with greatly increased amounts of quenching. Yet by extrapolating the fit of the curve back to time T equals 0, we come out with the same estimate of uranium content, which is basically the log of a number of counts that you would get at time 0.

Now, the first 40 μs or in some cases the first 50 are highly variable. These channels are ignored because this time period contains rather shortlived species, that is, fluorescence, from matrix effects; thus, we go out 50 μs or so, and then start plotting the data, essentially ignoring all of the earlier emissions.

I guess it also points up one of the differences we see between an integration over a fixed period of time and an approach such as this. If we were to integrate those curves over any segment of time, say, from channels 4 through 12 or 8 through 16 or on out, you would get widely differing amounts of activity. Of course, that can be corrected for by the standard addition method. Yet that means you've got to run more samples. So, this alleviates a lot of that and gets away from a lot of the effects of quenching.

Basically, again, it's a relatively simple instrument. It has a pulsed nitrogen laser that pumps a dye laser. Then, the dye laser output travels down through the reference cell and the sample cell. The emitted light is collected at right angles in the respective photomultipliers, and there's also a mirror there by the reference and the sample cells, by which you gain a little bit of efficiency, if you bounce back the light that is emitted is that direction.

It goes through a filter that removes unwanted wavelengths and passes 520-nm light. The light then passes through an aperture, that allows you to cut out some of the signal, so you don't saturate the photomultiplier at high levels of uranium.

From the photomultiplier, then, the signal goes into a pre-amp and a scaler, and out of there, we come to dual multichannel scalers, which just count the pulses. Then, those data are later fed into the computer.

Basically, with each pulse of the laser, you get X number of counts of light into the photomultiplier and we pulse the laser maybe 1000 to 2000 times, depending on the concentration of uranium. And you accumulate counts throughout all those pulses. At the end of a preset number of pulses, at the end of 2000 pulses, say, the accumulated spectra are dumped out into the computer.

It's a very compact little unit. It's about 2 ft by 2 ft or so, 24 in. square, maybe 3 to 5 in. deep.

We just add on a printer. The computer is contained within the instrument, the random memory and the accessible memory, and we interact with that through a standard keyboard printer apparatus. It also has an RS-232 port. So, if you want to plug into a PC, an IBM PC or some such thing, you could run the instrument through that and also acquire a spectrum. In fact, if you were going to do a lot of different matrices and a lot of experimental-type work, it would be advisable because you have a lot more accessibility to change the program and such....

The interior of the curette holder is painted flat black to cut down on scattered light. Basically, you just use a standard fluorimetry cell, and it fits into the same kind of holder you see in any spectrophotometer. All you do is add the sample to the curette and put it in the instrument, shut the door, then interact through the keyboard. It's a menu-driven program. You have several options, and the instrument does its thing.

To review, when the emissions are collected by the multichannel scalars, they accumulate counts in each channel. So, what happens is, the scaler at 10-s intervals takes a look at the emitted light. What you end up with is the emitted light as a function of time after the laser pulse. You've run a background and stored that already; now, the instrument subtracts out the background and plots the log of the net count. The program then runs a linear regression analysis on the data and compares the resultant zero-time intercept to a standard calibration curve based on your matrix (or what you expect you're working with, which you've also previously stored).

Q: Does this dual channel do two samples simultaneously?

A. ROBINSON: No. I'm glad you asked that. The reference is basically used as a means of measuring the power. The power of a laser varies somewhat as a function of time. So, by referencing the power level at the time you ran your standard curve, everything is normalized back to that same power level. The fluctuations in the power level, as you go through a set of samples, are all normalized. That's the function of that cell at this point.

Q: How many photon pulses can you count per laser channel?

A. ROBINSON: Are you talking about dead time now?

Q: Yes.

A. ROBINSON: The dead time--now that I've said that, I think it's around a nanosecond or a microsecond. Basically, at 2000 pulses at a low level, you will accumulate 3 or 4 thousand counts in a channel. I think Phil Friend was talking about that just the other day. I believe he said you could go up to 30,000-some counts before you would get into problems with dead time.

There is a dead-time correction that's built into the program. The appropriate correction is evaluated as you make the calibration curve and go higher and higher in uranium concentration. The dead time correction that you've programmed in is characteristic of a given instrument, and it does make correction for instrumental dead time. It is something that has been taken into account. Quite frankly, I can't give you a lot more detail.

We can see some of the types of spectrum that you get out of the instrument.... Basically, you store a background spectrum and you have a sample, say, at 7 parts per billion.... Or suppose you have a 25-part-per-trillion sample, which basically is 25 picograms in the cell. If you get down to that kind of level, the scatter becomes much greater and the uncertainty becomes much greater. Even so, we're able to handle those and there is a program within the instruments to calculate the uncertainty of the least squared fit of the data. So, it is taken into account.

I'll talk about some of the diagnostics later, but one of the things you can use to tell whether you're in trouble or not is a correlation coefficient. If that correlation coefficient starts to drop off too far, of course, you obviously know that you've pressed beyond the limits and there's something bizarre going on....

I think, next, I'd like to just go into the report format that we have programmed in at this point. It's a menu-driven program. There are a number of options that are very handy. Of course, you have the obvious, the sample i.d.--that's put in by a technician, a literal description that is a great help a lot of times. The phosphorescence lifetime is printed out from the data and that, as we'll get into a little later, is a very useful diagnostic tool for letting you know if you have difficulty with the kind of sample you're working with. We have the correlation coefficient and the reference intensity ratio, which relates back to the power level at that time, and the date.

The first time and date have to be entered at the first of the day, and then as long as the instrument is left on. It has an internal clock so that each sample is printed out with the date and time it was analyzed. Concentration is printed out in whatever format you choose. For example, we can choose parts per billion. We've also a lot of times used nanograms or micrograms. The uncertainty in the determination is given, as well as a couple of other parameters. The sample net count and the number of laser pulses are also given. After you run a number of samples, you start to look at those. It's just another possibility to recognize potential problems where more diagnostic information is printed out....

You can print out each channel's counts if you want. It's an option you have. The histogram is also printed out any time you have an r-squared value less than 0.96. It automatically prints out the histogram. Somebody looking at a histogram that has run a few samples may see a couple of things. First of all, the lifetime is longer than we routinely would like to see. We generally prefer the 200-300 μs range. Also, the correlation coefficient may be too low. So, the obvious thing would be to make a dilution.

For example, for a 1-to-10 dilution, you can get a relatively good straight line. The r-squared value is 0.998. No complaint there. However, if you look at the lifetime, you could see it's about 105 μs. That is less than desired. We're trying to set limits so the technician can look at these numbers and decide whether the sample needs to be reshot, diluted, or is okay. Yes?

Q: The concentration is three nanograms? Is that before dilution?

A. ROBINSON: The program calculates it based on 1 mil in the curette, so that it did not take into account the dilution. I should have probably written the corrected numbers on the viewgraph to take into account the dilution, but, yes, that gives the concentration in the curette. Normally, we run a standard 1 mL of a sample. We don't make concentrations. We just ash it down and bring it up to the same volume. Inspection of the data shows that we have still a lifetime that's lower than desired...

I'd next like to give a brief description of some of the data we have generated on actual urine samples. Most of the data was generated by technicians after a very brief training period. One of the things that we tried to do is set the instrument and the procedures up that we've written so that a technician can run it without having to make a lot of highly informed decisions.

If Dr. Miller runs the phosphorimeter or if I were to run it, or if one of you were to run it, we could make a lot of decisions often based on the look of the histogram, and a number of other things. But, for our purposes, we want to get the procedure so it's as automatic as possible. So, there are set points that you either accept the data or reject the data and urine.

So, we first trained a technician. I think she was trained for just a couple of days, basically, because there's not much to it except how to press the buttons and such. Then, we went ahead and started generating data. The general procedure that we use for all of our urine samples at this point is to take sample aliquots, typically 5 to 10 mL; place them in acid-washed, liquid scintillation vials; wet ash with nitric acid and hydrogen peroxide mixture; then, dry ash if needed at 500°C. Then, we dissolve the sample residue into a few tenths to 1-M nitric acid.

When we get ready to analyze, we take 1 mL of the ashed sample and add 1.5 mL of 1-M phosphoric acid and put the curette in, touch a few buttons, and then print out the results. Finally, we evaluate the diagnostics, that is, the r-squared and the lifetime, and evaluate the final results and go on to the next sample....

In one case, a single urine was divided up and spiked with varying amounts of uranium. Then, there were seven separate aliquots taken from each sample urine. They were ashed down separately and analyzed separately, so we got an idea of the variability of samples that have gone completely through the ashing procedure, and the instrument. So, it's not just instrument variation, but it's ashing and instrument variation in this case. The standard deviations

were extremely good throughout the range that we looked at. On the 0.25-microgram-per-liter sample, there was a recovery of 77%.

I think the one disturbing part of the system as of yet is that we do get occasional low recoveries, bias essentially.... I've been looking pretty closely at why this occurs. And, of course, you never know for sure. It could be a technician error, but I've looked back over a lot of data, and it does occur on occasion.

The problem should be minimal with efficient use of the diagnostics and also a closer look possibly at some of our calibration curves and the fact that the matrix does have an effect. The method is relatively matrix-insensitive, but it's not totally matrix-insensitive.

Water samples will read differently than digested urine samples. And they, in turn, are different than a soil sample or a vegetation sample.

We used to have a lot of variation down at the low end, in particular in the blanks. It turns out that when you develop an instrument like this, and you start using it, it opens up a new, full set of problems that you didn't recognize before. For example, we had a lot of variation in the blanks. We looked everywhere trying to decide what the problem was. It finally turned out that the technician would use hand lotion from time to time; even though she's very careful not to touch the curette, apparently, the vapors are enough to affect it. And, indeed, when we made very sure that no hand lotion was used, the variation went away. Now, that could come about from just greater care because we made a big deal out of it, or it could be the result of the lotion. I don't know. But we were able to get away from the variation at the low end. So I feel the precision is excellent now and the remaining problem is to find out why we occasionally get biases.

These are just some more samples. In the last slide, at the 0.056 μg level, the standard deviation for six samples was 0.003. I know that looks hard to believe from a set of ashed samples, but here are some other backgrounds with similar variation.

If you look at the number 2 urine in that group, we get 0.045 μg ± 0.007. But I have to say, in any group of samples, even at those low levels, we're more likely to have relative standard deviations of about 10% than 15%, but we're also not all that likely to have ?.2%. It's like any method, if you get down at a very low level, your standard deviation can vary, but for this method it is still extremely tight at low levels.

One problem that we've worried about, and we've seen some evidence of, is absorption. As you remember earlier, talking about the uranium absorption to the container wall, Dr. Singh stated he had not seen evidence of such absorption. The more I got thinking about it, I wasn't sure I had ever seen any definitive data on it, either. But I always sort of felt that it would absorb unless acid were present. One thing we have noticed is that if urine precipitates at all, uranium is very definitely concentrated in the precipitated fraction.

As you all know, once you receive urine samples, they can either be anywhere from clear to half full of flocculated precipitate. And if you don't shake it up well, there's a partition between the solids and the liquid. Normally, for our low-level analysis, we take a 100-mL or 1-L sample of that urine and digest the whole thing down; whereas, for the laser phosphorimeter, we would be taking 5 or 10 mL and doing analysis that way. With relatively small aliquots we wondered if there would be a problem with large flocculated precipitates, and so on.

So, we took a couple of terrible urines that were just about half precipitate, that had been sitting around for who knows how long, and we compared them. We ran five replicates out of each urine. The 5-mL-sized samples were analyzed in the laser phosphorimeter, as usual. The 100-mL-sized samples were prepared by digesting 100 mL down and then analyzing them on the laser phosphorimeter. We also had analyzed those same samples, using fluorimetry and a 100-mL sample. The agreement was very good between the 5-mL and the 100-mL aliquots and the fluorimetry results. We again see a slight low [illegible]as there, for unexplained reasons, really, in urine sample #2 with the [illegible]ze. Now, two of that gro[illegible] of samples were thrown out because the tech[illegible]an noted that she had dried them too fast and they spattered. So, it could be hypothesized, of course, that the others spattered, too. There was no way of knowing that. We have other data, also, and we are convinced that we would be able to obtain a representative sample with 5 or 10 mL out of the urine.

Q: How do you calculate the standard deviation that occurs?

A. ROBINSON: That is a propagated error calculation.

W. MOSS: It is not a model sample like the other ones?

A. ROBINSON: No, it is not. The other samples are actual separate aliquots.

For some data we generated, we began with the μg concentration of the samples at time equals 0. We then looked at them 10 days later, and observed a 16% decrease. We felt fairly sure that the uranium had been absorbed somewhere because they had not been acidified enough. We took those samples and reashed them thoroughly and we found about half of that 16%. We didn't find the rest of it.

So, again, we see a little bias problem that crops up there, and we have spent a lot of time looking at it in the last month or two. I think it basically lies in some of the calculation program. So it is something, I think, that will be fairly easy to remedy. But we will need to generate a little more data on that for urines. We don't see the bias for waters, usually.

We are in the process of making a fairly extensive comparison between fluorimeter data, laser data, and isotopic uranium data. It goes somewhat slowly because the lion's share of our urine analyses that we do for customers are very, very low level, where the fluorimetry data is the worst. So, we are not exactly sure how to interpret some of the data. And the isotopic data is,

of course, expensive. So you don't just willy-nilly run out and do isotopic on all the natural uraniums that are coming in.

But we do have a program going on. It is not yet completed, and I don't want to present that data yet. We have done some limited comparisons in the case of water, and we have done some limited ones for urine. But, basically, on the waters that we have done, we get an excellent comparison between the radiochemical and the laser phosphorimeter. We again get excellent precision and agreement on a set of samples that we have analyzed by all three methods. In general, I think there is good agreement, but we need a lot more data before I would really want to stand on it.

So, to just summarize then, some of the advantages that we see are that we do have a low detection limit for water and urine, in the range of 0.02 to 0.05 μg/L for urine. We have done some limited work where we have concentrated urines down, and we can go down to a factor of 2 to 3 below the 0.05 ng/l. With an extraction, we project, without great difficulty, a 0.005 to 0.0005-μg/L detection limit.

So, a detection limit wouldn't be a problem right now. We basically have to have about 0.04 nm of uranium in the curette from some source. If we can figure out a good way to concentrate and separate it out, we can measure 0.04 ng from 100 gallons. But that is kind of the bottom line. Excellent precision. You see less than 15% at a 0.05 μg/L, less than 10% at greater than five times the detection limit.

There is also a relatively low cost of the analysis, about $50 per sample or less. Most of that is technician time and, of course, amortization of the instrument costs. Diagnostic information is available and the data is corrected for quenching.

We have verified applications in urine, water, and air filters, with the reservations I have expressed. Air filters I didn't present, but we have done quite a bit there. We have studies in progress on vegetation, soils, and tissue. Vegetation has been a real bear. There are things going on there that we don't quite understand yet. Soils are not too bad. Again, there are some problems that need to be addressed. Tissue doesn't appear to be too much of a problem. And, of course, there are the overall advantages of low detection limits, broad dynamic range, excellent precision, and low cost per analysis.

Thank you.

R. ALEXANDER: Questions for Al before we let him get away?

W. MOSS: I may have missed it. What is the cost of the machine?

A. ROBINSON: About $25K or less. There is one more think I should say. There are a lot of negotiations going on right now between U.S. Testing and some individuals talking about forming their own company and going commercial with the phosphorimeter within this year. And we should know more in the timeframe of a month. But the estimated cost should be 25,000 dollars or less.

Q: Including the laser?

A. ROBINSON: Oh, yes, ready to go, and it could be run self-contained. If you want to hook on a computer, you can. But it has plenty of computer program in there to run the samples.

R. ALEXANDER: When you get that kind of sensitivity, 0.02 to 0.05 μg/L, and at that low cost, this particular technique won't tell you the isotopic distribution of the uranium in most cases. In some plants the people are exposed to varying varieties. The next two papers, if I am correct, present isotopic determination from isotope dilution mass spectrometry and also resonance ionization spectroscopy.

The next paper is by Dr. Dyer from Oak Ridge on an isotopic dilution mass spectrometer. This was some work that was funded by the Nuclear Regulatory Commission and that I believe was highly successful scientifically. We will have to look into the practical applications as Dr. Dyer presents his paper.

F. DYER: It is interesting. The costs are sort of converging at about the same level, no matter what method one is using: $50 to $100 a sample.

The first people to study thermal ionization isotope dilution mass spectrometry were Dupzyk and Dupzyk in 1979. They used a procedure in which they separated the uranium from the urine. They compared the results of thermal ionization isotope dilution mass spectrometry with fluorimetry back in those days, and the precision of this method looked very good compared with fluorimetry.

In 1983, we did the study that Bob just mentioned and published this in early 1984. It is Contractor's Report 3590. What I am going to say to you today is based to a large extent on what we found in that study.

The topics that I will be discussing today are the spectroscopy systems, the experimental method that we have developed--it looks pretty good and is used by others--the detection limit, the costs of analysis, and the commercial availability--the possible commercial availability of this method.

A little bit first about the experimental method.

The steps for the measurement of uranium by isotope dilution mass spectrometry are as follows. (See Figure 1.) Uranium-233 is added to the sample to serve as a uranium mass tracer. The uranium is then isolated from the sample and a fraction of the uranium is placed on a rhenium filament. The sample is dried, placed in the spectrometer, the spectrometer is evacuated and the sample is slowly heated to the analysis temperature (about 1700°C). The uranium is mass analyzed, and the results are calculated.

Now, in the case of urine, I have the separation shown in three steps in the figure. We take 10 mL of urine and we add 20 mm of high purity hydrochloric acid (Ultrex) and a small amount of ^{233}U. We let this sample sit overnight to obtain equilibrium among all the uranium chemical species so that

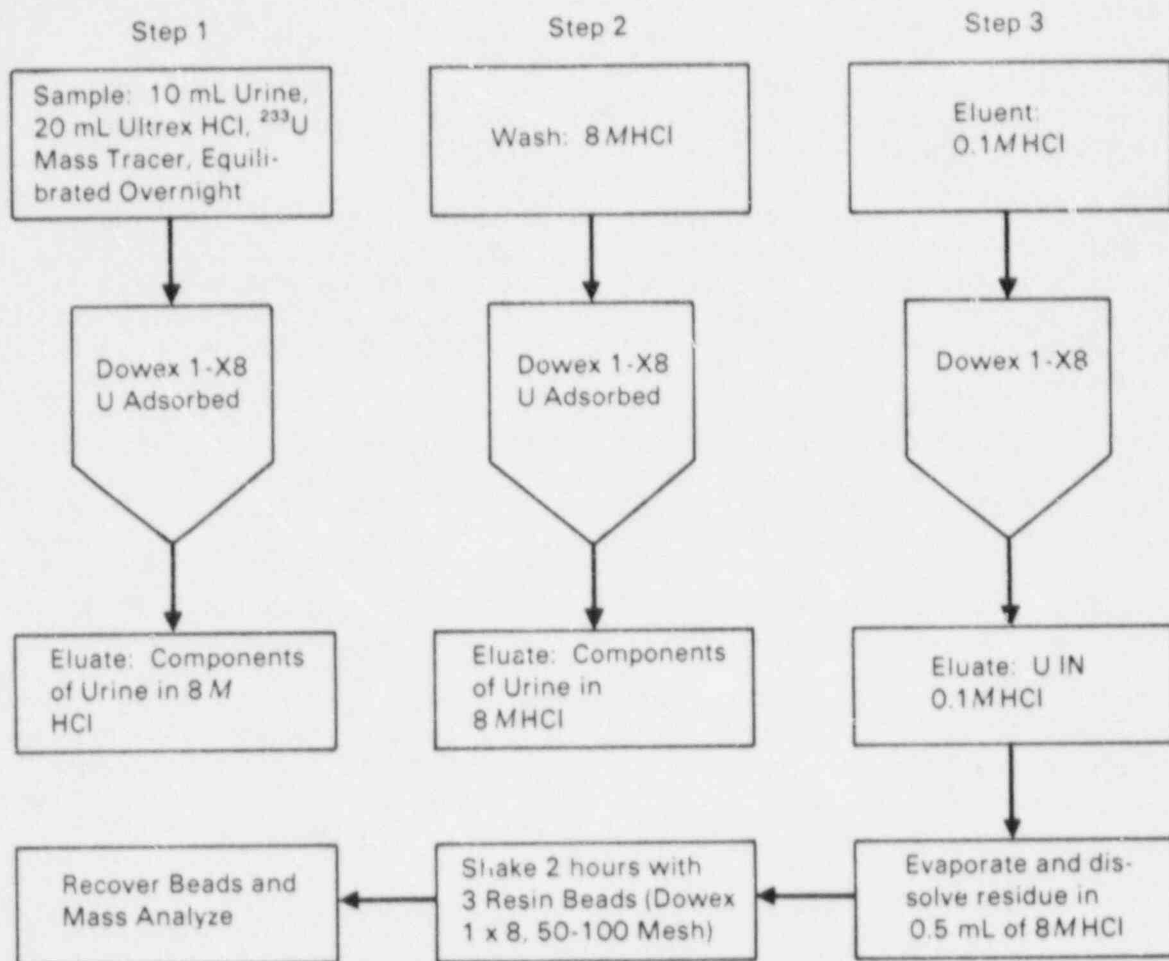

FIGURE 1. Separation Stages for Urine in Isotope Dilution Mass Spectromtry

the isotope dilution technique solution will work. Then, we run that solution through a Dowex-1 column to adsorb the uranium. We wash many of the non-uranium components out of the column in Step 2, and in Step 3 we elute the uranium from the column with 0.1-M HCl.

Next, we evaporate the small amount of 0.1-M HCl that we collect and take the residue up in about 0.3 to 0.5 mL of 8-molar HCl. We then add two or three small Dowex-1 resin beads and shake for about an hour with a shaker. We then recover and dry the resin beads. Finally, we place them in the mass spectrometer and analyze for uranium.

We can ask the question: What is required in this kind of analysis?

You need a pretty good chemical yield in the separation process. Our chemical yield here is of the order of 1%. You need complete isotope exchange with the ^{233}U and the natural uranium isotopes. You need a low blank or low background. (I will have some more to say about that later.) And you need a high-sensitivity mass spectrometer that has efficient ion formation and efficient ion transmission. If the amount of uranium on the resin beads that you mass analyze becomes much less than a nanogram, it becomes necessary to count the ions that you mass analyze instead of measuring ion currents. Those requirements.

We measured the chemical yield for the separation of uranium from the column and also the bead-loading experiment, where the uranium was loaded onto additional beads. All of these measurements were done by alpha-counting the ^{233}U that was used. In the case of the column separation, we recovered about 70% of the uranium that was in the solution to start with. Then, on the beads we recovered something like 1% and 2% of the uranium that went through the column. Thus, on the order of 1 to 1.5% is what we are talking about in terms of chemical yield.

In addition to these measurements, we also calculated the chemical yield according to the principle of chemical equilibrium. According to this principle, when equilibrium is established between the uranium chemical species in solution and the uranium adsorbed on the resin beads, the ratio of the uranium concentration in solution to the concentration in the bead is a constant. Based on published values of this constant and an estimate of the range of the sizes of beads with which we worked, we calculated a chemical yield that ranged between 2 and 14%. The calculated yield thus roughly agreed with measured values.

On the question of isotope exchange, Dr. Singh from the University of Utah will later address the question of having standards for this type of work. That has considerable bearing on the question of isotope exchange because how do you determine in an actual sample containing indigenous uranium if isotope exchange of ^{233}U and ^{238}U has occurred? That is a good question that might be answered if we had some standard urine samples containing known amounts of indigenous uranium.

We approached the problem of isotope exchange by analyzing samples, the same samples, by two or three different methods. Similar results from different methods indicate that exchange occurs. The first method is what I described before as the normal method that was shown previously in the second slide. You can see the results we found for this particular sample by the normal method.

By the way, I meant to mention that it was difficult for us to get urine samples in this study that had elevated levels of indigenous uranium, and many of the samples that we analyzed were samples to which we added uranium on a second procedure.

In a second procedure, we took aliquots of this sample and chemically destroyed the organic materials, after which we analyzed the resulting solution. Mean values of 2.82 μg/L for the normal procedure and 2.88 μg/L after the organic materials were destroyed were observed.

During the time that we investigated what we call the normal method, we also studied what we call a direct-loading method. Instead of doing preliminary separation, we just placed ^{233}U in the urine sample, made it 8 M in HCl, and put in two or three resin beads in a small volume of sample. After equilibration, the beads were taken out, dried, and analyzed.

A sample previously represented was analyzed by this method and the concentration observed, 2.7 μg/L, was in good agreement with that found by the normal method and by the procedure in which the organic components were destroyed. We, therefore, conclude that because the three procedures yielded essentially the same results, complete isotope exchange is likely to have occurred in each procedure.

I wish to point out that the direct bead-loading method has the advantage of being almost immune to the contamination problem. Except for the hydrochloric acid necessary to make the urine 8 M in HCl, no additional chemical or physical treatment is necessary. We believe that the technique of loading beads directly might be made the basis of sample collection in the field with almost no contamination problem.

I want to show the types of mass spectrometers that are necessary in doing high-sensitivity isotope-dilution mass spectrometry. The mass spectrometer one needs is not a simple $25,000 box that we saw in the previous presentation about laser phosphorimetry. It's an instrument that is about $400,000 or perhaps half-a-million dollars.

What we would like to have in a procedural blank is all the reagents that go to make up a sample; that is, the hydrochloric acid and the walls of the container that we have the sample in, everything that goes into a sample analysis except the sample.

The blanks that we analyzed consisted of 8-M HCl prepared by adding 20 mL of concentrated HCl to 10 mL of distilled water. Although the Ultrex HCl was used in the analysis of the urine samples, the distilled water was not. Thus, the distilled water was an extraneous part of the blank. If the distilled water contained small traces of uranium, a condition that we had no way of demonstrating, then we may have added a small amount of uranium to the blank that would exceed that of a true blank that accurately represented the combination of urine and HCl. We analyzed 10 blank samples for which we derived a mean value of 0.03 μg/L and a standard deviation of 0.0047 μg/L. We derived a detection limit of 0.02 μg/L from the standard deviation by multiplying the standard deviation by 4.65--a statistical factor used by Kurie....

There are several aspects about our results that are interesting and important, namely, the accuracy, precision, and bias that were found at each of

the levels of added uranium (as well as the blank urine). By the term bias we mean the difference between the observed value and the prepared amount. Concentrations measured at each level showed good accuracy and precision. For reasons unknown, the bias increased slightly with increasing uranium concentration. The uranium concentration in the blank urine was observed to be 0.032 μg/L. Since this value is larger than the procedural blank (0.02 μg/L), we believe that uranium was detected in the blank urine....

We'll start talking now about the cost of this analysis as it was presented in the report that we generated in '84. We looked at the cost of this method as well as the cost of some of the other types of methods for doing bioassay of uranium in urine. In Table 1, we broke the cost down into its various categories and evaluated the costs at several different rates of analyses (samples/ day). The first three cost items in the slide are labor costs: the technician costs for doing the chemical treatment of the samples, costs for the labor costs for operating the mass spectrometer, and costs for some supervision by technical personnel in overseeing this operation. The fourth cost item is for supplies and maintenance, the fifth is for the spectrometer and the laboratory in which it is located, and the sixth item accounts for down time and quality control. In figuring the costs of the spectrometer, an amortization period of 15 years is used. Thus, we see that at a sample load of 13/day, the cost for analysis is about $100/sample.

TABLE 1. Estimates of Analysis Costs

Cost Item	Annual Costs, k$ 12 samples/day	Annual Costs, k$ 39 samples/day
Chemistry	60	120
Spec. Op.	90	90
Tech. Pers	50	50
Supplies Maintenance	56	144
Spec. Lab	33	33
Down Time, QC	30	60
Total	319	497
Samples/Year	3,100	10,000
Cost/Sample	102	49

Because certain commercial mass spectrometers are perhaps able to analyze 39 samples per day in a nearly automated manner, the second and third columns of costs were figured for this rate of sample analysis. In the second column all costs are approximately double those of the first column, except the labor costs for the mass-spectrometer operation were not doubled. Although perhaps unrealistic, costs for the spectrometer were doubled in column 2. In the third column, except for supplies and maintenance, the costs were left as in column 1. Costs per sample drop to \$65 in the second column and to \$40 in the third column. The third column might be considered the attainable cost levels if nearly all aspects of the analysis procedure were automated. Although some of the values given here are slightly different from those given in NUREG/CR-3590, details for choices in the selection of costs are explained in some detail in that reoort.

Q: Frank, is the sample for all elements or per element? For all isotopes?

F. DYER: Yes. ^{238}U and ^{235}U.

Q: I have a lot of problems with that. You know, there is a sample, for example, a high-grade sample, that has a memory effect. It takes a long time before you go back to the base level.

F. DYER: We have the 39 per day.

Q: It is going to take several hours before you can back. Secondly, most of it is going to accumulate, and since it goes down for a long time, it is days before you can go back to the ordinary basis.

F. DYER: I don't believe anyone has any practical experience in analyzing this today. I have 13 there in the new computer-controlled mass spectrometer. The reason for the 39 today is, they tell me, that they can measure two batches in an eight-hour day and put in another batch at the end of the day. So, one can come out with 39 per day. Now, I don't know if one can run at this level and not have the problems that you are talking about. But that is the basis of the 39 per day. It is consistent with what we thought that people could handle from the standpoint of chemical separations.

Now, as I said before, if you automate quite a bit, you might be able to get the costs down to something like \$40 per sample. But no one has addressed the question of whether or not you can really analyze without some of those kinds of problems at this level.

Q: When you compare different techniques and the cost per sample, should there be several hundred samples per year on the cost of samples?

F. DYER: Yes. As to commercial availability, this technique is available in national laboratories and the Geologic Survey laboratories. I didn't mean to leave out the small laboratories that might have the potential for setting up to do this analysis, but I don't think that anyone is set up to handle a bioassay program at the present time....

Thank you.

R. ALEXANDER: Thank you, Dr. Dyer. We will now have a paper on resonance ionization spectroscopy. Dr. Jim Parks from Atom Sciences, Inc., will report on investigations on uranium urinalysis using the resonance ionization, or so-called single-atom technique.

Dr. Jim Parks.

J. PARKS: I think we will have to view this talk as a progress report on the work that we have been conducting and are continuing to pursue at Atom Sciences. I would like to tell you just briefly about Atom Sciences, the technology that we are developing, which is called resonance-ionization spectroscopy (RIS); an application of that technology, which we call sputter-initiated resonance ionization spectroscopy (SIRIS); and, finally, an application of that for bioassay measurements of uranium in urine samples.

I would like to describe to you the progress that we have made, and review our feasibility study and the ideas that gave us the encouragement to start this project. I would also like to give you our results thus far and where we think we can go.

Basically, at this point we think that we can measure--or we have demonstrated that we can measure--1 μg/L of uranium in urine.

The impetus for using the SIRIS technique for doing this kind of work is that it would work for all elements, and that it gives isotopic information. We anticipate that it would be an inexpensive method and simple to use....

Atom Sciences is a small company. It currently has 14 or 15 employees, depending on one technician's status right now. We started out with five employees, and basically we formed the company to develop the technology of resonance ionization spectroscopy and commercialize its applications to make it available to the private sector. H. W. Schmitt and G. S. Hurst are cofounders of the company. D. W. Beekman, W. M. Fairbank, E. H. Taylor, M. T. Sparr, C. R. Paice, D. E. Hurst, and R. Sangsingkeow are collaborators, and just recently Larry Moore in the audience has joined us.

I would like to review the RIS technology just briefly for you. RIS involves two steps: excitation of a selected atom, then photoionization of the excited atom. First, an element is selected, and the atoms of that element in their lowest energy state are chosen to be ionized by the RIS process. Once you have selected the atom, that atom is excited to a higher-lying energy state in an allowed resonant transition by the absorption of a photon of light of the correct wavelength or energy. Sometimes, the atom is excited to a second higher level in another allowed transition. Then, the final step ionizes the atom by the absorption of a photon energetic enough to remove the electron from the atom.

Key features, then, of RIS are its high selectivity based on the resonance, its allowed transition process, and its high efficiency in the ionization step. Basically, if the atom is in the laser beam, it has 100% ionization probability.

By "high selectivity," as I said, only atoms of a selected element are excited by the absorption of light of the proper wavelengths in one or more allowed transitions, and, secondly, excitations are resonant and can be easily accomplished with reasonable, commercially available laser wavelengths, power densities, and energies. I might emphasize the words "reasonable, commercially available lasers."

To illustrate the high selectivity, I show a Grotrian diagram for gallium (Figure 2). We have done a lot of studies on gallium, and this method that we developed is particularly suited to the semiconductor analysis work. In this process we generate light at 2875 Å to excite the atom from its lowest ground state to an intermediate state, and then the atom absorbs another photon of light at a longer wavelength, 5750 Å for the photoionization.

The selectivity then can be illustrated by a wavelength scan of the laser. It is a tunable laser that we use. You get only a single peak then, and nothing on the edges of the spectrum. The spectrum is very clean and other ionization doesn't occur.

The process is highly efficient. Using RIS, the ionization process can be saturated. In other words, all atoms of a selected element located within the laser beams can be ionized, and the excited states can be chosen, such that the photoionization of those states can be achieved with reasonable, commercially available laser systems....

There are a number of applications for RIS. All of these we are working on. There are dating techniques. There are ultra-sensitive detection techniques, including bioassays, basic research, and semiconductor materials analysis. We have spent a lot of time on semiconductor materials analysis, but we will only talk today about the bioassay studies.

One of the applications then for RIS that we are developing is called sputter-initiated resonance ionization spectroscopy. You begin with a solid sample. For the RIS technique to work you have to somehow atomize the sample; in other words, get the solid sample into atomic form in a vacuum system. We do that in our case by using sputtering. We use an energetic ion beam coming in and hitting the sample. The ion beam then sputters the sample. It is much like throwing a baseball into a sand pile. You get a little dust cloud kicked up.

Then, depending on what element we want to target for analysis, we use the tunable dye laser system to excite and then ionize just the element of interest; for example, gallium in silicon or boron or uranium, and whatever. See Figure 3. Once we ionize the atom of interest, we extract that ion and detect it with a particle detector. We may also have to reject secondary ions that

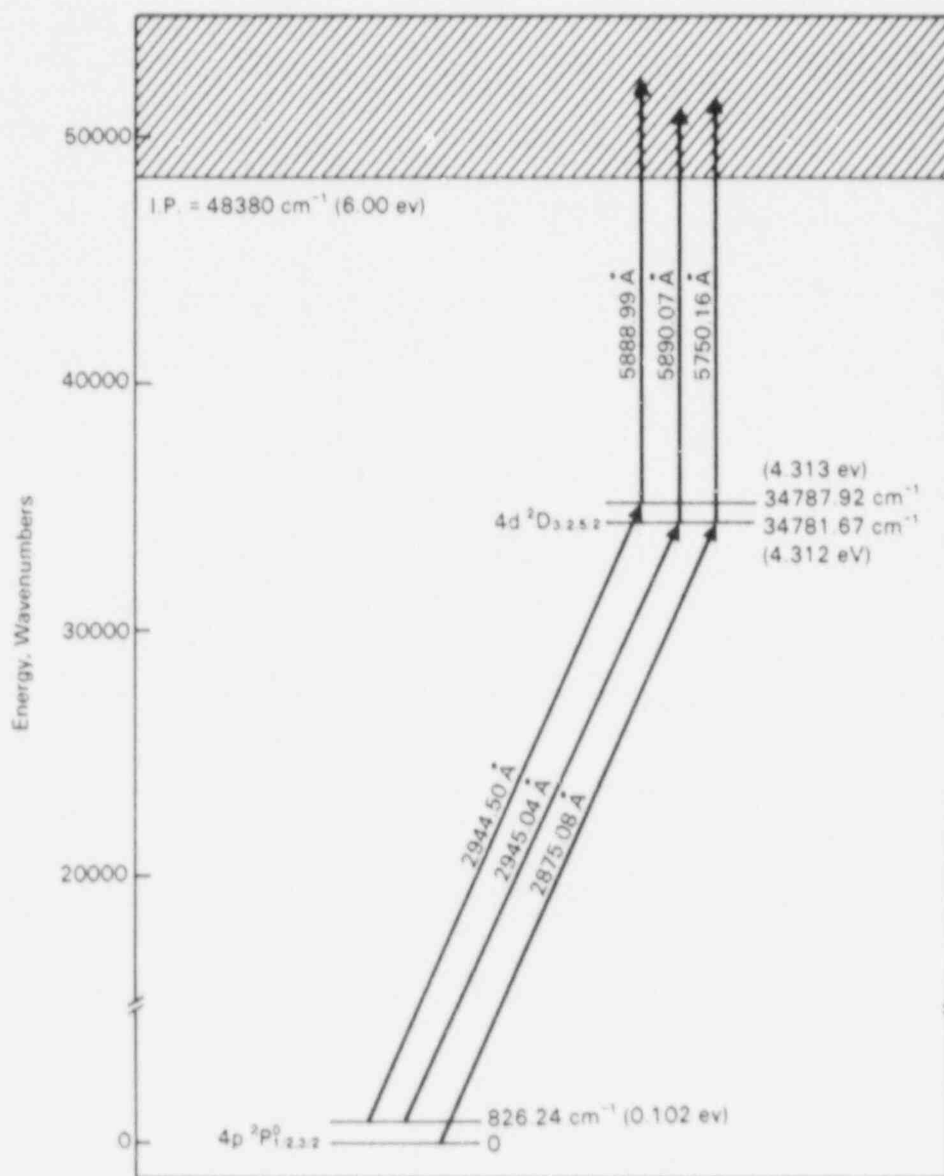

FIGURE 2. Grotrian Diagram of Gallium

are produced in the sputtering process. That can be done in a number of ways. We can also add a mass spectrometer to get isotopic information. In the RIS process that we use, we do not get isotopic information. We rely on the mass spectrometer to do that.

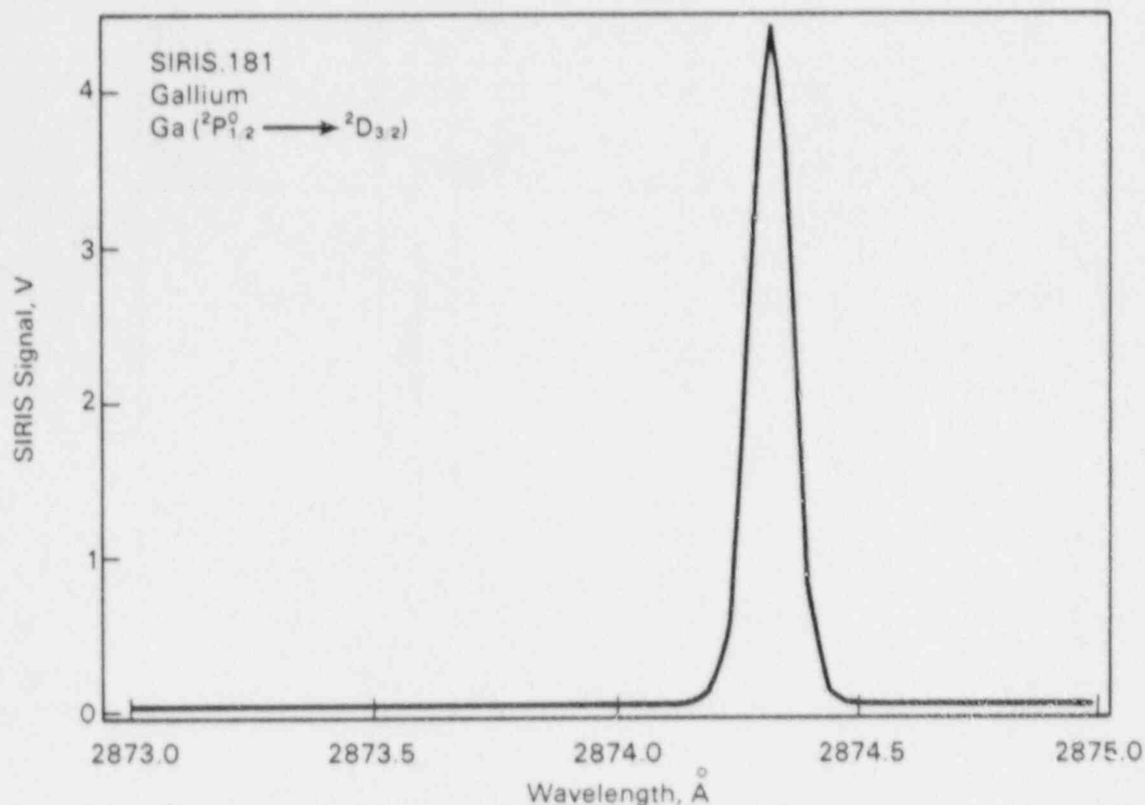

FIGURE 3. SIRIS Analysis Correlated with Laser Wavelength to Show Selectivity

Figure 4 shows a schematic diagram of the SIRIS apparatus that we have designed and constructed and have been testing for several years now at Atom Sciences. It consists of a duoplasmatron ion source in which you generate a beam of argon ions. They are put through an analyzing magnet to be sure that you have only argon ions in the beam and nothing else.

Because the laser system is a pulsed laser system, we require the ion beam to be pulsed. So, we focus the beam at a pair of chopping slits. Then, we sweep the beam across the slits in order to form a chopped beam. In our system we use magnetic focusing to get away from space charge effects. In the beam system we have a 4° bending magnet to eliminate scattered neutrals and other scattered ions from hitting the target.

The beam hits the sample, sputters the material, and creates a cloud of atoms that is representative of the material. Then, we use a neodymium YAG (Nd:YAG) pulsed tunable dye laser system to do the excitation and ionization.

We extract the selected ionized atoms from the cloud of atoms with a set of extraction electrodes. Then we put that through a double-focusing magnetic mass spectrometer consisting of an electrostatic sector for energy analysis and a magnetic sector for mass analysis....

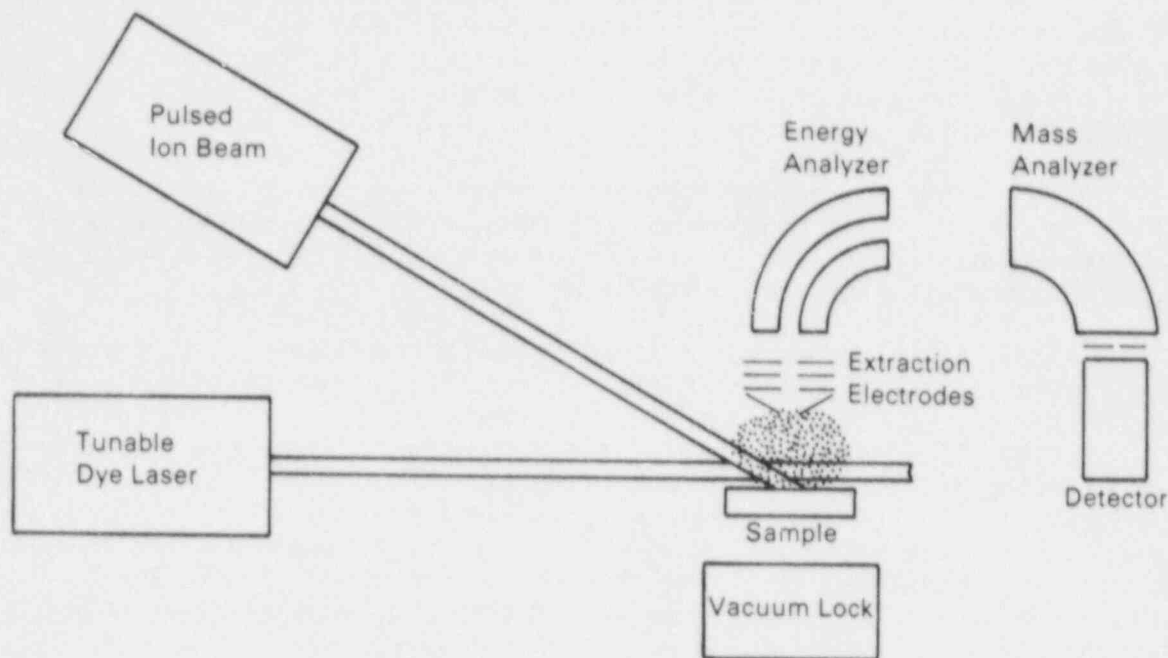

FIGURE 4. A Diagram of The Suputter-Initiated Resonance Ionization Spectroscopy (SIRIS) System

Before going on here, I would like to list some of the design aims of the apparatus and tell you some of the pertinent facts about the technique. You have to understand why we felt and still think that it would be a good instrument and a good technique to use for bioassays.

Originally, when we designed the instrument, the design aim was to have an instrument that could detect an impurity in a matrix of 1 part in 10^{10}. That's a tenth part per billion. That's the units I will try to work with here today. By that, it would have a sensitivity like that for a 5-min counting period, where we would count 100 atoms with a counting statistic of ± 10%.

The other thing about the technique I think we need to keep in mind is that it detects "parts per." What I mean by that is, that in the sputtering process, you kick atoms out of the matrix and the impurities in the matrix. The smallest number of atoms that you can detect in the cloud of atoms that you sputter out is one. So, your sensitivity is somewhat limited by how much stuff you can sputter.

So, for example, if you use a 1-mA beam and a pulse width of about 1 µs, then you can kick out about 1×10^{10}. You have inefficiencies in the mass spectrometer, but you make up for that by the length of time (5 min), and it turns out that the sensitivity that you get is 1 part in 10^{10}.

Now for the design aim we have not made. The design aim was to have 1 mA. At this point, we only have 50 µA. At the beginning of the bioassay project, we started with one hand tied behind our back. But what we have shown, though, with the instrument using 50 µA is that we should have a

sensitivity of 2 parts per billion. If you have residues on the order of 45 mg/L in a sample with a sensitivity of a few parts per billion, then you could measure 0.05 µg/L of uranium in urine if everything worked as well as some of the things that we've known about.

So, in the beginning in our feasibility study, we thought that we could do all the elements, not just uranium, but thorium, plutonium, and whatever else, and that we could get isotopic information. We thought that the process would be simple and that it would be inexpensive.

In our feasibility study, at that point, we did not have an instrument and had not tested it. We thought that the very simplest thing to do would be to take a small drop of urine and put it on a blank. We could use something that was high purity, like high-purity silicon, which you can get rather cheaply and just evaporating it. Based on our calculations, then, in Table 2 the ratio of the amount of uranium to other solid material in the urine after it's evaporated, would be in the ratio of 5×10^{-11} for a 0.05 µg/L sample. And remember, all we need--we thought at that time that our technique would give us a sensitivity of 1 part 10^{10}, ten times better than what we needed, in order to do that analysis.

So, that was really the driving force to try this technique. It would be the ultimate, I think, in simplicity for analyzing urine. Put a drop on the blank, stick it in the machine, and count it.

We thought then, if things didn't go real well, that we had a couple of aces up our sleeve. One thing we could do is do an oxidation and evaporation method to get rid of the organic materials. That would reduce the ratio to 4×10^{10}.

TABLE 2. Dilution of Uranium by Residue in Various Separation Methods

Separation Method	Residue	Number of Residual Atoms	R = U/Residue[(a)]
Evaporation	Total solids	2.6×10^{24}	5×10^{-11}
Oxidation and Evaporation	Inorganic	3.3×10^{23}	4×10^{-10}
Carrier Precipitation	$Ca_3(PO_4)_2$	7.6×10^{19}	2×10^{-8}
Anion Exchange	1 resin bead	5.0×10^{18}	3×10^{-7}
Same plus Elute	Impurities in HCl	1.4×10^{17}	9×10^{-6}

(a) Based on 10 mL urine at 0.05 µg uranium/L.

Then, using a carrier precipitation like calcium phospate, we could get the ratio down to 2×10^{-8}. We could really do that one very easily. Then we could do an anion exchange method, using a procedure like Frank just described, using an ion resin bead, in which the ratio would be 3×10^{-7}. And we should be able to do that with a thousand times sensitivity to spare. And if we wanted to go ahead and elute the sample, we could get that ratio down to 9×10^{-6}. That would be a million times, almost a million times, more sensitivity than we'd need.

Now, let me go into some of the problems that we've incurred and show you why we couldn't do the very first one.

I'll show you this just to give some credibility to the measurements. Figure 5 is a Grotrian diagram for boron. We used two excitation steps and an ionization step.

Using this process in the next two figures, I first show (Figure 6) some data of boron in some stainless steel or some steel that we obtained from the National Bureau of Standards for calibration purposes, for standardization. This shows the ^{10}B and ^{11}B in correct isotope ratio.

In Figure 7, I show a correlation graph from our measurements versus the NBS value for a group of those steel samples. They range anywhere from 110 parts per million down to 1.3. That happens to be the lowest of these samples for the boron, and what we show here is a very good correlation between our results and the NBS value....

Al Robinson said in his talk, when you do new things, you uncover new sets of problems. When we started measuring uranium, we learned some new things the hard way. One of the things we learned is that uranium did not behave the same way as gallium or boron or indium or a number of other things that we studied. The first thing we learned is that uranium has a very low ionization potential, and there are many, many excited states that could be formed. And, remember, in the case of uranium, we can only access an atom in a particular state, hopefully, the ground state. So, evidently, when we sputtered the uranium, the uranium could come off in any of these states and we could only access one of those. This shows you not the nice pretty spectrum of a single peak it shown with gallium, but the more complicated spectrum when we aim the laser at uranium. It turns out that we're using about the middle peak there for the ionization step (Figure 8).

So, we found that our sensitivity to uranium--that is, pure uranium metal, I remind you--was a factor of 40 less than it was for gallium or for boron or for indium. We found that uranium didn't give us quite the sensitivity as the other elements.

Not only that, just about any way in which you try to work with uranium, you wind up with uranium oxide. You might start out with uranyl nitrate. It will usually wind up uranium oxide, and when we cleaned our uranium samples, we

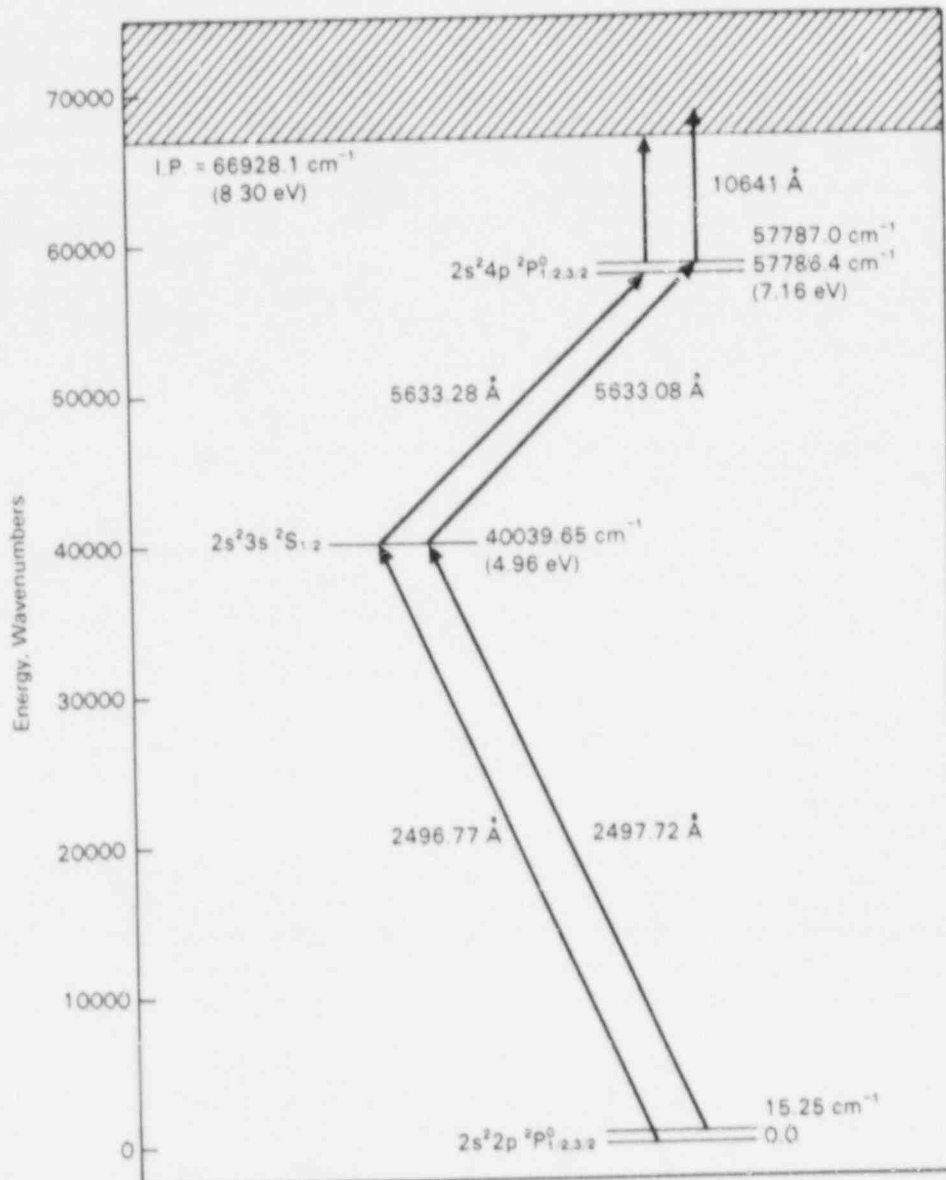

FIGURE 5. RIS Scheme for Boron

would see the signal increase. And correlating that with the uranium, which forms an oxide layer on the surface, it turns out, then, that our sensitivity for detecting uranium in uranium oxide is another factor of 40 down.

You know, we had a factor of about a million to spare, by doing a little chemistry, over using the straight evaporation technique. What we have demonstrated, though, at this point is that we can detect uranium in uranium oxide, which is what most of the bioassay samples would consist of, with a detection limit of about 3 ppm. Also, we have demonstrated that we can detect the uranium in a urine sample at the level of 1 μg/L.

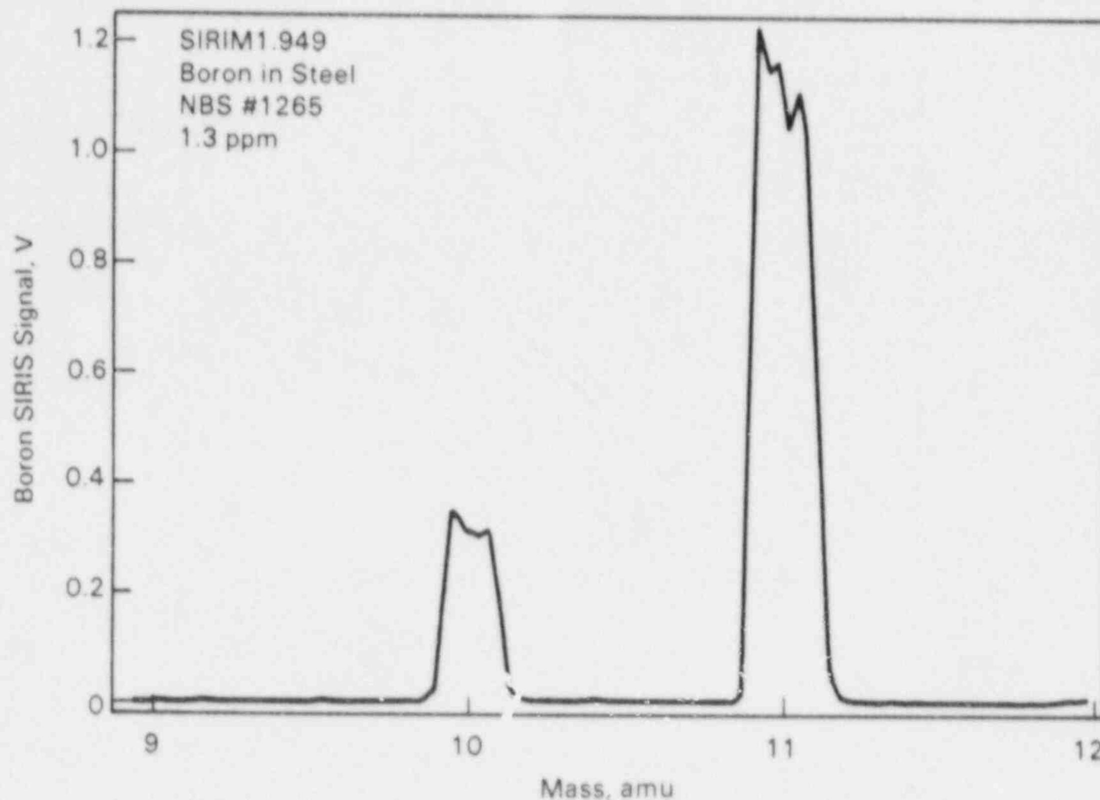

FIGURE 6. SIRIS Analysis of Boron in Steel

Table 3, I think, really sums up where we thought we were going, when we are, and where we think we're going now.

The first line at the top shows our design and estimation. In the first column, we anticipated the sensitivity of the machine to be one tenth part per billion for reference purposes here. We anticipated the elemental sensitivity to be the same for all elements. We said it would be 1 and the sensitivity for the solid was 1×10^{-4} ppm.

Remember that I said in our technique, we detected "parts per." So, if we have to do chemistry, even a minimal amount of chemistry using the same type of process that I'd like to describe to you, one of the things that we have to do is to eliminate the impurities, not so much the uranium impurity, as Frank pointed out, that was so important in his work, but in our case, it's just as important to eliminate the other impurities in reagents.

So, in our early estimates we estimated that the impurity level of the reagents that we would be using would be about 2 mg/L. And for that, then, we would have a sensitivity for uranium in urine of about 7×10^{-7} µg/L. But that's before we had the instrument and had really done experiments. We've done experiments now. We're a little bit wiser.

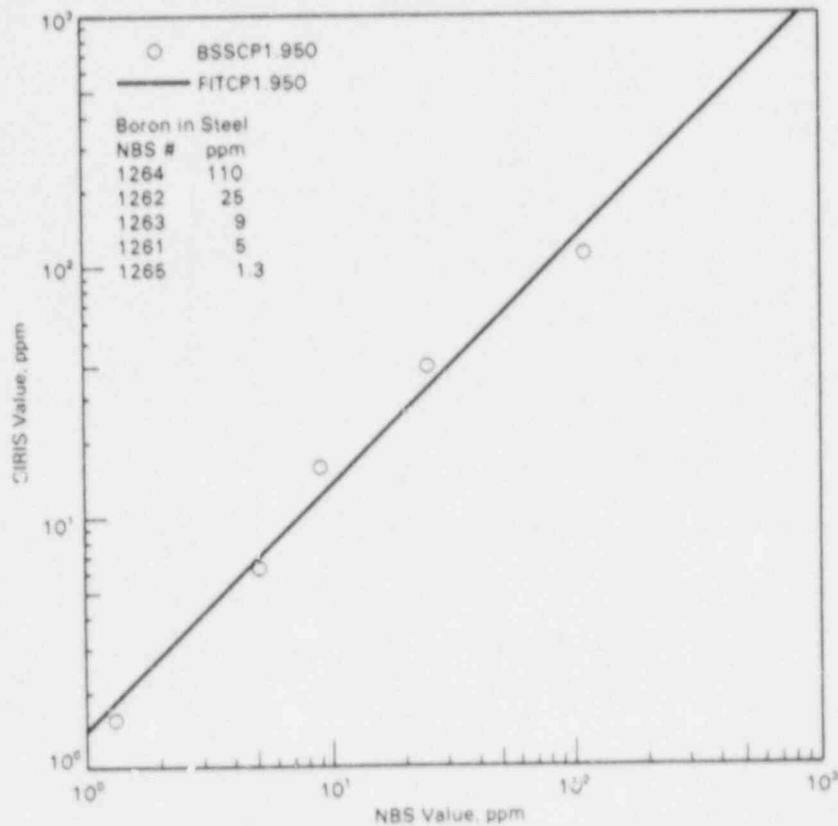

FIGURE 7. SIRIS Values Correlated with NBS Concentrations for Boron in Steel

We found out that our sensitivity to the machine is really not 0.1 ppb, but essentially a factor of 20 down because of the ion current. So, we have a machine sensitivity of 2 ppb. The element efficiency for uranium over the other things is a factor of 1/1500 down. That's essentially two factors of 40. The sensitivity for the solid (meaning for the uranium in uranium oxide, then) is only 3 ppm. For our impurity level in mg/L for the reagents that we have been using, we estimate that the impurity level is about 100 mg/L.

We do not have a clean lab in our laboratory. We have not used any specially prepared reagents. We just have electronic grade chemicals. So, that number is much higher, but despite all this, for our sensitivity right now, we have shown that to be 1 mg/L.

Now what can we do about that? Well, we might be able to improve the sensitivity of the machine, but we have not included that in the third line here. We might gain a factor of five there, but let's not consider that. Assuming that the element efficiency stays the same, the sensitivity for the solid is a way we might gain something, converting the uranium for uranium oxide, or the form from uranium oxide to some other form like uranium hexachloride, tetrachloride, or whatever.

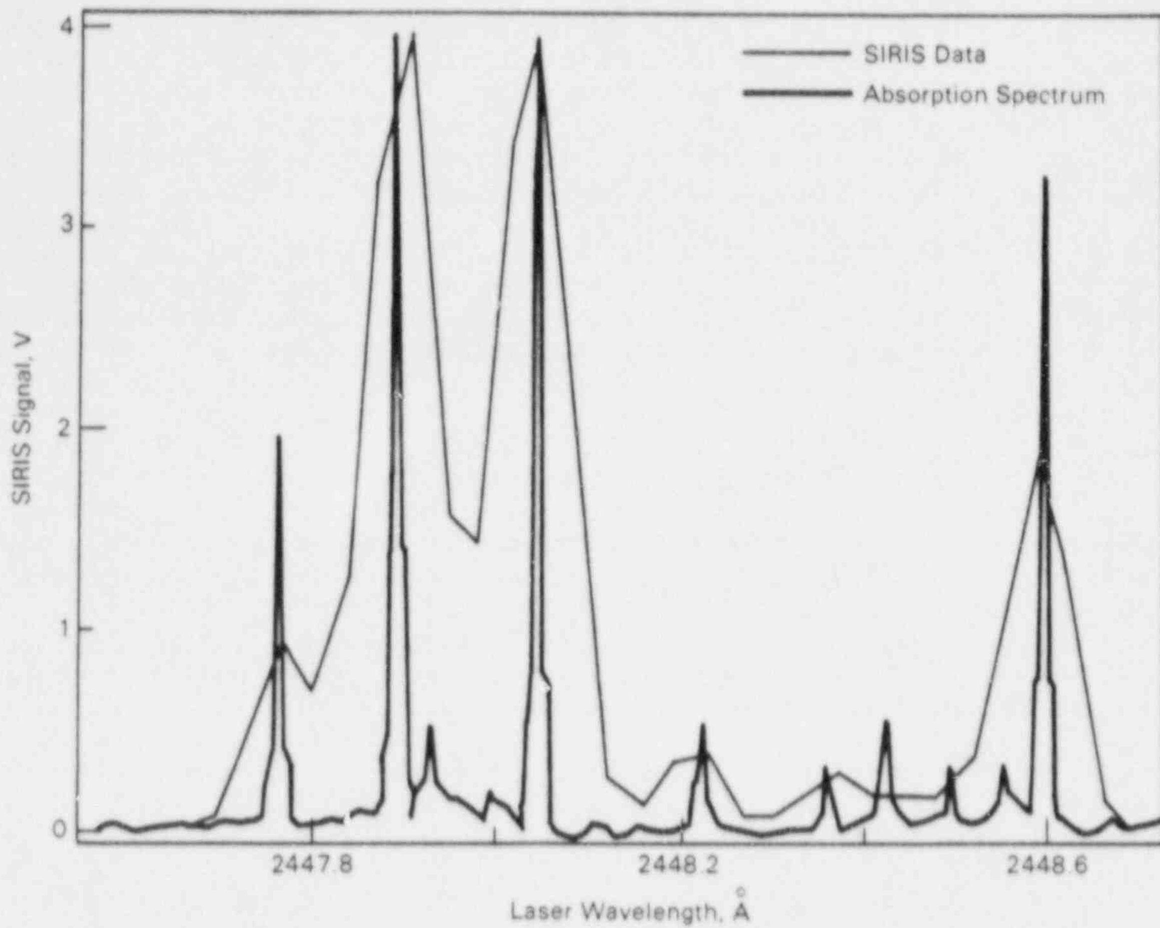

FIGURE 8. Complex Ionization of Uranium Using SIRIS Procedure

TABLE 3. Sensitivities for Uranium in Urine at Three Stages of Development

	Sensitivity of Machine, ppba	Element Efficiency	Sensitivity for Solid, ppma	Impurity Level, mg/L	Sensitivity for Solution, µg/L
Design and Estimation	0.1	1	1×10^{-4}	2	6×10^{-7}
Present Status	2	1/1500	3	100	1
With Improved Reagent Purity	2	--	--	2	0.02

But not even considering that, if we can reduce the reagents, as it would seem reasonable, to the anticipated level of 2 mg/L, then we should get our sensitivity down to 0.02 μg/L. And then I think that we would have achieved, or partially achieved, the goal of the project. We think that we can do analysis of uranium in urine and we could also get isotopic information and the same technique should be applicable to the other elements.

One of the other problems, though, that we ran into was that our measurements weren't very consistent. I haven't shown any samples of data where we've repeated measurements over and over and over again. But then, in order to get a handle on why we were getting so many inconsistencies, we turned to the technique of isotope dilution.

We then discovered something else that we really suspected. Although we thought if it ever happened we thought it would be the worst with uranium.

A wavelength scan for a sample that had a one-to-one ratio of ^{235}U and ^{238}U shows an isotope shift. It turns out, then, that generally speaking, in the RIS technique we use broad band lasers and the isotope effect never appears. It turns out, in the case of uranium, that uranium is just a bad actor. The isotope shift is quite large. So, for the broad band lasers that we're using, isotope shift becomes significant....

One of the big features of this technique was that it was supposed to be inexpensive. We still think that it's comparable to the figures that have been mentioned here today. The costs somewhat depend on the level that one is looking for because, essentially, what we do is count atoms. And so the more sensitivity that is required, the longer we have to count. So, that is taken into account.

We've also taken into account the isotope dilution that we use and we've taken into account that the anion exchange technique would be used. If the sensitivity at present (which is 1 μg/L) were used for a 5-μg/L sample, then 200 samples per day could be done for $15 per sample and, I should say, per element.

With the same sensitivity, if you got down to 0.05 μg/L, then the cost would be rather expensive for us to do that; it would get up to $428 a sample per element. However, if we continue to improve our reagent quality, that price could be brought down. When we've gotten the sensitivity of 0.05 μg/L that's desired, then we can do the sample for $32.

Another thing we are doing is that we're developing a simpler instrument, one that doesn't use a magnetic sector, but uses a time of flight. But, still, that instrument plus lasers would cost on the order of $300K. So, in capital equipment, this is an expensive proposition, but we think that it gives valuable information and will yield useful results for the bioassay project.

Thank you.

R. ALEXANDER: Questions for Dr. Parks?

(No response.)

R. ALEXANDER: Thank you very much. The next speaker is N. P. Singh, from the University of Utah, who will speak to us on the need for uranium standards.

N. SINGH: Thank you, Bob. Something that is missing is how to check if these machines are really good. For many techniques, you must know what is the precision of the technique? This is one of the most important aspects of any technique. To find the precision of the technique is very easy. You can take one sample and do an analysis and see how precise that is. But how do you know the methods if you have one sample and then you use different techniques?

Many labs don't have the ability to do analysis. It becomes very easy to check your technique if there is some kind of a standard; if you use that standard, you are very proud that it works.

So, at that point, actually, I get wonderful results. But I know that there is no way to prove that my method is very accurate. I do get 90% recovery. The question is, when uranium goes through the metabolic process, is that uranium in the same form as in the urine?

The question is actually: Is it really the best standard? I think it's a simple process. So, we very much need a standard. If all the agencies are supporting the federal government, some must support a uranium standard.

I'll give a good example. Two years back...

A. ROBINSON: Do you have any idea of what form it is though? That's the question, I guess. What form is the uranium in the urine? If so, would it be stable, or where would you get it, et cetera?

N. SINGH: Very good question. That, we don't know. There are all kinds of guesses out there. We don't know if we can get something through the development process; that is my point. We've got to have some uranium that has been through the metabolic process. To make sure that nobody questions us that the uranium and urine having gone through the metabolic process is different than others.

I'll give you an example. We've seen the situation in the case where there was really a mess and a lot of people were coming up with strange numbers. Finally, NBS people, Dr. Ken Ames here, freeze-dried and prepared a standard. And after that standard was available, they analyzed it and found out if it was right or wrong. At least, they knew where they were, so they were sure that the technique was working or not working....

So, I request that the NRC put effort and time, and also funds to have some standard of uranium in biosamples. If you really want to do analysis, rather than going to a trillionth part, what we really need is a good standard for uranium in urine.

Thank you.

R. ALEXANDER: Does anyone want to speak to that?

MR. WALTON: My name is Walton. We are making two biological reference materials from spruce material in northern Saskatchewan. The uranium levels are about 2 ppL. Also, there are four radionuclides that have been analyzed in these materials. And we'll have them available commercially shortly, in '86.

A. ROBINSON: Alan Robinson, U.S. Testing. I guess the question that comes to mind is, first of all, a lot of times, you can take natural urines from occupationally exposed people, people that are drinking a lot of uranium in water, and you can compare them via radiochemical methods. Therefore, I guess I would have to conclude that if you do any significant chemical treatment of it, first of all, that the biological form is immaterial, whether it's chelated to the DTPA, the citrate, whether it's some form hooked on to a protein, you name it, it really doesn't matter if it's reduced down to a uranium^{+6} state.

So, in a sense, I don't see the urgency for that. Plus, if you take a natural material and you run it through a freeze-drying process, a grinding process, a reconstitution process, the chances of your ending up with uranium in the same form as it was when it started seem to me to be zero.

N. SINGH: Of course, you said the question is how much chemistry is enough chemistry?

A. ROBINSON: I would say, from our experience, whenever we have compared fluorometry radiochemistry, just a simple boiling down, you know, with nitric and peroxide destroys all the organics. And you end up with the same compound.

N. SINGH: Uranium may not be that bad. However, there are times when you can use uranium chemistry. The cost goes down.

A. ROBINSON: That's the danger of that procedure. In the case of plutonium there were some efforts here a few years ago to simplify a lot of the effort--do direct distractions or direct reciprocations. The reason they failed was simply that. They were different chemical forms. What that tells you, that you will never match all chemical forms. It just tells you it may not be possible to make that shortcut. That's what it tells me.

N. SINGH: I think there is a mistake there. For example, with uranium, there is only one ester coming out. So, that's completely wrong right there. Now, plutonium has four, five, or six esters. Uranium does not have the product; uranium has only one ester.

A. ROBINSON: But in biological materials, I don't think anybody's ever demonstrated that, that there's a +4 or possibly +6 valence state.

N. SINGH: You said uranium +4?

A. ROBINSON: I said plutonium.

N. SINGH: I'm talking about uranium. That's exactly why I'm saying that. So, I'm saying that, for uranium, that's--if they understand that you could prove that it's working.

A. ROBINSON: But if you don't know what form it is to start with, you can't find the standard.

N. SINGH: What do you want me to say, Al? I want to know what form.

R. ALEXANDER: I hate to interrupt this because, for the first time since dinner, everybody in the room is awake, but we have to move along to the next speaker. You can get that afterward.

The next speaker is Dr. Moorthy from Brookhaven National Laboratory, who will talk with us about a technique that has not been discussed at this meeting, of the Fission Track Analysis. Dr. Moorthy.

A. MOORTHY: At the Brookhaven National Laboratory (BNL), we have been doing bioassay analysis, and in the past couple of years, we have been working on plutonium. We first used this technique in synthetic urine and we realized we didn't have the sensitivity. Thus, we are looking for some other technique to detect the desired limit. The calculations showed that we could have that sensitivity with the fission-track analysis. There were a lot of challenges in terms of purification of plutonium. It's probably not necessary but we're also doing it on the gallium and the plutonium, also.

I'm going to describe what we did for the plutonium. And you will see in a second that it's not different for the uranium oxide because the two neutron cross-sections are comparable. We have a statistical sample of plutonium and uranium. We have a 16-megawatt reactor which gives a fluence, 11.5×10^{14} neutrons/cm^2; the detection limit is 0.1 fg per sample. Usually, we analyze a liter in one day's output.

We collected 24 samples from 1981 to 1983. We enhanced the contaminants, and during this investigation we were surprised to see ^{239}Pu and nitric acid were enough to push our detection limit up to 0.1. We had about 25 mg per sample. So, after ashing the samples we still had samples enough for a bioassay. But that is a large volume to use.

At this point the most challenging contaminant was the separation of the radium. We removed the radium as much as possible, and we had a decontamination factor of 10. That is pretty good.

I won't go through how we did this, though we usually avoid the HCl and only use abo[illegible] 6 mL of nitric acid. So, we really don't need a whole lot of nitric acid. But this final separation accomplishes the maximum decontamination of the radium.

In the second state of ion exchange with the separation of plutonium, we do a little oxidation. We get about 35 mL, and we go to the separation chamber. At the end of this, we get, as I said, 10 or so. It is more than adequate. The samples are bathed in nitric acid. There is a reason for that. Nitric acid takes several days--we can usually treat about 6400 slides at a time. So, we can have a startup.

Typically, we irradiate a blank slide for 10 minutes. The total fluences are 10^{14} neutrons/cm^2, and we have 6 ± 6 photo tracks in the best area for sampling. Usually, when this happens, we can evaluate samples in the standard. For two samples, the flux is very stable. So, we really don't need a standard.

We can put about 16 samples in one area. We have a sample of uranium-free plastic wrap; we put this on top of a plastic sheet. We take another plastic sheet that is rather clear and very clean. We heat-seal around the sample. We have a chamber in which we can seal about 20 or more samples at a time in a vacuum. The seals will last for 30 days. Because a couple of times we have had something in the samples that has reacted, we are careful that water should not get in there. That is important. Once we have irradiated the sample for 10 minutes, we react it and do overnight cooling. The tracks produced are actually fission tracks. This can be applied to uranium.

We did some experiments with commercial reactors. The fluxes are 13 times less that what we have. Using 30 minutes irradiation time, I captured 100 μL and just put it on the slide, and again counted. For comparison we also calculated it for uranium using a 3-mg ^{252}Cf neutron source.

Again, if somebody is interested about access to thermal neutrons, they don't need the sensitivity provided by the reactor neutrons. Just for comparison, though, I can give the sensitivity for thermal neutrons, also. I can detect 5 ng. In the reactor, you can put them about 70 or 80 at a time, and you come back, as you can imagine, and that gives a pretty good sensitivity of 50 ng. I have the chemistry worked out.

I want to point out some of the advantages of this technique. First, we have experimentally demonstrated it. Second, we have used urine samples in large numbers. Third, the commercial reactor would charge $200. So, on the radiation there is a very small cost and high sensitivity, especially if reactor neutrons are employed.

In spark counting, you have three sparks. The equipment costs about $100,000, and it is really not expensive. So, the cost will be essentially a variation cost, about $10 to $15 per sample for the radiation plus the handling cost--one technician handles 16 samples.

We have a system that covers the whole reflector--the majority of them, 100 to 200 tracks per sample, per slide. Most of them are empty.

R. ALEXANDER: Thank you. That is a very interesting presentation. One question I would like to ask is--obviously, you are using the BNL reactor for your plutonium analyses, but is the reactor available for commercial use with uranium?

A. MOORTHY: This reactor is not really for commercial use, but I really have not asked for that. And some people are using commercial reactors.

R. ALEXANDER: You have a flux of about an order of magnitude difference.

A. MOORTHY: Yes. I also have ratios of 1300. It doesn't really matter for uranium, but for plutonium it is very important because you get damage, and you can get high damage when you use all of this chemistry. This technique can be used for uranium.

R. ALEXANDER: Does anyone else have a question for Dr. Moorthy?

F. DYER: I have a comment about this particular method. I think it probably does solve some problems for those of you who want to develop other kinds of things; that is, this is a physical method and it only has a background due to impurities on the track recorder and things like that. But it doesn't make any difference. I didn't understand whether you made that point or not, but that would be a way for us to establish standards for mass spectrometry or some elements where chemical forms are important.

A. MOORTHY: It has a definite long shelf-life.

R. ALEXANDER: Thank you very much. We will next hear apparently about a technique in which the fluorescence is measured following the laser beam excitation. That will be given by Dr. Zinger from Scintrex.

DR. ZINGER: Thank you. I will be talking about laser-induced fluorescence techniques and answer questions. Some of the topics in my presentation were discussed by Dr. Gray in his presentation this morning. I will refer you quickly to it, and I will show you one new development of the instrument that is especially important to applications where automated measurements are required.

The advantages of laser-induced fluorescence, LIF, over the conventional fluorescence measurements are that it is easier to carry out measurements with LIF techniques, that LIF allows direct measurements in solutions, and that LIF offers sensitivities which are 10 to 100 times better than the conventional fluorescence techniques.

When you use a pulsed excitation source, the fluorescence of species in solution will drop off at various rates for different species. The fluorescence due to organics decays quickly, in a matter of 4 to 10 ns. However, the fluorescence due to uranium species decays up to about 200 ns. Therefore, if one can delay the measurement of the fluorescence due to uranium, one will not measure the emission caused by organic materials, and that is precisely what we are doing in the UA-3 (the instrument that we have manufactured for six years).

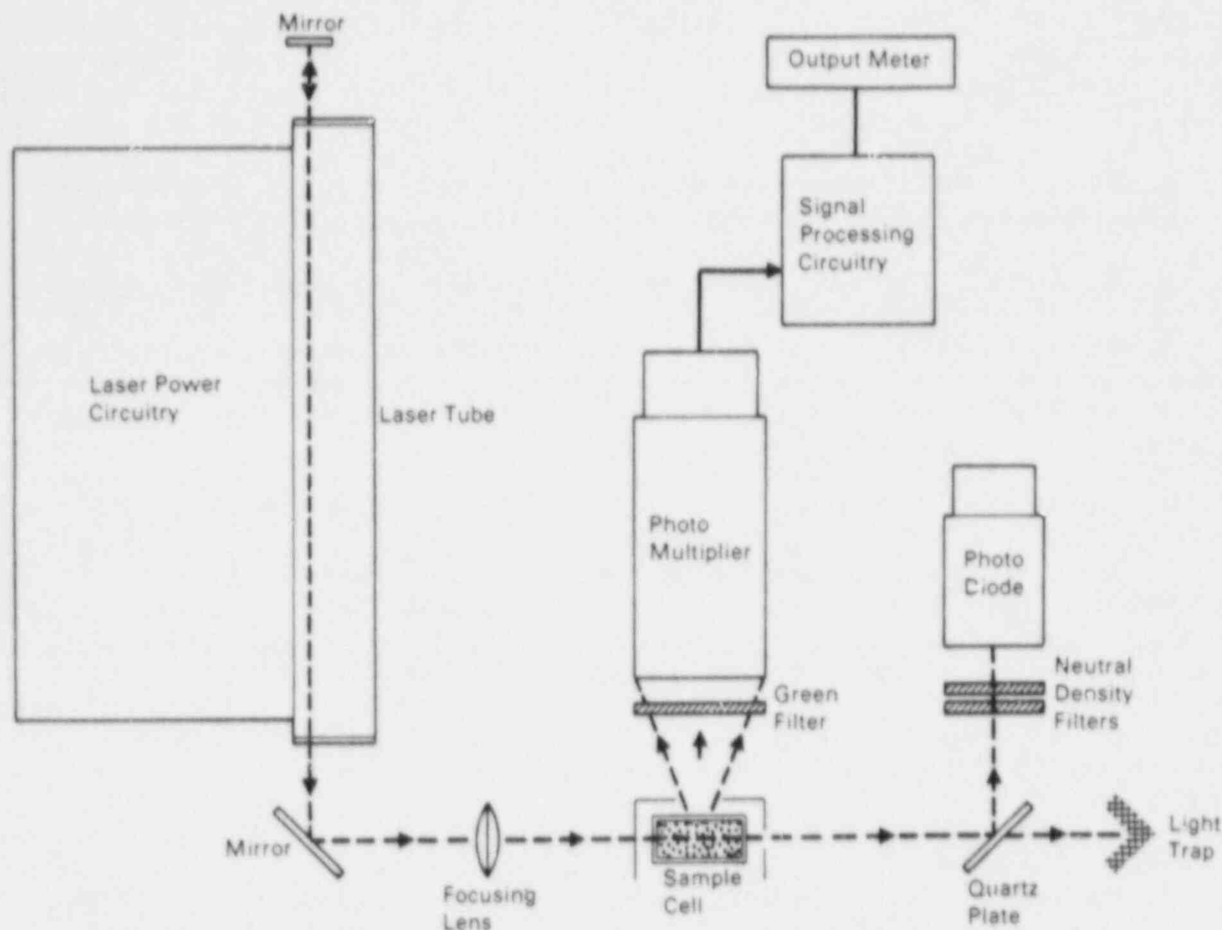

FIGURE 9. Diagram of Scintrex US-3 Uranium Analyzer

Initially, the delay time was about 30 μs. We have already reduced it to 10 μs, and by that time the emission from the organic species has decayed significantly.

We also developed a reagent which enhances the fluorescence of the uranyl species. There are various forms of this reagent available from Scintrex. The reagents consist of pyrophosphates and polyphosphates, which provide enhancements by factors of from 40-to-1 to 80-to-1, compared to the emission without this reagent. This reagent is called fluran, and it is based on buffered pyrophosphate solutions.

Here is the block diagram of the UA-3 uranium analyzer. On the left you see the sealed nitrogen laser, which has 125 mW peak power. The beam from that laser is focused on a sample cell which can hold up to 8 mL of solution. The emission from the UA-3 uranium is measured at a 90-degree angle. There is a green filter which will prevent any emission due to organic material from

reaching the photomultiplier. The measurement also is corrected for filtering effects in the cell, and you see a photo diode on the right side which will provide this compensation.

We've developed a number of digestion methods for urine samples. One avenue we've tried for removing the organic materials from urine was oxidation of organics with hydrogen peroxide. Traces of hydrogen peroxide ended in the final solution before we abandoned this approach. Hydrogen peroxide gets misused.

A second method is oxidation by ignition of sample with subsequent dissolution and analysis. We found high background, routings, and erratic results. So, we abandoned further investigation of that matter.

We also tried a solvent extraction using tertiary amine aliquot in xylene, followed by back extraction into dilute nitric acid. This technique proved to be very slow. You need three extractions and three back extractions. We had a lot of variations in blank levels and some emulsification problems.

For a fourth method we used a new method, in which oxidation of the sample used potassium persulfate. For the urine procedure recommended by Scintrex, the effective range is from 0.5 to 18 ng/mL. You basically take a 2-mL urine sample into a 150-mL beaker, add 1 to 2 g of potassium persulfate and 25 mL of dionized water, and boil it to dryness on a hot plate (that's a very critical step). You have to make sure that all the chlorides present are evolved as chlorine. Some of the chlorine will end up in the final solution and reduce the sensitivity. Add 45 mL of dionized water, stir, and dissolve the solids.

Adjust the pH with NaOH until just basic. Then make it slightly acidic with 10% nitric acid. The pH will end up slightly below 7. Transfer to a 50-mL volumetric flask and dilute to mark, and you're ready to analyze the solution of the UA-3.

By the way, this method was developed bv Chris Castledine from Scintrex, Ltd.

Basically, the whole analysis takes about one minute. We recommend that you eliminate the quenching effect or compensate for the quenching effect. This, although it's not time-consuming, involves one more step, and we were thinking of adopting this instrument in automated environmental and process control analysis.

Zero the instrument. Pour a 6-mL aliquot of the 50-mL aqueous solution into the cell. Zero the instrument with the sample cell in the sample compartment. Add 0.8 mL of fluran. Mix and record the meter reading. Add 10 μL of 250 ng/mL uranium (as a spike) to the cell and mix. For higher concentrations, 1000 ng/mL should be used. Record the meter reading. This reading indicates the uranium concentration of the sample plus "spike.". Then, calculate the concentration of uranium in the sample....

In the automated version of the UA-3 (designated the MU-4), a second gate delayed with respect to the first is used to correct the variation in sensi-

tivity due to changes in lifetime. On the assumption that the uranyl emission follows a single exponential decay curve, the lifetime can be calculated from the ratio of the signals from the two gates. Knowing the lifetime, the value of the first gate can be used to calculate the intensity of fluorescence at a time just after the excitation pulse and, therefore, when it is relatively unaffected by the changes in lifetime.

The gate widths and delays can be chosen to balance signal-noise considerations against an adequate range of quenching compensation. The number of gates could be increased, for instance, by using multichannel analyzers. However, the very small gains in signal-noise performance made by observing the signal further down the decay curve do not appear to justify the extra cost and complexity of the approach.

Thank you very much.

R. ALEXANDER: The next speaker is Chuck Mays from the University of Utah. He's speaking to us about fission track quantification of uranium.

C. MAYS: Thanks, Bob. This is not my paper. It's Fred Bruenger's paper. Fred is a chemist at the University of Utah, and he developed a technique of measuring plutonium in small biological samples. This involves separating uranium and everything else from it, so plutonium can be irradiated. He's worked the problem backwards, separating out the uranium and leaving everything else to the side. Then, he irradiates the samples and gets an enhancement factor of 1 million. And I'll explain that to you now.

If you have 1 ng of natural uranium, it produces about 1 alpha disintegration per day. It's kind of a limited detection by conventional means. If you take that same nanogram and expose it to a fluence of 10^{17} thermal neutrons per cm^2, you get about a million fissions. Proper chemical separation from bulk material is essential and the real trick is one in chemistry....

You start with the 1 to 200 mm of urine. You precipitate it to the rhodizonic acid. You oxidize the precipitate and dissolve it in concentrated hydrochloric acid. Then, on the ion-exchange column the uranium is adsorbed and comes over a negatively charged complex, and all the other garbage goes on to disposal. The uranium is then eluted and deposited on a polycarbonate detector, irradiated, and then the fission tracks are exposed are counted either visually or by automatic procedure. Their range is something like 15 to 20 μm.

A. ROBINSON: Do you just count those? One, two, three, four, five?

C. MAYS: Yes. They can be done this way if you don't do too many of them. We are developing automated procedures for counting these (a spark chamber).

A. ROBINSON: Is that used to count tissue culture cells? The problem is if they get too full like that, do you think they were resolved?

C. MAYS: It is a good way to go blind, isn't it? And there is a question of how many tracks you have and which ones you count. The problem is having too many tracks. A track density of 200-1000 tracks/mm^2 is presently considered ideal.

VOICE: You know, it depends on how many you have now. They have to be on something, and you give it about 80%. Some of them steam up.

C. MAYS: It is necessary to have standards that are run at the same time. So, you can see how this goes. With spikes, I think ^{233}U, he gets yields of about 80 to 85%.

If you have questions on this or if people are interested in this technique please give Fred a call. Or you can talk to Narayani Singh.

VOICE: We are supposed to interchange some samples with Fred.

C. MAYS: I think that would be an excellent idea. So, we will know that the picopicocurie is really a picopicocurie.

R. ALEXANDER: Thank you very much. I appreciate your sharing that information with us.

The final discussion before we end the day will be a summary of the sensitivity, cost, and availability of these techniques. That is being given to us by Dr. Bob Neel, who arranged this meeting.

R. NEEL: Thank you. I would like to thank the speakers and those who made public comments. After such an august presentation to such an assembled group of experts, I can't begin to summarize all the information we have. However, I do want to present to you very briefly what came from NUREG CR-3590, which is a report that was done for us by Oak Ridge National Laboratory.

I have updated this somewhat by communicating by telephone with each of the invited speakers. I did not contact those who made public comments. I have the notes that I have been able to augment with what I have on the slides. Please, if you see something in Table 4 that doesn't agree with what you presented earlier or you need extra information after I have shown it to you, you may want to comment about this.

All the slides are self-explanatory. Probably the thing that is of most interest, in my opinion, to our licensees would be the cost per sample. This is the cost to do a bioassay sample. The alpha counting numbers came from Mr. Melgard. The iodine numbers were taken from Dr. Dyer. The information on the radiochemistry came from Mr. Robinson. The cost range is necessary, of course, because we are talking about different numbers of samples and perhaps even different treatments, and for the details that are associated with this range in cost you will have to look at the transcript of the meeting or contact the speaker.

Fluorimetry, which is not an ultrasensitive method, is used for comparison purposes. For alpha counting, the numbers came from Dr. Melgard: 0.03 µg/L at the bare bones limit of detection, with a standard deviation at the limit of detection with 95% confidence level of 0.03.

The laser fluorimetry in the next column over, which is known also as laser phosphorimetry, is 1 µg/L of uranium with a standard deviation of 0.07 µg/L of uranium.

The IDMS method is 0.02 µg/L with a standard deviation of 0.02 µCi.

The laser kinetic phosphorimetry, 0.05 µg, with the highest precision of any of the methods reported.

The volume of sample is important if one has to mail the sample from the point of origin on-site a long distance to an individual who carries out that analysis for the supplier of the sample. The smaller the sample, in this case, the more rapid the handling. The largest sample is obviously required for the alpha counting, which uses up to a liter of sample. The laser fluorimetry is the next smallest and the laser phosphorimetry requires the least amount of sampling. The only sample size that I have reported here is one that has been reported to me as the best or optimum amount by the individual speaker.

TABLE 4. Factors for Comparison of Bioassay Measurement Methodologies

	Fluorometry	Alpha Spectroscopy[(a)]	Laser Fluorometry	IDMS	Laser Kinetic Phosphorimetry
In routine use	Yes	Yes	Yes	No	No
Experimentally demonstrated for bioassay measurements	Yes	Yes	Yes	Yes	Yes
Provides isotopic composition	No	Yes	No	Yes	No
Chemical processing required for urine analysis	No	Extensive	Moderate	Minimal	Minimal
Equipment Costs	Low	Low	Medium	High	$20-25K
Analysis Cost/Sample	Low	Medium $80-120	Low $25-30	Medium $60-102	Low $15-50
Reported limit of detection, µg/L of Uranium	1-10	0.03	0.6	0.02	0.05
Standard Deviation (95% C. L.)	--	0.03	0.07	0.02	0.003
Volume of sample needed to attain lower limit of detection (ml)	0.1	1000	10	10	2

(a) Following radiochemical separation. Limits of detection for natural uranium are based on counting sensitivity of 0.01 pC.
The source of this table is: NUREG/CR-3590, Evaluation of Isotope Dilution Mass Spectrometry for Bioassay Measurement of Uranium, Plutonium, and Thorium in Urine (June 1984), or personal communication.

R. ALEXANDER: Bob, before you change that, I think, is it correct that what we should really be saying is alpha spectroscopy?

R. NEEL: That is more correct.

R. ALEXANDER: That is not just counting from electrodeposition?

R. NEEL: The footnote--I guess I didn't refer to that. It says "following radiochemical separation."

R. ALEXANDER: There is one technical point that I would like to get cleared up in my mind. I may be the only one in the room confused about it or wondering about it. But using the term "phosphorescence" or "fluorescence," they seem to be almost interchangeable. Can someone elaborate on that? Don?

D. GRAY: It may be just a question of semantics, but we are calling the process at Scintrex phosphorescence.

R. ALEXANDER: So the terms are being used interchangeably?

D. GRAY: I noticed that in the presentation. I believe the terms are interchangeable. It is really just a question of semantics. The decay time puts it into the category of phosphorescence. That is really too fine a point.

VOICE: I think on this slide here the laser fluorimetry refers to the fluorimetric measure.

D. GRAY: Well, the third column says--

VOICE: That is laser fluorimetry. That is just looking at the fluorescence.

D. GRAY: The luminescence we have been looking at we are calling fluorimetry.

VOICE: That is the reason for the differences?

D. GRAY: Yes.

VOICE: Then that footnote ought to be taken off.

R. ALEXANDER: On these summary tables we really want to get them right. I am sure these will get wide circulation. So, be sure and let Bob Neel know of any correction that needs to be made in the summary tables.

R. NEEL: I would very much appreciate that because it is a little bit late today, and I may forget this. I might point out that the information on this first slide is a little more complete than the information I have on the following slide because these methods, as you notice up here on the first row, are in routine use, many of them.

IDMS, as indicated, is not in routine use. That is used routinely for our own purposes. That is from the contract we funded.

Now, the techniques I will show next are those that have been discussed in a little more detail by our speakers, and in some cases the costs are not available. I haven't really checked with the speakers because these are not in routine use.

R. ALEXANDER: I guess it turns out that the neutron activation analysis and the fission track analysis are the same.

VOICE: They are not the same. In neutron activation, they use ^{239}Pu. It is a real activation.

R. ALEXANDER: I see.

VOICE: So, they are different.

R. NEEL: Well, in any case, again I pointed out in the first row that these techniques, although they are under research, are not routinely in use, even the SIRIS method, which has more advantages in some ways than many of the others. It essentially has been experimentally demonstrated for bioassay measurements. The delayed neutron counting has also been demonstrated.

Some of the work has been described today. Some of it has been done at Oak Ridge National Laboratory and has not been described--the isotopic composition, the chemical processing. Equipment cost is given as high.

If any of the people who have made public comments would like to modify these words, we can do so. Even better yet, we would like to put in some dollar costs.

Again, the reported lower limit of detection is in micrograms per liter (µg/L). Delayed neutron counting was given as 1 µg/L. Neutron activation analysis is reported by Oak Ridge National Lab to have a sensitivity of 0.001, the fission track counting 0.01, the RIMS method 0.05, and the SIRIS method 1 µg/L. The volume of sample needed to attain the lower limit in any of these samples is small. Perhaps there is great promise here at this point in time, at least at the time we reported this.

Oak Ridge was unable to assign dollar values. If any of the speakers do have dollar values, we can add that to this table to keep it up to date, or any information about the lower limit of detection or the standard deviation at the limit of detection. We would appreciate that, also.

So at this point, I would like to complete my summary and thank you very much for coming.

R. ALEXANDER: Thank you, Bob. Is there anyone with us today who would like to make kind of a statement for the record?

Dr. Brodsky.

A. BRODSKY: I don't want to make a statement, but I would like to ask if Wirth Trapp would like to say anything. I am curious about his addressing perhaps the question that Dr. Singh raised. I know PNL is planning to check how the various procedures do, if nuclides are in process compared to these artificial spikes. I wonder if Wirth Trapp could say a word about that.

W. TRAPP: Yes. That is one thing that we are attempting to do with the bioassay project. There hasn't been anything like that yet, although we did do an experiment where we did spikes in natural urine and the artificial urine, and we said that would be analyzed. Just speaking from memory, for the spiked uranium and the artificial urine, we didn't see any differences for uranium and for plutonium. But we also did ^{90}Sr, and we did see differences there.

A. BRODSKY: Some time ago, before you were with the project, it was suggested that perhaps you could still get all of those good urine samples. There is a body burden that can pretty well measure americium. You would take some other urine samples and spike them and also take some of McClusky's urines themselves after analysis and add an additional amount by spike to see if you can get the same recovery. Also, perhaps the same thing could be done with some people who do have urinary excretions with uranium, if there are any around anymore.

VOICE: I thought that McClusky's urine was fine. I said that he could do the americium and then see if he could get any kind of numbers.

A. BRODSKY: That's one of the ideas. You can do a very accurate americium count. Of course, americium is not uranium, but it's not too different chemically in a lot of respects. Maybe you could find some people who also have uranium and are excreting it with these sensitive measurements. There should be plenty of people when you go down to the results of these sensitivities. There are events in the laboratory.

N. SINGH: In case we don't have uranium exposure, why don't we just give some uranium and do some exposures?

A. BRODSKY: I think you've got good ideas, but I've got no money.

(Laughter.)

R. ALEXANDER: Any other comments?

VOICE: I think the alpha spectrum should be called spectroscopy or spectrometry. Additionally, I think the tables are often just as good as their footnotes, which probably might be three or four times the number of pages that the tables take up.

I think it should be seriously considered whether or not an additional summary of the pros and cons of each of these procedures--never mind money in that respect, but at least some footnotes outlining what one procedure versus another procedure can do for the user. One never knows what the NRC is going to do with this information in terms of applying it to a fuel-fabrication facility or to decommissioning-type operations. And a procedure that may work

very well for one or maybe the other procedure, which may work for uranium bioassay-type mechanisms, might not be appropriate in the circumstance. So, I think the pros and cons of the procedures would be worthwhile.

R. ALEXANDER: Of course, what we hope is that what is said on the record today, the papers given today, would pretty much account for that. We don't plan to circulate the tables on them, only in connection with what's been said today. So, do you think that would be all right?

VOICE: I have a terrible feeling myself, and a lot of other people do, too, that there will be a lot of looking at tables and a lot of ignoring of pages of words that go with them.

R. ALEXANDER: Well, what we can do, then, is circulate before we release the tables, circulate it and let you work on it.

VOICE: Is there some way of cost estimation that people keep using? It seems to me we have to have some data on that. The tables should reflect that: how it is calculated after several years of lifetime?

R. ALEXANDER: When we circulate the tables, you might like to explain how your costs were derived.

A. ROBINSON: Al Robinson, U.S. Testing. I'd like to put in, I guess, a little plug also maybe for some standards, testing of standard to different matrices. In the case of the strontium, where we compared the artificial and natural urine, the difference there that was observed apparently related to the matrix of the artificial and natural urine, not the chemical form of the spike. So, I think that in our limited experience, various things often get into the matrix more than the chemical form, in the matrix of a given nuclide is the determining factor on how your analysis goes.

I guess my fear is that if you get standard materials, say, a standard urine, or a standard anything else, that the procedure will be developed using that and will be valid for that standard of urine and that chemical form which may have little or no relevance to the next guy down the road, who is eating rhubarb all day, or something like that, and excreting out a lot of oxalic acid, as opposed to something else.

That's the fear I have with standard matrices, or standard radiochemical compounds. They may be standard for that but they tend to override the chemical form, actually. So, we ought to be real aware of both.

R. ALEXANDER: I'd like to say that the only way our agency would recommend the use of a urine standard would be through adoption of a consensus standard. You would all have an opportunity to try to influence that decision.

N. SINGH: Today, I find there's a lot of emphasis on the techniques going lower and lower. The question is really: What lower limit do we really need for biological purposes? In my opinion, any method which can detect the uranium concentration in the general population--isn't that enough for bioanalysis purposes? Or do we need any more than that?

R. ALEXANDER: I spoke to the reasons that we're interested in lower techniques this morning. You may remember those two reasons. I'll mention them again for those of you who might have come in late. First, the probabilities are appreciable that the intake limits for both soluble and insoluble airborne uranium will be reduced possibly by as much as a factor of 5. And that is certainly the case with Class-Y material.... That is the principal problem. The other problem involves action levels that the NRC is imposing through licensing conditions that are too close to the detection limit. A lot of unnecessary investigative action is taking place. I think we'd all be more comfortable if that work was done only when necessary. There are two ways to accomplish that. One is for the NRC to raise its action levels. The other is to lower the detection limits. But we'd like the latter approach best. As we have seen, there are a number of practical ways to lower a number of detection limits for urinary uranium.

Very soon, if not already, lower detection limits will be a practical alternative, i.e., limits sufficiently low to accommodate the reductions in intake limits that are being considered. I really think it's going to go that way. Now, it may not go that way very fast, but I believe we can all look forward to that eventually.

I think a corollary change that is going to take place in our programs is de-emphasis on air sampling and emphasis on individual monitoring, i.e., urinalysis and in vivo counting (although you won't see it in the proposed Part 20 revision that will be published in a couple of weeks, or in the so-called compliance formula.)

Let me make sure you know what the compliance formula is. The compliance formula is the formula in which one adds the fraction of the external dose limits received to the fraction of the intake, or if more than one nuclide is involved, by the sum of the intake fractions. If the sum is less than one, then compliance has been demonstrated.

We're putting that same compliance formula into Part 20. But I think there will be a third component to the sum. And that component will be the organ dose ratio. So, the licensee will be allowed to use bioassay results and not have to back-calculate all the way to the intake just to calculate the dose--without introducing all these variances in the model and come all the way from inhalation to excretion.

And I just feel certain that if the emphasis on intake is removed, even in the compliance-formula regulations, then much more emphasis will be placed on individual monitoring through urinalysis and in vivo results. As things stand right now, based on what I've seen today and learned today, I don't see any reason why it shouldn't go that way.

I certainly thank all of you for coming and contributing to our public meeting. It's been very valuable for us. I wish that you would express to your employers our appreciation for permitting you to come and participate with us and help us today.

(Whereupon, at 5:00 p.m., the meeting adjourned, to reconvene at 8:30 a.m., Wednesday, December 4, 1985.)

PUBLIC MEETING ON

NEPHROTOXICITY OF URANIUM

General Services
Administration
Auditorium
18th & F Streets, N.W.
Washington, D. C.

Tuesday, December 4, 1985
8:30 a.m.

R. ALEXANDER, U. S. Nuclear Regulatory Commission, Presiding.

MORNING SESSION
(8:30 a.m.)

R. ALEXANDER: I'd like to welcome you all to this meeting on the important subject of nephrotoxicity of uranium. The subject that this meeting will be boring in on today is one that I believe is of considerable difficulty for the Nuclear Regulatory Commission and other agencies of the Federal Government.

It arises from serious questions that have been raised as to the adequacy of the nephrotoxic limit which has been used for many years. I have tried in the past to trace back the initial use. I know it predates 1955. I haven't been able to go back further than that. I know, as of 1955, the ICRP in a report that they published did use a nephrotoxic limit of 3 μg of uranium per gram of kidney. The maximum permissible concentrations (MPCs) that were calculated and published by the ICRP in 1959 are based on 3 μg of uranium per gram of kidney, and the American Conference of Governmental Industrial Hygienists use the same number.

Now, a question has arisen from animal studies conducted recently for the NRC at the University of Rochester. Dr. Paul Morrow, the central principal investigator, has concluded, based on examinations of animal kidney tissue, that damage does occur at concentrations considerably lower than 3 μg per gram. As a matter of fact, they detected kidney damage an order of magnitude lower than that. In the report that he prepared for us, Dr. Morrow recommends that the nephrotoxic limit be reduced by at least a factor of 5. His recommendations were repeated at the conference on uranium held at Richland, Washington, about a year ago.

So, when you are dealing with a chemically toxic substance like uranium, where there is no comfortable 20-year waiting period for the detrimental effect to show up, and apparently there is a threshold phenomenon to deal with, it seems to me that it's necessary for the government to make a decision rather promptly.

The purpose of this public meeting is to construct a public record on which the Nuclear Regulatory Commission can then make its decision. And we have provided people who can assist and provide that record, the best we could obtain. I trust that at the end of the day, when I get to the summary, we'll be closer to a decision.

This uranium-toxicity problem is a classic example of the difficulty of extrapolating data from animal subjects to man. The question of the sensitivity of animal kidney tissue, relative to that of man, is not a new one, but it is also one that has not been resolved.

In looking through the early records of studies that were done at the University of Rochester, I cannot find evidence to support a nephrotoxic limit of 3 μg per gram. The renal damage that was detected in those studies back in 1948 to 1953 were once again an order of magnitude lower than 3. On the other hand, workers--human workers--that were exposed in the 1940s to concentrations

of uranium much, much higher than we allow today experienced no clinically detectable damage, at least none that we know of at this time. We're not sure that those workers had kidney concentrations of more than 3 µg per gram, but there's certainly an indication that many of them must have.

As W. E. Harris of the AEC pointed out in 1959 at the first big meeting on uranium that we had in New York, of all of these hundreds of workers that were terribly overexposed by today's standards, nobody got sick, and he appealed for the agency to spend its safety money elsewhere than on the tremendously expensive ventilation systems that were being installed at those times.

So, the question was a great one then, and it hasn't been solved yet.

Although we have never been able to determine exactly how it was done, it is evident that the nephrotoxic limit of 3 µg per gram was established on bases other than animal studies. We're continuing with it in 10 CFR Part 20, the version in effect today. The MPC and intake limits for soluble uranium are based on 3 µg per gram. The same in the proposed Part 20, which I think we will publish for public comment within three weeks.

As things stand to date, at least 30 years after the decision was first made, we're still using 3 µg per gram; and repeated studies have shown rather extensive kidney damage at that level in animals if maintained over a sufficiently long period of time.

In all of those places where people have been exposed to soluble uranium, an extensive monitoring program has been conducted, so that if the kidney burdens became very large, I think probably the worker was removed from exposure until the burden was reduced.

The best I can tell from looking at the literature all these years is that the half-life of uranium in the kidney is about 15 days. So, since we had no record of anyone getting sick, it would appear that a kidney burden or concentration greater than 3 µg may be safe, as long as it isn't maintained too long and doesn't go too far above 3.

But now at the NRC, I think we're face-to-face with this problem. We're doing a major modification, an updating of our basic regulation. Probably, within about two years we'll have the new Part 20 in final form, and at that time, a decision has to be made about these concentration values for soluble uranium compounds. A factor of 5 is going to mean a tremendous difference in the operation of any sort of factory involving soluble uranium.

I had personal experience along that line a few years ago when I was the health physics manager for a uranium-fuel fabrication operation, and we had a factor-of-6 reduction in the intake limits. As a result of that, we had to abandon the facility and build a new one with glove boxes at a cost of millions of dollars. A decision to reduce the intake limit by a factor of 5 cannot be taken lightly by the government. We want very much to do the right thing with

respect to protecting the workers. On the other hand, we certainly do not want to unnecessarily injure the uranium industry in our country.

So, with that little introduction, I think you can see the dilemma we face at the Nuclear Regulatory Commission and why we have assembled you today to help us make a decision.

We certainly want as much public input as we can get. In that connection, let me urge you, all of you, whether you are scheduled to speak or are not, to participate. Very often at the public meetings that we hold for purposes like this, the best ideas, the best information we have is from the floor where people are inspired to enlighten us, and I certainly encourage you to do that at any time during the meeting.

You can see the importance, I think, of ultrasensitive techniques, if we're talking about reducing the intake limits for soluble uranium by a factor of 5. Prepared under the leadership of the EPA, new Presidential guidance will very soon reduce the intake limit for insoluble compounds by a factor of 5.

So, you'll be happy to know that the people who presented information about their research into the ultrasensitive techniques have determined that there are a number of ways that compliance by the industry would be practical under reduced intake limits.

I was very happy with the way the meeting turned out yesterday, but I certainly know that those of you who were here yesterday are very weary of hearing me. So, we've arranged for a much more attractive person to conduct the meeting today, Dr. Judy Foulke, who has worked for months to prepare for this meeting. I'll turn the podium over to her now to introduce the speakers. Judy?

J. FOULKE: Thank you very much, Bob. This is, indeed, a day we've been looking forward to for some time now, especially meeting some of our speakers.

One of the advantages of living in the Washington area is that we have such nice neighbors. The Commission has most of its offices in Bethesda, and up the street is an institution with a very fine reputation. We were able to call upon that institution to help us out today.

The first speaker, as you can see from your agenda, is Dr. Jeff Sands, who is going to give us the basic anatomy and physiology of the kidney. Dr. Sands graduated from Harvard College with a major in applied mathematics. He then got his M.D. degree from Boston University School of Medicine. He is presently a medical staff fellow at the Laboratory of Kidney and Electrolyte Metabolism of the National Heart, Lung, and Blood Institute at NIH.

Dr. Sands, would you come forward, please?

J. SANDS: The kidney is one of the major excretory organs in the body, and its main role is to filter the blood and thus to get rid of unwanted substances, and at the same time preserve within the body those substances which are needed.

It plays a particularly important role in the regulation of fluid volume, sodium and potassium balance and acid-base balance. The kidney is also intimately involved in the regulation of blood pressure, both through sodium and volume regulation, as well as by the elaboration of the hormone renin and the renin-angiotensin system. In addition, the kidney is involved in the production of erythropoeitin, which is necessary to stimulate bone marrow for the production of red cells.

The kidneys are located below the diaphragm at approximately the level of the lower ribs in the back. They receive their blood supply from the aorta by the renal arteries. Blood is returned by the renal veins through the inferior vena cava.

Descending from the renal pelvis are ureters, which carry the urine from the kidney to the bladder, where it is stored prior to excretion from the body. The kidney is divided into the cortex, which forms the outer portion of the kidney, and the medulla. In humans, the medulla is divided into several renal pyramids. The innermost portion of each renal pyramid is called the papilla.

During the formation of urine, blood enters the kidney in the cortex, goes through the medulla and emerges from the medulla into the renal calyces. From the calyces, it collects in the renal pelvis, prior to going into the ureter and descending into the bladder. In addition, there are structures called the medullary rays, which are extensions of the medulla up into the cortex.

The major functional organization of the kidneys is into nephrons. There are two major types of nephrons, the short-loop and the long-loop nephrons. Approximately 75% of nephrons are short-looped and 25% are long-looped.

Looking first at the short-loop nephron, it begins with the glomerulus, located superficially in the cortex, which then leads to the proximal tubule and then leads to the loop. In short-looped nephrons, the descending limb of the loop of Henle turns into the thick ascending limb and the border between the outer medulla and the inner medulla. It does not descend into the inner medulla. It then emerges into the cortex to form the distal tubule, and then next to the collecting duct system.

Long-looped nephrons have a glomerulus located near the cordical-medullary junction, so-called juxtamedullary glomerulae. This nephron also has a proximal tubule, which then leads to a much longer loop of Henle. Unlike the short-looped nephrons, long-looped nephrons have two types of ascending limbs in their loop of Henle. Initially, a thin ascending limb, then a thick ascending limb before reaching the distal tubule, and again emerging with the collecting duct system. The long-looped nephrons are particularly important for the ability to concentrate one's urine. In various animals, those with a greater ability to concentrate the urine have longer and more numerous long-looped nephrons.

The glomerulus is composed of Bowman's capsule, consisting of three structures. There is the basement membrane, the parietal epithelium, and the visceral epithelium. In between the two epithelia is a space called Bowman's space.

The visceral epithelium is in contact with the blood capillary. Blood enters the capillary through the afferent arteriole. It is then filtered across the epithelial membrane and an ultrafiltrate of plasma enters Bowman's space. This ultrafiltrate is similar in composition to blood plasma except that blood proteins are not able to penetrate the endothelial-epithelial border. This is due to the size of plasma proteins and the negative charges that are on plasma proteins.

The blood then leaves the glomerulus to the efferent arterioles. This is a fairly unique system in that there are two arteriole systems, both entering the glomerulus and exiting the glomerules, from which the blood continues to the proximal tubules located in the cortex.

The so-called JG, or juxtaglomerular, cells are the cells which, by sitting near the afferent arteriole, are able to sense changes in blood pressure and blood volume entering the glomerulus; if these go down, the JG cells secrete renin, which then leads to increases in blood pressure.

The blood supply of the kidney comes from the renal arteries. These are divided into interlobar arteries, which send the blood to arcuate arteries. The arcuate arteries form bridges between the interlobar arteries. The arcuate arteries, in turn, give rise to interlobular arteries, which supply blood to the glomerulus and to the medulla.

The kidney has a very large blood supply. Approximately 25% of the cardiac output goes to the kidneys. Thus, in four minutes the entire blood volume of the body has been filtered through the kidneys. There is a very rich blood supply to the glomerular and tubules. In the medulla, however, there is a very different blood supply. In this case the vasa recta run down in parallel fashion to the inner medulla and then turn around and reascend. Thus, there is a parallel arrangement of the descending and ascending vasa recta. This gives a very poor blood supply to the medulla, rendering this area of the kidney rather hypoxic. However, this arrangement is very useful in urinary concentration.

There are basically three ways in which the urine is formed from the blood. The initial process is glomerular filtration. Once the ultrafiltrate has entered Bowman's space, it is then further modified by the processes of tubular reabsorption and tubular secretion.

It is useful to have a measure of the glomerular filtration rate. This is one of the prime indicators of how healthy the glomeruli are. The factors involved in determining glomerular filtration include the permeability of the endothelial-epithelial junction. A process which injures this membrane would lead to a change in glomerular filtration. Another factor is the difference in hydrostatic pressure between the endothelial capillaries and the pressure from

Bowman's space. Under normal circumstances the hydrostatic pressure is greater in the endothelial capillary, that is, the blood side, and thus favors the filtration of fluid into Bowman's space. This is opposed by the colloid oncotic pressure of blood compared to Bowman's space. The blood side is much higher, as the plasma proteins which are normally prevented from crossing the space exert a pressure to retard fluid flow. The net result is the balance between these three factors.

One measures glomerular filtration in practice using the clearance principle. Clearance is the way of measuring the volume of blood that has a certain substance removed from it per unit of time. Experimentally, one wants to use a substance which is filtered but that is not changed as it goes through the tubules so that one has a pure measure of glomerular filtration. Inulin is such a substance frequently used in experimental work.

In human beings it is not very practical to administer a foreign substance to measure glomerular filtration. Fortunately, there is an endogenous product in the blood called creatinine, which is a natural breakdown product of muscle metabolism. In the steady-state, the concentration of creatinine in the blood remains fairly stable. Since creatinine is basically filtered with very little reabsorption or secretion, one can use creatinine in place of inulin.

In order to do this, one can measure the concentration of creatinine in the urine plus the urine flow rate. Their product is equal to the glomerular filtration rate times the serum creatinine level. As one can measure the serum creatinine, one can measure glomerular filteration.

There are times, however, when this is not a valid indication of glomerular function. Anyone who has concomitant muscle disease will have elevated serum creatinines that are not reflective of the process in the kidney. Additionally, as people age, their muscle mass changes. Fortunately, as one gets older there is an equal decrease in muscle mass, hence, in the production of creatinine, and a decrease in the glomerular filtration rate and the excretion of creatinine, such that the serum creatinine stays the same with age.

However, in going from the age of 21 to 65, the glomerular filtration rate has decreased about 50%. If one calculates the creatinine clearance, one will see this. However, just looking at the serum creatinine does not give one an indication of this decreasing glomerular function that normally occurs with age.

Due to the large blood flow through the glomerulus, the glomerulus is uniquely susceptible to injury. Any toxins that are in the blood, whether they be exogenous substances or endogenous products of other diseases, can hit the endothelial-epithelial border, thus injuring the glomerulus. This is manifest by the appearance of proteins in the urine, which are normally prevented from entering, as well as by a reduction in the glomerular filtration rate reflected in the creatinine clearance for an elevated serum creatinine level.

Once the blood has been filtered, it enters the tubule, initially the proximal tubule, and at this point the processes of tubular reabsorption and tubular secretion will form the urine into the final product. Tubular reabsorption is the process by which substances are reabsorbed from the urine back into the bloodstream and thus taken back into the body.

The process of reabsorption involves the transfer of these materials, which can either be a passive process or an active process. Passive transport is when a substance is in a higher concentration in one place than in another and just flows passively down its electrochemical gradient from the place of higher concentration to the place of lower concentration. No energy is expended.

Active transport requires the expenditure of energy, usually hydrolysis of ATP. Active transport takes a substance which is at a lower electrical-chemical state and moves it into a higher state. This is done for substances which the body wishes to preserve.

Transport processes involve carriers located in the membranes, and these processes usually have a threshold, at which point all the transporters are working as hard as they can and cannot transport any faster. For example, the body normally reabsorbs all of the glucose which is filtered from the plasma into urine. As the filtered load increases, the transport rate can initially keep pace with the filtration rate, and the excretion is zero. However, if the filtered load of glucose runs too high, as often occurs in diabetes, there is more glucose entering the urine than the kidney is able to reabsorb. At this point the transport rate has plateaued, and above this rate any additional glucose will be excreted.

Tubular secretion is the reverse process. Tubular secretion is the process by which substances in the blood are secreted into the urine for elimination from the body. Secretion, like reabsorption, is across transporters and can be either active or passive, and again they demonstrate threshold phenomena.

Para-aminohippurate secretion (PAH) is commonly used to measure renal blood flow because it is both filtered and then secreted. Initially, there is a filtered load added to this. It is the transported--or secreted--material; thus, the excreted quantity is the sum of these two. Above the plateau value, however, the excreted amount of PAH rises with the increasing filtered load. Renal blood flow is approximately 1200 mL/min.

Urea is a substance which is passively excreted. As the renal blood flow, hence the urine flow, increases, the amount of substance able to be passively reabsorbed decreases. With increasing urine flow, as it spends less time in contact with the transport, it is able to move it passively from one place to another.

The quantities of materials that are handled by the kidney in a given day: Water has about 180 L per day which are filtered. Of this, over 99% is

reabsorbed. The same is true for the other substances shown: glucose is virtually 100% reabsorbed. Thus, the kidney does a lot of work of filtering and then reabsorbing important bodily substances.

It also makes use of this large quantity of sodium which is filtered. By filtering all this sodium, it builds up a concentration gradient for sodium which is much higher in the tubule than it is back in the bloodstream. Out of the 2500 meq per day of sodium which are filtered, approximately 67% is reabsorbed in the proximal tubule.

The proximal tubule is a high-flow reabsorption site, taking back most of the vast quantities that need to be reabsorbed. The proximal tubule takes particular advantage of sodium by coupling onto transporters other substances chosen for reabsorption. Specifically, sodium and glucose are often coupled, as are sodium and amino acids. By coupling, I mean there is a membrane protein which will take one sodium and one glucose and carry them together across the epithelium.

In this way, the kidney is able to take advantage of the electrochemical gradient for sodium to move a different substance against its electrochemical gradient. As long as the sum of the two still favors the movement, one is able to reclaim glucose and amino acids, which are important for the body by taking advantage of the gradient established for sodium. This is called a secondary active transport phenomenon, since the energy is not expended to move the sodium and glucose or amino acid, but the energy is expended to establish the initial sodium gradient.

As one then moves around the nephron, one sees an additional 25% of sodium is reabsorbed in the thick ascending limb of the loop of Henle. This is an active process which plays a key role in urinary concentration.

The distal tubule only reabsorbs 5% of the filtered load. However, the distal tubule's role is very different from that of the proximal tubule. The distal transporter is important for fine-tuning the exact concentrations in the blood. By having one side which reabsorbs most of the work, sort of coarse-tuned, and then having the distal side, which is fine-tuned, the kidney is able to control the quantities of sodium, postassium, and acid-base in the blood.

Needless to say, the disease processes which affect the proximal tubule or distal tubule will be different. In proximal tubular diseases one finds the appearance in the urine of substances which are normally completely reabsorbed, so that one would find large quantities of glucose, amino acids, and bicarbonate appearing in the urine.

The kidney is also important for hypertension. When the kidney senses a drop in blood pressure, it secretes the hormone renin, which then stimulates the production of angiotensin. Angiotensin is a potent vasoconstrictor and directly increases blood pressure. It also acts on the adrenals to produce aldosterone which stimulates the kidney to reabsorb more sodium.

With an increased intake in sodium, there is arterial pressure; one has an increase in the hydrostatic pressure in the peritubular capillaries. One also gets an increase in extracellular fluid by increasing sodium, and the plasma oncotic pressure in the peritubular capillaries decreases. These processes are also stimulated by changes in the resistance in the arterioles. The net result is a decreased uptake of fluid into the peritubular capillaries, a decrease in total tubular sodium absorption, and increased sodium excretion. Thus, the system works. If you increase your sodium intake, you increase your excretion.

Next, I want to turn to the process by which we concentrate urine.

As a model of this, one could view the medulla as a box, where the basa recta come in and out, the loops of Henle come in and out, and the amount of water and solute entering will be equal to the amount of water and solute leaving, thus there would be no net change. However, if we had a process where the amount entering was greater than the amount able to leave, one would accumulate solute in the box or in the medulla.

Suppose there is a partition in the box, more or less like the loop of Henle, which has a descending and ascending loop with interstitium in between. In the steady state nothing would really change. Solute in would equal solute out. However, if this barrier were a semi-permeable barrier which allowed solute to pass from the ascending side of the box to the descending side of the box but not the other way around, one would accumulate solute in the box. In addition, one would form a gradient of solute, as the solute which is transported from the ascending side of the box to the descending side of the box would continue to be pushed down, and more solute would accumulate in the bottom of the box than at the higher parts of the box.

In the kidney, the ascending limb is able to move a fixed amount of solute from the ascending side to the descending side by transporting a small amount of solute, usually 200 milliosmoles, from one side to the other. Initially, the fluid is equal on both sides, say 300 milliosmoles, and one is able to generate a gradient of 200. The ascending fluid would be at 200 and the entering fluid would be at 400. Or if it is fluid at 400, it would then move down the box and eventually appear on the ascending side, so that it would now be able to take it from 300 to 500, to get the same 200 gradient but by having a higher starting point. Due to already concentrating solute coming from above, it is able to make the total concentration higher. The net result in the kidney is that one is able to take the 200-mdsm gradient and establish an axial gradient of 1200 milliosmoles.

There are two key features to this model. The first is the ability to establish a very hypertonic inner medulla, which one can later use to concentrate the urine. But equally important is that as the fluid comes back up the ascending limb and sodium is actively removed, we end up with dilute urine. Thus, the fluid entering the distal tubule is actually hypotonic, or less concentrated than is the blood.

Thus, you have the ability, by having this dilute urine entering the distal tubule, to determine whether you want to have a dilute urine by just not

changing this dilute urine or whether you wish to reconcentrate the urine and thus conserve water and salt, depending upon the status of the person.

The isolated perfused tubule studies performed in rabbits basically confirms the theory. If you look at the thick ascending limb, it actively transports sodium but is unable to transport water; thus, one of the requirements. The ascending part of the box removes salt but does not allow water to come back in. The descending limb, on the other hand, does not transport salt, but is able to transport water. Thus, salt is actively removed from the ascending limb, causing increased concentration in the interstitium, and water then flows out of the descending limb by osmosis.

In order to take advantage of this gradient, one needs to have a third tube which runs in parallel with the first tube. It starts at one end and comes out at the bottom. This is provided by the collecting duct. The fluid from the distal tubule, which is hypotonic, enters the collecting duct. If the person is dehydrated and needs to conserve water, this leads to an elaboration of the hormone ADH.

There are osmo-receptors in the brain and volume receptors in the heart which monitor the blood osmolality and the blood volume of the person. If these are low, it sends a signal to the hypothalamus to elaborate ADH. The ADH acts on the collecting duct to make it highly permeable to water, urea, and sodium. With ADH present, as the urine enters the collecting duct, it is able to come into equilibrium with the surrounding hypertonic interstitium; thus, water and, to a degree, salt are reabsorbed from the collecting duct. Then, urine emerges as highly concentrated.

On the other hand, if the person is in a water diuretic state, that is, if there is fluid or water overload, the osmoreceptors and volume receptors turn off the production of ADH, and the collecting duct becomes essentially impermeable to water, urea, and salt. In this situation, as the urine from the distal tubule flows down the collecting duct, it is prevented from coming into equilibrium with the surrounding interstitium, and the dilute urine that was present in the distal tubule emerges as the final urine.

The vasa recta, which we noted before, are also shaped in a countercurrent fashion. They serve a very important purpose. If the vasa recta were shaped like the collecting duct, starting at one end and coming out the other end, it too would be concentrated by passing through the medulla. It would dissipate the gradient that the loop of Henle had generated. This would defeat the purpose.

If you concentrated blood and then let the highly concentrated blood go back into the bloodstream, one would not accomplish useful work. By having this hairpin configuration, one can concentrate the blood as it comes down and loose the gained solute as it goes back up. So, the net result is that the blood emerging from the ascending vasa recta is slightly concentrated, by about 30 milliosmoles, but not concentrated to 1000 milliosmoles, and most of the work of the gradient is preserved by having the blood flow in this hairpin

fashion. The permeabilities of the vasa recta are similar so that solutes are not prevented from exiting and entering as they are in the loop of Henle.

The last major function of the kidney is to regulate the acid-base balance of the person. Two organs are primarily involved in acid-base balance. These are the lungs and the kidneys.

The lungs are involved in achieving short-term changes by changing the respiratory rate and thus increasing or decreasing the amount of carbon dioxide which is removed from the bloodstream. Carbon dioxide is an acid, and in the case of metabolic acidosis, one is able to hyperventilate, thus blow off CO_2, and help to return pH towards the normal value of 7.4. In the opposite condition, metabolic alkalosis, the lungs respond by decreasing the respiratory rate. This is not as effective as hyperventilating since one must also breathe to avoid hypoxia.

In order to deal on a more long-term basis--that is, one cannot continually change one's breathing habits--the kidneys are also involved in acid-base, and over the course of a day or two are able to actually remove acid or base from the bloodstream and thus return the person back towards normal acid-base balance. The other important part of this is that the blood contains many buffers. Buffers are substances which, when there is a change in acid-base, will tend to take up or lose protons, so that the pH remains closer to what it started.

The most important buffer in the body is the bicarbonate-carbonic acid system. The Henderson-Haselback equation relates pH to the pK of the acid, plus the logarithm of the base form divided by the acid form. In this case, bicarbonate to carbonic acid:

$$pH = pK + \log \frac{(BA)}{(HA)}$$

where Ha = the concentration of a weak acid,
BA = the concentration of a weak salt of this acid,
pK = the buffer system.

A buffer should have a pK that is equal to the pH that you wanted the blood to be at. Although the bicarbonate system has a pK of 6.1, which is a reasonable amount away from the pH of 7.4 (as both pK and pH are measured in log units), the amount of bicarbonate is so large in the blood that it remains the most effective buffer. Hemoglobin is also able to buffer blood, as is the phosphate system.

There are three principal renal adaptations to changes in acid-base. The first is bicarbonate reabsorption. In a condition of acidosis, bicarbonate is used up in the body as the extra protons combine with bicarbonate to form carbonic acid, thus neutralizing the acid protons.

The first kidney response is to regenerate the lost bicarbonate. The reabsorption of bicarbonate or a unit of base is equivalent to losing a unit of

acid. In the lumen, which is equivalent to the inside of a tubule, one has filtered bicarbonate which can combine with protons in the urine to form carbonic acid. Under the influence of carbonic anhydrase, the enzyme which catalyzes the dehydration reaction, water and CO_2 can be taken back into the cell. Carbonic anhydrase then forms carbonic acid, and this can break down to a proton plus bicarbonate. Bicarbonate can then be transported back into the bloodstream.

Usually, this is accomplished in conjunction with sodium, and the proton can then return to the urine, where it can combine with another bicarbonate or other buffers in the urine to be excreted. The net result is that bicarbonate, which started off in the urine or lumen, ends up in the blood or interstitium.

The second process is the formation of titratable acidity. Titratable acids are those acids which remain as acids. That is, one can take them in a beaker and titrate them as one would do in a chemistry laboratory.

The most important of these is phosphate. As phosphate enters the urine, protons which come from the cell are able to combine with phosphate, and then these are excreted in the final urine. Again, for each proton that comes from the cell to the urine and is trapped there, a bicarbonate has been formed, which is then reabsorbed into the blood.

The third process is that of ammonium secretion. As the acidosis continues, the renal cells, particularly the proximal tubule, generate the production of NH_3, which is a base. Ammonia is uncharged and is able to diffuse into the tubule lumen. It can there combine with protons, and once appropriated to form ammonium, NH_4, it is not trapped in the urine; thus, it is able to be excreted. Since this process occurs over two or three days, the kidney can increase dramatically its production of ammonia, thus providing a source of base for the elimination of acid.

Another mechanism to deal with acid-base abnormalities is that the three major cations which occur in the body--sodium, potassium, and protons--are able to be moved from the inside to the outside of cells. In the short term, one is able in the body, in acidosis, to have the cells take up some of the protons, releasing sodium or potassium, and then reverse the process in alkalosis. This provides a short-term remedy. The distal tubule is able to do the same thing. It notes the relative concentration of sodium, potassium, and protons. It is able to selectively excrete one or the other cation in order to have the balance of all three be in the best interest of the person. While there are certain priorities, such as maintaining blood pressure with sodium when a bodily emergency is not present, the kidney alters the contribution of all three to what it considers the best combination.

J. FOULKE: Dr. Sands, could you talk a little bit about the nephron? What's the turnover rate? How many cells are there?

J. SANDS: The nephrons do decline with age. There's approximately a 50% decrease in the number of nephrons as one goes from approximately the age of 20 to the age of 65. This was learned by a German nephrologist who took kidneys

at autopsy from people who had died at various ages from nonrenal-related diseases and actually counted the number of nephrons. And there's a linear dropoff with age, such that by the age of 65, one has about 50% of the nephrons that one started with.

The number of nephrons is on the order of 1 million in the two kidneys to start with, and one can lose 75% of one's nephrons and still have adequate renal function. That is, one can live on one-half of one kidney. So, at least initially, one has a very large reserve of renal function.

Now, the types of cells present in the kidney. In the proximal tubule one has the brush border cells. These cells have on their inside surface, that is, the surface facing the urine, a brush border, which is a very elaborate way of increasing the membrane. There are enormous numbers of transport sites available, not just simply the amount one could measure, if one measured the length of the cell. But by having multiple foldings of the apical membrane, one can bring large numbers of transporters in contact with the urine.

The cells in the loop of Henle do not have this brush border. They are simpler cells. The thick parts of the loop of Henle have large numbers of mitochondria. This is where most of the work, the energy for the work is done. The thin limbs are very thin cells without much detail. They do not have many mitochondria. There really does not seem to be a source of energy within the thin limbs. One of the continuing controversies in urinary concentration involves the thin limbs and where the source of energy for their transporting concentrated urine comes from.

The distal tubule cells are somewhat similar to the proximal tubule cells except, again, there's not a brush border, this not being a high-volume source of reabsorption.

The collecting duct cells involve two types of cells. There are the intercalated cells and the principal cells. The intercalated or dark cells have large numbers of mitochondria and are involved in producing energy for the transport processes. Acid and base transport is carried out by these cells. Interestingly, there seem to be two populations. There are the acid cells and the base cells, which seem to be mirror images. By changing the relative number of acid or base cells, one theory holds, one can control acid and base by just taking essentially the same cell and flipping it around, so it moves in opposite directions.

The turnover of cells is fairly rapid, such that if you have 30 minutes of ischemia, 30 minutes of cutoff of blood supply, one usually has irreversible renal damage. Thus, the cells do change fairly quickly.

The cells in the cortex in the area of the high blood supply are more susceptible to anoxic injury then are the cells in the inner medulla, which have such a poor blood supply that they are more impervious to injury and anoxia than are the cells located more superficially, which usually have a good blood supply.

Kidney cells are capable of gluconeogenesis, as are liver cells, that is, of forming glucose from other substrates. The medulla runs heavily on glucose. The kidney is more dependent on glucose than most organs are, the brain being most heavily dependent on glucose. This makes it susceptible to a cutoff of blood supply, in that the kidney needs the supply of glucose from the blood in order to metabolize and keep the cells alive.

R. ALEXANDER: Can you explain the scenario by which uranium passes through the kidney? Where it's absorbed, how it's excreted?

J. SANDS: Actually, I'm not really familiar with how uranium is handled. I think there are probably people who know that better than I do.

J. FOULKE: To keep on schedule, we'll proceed to our second speaker. Gary Diamond from the University of Rochester is going to speak on the effects of heavy metals on the kidney.

G. DIAMOND: The title of this talk is "Renal Toxicology of Metals," with a special emphasis on the effects of metals on the kidney. I want to preface this with a few general comments.

To summarize renal toxicology of metals and the effects of metals on the kidney in 40 minutes is difficult at best. This is because of two problems. The first is the enormity of the amount of literature and the amount of work that has been done on the effects of metals on the kidney. I think the papers on the toxicity of lead number in the tens of thousands; that's just one metal. Furthermore, the history of these studies go back to the turn of the century. This large amount of literature makes it very difficult to summarize all of the data on the toxicity of metals with any degree of accuracy.

Secondly, all metals are not alike. They're not alike chemically, structurally, and biologically, and this presents some difficulties in speaking about specific metals, because the biology, biochemistry, and toxicology of one given metal may be quite different from other metals.

Therefore, what I'm going to attempt to do is to take a broader look at the metals themselves and attempt to describe some general concepts about the renal toxicology of metals, what makes the metals unique, which of their special characteristics are related to their toxicity, what distinguishes metals from other kinds of nephrotoxins, and what common factors are involved in the renal toxicology of metals.

To begin with, when we look at the effects of metals on the kidney, we have to immediately distinguish two phenomena: indirect versus direct effects. The direct effects of metals are those effects that occur as a result of an interaction between the metal and the kidney. We'll discuss those in greater detail later.

Indirect effects are effects on kidney structure and function that do not result from direct interaction between metal and kidney but interaction between the metal and other tissues. I've given two examples of this. One would be

the hemolysis caused by arsenine compounds which can result in structural abnormalities in the kidney, simply because of the delivery of a large filtered load of hemoglobin and hemoglobin by-products to the kidney. A common example of an indirect effect would be ischemic damage due to shock produced by acute doses of metals.

Metals, by virtue of their chemistry, can react, as you'll see, with many important biological systems. For example, cadmium in high enough concentration is a calcium channel blocker and can actually perturb the function of the heart, e.g., stop the heart.

Thus, high acute doses of certain metals can cause a fall in the blood pressure and renal damage can result, secondary to cardiovascular collapse. This has some relevance to the study of the toxicology of metals. When you use high doses of nephrotoxins and your end point for nephrotoxicity is not an index of kidney function or an index of kidney pathology but say, death, you may not, in fact, be looking at direct kidney effects at all. An animal given a sufficiently high intravenous dose of cadmium chloride will die before you withdraw the syringe, but not from kidney toxicity.

The direct effects of metals on the kidney are those which I want to concern myself with during the remainder of this talk. These effects can be divided up, I think, into two general categories: 1) damage to the cells of the proximal tubule, and 2) damage to the glomerulus.

A schematic diagram of the nephron shows the glomerulus, proximal tubule, loop of Henle, distal tubule, and collecting duct. The collecting ducts coalesce to form the ureter, where the urine leaves the kidney.

Generally speaking, most heavy metals are proximal tubule toxins. That is, they damage the proximal tubule, primarily. The actual site of damage within the proximal tubule is not the same for all metals. That's an important point. The proximal tubule is not a homogeneous tissue. There are many subtypes of cells in the proximal tubule. There is heterogeneity with respect to function, as well. For example, cadmium chloride, when given chronically to rats, produces damage to the proximal tubule, tends to be localized or more intense in the areas of the early proximal tubule, what is called the proximal convoluted tubule, located in the cortex. At a high enough dose, of course, you'll begin to see damage in other parts of the nephron, as well.

By contrast, mercury and uranium compounds damage the more distal segments of the proximal tubule, the straight segments (pars recta) that are located deeper in the inner cortex and outer medulla. As with cadmium, mercury- and uranium-induced damage can be seen in the proximal convoluted tubule, as well, and in other parts of the nephron when the dose is high enough.

Thus, there are important distinctions, in terms of where the damage occurs.

As we just heard from Dr. Sands, the proximal tubule is a very important site for the absorption of large volumes of fluid, sodium bicarbonate, and

various other organic substances. Substrates that are filtered at the glomerulus, including glucose, aminio acids, and proteins (e.g., albumen) are reabsorbed in the proximal tubule. Thus, damage to this area of the nephron leads to several consequences, one of which is the loss of these substances into the urine. This is because if the proximal tubule is not able to reabsorb these solutes, the distal tubule just cannot replace completely the function of the proximal tubule.

The glomeruli are located in the cortex, the proximal convoluted tubule on the outer surface of the kidney. When you look at a kidney that has been damaged by cadmium chloride, you would, indeed, depending on the dose, find structural damage, even loss of the proximal tubules in the very outer sections of the kidney. The damage would become less and less as you move into the cortex. With a metal like uranium or mercury, damage would be seen in those segments of the proximal tubular that are a little bit deeper in the cortex, proceeding even up into what we call the medullary rays. This can actually be a remarkable visual effect when you see an animal that's been given mercury chloride. You can clearly see a band of recrosis process that appears right at the junction between the outer medulla and the cortex.

There are several consequences of proximal tubular damage.

First of all, because the proximal tubule is a site of reabsorption of solutes, as a result of proximal tubular damage, we often see the impaired tubular reabsorption of these solutes and the appearance of these solutes in the urine.

Another important function of the proximal tubule is the secretion of organic ions. By secretion, I mean the movement or transport of substances from the blood across the tubular epithelium to the urine. This is a very important mechanism for the elimination of a wide variety of anionic and organic drugs, as well. So, when this section of the nephron is damaged, it can have profound consequences, not only on the physiological function of the kidney but on the distribution excretion of drugs and other xenobiotics.

In addition, another phenomenon associated with proximal tubular damage is the release of enzymes from the proximal tubular cells. We'll discuss this issue later a little bit more in detail.

If the damage is severe enough, to the point where, in fact, the entire tubule becomes necrotic, pieces of the cells actually slough off the tubule, move into the lumen of the tubule, and simply clog it up. Thus, at a sufficient level of damage, the release of debris into the tubular lumen can result in tubular blockage, which by itself can have important consequences.

Finally, a newer concept that has developed over the past few years has been the acknowledgment that when the proximal tubular cells are damaged, certain proteins or pieces or parts of the proximal tubule can enter the bloodstream and apparently generate antibodies to these proteins. These antibodies can then either bind to the proximal tubule or to components of the glomerulus.

These immune complexes then, themselves, can cause structural damage and impaired kidney function.

I mentioned the consequences of impaired reabsorption and secretion of solutes. I will reiterate, this essentially includes the excretion of most of the substances that are normally reabsorbed by this section of the nephron. So, we would expect to see decreased tubular reabsorption and increased excretion of glucose, increased excretion of amino acids, and increased excretion of low-molecular-weight and high-molecular-weight proteins.

In addition, Dr. Sands described for you how the loop of Henle is a very important site for the formation of concentrated urine. The loop of Henle can only concentrate urine, however, if the volume of solute and fluid leaving the proximal tubule is sufficiently low.

If it's too high because of proximal tubular damage and impaired resorption in the proximal tubule, then the loop of Henle cannot properly perform its function. Under these conditions the urine cannot be concentrated. What we call a concentration defect develops in which the animal or individual cannot form a concentrated urine. This can have an important impact on the water content.

Another consequence of the loss of reabsorbative capacity of the proximal tubule is that the solutes--well, first, let me back up. The reabsorption of solutes in the proximal tubule is important for the reabsorption of fluid, simply because the proximal tubule is completely permeable to water. As the solutes are absorbed either actively or passively, water follows because of the osmotic forces produced.

So, if solutes are not reabsorbed sufficiently in the proximal tubule, and those solids are not reabsorbed in the distal tubule, the solutes along with water will leave the kidney and you will have polyuria. That is an increased urine flow rate, i.e., diuresis.

In one particular model, we gave various doses of mercuric chloride to rats and examined the composition of the urine during the following 24 hours after the dose.

We gave adult rats three doses: 0.3 mg of mercury per kilogram i.v., 0.4 mg Hg/kg, and 0.5 mg Hg/kg. There was a very steep dose-effect curve for mercuric chloride. Going from 0.3 to 0.5 mg Hg/kg, you get a tremendous change in the excretion of solutes. As the dose of mercuric chloride increased, we saw the appearance in the urine of a number of solutes. The increased excretion of albumin may represent the impaired absorption of filtered albumin, or may possibly represent direct effects on the glomerulus so that more albumin is being filtered.

We also saw the appearance of amino acids in the urine, as exhibited by the appearance of reactable alpha amino groups, which are excreted in excess in the urine at the higher doses of mercuric chloride.

There was also the increased excretions of three enzymes. The first enzyme, gamma gt, is actually called gamma-glutamyltranspeptidase. This enzyme is located in the proximal tubule. Its highest concentration is in the more distant segment of the proximal tubule, which we call the proximal straight tubule or pars recta. It is located in the luminal membrane, i.e., the membrane that interfaces between the tubular fluid and the cell. It's a very important enzyme for the metabolism of glutathione. The next enzyme is lactate dehydrogenase (LDH). This enzyme is located within the cell and is important for the metabolism of lactic acid. NAG or N-actylhlucosaminidase is an enzyme located within a lysosomal vesicles within the cytosol.

So, we see the appearance of enzymes in various compartments within the cell, escoping into the urine in response to mercuric chloride; enzymes from the luminal membrane enzymes packaged in lysosomal vesicles as well as enzymes which are free in the cytosol.

I want to point out also that in these same animals, histologic abnormalities, i.e., necrosis, was apparent at the lowest dose of mercuric chloride (0.3 mg Hg/kg). However, the only index that is significantly elevated in these animals at the 0.3 mg/kg dose is the excretion of LDH. All other indices were negative. Yet, at that this lower dose, the proximal tubule cells were damaged.

So, the fact that you do or do not observe these biochemical indices in a urinalysis does not mean that the kidney has not been damaged, and that there is no loss of functional capacity. At the 0.5 dose, more than one half of the straight segments in the outer medulla are destroyed completely. They're entirely necrotic.

Just let me make a few more general comments on enzymuria. It's not clear to me exactly what enzymuria means--that is, the increased excretion of enzymes as a result of nephrotoxic insult.

There is some controversy over this. Perhaps the appearance of brush border enzymes like gamma-gt and alkaline phosphatasee represents membrane damage. Perhaps the membranes are sloughed off and fragments of membrane are excreted. This may explain the increase in excretion of enzymes located in the brush border.

Perhaps, however, the binding of the metal with the enzymes (it is known that metals can't interact with certain enzymes) leads to a change in the turnover of the enzymes or a turnover of the luminal membrane itself. This normally occurs in all cells undergoing metabolism and resynthesis. Perhaps this process is accelerated in the metal and results in the release of those enzymes into the urine. Clearly, if the cell is ruptured, as in the higher doses of mercuric chloride, cytosolic enzymes will appear in the urine. Thus, the appearance of enzymes that are packaged within the cell may represent a leakiness of the luminal membrane to these rather large-molecular-weight enzymes, or perhaps simply rupture of the membrane so that the cell contents

are actually extruded into the lumen and excreted in the urine, or perhaps more complicated mechanisms. It's not exactly clear to us how enzymes are terminated.

I mean, what are the signals and the regulation steps involved in degrading an enzyme? Part of the process clearly involves the interaction of the enzyme with lysosomes. Perhaps a change in the turnover of the lysosomes or a change in the transport of the lysosomes is involved in the extrusions of enzymes that are normally found in the cytosol.

That's an area of increasing interest, and it's obviously very important for toxicology because these enzymes hold promise for use as easily measurable, noninvasive indices of nephrotoxicity in humans. The more we understand about the process by which they are excreted, the better off we are.

I mentioned that if the damage is severe enough, one can then begin to see the extrusion of cellular debris into the lumen. These can appear as actual casts and can be visualized under a microscope. Eventually, this can be severe enough to the point where the tubule is blocked. Fluid cannot flow through the proximal tubule anymore. If filtration continues and eventually the intertubular hydrostatic pressure rises and net filtration pressure decreases, this can cause the glomerular filtration rate to decrease. When the hydrostatic pressure in the tubule rises to the point where it equals the net filtration pressure in the glomerular capillaries, filtration will stop. This is a very common experimental observation in studies in which large doses of metals have been given to produce severe structural damage.

If the glomerular filtration is sufficiently lowered, then we can actually see a decrease in the urine-flow rate. I mentioned earlier that the impaired reabsorption of solutes in the proximal tubule can result in diuresis; if the tubules become blocked, or for other reasons the glomerular filtration is sufficiently low, you can actually observe, can see low urine-flow rates in animals poisoned with heavy metals. In fact, eventually, the animals become anuric, where there's no urine flowing at all. At this point, all excretory function is stopped and we would consider that animal in complete renal failure. In fact, depending on the toxin and depending on the dose and depending on the time at which we look at the animal, one can observe these phenomenon, as all phases of polyuria, anuria, or oliguria.

In severe and perhaps even mild damage (I shouldn't say necessarily only severe damage), there is evidence now that when the tubule is damaged, components of the tubule, perhaps proteins, can serve as antigens for the formation of antibodies within the body. The body is essentially forming antibodies to itself. These antibodies can deposit in the glomeruli and change the filtration characteristics of the glomerular filter.

In fact, there is some evidence that they can directly affect the tubular transport function of the proximal tubule. For example, if you take the kidney cortex and you homogenize it and inject it into a rat, this injection of proteins from the proximal tubules will generate an autoimmune response, which can have profound effects on the tubular secretion of anions in the proximal

tubule. In addition, there may also be a component of direct glomerular structural dam[illegible] which can result from metal toxicity.

Let me mention one very intriguing subject. That is the phenomenon of tolerance to the proximal tubular effects of metals. By "tolerance," I mean that if animals are challenged with a low dose of a nephrotoxin, say, mercuric chloride or uranium compounds, and then are given a large dose of the toxin, which is definitely a high enough dose to produce structural damage and toxicity, the animals given a second dose of the toxin will respond less than other animals given only one dose.

We call this tolerance. It's not clear exactly what the mechanism is, but there are at least several possibilities. In the case of the tolerance to mercuric chloride, it's been demonstrated that a component of that is an altered disposition of the metal. That is, if the animal is given a second dose of the mercuric chloride, a smaller fraction of the dose actually ends up in the kidney. Whether this contributes to the decreased response is not clear.

Secondly, within days, or even hours after structural damage occurs to the proximal tubule, the tubules can begin to regenerate. Eventually, the remains of the proximal tubular cells are regenerated.

It's possible that these regenerated tubules either handle the metal differently from normal, undamaged cells or are simply less responsive to the toxic effects of the metal. It is clear they are structurally distinct from the normal proximal tubular cells. They lack, for instance, a brush border. They may lack other transport processes or contain ligand reactions that are important for the expression of the toxicity within the cell. The mechanism is really not clear.

However, it is an important issue because we can demonstrate in animals that the indices of toxicity that we're looking for in the urine may not be there in an animal that has been challenged previously with a dose of a metal toxin. This is another reason to be conservative and concerned about the lack of appearance of positive indices of damage in urinalysis data; it does not mean there is no damage to the kidney.

Another interesting phenomenon is cross-tolerance. You can make an animal tolerant to mercuric chloride by pretreatments with a wide variety of proximal tubular toxins. So, one requirement for producing cross tolerance appears to be the proximal tubular damage. Damage to the tubule probably results in the repopulation of the nephron with cells that have a cultured sensitivity to further damage.

I mentioned that in addition to effects on the proximal tubule there are also effects on the glomerulus. We can classify these as indirect or direct effects. An example of what might be an indirect effect would be the proximal tubular damage resulting in release of antigens, the formation of the auto-immune complexes, and the change of the glomerular filtering characteristics. That would be one example of an indirect effect.

In addition, it is known that metals probably can produce direct adverse effects on the glomerular endothelium. Many metals--cadmium, for instance--in certain tissues is a notorious endothelial toxin and can change the permeability characteristics of vascular endothelium. It is possible that a component in the change in glomerular function that one sees in animals exposed to nephrotoxins might be due to direct effects of the metal interacting either with the endothelium or the epithelium in changing the permeability characteristics.

One consequence of decreased permeability of the glomerulus is a decreased glomerular filtration rate and oliguria. If the pores in the glomerulus which restrict the entry and filtration of proteins across the glomerulus are sufficiently widened by a change in, say, the charge in the basement membrane of the glomerulus or because of damage to the actual glomerular epithelial cells, one can produce an increased filtration of large-molecular-weight serum proteins--albumin, for instance. This may contribute to the albuminuria that one sees in animals given nephrotoxins, in addition to the decreased tubular reabsorption of filtered albumin. So, the decreased glomerular filtration rate and the increased glumerular sieving of proteins would be two consequences of glumerular toxicity.

You can see that the effects of metals on the kidney are complex. They involve many components. But certain things are also very clear.

It is clear that with doses of mercuric chloride or cadmium chloride you can get proximal tubular damage, and it is clear that there are several consequences of proximal tubular damage. First, it is clear that the result of the proximal tubule is decreased tubular absorption of solutes, including sodium. Either as a result of the direct increase in the delivery of sodium to the distal nephron or perhaps due to the loss of solutes in the body, the extracellular fluid volume can become contracted. This can lead to vascular constriction. This could be due to a stimulation of the release of the enzyme renin from the juxtaglomerular cells, which were alluded to earlier by Dr. Sands. That is, as a response to a decrease in the extracellular fluid volume or an increase in the sodium concentration in the distal tubule, renin secretion is stimulated. This can result in the production of some very potent renal vasoconstrictors, which can constrict the arterioles of the glomerulus and decrease blood flow. And they can decrease renal blood flow sufficiently to initiate ischemic damage to the kidney.

The proximal tubular damage can also lead to auto-immune reactions which might cause damage to the glomerulus, increased protein sieving, and delivery of the protein to the proximal tubule in the urine. It can also result in total obstruction of the lumen. Furthermore, the changes in tubular hydrostatic pressure can decrease the glomerular filtration rate and essentially lead to oliguria or complete renal failure.

This concludes the general summary of the kinds of effects that can be produced by metals.

At this time, then, I would like to just briefly consider certain other special characteristics of metals that make them unique as nephrotoxins and are important in terms of the understanding of why metals are nephrotoxic. There are four characteristics important to consider: metals as elements, oxidation reduction reactions, organometallic complexes, and reversible reactions with ligands.

My point about metals being elements is that, unlike organic toxins, metals are virtually indestructible in the body. Once they are in the body, the metal itself cannot be transmutated. This means that detoxification for metals involves two processes: excretion for elimination of the metal from the body, or complexation of the metal with a ligand which might render the net less toxic than the free metal. That is not true for organic toxins. For many organic toxins, the detoxification process can involve the enzymatic altering of the organic structure in addition to excretion.

Oxidation and reduction states of metals. An example is the redox states of mercury which can exist in biological solutions: Hg^0, Hg^+, Hg^{++}. The oxidation state of the metal can have an important impact on the disposition of the metal in the body and on the toxicity. Hg^0 is a relatively lypophyllic substance. It can permeate lipid membranes of cells relatively easily, and enter into the brain, and is therefore a notorious central nervous toxin. Hg^+, which would perhaps be exemplified by methyl mercury, is not as soluble as mercury vapor but can also enter a variety of tissues in addition to the kidney and has its own specific pharmacological properties. Mg^{++} is relatively lipid in soluble compound. In fact, when given to animals as, say, in mercuric chloride, it is distributed to the kidneys primarily. This memory can exist in any one of these oxidation states in biological systems, and this can have great impact on the toxicity.

Organometallic compounds. Again, I am going to use a mercury metal as an example here, but one can find examples for other metals as well. For, RCCC-mercuric-plus-1 compound, one of its important characteristics is that it is a diuretic. It promotes sodium reabsorption in the loop of Henle. I am not talking about destruction of the loop of Henle here; I am talking about a pharmacological effect that occurs in minutes after the substance is injected. That is because this substance can interact with the sodium transport mechanism in the loop of Henle of the renal tubule. On the other hand, methyl mercury, also an organometallic compound, is notoriously a central nervous system toxin. The divalent mercuric ion, exemplified by mercuric chloride, is a very weak diuretic, but it is a proximal tubular poison, as we saw, and it can produce diuresis if the proximal tubule is damaged sufficiently. Thus, metals can undergo complex organic complexations, either outside the animal or inside the animal that can alter the toxicology of the metal.

Metal ligand complexes are extremely important in the chemistry and biological chemistry of metals. They are very inte[illegible]ly studied, and there is an enormous literature on this subject. I am jus[illegible]ng to discuss, briefly, a

few points about this because, for one thing, this bears very directly on the issue of uranium toxicity and probably--not probably, definitely--on the toxicity of most metals.

What I am referring to is that metals can interact with a variety of biological molecules in the cell in reversible reactions. Cd^{++} can interact with thiol groups of proteins and nonprotein thiols and do so reversibly. These ligands may be critical target sites of enzymes or transport proteins involved in maintaining functions. They may be important in the development of toxicity or may be important for the detoxification of metals. These ligands can occur, of course, in the circulation within the cell membrane and also within the cell.

This is an example of a potential ligand interaction that is important in the renal handling of uranium. It was reported many years ago by Newman. He hypothesized that uranium might exist in the plasma as a complex of uranium with proteins and bicarbonate. This is a reversible complex, so that the compound in equilibrium with this would be a uranium bicarbonate complex.

The properties of the glomerulus restrict the glomerular filtration of proteins. Uranium associated with bicarbonate would be expected to be highly permeable and would thus gain entry to the tubular lumen.

It was suggested--and there is evidence to support this--that as long as the uranium remained as a complex with bicarbonate, it enters the kidney and associates with the kidney rather poorly. But as Dr. Sands discussed, as the tubular fluid is processed in the proximal tubule, the bicarbonate in the tubular fluid is titrated with protons, resulting in the production of carbonic acid, CO_2. When this occurs, the uranyl ion is then liberated and in this form is able to bind to or enter tubular cells. In support of this, it can be shown that an increase in the filtration of bicarbonate increases the amount of injected uranium that accumulates in the kidney. If the urine is acidified so that the filtered load of bicarbonate is low, less uranium is accumulated in the kidney.

Another example of this would be the interaction between cadmium chloride and cysteine. CdO_2, when injected into the rat, is a very potent testicular toxin. But if CdO_2 is injected together with the cysteine, it becomes a much more potent nephrotoxin. Presumably, there is some interaction between cadmium and cysteine that produces an altered disposition of cadmium or an altered toxic response.

Within the cell, of course, the metals can interact with a variety of ligands. The interactions of cadmium may bear on many other metals--mercury, zinc, and perhaps uranium, as well. Cadmium, like many divalent metals, is a thiol reagent, and it can interact with glutathione or cysteine. Cadmium can also interact with protein thiols, such as those of metallothionein.

If I had another hour, I would talk to you about metallothionein. This is a fascinating protein. If you wanted to build a protein for the purpose of

binding metals, this is the way to build it. But nevertheless, the interaction with metallothionein may represent an important toxification mechanism.

Finally, cadmium combines with membrane proteins. One of the most important groups of proteins would be thiol containing transport proteins in the mitochondria and the cell membrane, such as the transport ATPases. These proteins are vital to the life of the cell, since they create gradients necessary for establishing the energy for all transport processes. They all have thiols located at the active centers. Mercury, cadmium, and many of the other heavy metals that interact with thiols can interact and inhibit the activity of these enzymes.

I will just summarize with what is left, which I believe are at least some of the important issues facing the metal toxicologist today. Some of them we discussed in a portion of this paper.

First of all, in what form is the metal delivered to the kidney? That is an important consideration in terms of how it exerts its toxicity, e.g., in what site in the nephron it exerts its toxic effect.

Where and how is the metal accumulated in the kidney?

What transport processes are involved? What ligand interactions are important for toxicity?

And, finally, because metals are indestructible and the only process for detoxification is elimination of the metal from the kidney, a critical point in our understanding of the toxicology has to be the understanding of how that metal eventually leaves the kidney and exits the body.

Thank you.

R. ALEXANDER: Dr. Diamond, going back to Dr. Sands' presentation this morning, he showed us how in the proximal tubules the uranium could essentially circulate. Then, on the other hand, I'm sure uranium is incorporated into the cells to some extent, also. Is it true that the chemical toxicity of uranium, then, would be due to the circulating uranium in the proximal tubules, and that the intracellular uranium kidney cancer would be the principal problem there?

G. DIAMOND: I would envision, and this is my own perspective, but the toxicity of the metal, of any metal, would result from some interaction between the metal and some ligand in the kidney. Whether that ligand might be an enzyme located at the interface between the blood and the cell, or the tubular fluid in the cell, or a ligand within the cell is difficult to assess for any metal; because one of the ways one would assess that is to look at how a function of the cell and the nephron changes in response to the metal and openly tries to isolate this component. Those proteins are enzymes that might interact with the metal. That affects that function. So, you have an ATPase, for instance. In response to metals one sees changes in the sodium reabsorption and can see in the isolated epithelia changes in sodium reabsorptions. Well, if you take out the ATPase and look at the effects of metals on it,

they're inhibitors. In fact, they will inhibit any enzyme. The problem is translating that into what the target site is in the cell or on the cell. I'm not sure I answered the question. Was that what you were getting at?

R. ALEXANDER: Maybe I can come at it from a slightly different way. For the retention function that we use for uranium in the kidney, then, are we describing a complex phenomenon that involves intracellular uranium and the release of intracellular uranium and the excretion from the circulating uranium in the proximal tubules?

G. DIAMOND: I would think that would be the release of uranium from the tissues. Certainly.... Oh, I see. Okay. Are you asking whether the uranium that comes in the urine is due to uranium leaving the extracellular fluid plasma circulation, as opposed to the uranium which flows directly from the tissue?

R. ALEXANDER: Or more likely, a combination of both.

G. DIAMOND: I'd have to say, possibly, a combination of both. For mercuric chloride, mercury, there have been attempts to identify the source of mercury that's excreted in rats and dogs that have been given mercuric chloride. I'm not sure really an attempt has been made in uranium research.

One approach to this has been to give an animal, say, $^{200}HgCl_2$, the nonradioactive isotope, and then follow it with a tracer dose, and then progressively in time measure the specific activity ratio of isotope ^{203}Hg to ^{200}Hg in the tissue and in the urine. One finds that after about three or four days, or actually less than that, the urine-specific activity reaches a plateau which is equivalent to the specific activity and different from the specific activity in the literature.

This doesn't give you a specific answer but one interpretation of that is that it's consistent with the idea that most or all the mercury in the urine is in the kidney, and is coming from some pool that is in the kidney tissue. And I just don't know whether that's been done or not for uranium.

The problem that is a very important issue, that is, the localization of metals in the kidney, is a tough thing because, again, if you want to look at an enzyme cell, you isolate various components of the cell and centrifuge them and find out where the activity is. But these metals are so reactable to thiols that just because you find them associated with the thiol protein does not mean that it had access to that protein in the cell. Perhaps the cadmium came in some other area.

R. ALEXANDER: One additional way to look at the same problem: Would you say, then, that all the damage mechanisms that you discussed with us this morning occur as a result of the metal in extracellular fluids?

G. DIAMOND: No. I would suggest that these are due to interactions of the metal with some ligand. Now, clearly, there are these indirect effects which can occur if the calcium is sufficient to block channels to the heart;

then, indirect cardiovascular effects might be observed. And I think, with these large overdoses, we're talking about interactions between ligands on the cell and in the cell. That's my own...that's where we're looking, and that's where I would look if one wanted to look at that and research it.

J. FOULKE: Isn't this complicated by the fact that the other heavy metals exist as the simple ions, but the uranium as the UO_2^{++} the radical?

G. DIAMOND: I think that's true. I'm not an inorganic chemist. I wish I was, but inorganic chemists tell me this is Cd^{++}. It does not exist in the biological fluid. It will always exist as a ligand with something. I'm told that mercuric is actually difficult to see in biological fluids, first of all because of its affinity for chloride.

So, in the matters that I'm more familiar with than cadmium and mercury and zinc, the divalent, freely ionized metal species are probably a rare form of metal because of the tremendous abundance of ligands. The free thiol group on albumin alone is 1-million mmol. If you add up all of the potential nonprotein thiols, the potential reactions that cadmium can undergo, you've got concentrations in the 5-10 mmol range for cadmium and other metals. So, I don't see that as a distinction for uranium per se. I think most metals probably interact with a variety of ligands.

R. ALEXANDER: Dr. Paul Morrow, whom we had planned to have with us today, was not able to be with us from the University of Rochester. However, Dr. Diamond, whom we've just heard deliver his paper, has agreed also to deliver Dr. Morrow's paper. So, let me introduce again Dr. Gary Diamond from the University of Rochester, to talk to us now about the nephrotoxicity of uranium as determined from animal studies conducted at the University of Rochester.

G. DIAMOND: Animal studies have provided an invaluable data base for understanding the metabolism and toxicity of uranium. The largest effort in these regards was accomplished during World War II and has been reported in the monograph series edited by Voegtlin and Hodge (1).

Largely from these studies, a number of general findings and conclusions were made which continue to have a major impact on the control and interpretation of human exposure to uranium. Some of these will be described in this presentation.

More recently, Hodge, Stannard, and Hursh published a book which contained several review articles of pre-1970 studies of uranium in both laboratory animals and man (2). In 1975, Durbin and Wrenn also prepared a review of uranium metabolism and the bases for evaluating acute and chronic uranium toxicity (3).

Since 1970, there have been additional studies, mostly in laboratory animals, which have been important in reconfirming and enlarging much of the extant informational base. In a few instances, these recent studies have provided new data and raised some questions about previous findings and interpretations (4-15).

This presentation will briefly summarize some of our general understanding of uranium metabolism and toxicity before examining in more detail the nephrotoxicity of uranium.

GENERAL STATEMENTS REGARDING URANIUM TOXICOLOGY

First, there are two broad classes of uranium compounds which distinguish both their chemical and metabolic behavior, viz., the uranic compounds in which uranium exists as a U^{+4} ion and the uranyl compounds in which uranium is a UO^{++} ion. These are the only stable forms of uranium in aqueous solution. There are several uranium compounds which appear to lie somewhat ambiguously in between these two major classes: uranium tetrahalides and triuranium octoxide (U_3O_8) are two notable examples (2,10,12).

Secondly, most of the uranic compounds have relatively limited water solubility and in the body are typified by relatively limited absorption and transportability. Consequently, in either acute or chronic exposures, their dosimetry is largely limited to their route of administration, i.e., to the gastrointestinal tract or lungs when injected or inhaled, respectively. Of the two, the inhalation-exposure route is far more important. In chronic exposures, cumulative translocation processes also result in significant uranium doses being delivered to other sites, e.g., lymph nodes, bones and kidneys, but almost invariably the exposure limitation is based on lung dose. The translocation processes which affect the retention of uranic compounds appear to depend upon particle transport, such as in the case of the lymph nodes, and the oxidative conversion of the uranic state to the uranyl state whereby absorptive transfer and systemic uptake occurs, such as in the skeletal and renal accumulations.

In contrast, uranyl compounds are generally water soluble. In biological systems, they manifest a much greater transportability so that the renal and skeletal accumulations have the greater toxicologic significance than sites associated with the route of administration.

Thirdly, the general toxicology of uranium compounds is a composite of chemical and radiation effects. If one considers either depleted uranium, or natural uranium on an acute or subacute basis, then it is possible to generalize that one cannot produce a radiologic limitation, that the chemical toxicity prevails with the kidney effects being the most restrictive. On a chronic exposure basis, the picture becomes fixed. In the lungs, the effects may be due to a combined toxicity (fibrosis and malignant tumors), whereas the renal toxicity appears to remain chemically induced.

When the specific activity of the uranium is increased, the toxicity picture shifts toward radiation-induced effects, and this is clearly so when the isotopic mixture is equivalent to about 6 or 7% enrichment (^{235}U). This does not infer that the chemical toxicity disappears, but that the dose limitation is based on the maximum permissible radiation dose to the organ, not the chemical dose.

Fourthly, the behavior of both uranic and uranyl uranium compounds appears to differ among several species. These differences are almost entirely quantitative not qualitative. With few exceptions, therefore, it is possible to scale one species dose-effect relationship to another by a simple numerical factor. This general finding gives us confidence to extrapolate findings from animal studies to man. We must keep in mind, however, there are known exceptions to this generalization which occasionally are important.

NEPHROTOXICITY OF URANIUM

Inasmuch as the prevailing view of the chemical state of uranium entering the mammalian kidney is the uranyl ion, UO^{++}, it would seem, from the nephrotoxicity viewpoint, that the initial form of the uranium entering the body is relatively unimportant except for the quantitative distinctions produced at the renal level. From this same perspective it follows that various uranyl compounds should have a very similar nephrotoxicity in a given specie. For the purpose of this presentation, both of these concepts are assumed to be valid: this allows us to focus our attention on UO^{++} and to generalize the biological information.

Despite the attention given to this subject experimentally, many aspects of uranium toxicity to the renal nephron are not understood. To put these into context, this portion of the presentation will begin with a resume of past studies and will be followed by a description of important areas where the information is uncertain. Both of these summaries will also be interpreted in terms of present NRC procedures and concerns.

Findings of Early Studies (pre-1970)

Based on early animal studies, an 11% uptake value was accepted for describing the fraction of the absorbed uranium which goes to the kidney. Also, a 15-day effective half time was determined for the retention of U^{6+} uranium in the kidney. This implied that 4.6% of the renal uranium level would be excreted daily. A review of the early animal data also led to the decision that 3 g U/grams of kidney was the threshold concentration for chemically-induced renal injury [Spoor and Hursh (16)].

From these early determinations, the worker protection criteria were developed for U^{6+} or the so-called "soluble" uranium compounds. The essence of these standards was that at the MPC levels, the intake of U^{6+} would equal the urinary output of U^{6+} when the steady-state renal concentration was 3 g U/g.

Subsequently, investigative and action levels based on urinary U^{6+} excretion were derived by the NRC for uranium conversion plant and mill workers on the foregoing criteria, but the "...more highly transportable compounds UF_6 and UO_2F_2..." were excluded from these bioassay criteria [USAEC Regulatory Guide, June 1974 (17)].

Findings from Recent Studies

1. Uranium (U(VI)) Retention

The renal retention of uranium has been found to be more complex than inferred by the 15-day half time obtained from early studies. For example, studies with UO_2F_2 in rats [Morrow et al. (1981)] showed that the renal retention of uranium was more prolonged following multiple administrations ($T^{1/2}$ = 32 days) compared to a single administration ($T^{1/2}$ = 16 days). This study and some of the early studies indicated that the uranium retention time in the kidney tended to increase with time. This effect was vividly demonstrated by the two-year study of Stevens et al. (4) with uranyl citrate in dogs in which a 79.5-day renal half time was measured for about two-thirds of the renal burden.

2. Nephrotoxic Threshold

The UO_2F_2 toxicity studies performed by Morrow et al. (6) in dogs and rats confirmed the earlier finding with U^{6+} compounds of different species susceptibilities. In the UO_2F_2 study, the dog was found more susceptible to the nephrotoxic action of uranium than the rat, but otherwise the course and nature of the nephrotoxic response were similar. In these short-term studies, peak uranium concentrations of around 0.5 g U/g in the canine kidney were associated with definite histologic and functional abnormalities. From these studies, the earlier rat studies (1) and the human studies with intravenous uranyl nitrate (2) one can deduce that humans probably lie in an intermediate position to the dog and rat regarding nephrotoxic susceptibility.

3. Urinary Indicators

In the UO_2F_2 studies of Leach et al. (11) and Morrow et al. (6), a relatively extensive examination of urinary indices of renal injury was undertaken. These studies stressed the important distinctions found in naive and previously-exposed subjects. These distinctions were especially apparent in relation to the urinary indicators of acute uranium-induced renal injury.

An example of these studies is presented in Table 5 where urinary volume, glucose, lactic dehydrogenase, N-acetyl glucosaminidase, and catalase excretion are represented temporally and in relation to concommitant histopathologic evaluations following an acute inhalation exposure to UO_2F_2. The absorbed uranium dose averaged 220 μg, indicating about a 30-g U/g renal level. Significant changes (p 0.05-p 0.001) are indicated by the asterisks. Questionable changes by the (?) symbols. Incidentally, the more "traditional" indicator, proteinuria, was significant only between 4 and 6 days post-administration.

Repeated U^{6+} administrations affected selected urinary indicators as follows. In each case, repeated small administration of UO_2F_2 produced either equivocal changes in some indicators or small but significant changes. When a "challenge" dose was administered (a dose of known injury potential), some indicators proved to be relatively refractory as though a "tolerance" had

developed. The same challenge given to a naive animal produced a pronounced response in the same indices. With other indices, the challenge dose induced a major response in the multiply-dosed animals as though no tolerance existed.

Besides these complexities in urinary indicator responsiveness, these studies also examined a number of additional indicators and compared them to the more conventional urinary criteria, e.g., proteinuria.

4. Sites of Nephrotoxicity

By virtue of urinary indicator studies, it is possible to determine the sites of uranyl ion toxicity in the nephron (5,6,11). Collectively, these studies indicate that almost all segments of the nephron can be involved acutely. These studies and recent histologic studies (13,15) confirm the general finding that the earliest changes occur in the inner renal cortex which can be interpreted as involving the distal or terminal portion of the proximal convoluted tubule.

The pars recta of the proximal tubule (P2 and P3C segments) were the areas most consistently damaged by uranium nitrate, according to Haley and coworkers, exhibiting diffuse focal brush border loss and increased vacuolization as early as 1 hour post-administration (13). By five days, cells in the P2, P3C and P3M segments had become necrotic while some cells of the P1 segment were vacuolated and the distal nephron segments exhibited swelling and vacuolization. Many of the tubules became dilated and lined with squamous epithelial cells and by eight weeks, tubular regeneration was virtually complete, but atrophic proximal tubules, interstitial fibrosis, and mononuclear cell infiltration, consistent with a chronic type of injury, continued beyond this time (15). These histopathologic studies in rats by optical and electron microscopy were based on a single 10-mg/kg uranyl nitrate injection and eight weeks of follow-up studies.

Some renal function tests (5,6) and electron microscopic findings (13,14) both indicate glomerular injury by uranyl compounds. Glomerular damage has also been described by others (18,19).

Finally, there is also histologic and functional evidence that the distal tubules, Henle's loop, and collecting tubules are adversely affected by uranyl compounds (5,6,15).

5. Regeneration and Repair

As indicated in III.B.4., the injury and regeneration patterns induced by UO^{++} overlap in time. With minimal acute injury, there is evidence of regeneration histologically as early as 48 hours after UO^{++} administration. The pattern of regeneration is spotty and occurs with co-existent necrotic nephrons and concommitant evidence of renal dysfunction (6).

Several observations stand out regarding repair in the histologic studies of UO_2 nephrotoxicity. The regenerated tubular epithelium is squamous in character and basophilic with short microvilli on the terminal surface often arranged in clumps. Many tubules remain dilated after regeneration is

TABLE 5. Sequential Data Following UO_2F_2 Inhalation

Time, days	Histological Findings	Urinalysis Findings: Urine, mL/24 hr	Glucose, mg day-1	LDH, units/day	NAG, units/day	CAT, units/day
0 (control)	Normal	7.2±4.5	4±5	0.07±0.02	7.4 x 10^3 ±6.4 x 10^3	11.7±7.4
2	Rare degeneration of tubular cells	4.8±1.5*	88±107	0.87±0.68*	21 x 10^3* ±18 x 10^3	29.2±21.7
3		11.3±2.5(?)	946±308*	0.75±0.71	33 x 10^3* ±15 x 10^3	84.8±48.9*
4	Severe tubular necrosis (inner cortex)	23.4±6.2*	2366±612*	0.79±0.40*	47 x 10^3* ±17 x 10^3	138±58*
6	Several tubular necrosis some early regeneration	30.3±3.7*	692±656(?)	3.5±1.4*	22 x 10^3* ± 6 x 10^3	386±132*
11		12.6±4.0*	1±0.9	0.27±0.04*	12 x 10^3(?) ± 8 x 10^3	22±3.6*
14	Extensive regeneration Dilated tubules lined with low cuboidal epithelium	11.1±0.9*	1±0.3	0.16±0.04*	12 x 10^3 ± 4 x 10^3	23±10(?)

advanced. The collective evidence suggests that nephron loss may normally occur so that renal repair may be incomplete. Injury characterized by interstitial infiltrations, and fibrotic changes and the flattening and broadening of the podocytic processes of the renal corpuscle may also represent persistent changes which conceivably have importance in the long-term status of the kidney, but this has not been established (13,14).

SUMMARY AND CONCLUSIONS

Despite the fact that uranium is one of the most thoroughly studied heavy metal toxins, there remains much we do not understand about its nephrotoxicity.

New experimental studies of uranium retention in the kidney and of the threshold level for uranium-induced nephrotoxicity both indicate that our currently accepted exposure levels are too high.

Because of species distinctions and the differences in uranium excretion and metabolism related to the exposure history of the subject, we are not presently able to determine how excessive our present levels are. Dog studies, for example, indicate a factor 10 whereas rat studies suggest a factor of 2 or 3 may be more appropriate.

Findings related to urinary elimination of uranium and urinary indicators of uranium injury are also complicated by the exposure-history effect and the so-called tolerance phenomena; consequently, although current bioassay procedures are brought into question, few specific modifications for improved procedures and interpretation are available at the present time. Nevertheless, the current armamentarium available for measuring renal injury through urine analysis provides convincing evidence that uranium produces a diverse, temporally-varying pattern of nephrotoxic actions. This strongly suggests that improved urinary indicators of renal injury will be forthcoming.

One of the serious limitations of the animal studies is in the chronic toxicity area. Virtually no studies have reckoned with prolonged exposure effects and the concurrent regeneration of the kidney.

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J. FOULKE: I guess there are no questions for Dr. Diamond.

We would like to thank -- oh, there are.

Q: You mentioned somewhere that Morrow said the human fell between the dog and the rat, and I missed what human data he was basing that on.

G. DIAMOND: Besides human data, there are many references.

Q: Are they the injection data?

R. ALEXANDER: Those people were terminal, I think.

G. DIAMOND: Let me see. Bob, the injection studies were of terminal cancer patients.

Q: More or less terminal, yes.

G. DIAMOND: They are summarized in the review. I am personally not familiar with these studies. I assume they must have had data on the uranium tissue content, which would only be obtained by biopsy or necropsy. I am not familiar with them, but I can give you the citation.

R. ALEXANDER: It is a very significant question because Dr. Morrow has pointed out that the extrapolation to the dog would involve a factor of 10 for dog and a factor of 2 to 3 for rat. Then, when he says that man lies somewhere in between, that tracks the factor of 5 that he is recommending that we reduce our limit by.

J. FOULKE: There will be new human data coming to light at this meeting today.

R. ALEXANDER: I have one question to Dr. Diamond, if you would happen to know about the indicators. Dr. Morrow has pointed out to me several times that he feels that proteinuria is not the best indicator. Studies he did for us with UO_2F_2 verified that and further work needs to be done. The slides you just showed reveal that glucose, urinary glucose, would be a much better indicator than proteinuria. But here is the question. Proteinuria, I believe, results from actual destruction of tubule cells. So it is a direct indicator of kidney damage from uranium. Is glucose a direct indicator of kidney damage?

G. DIAMOND: I am not so sure that proteinuria has to result from destruction of a cell because a major component of proteinuria can be and often is serum proteins, which may escape into the urine because either the barrier for filtration has been altered, in which case there may not be any destruction of the tubule, or the resorptive process for the protein is here.

R. ALEXANDER: Do you think glucose is an indicator of kidney damage or an indicator of the presence of high concentrations of uranium in the kidney?

G. DIAMOND: It would be an indicator of an impaired transport of glucose, an impaired function, whether that is secondary to or a structural alteration or just the emission of the transfer protein by interaction with uranium. I would suggest that also may be related to the urinary uranium content at that specific location in the kidney, although that is kind of difficult information to get. It is one thing to be able to get the cortical content in grams, and it is another thing to ask what the content is at the site of glucose transport. It is a very difficult problem. So, I would suggest that it reflects impaired function of the tubule as long as the glucose levels in the blood have not gone up, and in these studies you can demonstrate that the glucoseuria occurs without concentrations of the glucose in the blood.

He finds, from my latest conversations with him, that the earliest indication that coexists with structural damage is LDH excretion. It may vary with the metal perhaps as site of damage.

J. FOULKE: Thank you very much.

Q: Excuse me. Is there a reference to this paper, the one that you just gave?

G. DIAMOND: There is NUREG/CR-1045 from 1980. That is the second of the two.

J. FOULKE: So, I would like to introduce now Dr. Robert Moore, who is Director of the United States Uranium Registry. He is going to talk on some human findings. We have all been waiting for you.

R. MOORE: Thank you, Judy.

The United States Uranium Registry (USUR) was established in the spring of 1978 by the Energy Research and Development Administration (ERDA) and funding has been continued by ERDA's successor agency, the Department of Energy. This

program is managed by Hanford Environmental Health Foundation with health physics support supplied by Pacific Northwest Laboratories (operated for the Department of Energy by Battelle Memorial Institute), and tissue analysis performed by Los Alamos National Laboratory. When established, the USUR was given three mandates: 1) to characterize the uranium fuel cycle as related to electrical power production, 2) conduct a feasibility study regarding a United States registration of all uranium workers, and 3) to establish a tissue-donation program patterned after the successful program operated by the U.S. Transuranium Registry.

The current thrust of the USUR is this third mandate and this paper, which compares kidney histology among nine individuals, four of whom had known occupational uranium contact, comes from that effort.

TISSUE DONATION PROGRAM

The U.S. Uranium Registry tissue donation program began in 1980 when premortem commitment for postmortem tissue donation was obtained from six individuals. This is a voluntary program, and the agreement is signed between the individual and the U.S. Uranium Registry. On occasion, individuals have been interviewed at the work site but the employer is in no way involved in this commitment. Indeed, an employer knows of an employee's participation in the program only if the employee tells him. Participants also sign permission for release of medical and health-physics information, and such information is obtained at the request of the registrant. It should also be noted that the postmortem tissue-commitment agreement is also signed by an individual's next-of-kin.

This paper represents a specialized study from material from one segment of our registrants.

MATERIALS FOR STUDY

The four individuals forming the basis of this study were involved in fuel production for reactors designed to produce plutonium in the Cold War effort established in 1951. These individuals had worked with uranium for an average of 23.5 years (range 17-27 years). All four individuals worked in this capacity until retirement. Three of the individuals worked exclusively in fuel fabrication while a fourth was in supervision and management in a number of capacities, including fuel research and development, engineering, and production. Table 6 shows age at death, year of death and cause of death of the four uranium workers.

For comparison, five individuals were selected from autopsy cases at a local area hospital. Table 7 shows the age at death, year of death and cause of death of these five individuals who had no known uranium work history. All subjects were male and none died as a result of kidney disease. Kidney tissue blocks on all nine cases were stained using a hematoxylin and eosin technique and a trichrome (connective tissue) technique (a trichrome section could not be

TABLE 6. Status of Death of Four Uranium Workers

Worker	Age at Death	Year of Death	Cause of Death
T-54	68	1982	Amytrophic lateral sclerosis
T-51	68	1982	Lymphocytic lymphoma
J-45	71	1982	Mesothelioma
W-51	63	1984	Acute myocardial infarction

TABLE 7. Status at Death of Five Control (non-uranium) Workers

Case	Age at Death	Year of Death	Cause of Death
A-12	75	1983	Acute myocardial infarction
A-13	60	1983	Streptococcal pneumonia with sepsis (diabetic)
A-15	70	1981	Aortic valve replacement congestive heart failure, Gr IV
A-17	69	1983	Acute pancreatitis
A-47	64	1981	Acute trauma, fall from horse

obtained on one of the comparison cases). All kidney sections were sent for review to a pathologist skilled in determining histologic kidney changes as a result of uranium in both man and laboratory animals. The only additional information given the pathologist was that four cases were uranium workers, but the specific slides were not identified.

ESTIMATED URANIUM EXPOSURE TO THE KIDNEYS

Uranium exposure to the kidneys of the four occupationally exposed individuals was estimated from their health physics records and occupational histories.

The first case, T-51, is likely to have received little or no occupational exposure to uranium. Although this individual was employed at various jobs involving uranium for almost 27 years, his potential for exposure is considered minimal, a conclusion supported in part by the fact that he was not placed on a routine bioassay-sampling program and by his job titles, which at one time or another were professional, supervisory, or administrative in nature. However, he did work in uranium recovery operations as an engineering shift supervisor from 1951-1958, including three years in the UO_3 recovery plant. From 1959 to his retirement in 1978, he was involved in fuel fabrication, primarily in engineering or administrative capacities rather than directly involved in operations.

The only in vivo uranium bioassay data for this individual were obtained in May 1975, when both a uranium urinalysis and in vivo counts (whole body, head, and chest) were performed. Uranium was below detection limits in all cases (1 g/L for uranium in urine; 1 g for in vivo counting). Postmortem radiochemical analysis of various tissues indicated concentrations of uranium at levels typically associated with normal dietary intake--i.e., so-called "background" levels (ICRP 1975; NCRP 1984; Wrenn et al. 1985). Hence, it is reasonable to assume that occupational exposure to uranium in this case was at or near zero or in any case below detectable limits.

Case T-54 was employed from July 1954 to October 1970 in several jobs involving uranium fuel production. Review of this occupational history and health-physics accords reveals no evidence to suggest that this individual incurred a significant intake of uranium or any other radionuclide. The urinalysis schedule and work history suggest that exposure, if any, was most likely during the early years of his employment when he was largely engaged in fuel-finishing operations. There was no indication in the health physics records of accidental or other high-level exposure to uranium. During the first four years of his employment (mid-1954 to 1958), 40 urine samples were analyzed for uranium. Uranium concentrations in these samples were in all cases 4 g/L with values typically on the order of 1 g/L or a daily excretion of 1.4 g based on the Reference Man urinary volume of 1.4 L per day. This is approximately the amount attributable to dietary intake (NCRP 1984). Moreover, many samples were "paired," i.e., collected at the end of one work week and the start of the next. In general, there was no difference in these paired samples, suggesting that the output of uranium was more or less constant, hence from dietary sources rather than occupational exposure. Thus, based on this urinalysis data, it is unlikely that this individual incurred any significant occupational exposure to uranium. This conclusion is supported by postmortem radiochemical analysis tissues which reveal no significantly elevated concentrations of uranium relative to the range of levels reported as "background."

Case W-51 also had long-term employment with potential for exposure to uranium, largely during the years from 1952 to 1958, when he worked in a number of jobs associated with uranium fuel fabrication. During this period, 86 urinalyses for uranium were performed with concentrations typically on the order of a few g/L. Maximum levels were between 10-20 g/L and were observed in six samples during this period. Urine samples obtained subsequent to this period, largely during the 1960s and an in vivo count in 1973, were below detection limits. There were no indications in the health-physics records that this subject had incurred any accidental or high-level exposure to uranium. Excretion of uranium by way of the kidney can be estimated from the urinary excretion data. Assuming a daily concentration of 2 g/L over a six-year period (1952-1958) and a Reference Man total daily urinary volume of 1.4 L, the total excreted would be about 6 mg. The rest of his occupational exposure is unlikely to have contributed significantly to this amount, and so this individual is estimated to have excreted several milligrams of uranium based on his occupational exposure.

The final case, J-45, was employed from September 1946 through April 1979 in several positions involving uranium, including canning, process control, and

fuel finishing. His uranium exposure potential was greatest during the period from 1948 to August 1957. During this period, he submitted a total of 102 urine samples for analysis. Elevated levels of uranium in urine were observed in samples submitted prior to mid-1952, with an occasional subsequent significant elevated sample. The highest concentrations were observed during 1948-1950 while this subject was employed in the uranium mill plant. Urinary uranium concentrations during this period ranged from a low of 1.5 to a high of 72 g/L with levels typically greatest during the early part of the time. An estimate of the amount of uranium passing through the kidneys can be relatively easily made from the urinalysis data. For these five years of his employment, uranium concentrations in urine averaged about 5 g/L or about 7 g per day based on Reference Man urinary excretion of 1.4/L per day. For the five-year period, this would amount to 7 g x 5 yr x 365 d/yr = approximately 13 mg (1.3 x 104 g). Exposure during the remainder of his career probably added no more than 2 mg to this total. Thus, this individual is estimated to have excreted a few tens of milligrams of uranium as a result of his occupational exposure. Postmortem tissue concentrations are consistent with this estimate.

In summary, Table 8 gives estimates of uranium passing through the kidneys for these four cases.

It should be noted that permissible levels of inhaled uranium typically are in the range of a few hundred mg/yr. Clearly, the estimated exposures of these individuals based on routine surveillance procedures were well below these limits.

SUMMARY OF SLIDE REVIEW

In this review the pathologist concluded that five cases were either normal in appearance or showed minimal changes consistent with the age range. The remaining four cases, in the opinion of the pathologist, showed residue which could be attributed to a mild chronic toxic process. These included "atrophy, patchy chronic inflammation, tubular dilatation, calcified tubular casts, and hydronephrosis of undetermined cause." The pathologist concluded, "If I had to guess which cases had been exposed to long-term inhalation I would pick cases A-15, A-47, T-51, and W-51." When one decodes these cases from the slides, one can see that two cases were uranium workers and two cases were not.

TABLE 8. Estimated Kidney Exposure to Uranium from Occupational Sources

Case Number	Results
T-51	below detection limits
T-54	below detection limits
W-51	few milligrams
J-45	few tens of milligrams

DISCUSSION

In this small study it is of interest that the pathologist was divided equally in correctly and incorrectly choosing the uranium workers on the basis of histologic kidney changes. It is of further interest and perhaps more important that among the group considered normal was the uranium worker who over the years excreted the largest amount of uranium, J-45. It is also of interest that Case T-51, who had the least excretion, was "guessed" to be a uranium worker. This error may be artifactual, however, since Case T-51 had lymphocytic lymphoma with chemotherapy which may have been toxic to the kidney.

CONCLUSIONS

Histological kidney sections stained by both hematoxylin and eosin and trichrome techniques were submitted to a pathologist skilled in recognizing uranium changes in both humans and laboratory animals. These sections were from four individuals averaging 23.5 years work in uranium fuel fabrication and compared with histological kidney sections from five individuals who died at nearly the same age and who had no known occupational uranium exposure. The results of this study are summarized in Table 9.

This small study of only four registrants suggests that long-term, retired workers occupationally exposed to uranium within permissible limits lack specific histopathological kidney changes, which will reliably discriminate between uranium exposure and the tissue alterations present in unexposed individuals of a comparable age.

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TABLE 9. Uranium Toxicity in Former Uranium Workers Compared to Non-Uranium Workers

	Verified Uranium Worker	Verified Non-Uranium Work History
Chronic Histotoxicity Found	2	2
No Histotoxicity Found	2	3
Total Workers	4	5

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R. ALEXANDER: There's lots of questions, Dr. Moore.

(Laughter.)

R. MOORE: An hour later...

R. ALEXANDER: Do we have any information about the compounds? The uranium compounds that these people were exposed to?

R. MOORE: Yes. They worked with uranium metal, primarily. Now, there's the old question of what comes up. Basically, these people were working with low-enriched metal for fuel fabrication.

R. ALEXANDER: In a case like that, you probably have the lung, then, acting as a source of uranium, a chronic source of uranium for the kidney exposure?

R. MOORE: They would have inhaled, yes, that's correct. Is that what you're saying, Bob?

R. ALEXANDER: Yes.

R. MOORE: Yes, they would have inhaled and it then transported to the kidney.

Q: Either that or contaminated the urine samples. You don't know what happened.

R. MOORE: As a matter of fact, these were metal handlers, uranium metal handlers.

R. ALEXANDER: You're saying it was not oxide?

R. MOORE: No, it was not oxide. These were metal handlers.

Q: Do you have measurements on the lung?

R. MOORE: Yes. And when they were measured by the lungs they were less than detectable. They did not have lung burdens.

Q: Were those in vivo or radiochemistry measurements? Was that counted by an in vivo technique in the lung group, or were those chunks of lung that were analyzed?

R. MOORE: We have both data. But in answer to your question, three of them were on routine surveillance procedures because they were metal handlers. The administrative person apparently had one urine and one count.

M. THUN: Michael Thun from NIOSH. I'm very interested in the idea of a registry. One of the things that you pointed out that limited the informativeness of this was that, of your four exposed, three of them were practically unexposed.

R. MOORE: That's right. We're happy about that from a Hanford standpoint. But, for purposes of the study...

M. THUN: That's right. That's good for the controls, but it reduces the informativeness of your study. At NIOSH, we evaluated two depleted uranium facilities, two mills, and we're currently evaluating the Fernald plant. There are workers out there with much higher sustained urine uranium levels than you've been able to include.

Is the registry going to be able to actually include some exposed people? Or what is the problem in recruiting those people into participating in your registry?

R. MOORE: That's one of the very important things about a gathering like this. We would be very happy for people to identify such individuals for us, and we would make every effort to enroll such individuals. In fact, I said in my conclusion, ideally, we would like to have people who have had exposures in excess of what are presumed, at least as one caveat. The other caveat is, of course, that we would like to have them while they're actively working with uranium. Yes, if people in this audience can identify individuals for us, we would certainly be delighted. Does that answer your question?

M. THUN: Yes.

Q: We've got a slightly different situation. What is your program for attracting involvement?

R. MOORE: We have primarily depended on the medical and the health physics departments at various facilities. When we were working on USUR-1, we investigated 39 different facilities. That's quite a number, but it's far from all of them in the United States. So, part of that effort was to get the populations. But, basically, we have depended on the medical and the health physics people in the facility to identify people for us.

A. BRODSKY: I congratulate you on attempting to do this very difficult work. I wish you could have been funded 20-30 years ago to start this registry. But we haven't been able to dig up many of these old cases.

In the studies that you're doing now, have you looked at the bone content and the lung content?

R. MOORE: We are in the process of starting with that. We don't know what background is, so we're attempting to study some unexposed individuals. It's like the old psychiatrist joke. He says, "How is your wife?" The patient says, "Compared to what?" So, we're in the process of developing some background data and then starting to make just the type of comparisons you outlined.

Our friends at Oak Ridge who do the epidemiology work for the Department of Energy are beginning, as they get into their work, to target some populations. And they're calling it to our attention, and we should see if we can find somebody.

Q: Did you get any bone tissue and lung tissue samples from these cases?

R. MOORE: Yes, we have.

Q: Do you have it?

R. MOORE: Yes.

Q: Will you be looking at that?

R. MOORE: You bet. And comparing this with some nonoccupationally exposed. Jim McInroy is doing some analyses on this right now on individuals who live in the same area that these people did, but did not work, had no occupational exposure to uranium. We're excited about that.

N. SINGH: I have to make a comment on Alan's comment regarding the general population. I still maintain that it depends on a factor of 20, depending which bone. Which one would you take for your purpose? It varies by a factor of 20 already, so which one are you going to take?

R. MOORE: That's an excellent question. We've talked about this at other times, and we both recognize the problems. For instance, if you look at American data, if you look at the Russian data, if you look at people from Colorado or Southern Utah, they're all different. So, as he says, compared to what? That's why we are attempting as we go along to analyze these cases to develop some "background analyses" on individuals from the some area. And for the people who live in the Colorado Plateau, we'll be able to use Naranyi's data. That's what Naranyi and I were talking about. We're suspicious that the Richland background is going to be different than the Colorado Plateau background. I don't know if I've answered your question or not. I think Naranyi raised a very important point.

Yes, sir.

Q: How many people have signed up for your registry?

R. MOORE: Forty at present.

R. ALEXANDER: Dr. Moore, Alan Brodsky, I think, metaphorically, used the term "dig up." Are you prepared to exhume workers who have already passed away, whose families would be amenable to a tissue study?

R. MOORE: To date, we have felt that the legal problems in an exhumation plus the emotional problems of a family are beyond our resources. What we have tried to do is to target individuals who fit various categories. In this particular group of people, to our knowledge, they have had no known incidents or known uranium incorporation.

We do have another group of people we're working with who have lung burdens. So, we have zeroed in on certain groups to enroll and we have done that rather than considering exhumation. I am sure, if there is a scientific value in a specific case, I don't think we'd be reluctant to try. But at the moment we feel we can better use our time and resources in targeting specific cases.

R. ALEXANDER: At the DOE and at the NRC we have records of probably everyone who has been overexposed to uranium in this country. Whether or not that information could be revealed to you or not, I don't know. But if you would be willing to make such a formal request of the registry, probably to both agencies, that could be explored. We might be able to make a number of the names of a number of overexposed people available.

R. MOORE: That is great news to hear. That is great news to hear.

R. ALEXANDER: It is not great yet. It is interesting.

Q: If you want a little help in that, at Argonne we do that kind of business. We do exhumations routinely. We have 20 years experience.

R. MOORE: I am glad to know that.

AFTERNOON SESSION
(1:10 p.m.)

J. FOULKE: First on this afternoon, we have Max Scott. Dr. Scott has over 24 years of uranium health physics experience. He is on the facility of Louisiana State University and is a member of the Health Physics Society's Committee on Uranium Bioassays. Dr. Scott will now talk to us about the uranium experience at Oak Ridge.

M. SCOTT: Before I get into my paper directly, I would like to make a few observations concerning some of the things that have been discussed yesterday and today. I would like to discuss a little ALARA in the form that Bob told you about yesterday. I think we need to concern ourselves greatly about what impact a lowered urine limit is going to have on such things as investigations, resampling, upset of a worker, and this sort of thing.

I don't quarrel with Bob or anyone else. I want to do it right, whatever right is. But, certainly, there are impacts completely independent of a person's health that will occur if we lower these limits. You will get many more false positives. As you know if you have ever run a urine program, you will realize that you get a great number of high results that do not confirm. I don't know what high is, but it is going to be a lot lower if we lower the limit by a factor of 5. I think that the regulators certainly ought to keep this in mind. There is an impact, and it should be a value judgment on setting any kind of resample value, restriction value, the fining of a licensee, whatever. I think that is very important.

Also, I think there are three questions that the Nuclear Regulatory Commission is going to have to answer before they change anything.

The first one is--and Bob asked the question this morning, and I am going to ask it in a little different vein--what is important as far as kidney damage is concerned? Is it the concentration in the blood, or is it the concentration in the kidney or kidney tissue? I think it is very important that we know this before we start changing limits.

It brings me into my second question. Although we spent a day yesterday hearing about different ways of reducing the limit of sensitivity for an analytical technique, nothing was said about excretion. Unless we really know how the uranium is coming of of the body and unless we know what organ it is coming from, I don't know that a very sensitive technique does much for us.

So, we have got to establish some excretion models if you believe ICRP-30, where uranium comes out of several tissues with at least a couple of half-lives. The old limit of 42 μg per day or 30 μg/L that all of us have used, particularly in the uranium mining industry, assumes that everything that was seen in the urine was coming from the kidney deposit. I don't believe this is true. I don't think that anybody really believes this is true. So, we have

got to have a well-established excretion model to go along with the lowered limit, and I think it must recognize these other tissues besides the kidney.

The third question: although kidney changes have been detected, is there any evidence that these kidney changes lead to any deleterious health effect?

With that, now I will start my paper.

I had the fortune, or misfortune, of showing up in Oak Ridge in 1961, just about the time that the whole body counter was placed into routine operation. From that time through 1977, I was responsible for the operation of that facility, and we detected a great number of elevated lung deposits of enriched uranium.

The first ones that we detected were employees of the Y-12 plant. As these elevated measurements become known, other people started sending suspected exposures to us. During that period, we did counting for 20 or 30 facilities all over the country, including Atomic International from California. The Y-12 plant management proposed that Y-12 have a mobile body counter to go to other ERDA, DOE, and NRC facilities.

I am giving you all this background to say that I think I have been involved in looking at most every exposure to enriched uranium that occurred in the U.S. from about 1961 through about 1977. I am only going to show you three cases, but I think they are typical of what occurred in that period. I think it is typical of what uranium does when it gets into the lung, at least, and I will tell you what my feeling is as far as what we need to do and not do in resampling values, and this sort of thing.

I have compiled a great number of cases of people who were restricted from uranium processing, based on elevated urine data. Most of these cases occurred back in the 1950s. If you look at the paper by West that was in Health Physics about two years ago, there seem to have been next to no restrictions in the last few years, but back in the '50s there were quite a few.

I have summed urine excretion from some 150 individuals that were restricted from further uranium processing. Most of this uranium was probably as dust, probably either U_3O_8 or UO_2. But I do not have any definitive data that says this was U_3O_8 or UO_2. Excretion is a two-component exponential with a half-time of about 2.8 days and about 118 days. This does not fit anything that I have seen in the ICRP-30 lung model. The 2.8 days isn't too far off the 2.9 days that we saw yesterday from the Bear Creek uranium mill.

R. ALEXANDER: Would you mention those two again?

M. SCOTT: 2.8 days and 118 days. In the first case, a man was drilling holes in a uranium plate. Supposedly, there was local ventilation, and the ventilation failed. The case was picked up by an elevated urine sample. Then, they re-created the incident and took air samples. The analysis of the air samples said it was U_3O_8. That seems reasonable, but I can't swear to it.

The urine excretion data for an exposure in a re-creation of an incident with U_3O_8 shows three excretion patterns--an 8-day half-life, an 85-day half-life, and a 240-day half-life.

I should digress just a minute and say that all the cases that I am going to talk about you can find referenced in the Handbook of Pharmacology, which was talked about this morning, edited by Hodge and others, published by Springer-Verlag, "Uranium, Plutonium, and Transplutonic Elements." Either that or a paper written about two years ago by West in Health Physics. So, I am not going to reference any of these cases. I think you can find it in one of those two articles and other cases, too, if you are interested.

What I did when I took this case and took the urine data for Day 1, Day 30, and Day 100, I plugged it into the lung model, assuming it was either W material or Y material, and predicted what the lung burden would be, based on that data. There was an extreme overestimate. Obviously, the further you get out from the exposure, the better agreement you get, but it is still not very good. I don't know how I would use data like that to come up with any kind of resample value based on the ICRP lung model.

In another case of uranium exposure, I didn't know the exposure material. There was a fecal half-life of 144 days, a urine half-life of 138 days, and a chest retention half-life of 245 days, and a good agreement between urine and feces, about a one-to-one ratio....

In the third case, one component had a 70-day half-life, but we didn't have fecal data very soon after the exposure. It was the author's opinion--and I agree with that opinion--that it probably would have shown a fast component if the data had been collected. The urine shows a two-component exponential. You have an 11-day and a 70-day component. The chest retention, 380 days--not all that far off of the lung model as far as Y-class material goes.

R. ALEXANDER: Max, was the actual burden determined by in vivo counting?

M. SCOTT: In vivo counting, actual. So there is no limit of error around that. Now, in the Y-12 plant, over the period through the '60s and '70s, there were some 49 people restricted from uranium due to elevated urine or body-count results. The majority of these were based on high body counts or high lung-count measures. Of these cases, 44 of them fell off rather fast with half-times of 100 days or less. Now, it was our opinion back in those days--we didn't know what Y-class material was--that this was insoluble material. We didn't even know if it was transportable or nontransportable. It was our opinion, then, that this was insoluble or nontransportable material. It was dust. It is UO_2, U_3O_8, this sort of thing in 90% of the cases. I think we could say today with certainty that it was not Y-class material. In 40 of the 49 cases, they had a half-life of less than 90 days. Now, the remaining five cases showed half-lives of several hundred days, and in three of these cases we don't even know whether there was a fall-off.

Essentially, the burden is fixed. I don't know where this burden is. Very likely it is in the lymph nodes, but I don't think that we can call them

typical, and I don't think that we can justify using them to support the lung model or some unreasonably low urine-excretion value. If you believe the ICRP lung model, there should be a set urine-feces ratio. Now, in four of these cases we collected fecal samples over periods of up to six years and got ratios ranging from 0.3 to 1.7. That is fecal over urine.

So I don't know what you would use.

R. ALEXANDER. There is a slope, though. So quantity-wise, you would expect to get more uranium in the feces than you would in the urine.

M. SCOTT: I don't know if you would expect that, Bob, beyond the first few days after the exposure. I am of the opinion that the "true"--whatever that is supposed to mean--fecal-to-urine ratio is about 1-to-1. The model will tell you there is more in feces than there is in urine, but in my opinion it is about 1. I think it is equally important in the urine, particularly in evaluation of exposure.

The way we got into this was that the lung counts were dropping but the amount being excreted in the urine did not account for the reduced lung burden. And as it turned out, when we finally got smart enough to start taking fecal samples, they were excreting a significant amount in the feces. I don't necessarily mean high, but compared to what was in the urine we saw a significant amount and began to get a reasonable material balance. So, I think the feces is important, but I don't think it is as important as the lung model says it is.

I was afraid to go as far as doing a correlation between urine and air samples. But I wanted to do a correlation between the urine and body count. So, we took all the cases that we had with four or more sets of urine and lung counts and calculated a correlation coefficient for the cases. Most of you who know statistics know that the variance is in the denominator in correlation coefficient calculations. You can find any kind of correlation coefficient you want to choose, -1 to +1. So, it really didn't help us much.

I don't know if a correlation coefficient does much, because it's individuals that get exposed. We can take workers, for instance, the average for the metal fabrication workers, their body count average and their urine average, and then we can get reasonable correlations from lung count and urine results. So, in large groups, I think you can get correlations like you saw with the air samples and the urine data.

I really don't know what this does for you, though, as far as the correlation. It's an individual that's exposed. It's not a group. One of these five cases that had such long half-lives, when we picked her up, the Operation Management said, "Well, if she's high then So-and-So's high because they work right beside each other. They do the same work." But this other person was not high at all. We did quite a bit of work on that individual; she wasn't exposed.

I felt like jumping up and hollering, where were you 20 years ago? This lady worked with several different commissions on ^{235}U, and we collected gallons of urine, trying to get enough and boil enough down to do some kind of a mass spectrum to try to determine what the isotopic distribution was. We never were successful. I certainly would have liked to have some of these mass spectra analysis about 20 years ago. So, I think, at least as far enriched uranium goes, exposure is some very small isolated incident that happens, and I don't really think there will ever be much or any correlation with any kind of air sampling, just because it's such an isolated event.

Essentially, I think this covers what typical exposure to enriched uranium looks like, of a nontransportable type, and I'd like to give you what I have learned or what I think I know, based on these cases.

It's my opinion that any exposure probably is made up of all three components. If you're working with U_3O_8 and UO_2, there's certainly a W component and very likely there's a D component, also. So, I don't think you can use the one model very well to come up with a resampling value. I think urinary uranium analysis is a very good method to screen people for potential exposure, even those working with a Y class of material. I also think we should consider maintaining a urine program. However, it should strictly be a flag: "If there's something there, you should probably take another urine sample," "Maybe you ought to get a body count," or something like this. I do not believe that you can pick a value that will be fair to the employee, the regulator, and the operator of the facility so that you can say, "Okay, use this." We're going to restrict the employee and give him a lifetime income, and we're going to fine the operators, and we're going to criticize the regulator.

R. ALEXANDER: Is it okay, on the other hand, to demonstrate compliance with the urinary uranium data?

M. SCOTT: I don't know what kind of answer you want, Bob, but I doubt it. I don't know.

R. ALEXANDER: I'm always seeking the truth.

M. SCOTT: For what I call nontransportable uranium, there would be no level that you could have demonstrated compliance and remained in compliance. When you look at the lung half-life in those five cases that we have, two or three of the cases based on urine data would never have been pulled out of the area. I mean, their urine was almost background. You know, if you didn't have a body count, I just don't think you could come up with it. So, I just don't really think that there is a reasonable value that could demonstrate compliance.

R. ALEXANDER: If you have a highly insoluble deposition in the lung, it may not show up in urine for six months to a year.

M. SCOTT: So, how do you demonstrate that? I don't know how. I think urine is good now. You know, I would use it. The way I would use urine is to start by scratching my head--I mean, we've got a high urine. The first thing's

going to be, well, let's get another sample. That's the first thing I'd say: one high urine means that we ought to get another urine sample. You might want to investigate the case, but the sample ought to be high enough so that you're not spending valuable health physics time, tying them up with unproductive work. You can't have all these people running around trying to figure out how the exposure took place.

R. ALEXANDER: Let me keep bugging you for just a minute. Since you're sort of ruling out your analysis for either demonstrating compliance or demonstrating violations, you'd probably agree with us that air sampling is worse. So, are you saying that you should rely altogether then on in vivo counting?

M. SCOTT: If I were working in a facility, I would not be comfortable demonstrating compliance for nontransportable material if I wasn't doing in vivo counting. I think urinalysis is fine. I think air sampling is fine. I don't say do away with it. It's just how you use it. You might say you use them as a screening technique and as early warning signals. Certainly, air sampling is an order of magnitude (or some order) less superior than urine. But neither one of them is very much good for nontransportable material, in my judgment.

Obviously, there has to be a relationship between air sampling and urine sampling or individual sampling. But I don't believe that we're smart enough to say what the relationship is. And I really don't believe we'll ever be smart enough to sample air effectively enough to estimate any kind of further burden. It's bad enough trying to do it with urine and assume the excretion model. With air sampling, you've got to learn to sample it correctly. Then, you've got to assume some kind of deposition and excretion model. So, it's just so complicated, I don't think you can do it with any degree of accuracy, to the degree that you should allow people to demonstrate compliance or noncompliance.

R. MOORE: This information is only about 60 days old. You'll be happy to know that I have one of your cases signed up in our program, and I hope to get one or two more.

M. SCOTT: That's definitely good. One thing that's always bothered me, of course, is that I never had an opportunity to prove what I was really seeing, what I said I was seeing. One of the cases that had a burden died and I tried very, very hard to get his lungs, but management wouldn't support me. I've always been a little bitter about that fact. That would have been a perfect check of my calibration. So, yes, I'd like to keep in touch with you on that case.

R. MOORE: I appreciate it.

M. SCOTT: I support the ICRP-26 philosophy and ICRP-30. I think it's much better than the ICRP-2 publication. As I have done here, I've previously criticized the lung model rather seriously. I think it's better now. I think probably we should develop some air limits and some excretion values, but I hope that the Enforcement Branch of the NRC is well enough attuned to under-

stand how inaccurate these can be and use them, certainly, as just general guidelines--not looking at the table at a 1-μ particle and saying, therefore, their air sample is over this value and they're in violation. That plant may not be working with 1-μ particles. It may be something else. I simply endorse the concept in the proposed Part 20, but it's got to be tempered with reason, it's got to be tempered with plant experience, it's got to be tempered with the fact that none of the exposures I have seen follow that lung model.

R. ALEXANDER: Let's do something unusual here and ask my friend Doug Collins to respond to that. Doug Collins is Section Chief of our Inspectors in (NRC) Region 2 in Atlanta. There is another side to this coin, and I'd like Doug to put it on the record, if he would.

D. COLLINS: What we've been telling people is that urine and in vivo counting--which, of course, have to be performed by licensees who process uranium--both urine and in vivo counting are elements that have to be considered in the total evaluation of individuals' exposures. You can't just say, "Oh, well, I know that I'm going to have complexities with the deposition and excretion models, so I'm not going to consider that. I'm just going to go with the air samples."

We've said that's really not an appropriate survey. An appropriate survey is to take all the information, including bioassay results, and in your professional judgment--your knowledge of the plant, your knowledge of the individual, and your knowledge of the job--consider everything to come up with a full survey. You want to answer [illegible] questions: Do we need extra controls? This is the most important thing [illegible]d, also, what was the individual's exposure?

M. SCOTT: I think we're saying the same thing, Doug. At least in terms of Y-12, until the case was almost over. In other words, it was almost time to remove the restriction before you really knew whether he was overexposed. I think what I'm trying to say is that just because someone exceeds 40-MPC hours doesn't mean that they've been overexposed. My feeling is that you ought to follow that case to its completion. Then if he's over, and then if you have to cite somebody, cite somebody at that time, but don't cite somebody until you've got all the data and all the facts, and do it based on urine and in vivo.

R. ALEXANDER: What do you do, apologize to the other workers who were overexposed during that study period?

M. SCOTT: Like I said, exposures are isolated events. If they were overexposed, then the workers are going to be having high urine and high body counts, and then you're going to be doing something. But just because the air samples are high, in my judgment, at least, no apology is necessary because you haven't proved they've been overexposed yet.

D. COLLINS: Max, let me add one other thing. Most licensees also have accident plans for investigations, based on air-sampling results, individual air-sampling results. So, as Bob said, that is an indication that you do have a different containment situation that you might decide for.

M. SCOTT: Again, I'm not saying to not use air. It is a first line of defense. And I'd use it. But in my mind, it's very difficult to take an air sample and calculate burdens for lung, kidney, and bone based on that air sample. I'm not even sure I can do it with urine and body counts. Of course, I'm sure there are people who can.

That is pretty well everything that I have to say. There's a question back here.

MR. SPITZ: I wanted to make one small comment. I'm with Monsanto Research Corporation. Just a few weeks ago, the Department of Energy ran a meeting to look at some of the problems associated with the health physics of uranium at DOE facilities. We did not come up with very many answers, but lots of additional questions.

One of the things we did indicate, however, was that models--whether they're the lung model or the metabolic models that look at amounts of uranium that are expected to be in the kidney based on urinary excretion--these models are very good in a prospective manner to help us with regulations, to help us determine whether we're in compliance. Once an exposure does occur, once one of our indicators tells us that something unusual has happened, then we should not necessarily consider the lung model or any other model to be sacrosanct. We should take the measurements we have from that individual and use those measurements to describe the dose, the uranium burden or whatever other health physics item you're looking for, rather than going back to the model because the models are very general. Their major use is to be prospective, not necessarily retrospective. I like the ICRP lung model because it's been very useful in a prospective manner to help me establish guidelines when someone might have been exposed.

So, if the ICRP-1 model or the new models for uranium metabolism don't fit a particular exposure case, that's not any great harm, because we're going to go ahead and take measurements. A lot of professional health physics judgment is very important to determine what an individual's exposure is.

M. SCOTT: I agree with you. And that's what we ought to call them. We ought to call them guidelines, and I hope the regulators will call them guidelines.

Q: You expressed the point eloquently but I just want to say again, since this is a public forum, that I completely agree with you that when the NRC or anyone else comes up with a limit, that all the organs have to be considered. So, if the nephrotoxic limit were changed by a factor of 5, for instance, it does not necessarily mean that the urine limit, that is, the existing urine limit, will also be cut by a factor of 5.

I have one question for you. And that is, with your experience at Oak Ridge, when people were submitting relatively low-level urine samples, say, below 15 μg/L, did you do any testing, or did you have any data to indicate whether or not there might have been some nephrotoxic limit exceeded?

M. SCOTT: No, we never did anything at all. There was only one case I was aware of that had a soluble exposure, a highly soluble exposure. To the best of my knowledge, well, I know the case was never written up, and to the best of my knowledge, there was no kidney damage.

Q: Just a point on your long-term lung burden, that they weren't changing or something. When you look at the long-term dog experiments and the information from, say, plutonium dioxide coming in these days, if you look at each annual report, it gets a little bit longer. All it says is that it's nice to use first-order kinetics, but we're probably really dealing with power functions here.

M. SCOTT: I don't know how to work a power function, Bob.

Q: It's going to take the biologists another 40 years.

(Laughter.)

G. DIAMOND: May I just make one point with regard to that comment? From the theoretical aspect, did you consider that it's unlikely that uranium or any metal does not undergo at least one, if not many, many interactions with ligands? Many different kinds of ligands in the body? You may be able to fit the data to a first-order relationship, but, theoretically, it would be highly unlikely that it in fact would be first order because there has to be second- or higher-order interactions between the metal and certain proteins and nonprotein ligands. So, I would agree with your comment.

J. FOULKE: Thank you very much, Dr. Scott, for sharing your conclusions and. recommendations with us.

Next on our program, we'll have more human data. Dr. Michael Thun is a graduate of Harvard College and the University of Pennsylvania School of Medicine. He served as a resident in internal medicine and subsequently as an epidemiological intelligence officer at the Centers for Disease Control, National Institute of Occupational Safety and Health. That's where he is now as Section Chief at NIOSH. Dr. Thun is going to give us some information on renal toxicity in uranium workers.

M. THUN: Thank you very much. I appreciate the opportunity to be here and speak with you today. Elizabeth Ward and I from the National Institute for Occupational Safety and Health put together this talk to summarize research at NIOSH, both past and ongoing, on Renal Toxicity in Uranium. The second half of our talk will describe a study of renal effects among uranium mill workers that we did a few years ago in Colorado.

Before describing that study, I would like to step back and address some more fundamental issues, most of which have been touched on, this morning.

The first question has already been addressed. It's quite clear that the kidney plays an important part in the toxicity of compounds of uranium. I won't dwell further on that point.

The second point is a restatement of a comment that Bob Alexander made in his opening comments. The basic question is: "If uranium really does cause kidney problems, why aren't we seeing them?" The supposition is that 40 years of industrial experience would have detected a problem if one actually existed. This belief has been stated several times during the day and I'd like to express an alternative interpretation about the conventional wisdom.

The third point concerns the tests that should be used to detect a renal effect from uranium. What I'd like to do is to draw upon some of the ideas from the nephrotoxicity talk that Dr. Diamond gave this morning as they relate to practical measures for medical monitoring. The field of monitoring renal effects has changed a great deal since the 1940s and it's still changing.

Finally, I'll describe the NIOSH study that we did in Colorado.

The first question I raised was: Could important renal effects have been missed in industrial populations? Could the perception that no renal toxicity has occurred be wrong? I think there are these four reasons why the answer is yes.

The first is that the kidney is an organ that has large reserves. You heard this morning that loss of overall renal functions, by which I mean loss of glomerular function or decreased glomerular filtration (GFR), won't be detected by conventional testing until the damage is relatively advanced, somewhere between 75-80% of the functioning nephrons lost.

For example, the two tests that are most likely to be included in a worker's annual medical examination are a serum creatinine and blood urea nitrogen. These tests are unable to detect loss of renal function much less than 75%. More sensitive tests, such as measurement of creatinine clearance or the more elaborate tests used in clinical and experimental conditions, are impractical in a field setting, since they require collection of a timed urine sample. The results of even timed samples are difficult to interpret unless the subject is at rest for part of the collection period. The result is that workers can experience substantial glomerular injury and we can easily miss it.

I think that the second point is even more important. That is that the tests which have been conventionally used for surveillance of workers who are acutely overexposed have been more sensitive to glomerular injury than to renal tubular injury.

The recommendation following acute overexposure is that a dipstick test of the urine for albumin be performed. Although albumin excretion is affected by tubular function, it is more profoundly affected by increased glomerular permeability. In order to have a high enough concentration of urine albumin to detect by dipstick, you would probably need enough glomerular injury to increase glomerular permeability, cause leakage of large proteins, and overwhelm the reabsorptive capacity of the tubules. The most sensitive part of the kidney to uranium injury is the straight portion of the proximal tubule. Only as the dose of uranium increases does glomerular injury occur.

I think the fourth point is that most kidney disease is idiopathic. About 90% of cases of renal disease are not explained by any known etiology. They're

usually just described morphologically. So, if a current or former uranium worker, particularly a former uranium worker, developed serious renal disease, his illness would probably not be attributed to uranium. This is because most physicians would attribute it to some accompanying diseases such as diabetes or high blood pressure, or label it idiopathic. Most physicians have no awareness of the nephrotoxicity of uranium, regardless of their general training.

How should the renal effects of uranium be detected? I'm really going to be talking mostly about the special studies; I'm not talking about routine monitoring because I really don't think that we can make recommendations yet about the appropriate tests for routine monitoring.

Research into the development of noninvasive tests of renal function is a very active field. There's a lot of activity going on because of renal transplants, renal transplant reactions, and drug reactions. The goal has been to develop tests which are more convenient that the traditional tests which must be performed in very controlled clinical or experimental settings. The ideal screening tests would be highly sensitive, in order to detect effects that are less than major effects, and also highly reliable.

The first principle in choosing a screening test is the one I alluded to. The test should be directed at the site of suspected injury. Toxic substances can affect a variety of sites within the kidney, including the glomerulus, different parts of the tubule, the interstitium, and the vasculature. Uranium preferentially injures the straight portion of the proximal tubule. That's where, in theory, we ought to focus our testing.

Knowing the anatomic site one is interested in does not really remove all the mystery. Our experience with other heavy metals suggests that the effect one is looking for may change depending on a number of other features apart from the metals, such as the rate of exposure and the age of the subject. Mercury, uranium, lead, and a number of solvents and drugs all affect the proximal tubule. The effects are quite different. They vary with the metal, the rate of exposure, the total dose received, and, in some cases, with the age of the recipient.

An interesting example is acute lead poisoning. Acute lead poisoning in children causes the Fanconi Syndrome in children but not in adults. In adults, chronic exposure affects the renal interstitium, not the tubules. The tubules of adults develop inclusion bodies containing lead, but they don't develop the full Fanconi Syndrome.So, even once you identify the site of interest, the field is full of unknowns.

We still rely largely on the process of trial and error to determine which tests work best. Fortunately, we have quite a few tests of proximal tubular effects to choose from. There are two general categories of these tests--those listed under tests of impaired protein reabsorption, and those listed under markers of cell injury.

There is a whole variety of other markers that reflect impaired reabsorption of other substances that were mentioned this morning, such as glucose,

bicarbonate, urea, and so forth. Most of these are difficult to measure in field settings because of variations due to diet and other extraneous factors.

In the Colorado study that I'll be describing to you, we looked only for two types of proteins. We looked for amino aciduria and beta-2 microglobulinuria, both markers of low-molecular-weight proteinuria, suggesting that these proteins have been filtered through the glomeruli normally, but that reabsorption by the proximal tubules is impaired.

Since the Colorado study, NIOSH has obtained the capability of examining various enzymes and antigens in urine. As part of a study that's still in progress at the Cincinnati Fernald plant, we are using an extensive battery of laboratory tests. The analyses have been developed in collaboration with the laboratories of the Centers for Disease Control....

I'm going to now describe the Colorado study. In 1981, NIOSH was contacted by workers at a Colorado uranium mill, who were concerned about past high urine-uranium levels and about possible kidney damage. The mill is on the Colorado plateau in the uranium-vanadium belt, about one hour south of Denver. It receives raw ore from a mine.

The ore, as in all mills, is first fed into crushers, which grind it up in preparation for extracting uranium. In the crushing area, the exposure is to uranium oxides, which have relatively low biologic solubility. The crushed ore is then leached chemically to extract a product called yellowcake. When inhaled, highly soluble uranium diurinates in the yellowcake are rapidly absorbed into the bloodstream and excreted through the kidneys.

Air-uranium measurements that were collected by the company showed that air concentrations of soluble uranium were highest in the yellowcake drier area until 1979, or rather through 1979. Before that date, monthly air samples in this area averaged above the present occupational standard of 1×10^{-11} µCi/mL. In 1979, a new mill was constructed and exposures declined, due to enclosure of the yellowcake drying and packaging area.

Measurements of urine uranium showed similar patterns although there's a slight difference in the time course. From 1975 through 1978, urine-uranium levels were high. Twenty-two percent of samples exceeded the NRC action level of 30 µg/L. This level, as you know, is considered to be the concentration in urine above which kidney damage can occur.

For the medical study, we selected as our subjects only workers who began production work prior to 1979, when the exposures were relatively high, plus those workers who were presently in yellowcake. We identified 27 yellowcake workers. Three of them were current and the rest of them were senior. In addition, 12 workers in the crushing area participated, totalling 39. There were 42 who might have participated. We lost three.

Workers at a nearby cement manufacturing plant with no known exposure to nephrotoxins agreed to serve as controls. We matched the subjects and controls on race, sex, and age, plus or minus two years. We systematically selected 36

controls to match the 39 uranium-exposed subjects. Thirty-three of these comprised matched pairs. The analysis of the matched and unmatched were equivalent.

To test renal function, we collected timed eight-hour urine samples during the workday. The layout of the plant provided an excellent setting to begin and end the urine collection. The workers had to shower, and we could begin and end the timing as each worker passed through the change room.

On each urine sample and on the midshift blood sample, we measured the following parameters. Excretion of beta-2-microglobulins and amino acids was measured to assess the capacity to reabsorb small metabolites. Serum creatinine and creatinine clearance were tests of glomerular function. Because we measured these under field conditions, and because of the limitations I mentioned earlier, these tests were useful primarily to standardize the renal tubular function test for glomerular function.

We also measured blood lead and cadmium, heavy metals which could potentially confound our results. We calculated clearances and used the clearance of beta-2-microglobulin, relative to creatinine clearance as the variable most closely reflecting the tubular component of beta-2 excretion. We also collected information on a number of variables potentially related to renal injury.

All of the exposed and control populations were white male Colorado residents of similar age. We noted a small, but statistically significantly higher blood lead level in the controls relative to the subjects. This difference would tend to obscure a renal effect among the uranium workers if these low levels of lead would have any effect at all. The lead levels were low, reflecting blood lead levels in the West. Blood cadmium levels were similar in the exposed and control groups.

I will present the results of the medical tests as they pertain to tubular function, glomerular function, or effects outside of the kidney.

The excretion of beta-2-microglobulin expressed in a variety of ways was significantly higher among the uranium workers. None of the urinary beta-2 concentrations exceeded the published upper range of "normal" of 370 μg/L; however, there was a between-group difference. There was a statistically significant difference between the exposed and the unexposed groups.

We can plot the relative clearance of beta-2-microglobulin, that is, the clearance of beta-2 relative to creatinine clearance versus the years in the yellowcake area. On the scatter diagram, and also on the regression line, is a significantly positive slope in relation to years in yellowcake that is not explained by age. Years in yellowcake was the only variable that contributed significantly to this slope. It was also highly significant that r^2 is 0.3 and $p < 0.001$.

Moving to the amino acids, we found statistically significant between-group differences for five of the amino acids measured. Of the 26 amino acids measured, excretion was higher among the uranium workers for all of the amino

acids. It was significantly higher for the top five.

We also grouped the amino acids because amino acids are reabsorbed by specific transport groups. We then compared the uranium workers with the control workers by groups. In fact, the most significant difference was for the neutral amino acids. Most of that was contributed by the amino acid methionine. The amino aciduria that we observed, like the beta-2-microglobulinuria, was mild.

We found another interesting thing, that is, that the serum level of the protein beta-2-microglobulin was higher among the exposed than it was among unexposed workers. This has also been seen in cadmium workers and mercury workers. It did not explain the increase during excretion. It was a separate phenomenon that we really don't have an explanation for.

To show you the effects on glomerular function, we found the exposed worker actually had a lower serum creatinine and a higher creatinine clearance than the unexposed workers. This would indicate that during the time period we measured them, their glomerular function was actually better than the unexposed workers.

As I mentioned, though, there was a problem of interpreting the creatinine clearances and serum creatinine. The exposed workers spend a period of their time sedentary in control rooms in the plant, whereas the unexposed workers were mostly truck drivers and mechanical people at the cement plant. Thus, the control workers were much more physically active. Physical activity would tend to shunt blood away from the kidneys and reduce glomerular function. So, we used the glomerular parameters to adjust for the glomerular contribution to the tubular tests.

To summarize the positive findings of the Colorado study: As I mentioned, we found high urine and air levels of uranium prior to '78. These levels exceeded current NRC action limits. Measurements after 1978 were generally within NRC limits. We also found a mild increase in beta-2-microglobulin and amino acid excretion among the exposed, and we found that this increased in a dose-dependent way, suggesting to me reasonable evidence that the effect was associated with uranium. The conclusions we drew from this were, one, that at least at this mill, the exposure to uranium did exceed a no-effect level. The second interesting point is that we found low-molecular-weight proteinuria along with nondetectable urine albumin by dipstick.

We also had some ideas for future studies. One is that future studies should look at other markers of tubular effects to see if things such as enzyme excretion might be even more sensitive than beta-2-microglobulin in detecting the renal effect of uranium.

In our population, there were very few workers who were senior. Only one had over 20-years experience. In cadmium occupational studies, it's normally the workers with over 20 years of exposure who develop marked nephropathy. We were interested in following exposure with a population with more sustained exposures. So, we subsequently did look at a number of facilities where we

were asked to do hazard evaluations, and we looked at two depleted uranium facilities, one of which was still operating. The exposures were extremely high, but the workers' employment was short, partly because of the intermittent nature of the orders for military parts that they were processing and partly because of worker dissatisfaction with job conditions. We are currently studying renal effects in the Fernald plant, senior workers who have been at the plant for a sustained period of time. We have added to that study a number of markers, such as the enzymes that were discussed earlier.

One of the problems in detecting the renal effects of uranium in field settings is that the distal portion of the proximal tubule is a relatively silent area. Much of the reabsorptive activity occurs prior to the distal proximal tubule, in the convoluted proximal tubule. We are still looking for the optimal marker to sensitively and reliably detect an effect.

Thank you.

Q: Two questions. The first one is, you've done a type of protein analysis, and it's my understanding of the uranium renal effect that one of the better indicators is glucose. I was wondering why you didn't include glucose?

M. THUN: Sure. The main reason is that it's so much affected by diet. We considered a number of potential markers that had been useful in the University of Rochester studies. One such test was the wine catalase. Excretion of catalase was one of the most sensitive markers of uranium effect in the Rochester studies. The researchers subsequently tried to use catalase in one industrial population, and found that the test was inhibited by some pollutant in the workplace. It didn't work. The Rochester investigators did report one occupational study that was positive for a renal effect. However, the findings were questioned because it hadn't controlled for urine concentracion. Glucose excretion is one of several parameters that would be very worthwhile to monitor, such as glucose phosphate, but which are hard to measure in field settings.

Q: They mentioned glucose, because it was so substantially above the normal levels and so on. My cursory reading, and I don't have a medical background, I have a biological background, is that there really weren't all that many things that were very high in glucose.

M. THUN: The problem with glucose, the reason why I haven't tried to measure it is, that you must have a timed sample to assess exertion, and the timing has to be accurate.

Q: I believe the literature indicated that the glucose was elevated relatively quickly and remained high for a fair period of time.

M. THUN: In rats, and the investigators collected a timed sample. You see, to measure glucose reabsorption, one computes a function called the tubular maximum reabsorption for glucose. Time is one ingredient of the equation. So, unless you can get an accurately timed sample, you can't do it. Now, in this case, we have an accurately timed sample. I didn't include glucose measurement because of diet. In the Fernald study, the timing was more

problematic. Whenever you measure time in a field study, you have a problem.

Q: The second question is, you're indicating that certain types of proteins show a significant difference between those and your control group, but are you saying that because there is a difference, it means that there is kidney damage?

M. THUN: I'm saying it's consistent with what uranium nephrotoxicity would cause. The distinction between damage and dysfunction was bantered around this morning. In the kidney, injury is most clearly visible as morphologic injury. We could not examine morphology without biopsying these workers' kidneys. What we did look at was altered function. Altered function may have many determinants, one of which is renal injury.

A. BRODSKY: How did you correct for age, when you got the correlation of the beta-2-microglobulin?

M. THUN: We did a multiple regression. We put age in the equation, and age didn't eliminate the statistically significant difference from zero of the slope.

A. BRODSKY: So, you've got enough data for varying occupational periods but different ages?

M. THUN: Right. We had enough data, even limiting the analysis to the uranium workers. You see, if we'd included the cement plant workers in the regression analysis, we would have had a much steeper slope because we already had a difference between the cement workers; all of them had zero years in yellowcake. The analysis presented here considers only the uranium workers and reduces the chance of detecting the effect. The older unexposed people were the people who had always been in the crushing area and had minimal exposure to soluble uranium.

R. ALEXANDER: You pointed out that no systematic surveillance of renal effects in occupational populations has been done. I understand from Dr. Jerome Wilson at NIH that he has done such a study.

M. THUN: I should say "until recently." I haven't seen the results of Jerome Wilson's study. It is true that he has completed that study, but this is the first one.

R. ALEXANDER: You have not seen the results?

M. THUN: No.

R. ALEXANDER: He has indicated to me that the results are of interest--will be of interest to us, and he was scheduled to give his paper at this meeting, but he doesn't have it approved yet. So, he couldn't give it. But I think that correction should be made for our record.

M. THUN: Thank you.

G. DIAMOND: The kidney--I just wanted to make one point. The kidney does have a large functional reserve, which may mean that there could be substantial loss of renal mass without detecting impaired function. The remaining renal mass may be sufficient and may have adapted sufficiently to maintain the normal physiologic function of the kidney, so that you might not detect that loss of mass had occurred.

But in addition to that, when that organism is stressed by another toxic insult, that person or individual may in fact be potentially more highly susceptible than a naive individual because they are essentially functioning with a small renal mass. So, there is another component to the idea of large functional reserve. You may not be able to detect the damage. In addition, that person may in fact be potentially more at risk than the individual who has his entire renal mass.

Just a comment.

M. THUN: I think it is good to underscore that just because the reserve exists, it need not be desirable to use it up.

J. FOULKE: We are going to switch now and get back to some of the animal studies. Our next speaker is Dr. Glenn Taylor, from the University of Utah. He has been there, he tells me, since 1956. He is going to talk on kidney damage in the dog from ^{233}U and ^{238}U.

G. TAYLOR: The few data that I have are based on a relatively small study from the toxicity point of view. It was conducted at the University of Utah. The primary objective of these studies was to evaluate the metabolism and anatomical distribution in beagles in order to predict the relative toxicity of uranium as compared to some of the other actinide elements. Nevertheless, we did make some toxicity experiments. I will touch very briefly on some of these.

The uranium in this experiment was given by a single intravenous injection, 2.8 μCi of ^{233}U per kilogram in 9 mm of 0.08 citrate buffers with a pH of 3.25, prepared by dissolving the uranyl nitrate in the citrate buffers. This dose is equivalent to about 3 mg of uranium for a 10-kg beagle.

The initial retention as the percent of the injected dose was highest in the kidney, next highest in the skeleton. About 60%, in fact, of the uranium was excreted during the first day. There are very significant differences in biological half-life between the kidney and the skeleton. In fact, by seven days the retention in the skeleton was actually higher in spite of the very high initial uptake in the kidney. The biological half time in the skeleton is almost 900 days. If we go back to the statement that was made this morning--this is based on data from Fred Bruenger's study--if you took long-term studies, it would be about 79 to 80 days. Ultimately, the retention after one year is about a tenth of 1%. If we consider the biological half-time for the skeleton at two years, the rad dose was 2.8 μCi/kg.

With the anatomical distribution of the uranium in the beagle kidney, retention actually occurred in less than one-half, in about one-half of the nephrons. Within the kidney those nephrons involved were principally located in the deep aspect. The fact that only about half of the nephrons were involved is probably the reason why we didn't lose any animals.

The distribution within the nephron itself is very meaningful. Selected deposition occurred in the top of the tubule. It involved the straight aspects as well as the convolutions. Very little of it occurred in the glomerulus, and the desposition in the thin part tended to essentially zero.

The lesser amount of uranium was deposited in the distal tubule. The maculate density does represent a moderate amount of activity after a fairly long term. The uptake was just a few days later in the distal tubule as compared to the proximal. Deposition in the collecting tubule was lower, except down near the renal depths. We actually had the formation of hot spots in selected cells. This actually reflects the fact that there are two cell types in the epithelium at these deep depths. By far, less than half of the cells of the distal tubules were involved.

The most obvious lesion that we saw was the formation of very highly radioactive casts in various aspects of the nephron at various levels, but especially in the proximal aspect. Again, there was still very little uptake in the glomerulus.

By 94 days post-injection, we have the chronic interval. There was a loss of tubules, fibrosis, even in the cortical-medullary area. There are multiple foci of fibrosis. There were some tubules with very low epithelium, almost squamous in nature. Presumably, this did represent a regenerative response on the part of the convoluted tubules. This condition persisted almost unchanged. We looked at this out to approximately three years, in spite of these very significant morphological changes with respect to the toxicity.

Of course, one of the obvious questions was addressed this morning: are these chemically-induced or radiation-induced or a combination of both? What is the etiology of these lesions? In an effort to get some idea of this by injecting an animal with ^{238}U; another was given the identical mass of ^{233}U, essentially 3 mg. We made our comparison using several very easily determined trends. One compared the BUN. There were actually two phases that we observed. One appeared at about nine days. Then, by three weeks most of the animals had returned to a normal BUN.

Then we saw the second rise. That was at about 12 weeks. That was a sustained rise in the case of ^{233}U, and it never did come back to normal values during the period of time we followed the dogs. In the case of ^{238}U, we did get the initial rise, a very slight one, up to about 30-mg%, then dropping back down the same to normal levels.

The standard deviation was about 20-mg%, so that the ^{238}U animal, with the exception of a very transitory rise, remained pretty much within one limit throughout the entire observation period. This period was for five years. The

secondary rise observed in the case of the ^{233}U animal did not occur in the case of the ^{238}U. We can conclude from this that the initial rise was chemical in nature and that the secondary and sustained rise was related to the greater toxicity.

R. ALEXANDER: Maybe you have already said so, Dr. Taylor, but the gravimetric unit--is the quantity of ^{233}U and the quantity of natural uranium about the same?

G. TAYLOR: It was identical.

Looking at creatinines, again with the same dogs, essentially the same picture is observed, except that the secondary rise in creatinine was not nearly as high relative to the case of the blood-urea nitrogen. It did occur and went up to a sustained level. There is a significant amount of variation in these values. And, of course, there is a real caveat that should be mentioned: as in the case of ^{238}U, there is only one dog. It would have been nice to have had a dozen.

Looking at the urine volume in these animals, the effects were essentially similar. Although the rise in the irradiated dogs was higher and occurred slightly earlier and then came back into the baseline somewhat later, the high point of the urinary increase was essentially the same in both the ^{233}U and the ^{238}U. One could conclude that their increased urinary output was probably chemical and not radiation-induced.

I think I would state in conclusion that very significant kidney lesions were induced by the ^{233}U in the beagle, and tentatively at least, the data indicate that the long-term effects were probably radiation-induced, but with at least superimposed effect caused by the chemical factors in these particular animals. In spite of the very significant kidney lesions, probably the greatest risk the dogs had who were injected with ^{233}U was relative to bone cancer. In fact, in this case the risk of bone cancer was very high.

From the data taken from a study by Stevens primarily on the bone surface, uranium deposits in the skeleton remain there for a relatively prolonged period, and then gradually become involved. So, essentially, uranium from the standpoint of the skeleton is somewhere in between. Plutonium is primarily a surface seeker, and radium is essentially a deeper-seeker.

We have, of course, seen a high incidence of bone cancer in animals that have a rad dose significantly less than we have shown here, at least for ^{233}U animals. Certainly the skeleton, with regard to ^{233}U, would be perhaps one of the elements that had potential for trouble.

R. ALEXANDER: You mentioned that all eight of the dogs survived these tests. I wonder if any recovery information can be extracted from the data that you selected. The recovery time is particularly important to us in establishing criteria for uranium bioassay programs.

G. TAYLOR: Recovery clinically speaking?

R. ALEXANDER: Yes, clinically, I suppose. I really don't know if it would be clinically or not. Certainly, observable effects in one way or another. It is very important to assume a time of recovery in establishing a model on which to base a bioassay program. So, anything you could add to the recovery time that you observed in any way might be helpful to us.

G. TAYLOR: The biochemical parameters, at least with ^{233}U, never did come back. For example, the BUN was above normal throughout the entire observation period. The creatinine was also above throughout the observation period.

R. ALEXANDER: Is that still true today, that the BUN has never returned?

G. TAYLOR: The animals have all been sacrificed. As long as the study went on, in the case of ^{233}U, it was 730 days. In the case of ^{238}U, we carried it out for about five years. Now, those dogs were normal histologically. They were also normal from the standpoint of the biochemistry, relatively crude biochemistry--BUN and creatinine. The urine changes came back to normal in both cases and stayed down. We did not see any return of the oliguria after the initial phase. So far as clinical illness was concerned, it wasn't there. These were animals with normal weight. You'd never pick them out clinically. We did not see detectable microscopic changes in the ^{238}U animal. This went on five years. The histopaths on that particular animal were perfectly normal.

R. ALEXANDER: But the ^{233}U animals?

G. TAYLOR: There, they were essentially the same for 94 days. We didn't have one, say, at 50 days but they were essentially the same from 94 days out to the time we sacrificed the animal.

R. ALEXANDER: Is that why you attributed it to the radiation?

G. TAYLOR: That we did because we did not see that in the ^{238}U animal.

Q: Do you know what the doses were in terms of microcuries or micrograms of uranium per gram of kidney? Do you know how much was in the kidney? How much uranium was actually absorbed in the kidney?

G. TAYLOR: We didn't do that. We could if you just took the average amount of uranium in the kidney; it would be, say, 2.8 μCi or 3 mg. It would be somewhere on the order of 45 μg/g of kidney weight. But this would just be simply a very crude average, and you can see the very focal nature of this radiation would be off by a lot. But one could take the radiograms and come up with a dose that goes out to infinity. We haven't done that yet.

Q: The average value is already interesting because, in a lot of cases, you can resolve the kidney and measure the average values. It's already useful to know.

A. BRODSKY: Allen Brodsky, NRC. Dr. Taylor, you did see some risk of bone cancer. But was there any bone cancer in ^{238}U-exposed dogs on sacrifice?

G. TAYLOR: No. Bone cancer, of course, in the beagle is rare. We've only seen one tumor out of 150 controls. Also, these dogs were not carried out long enough. We looked at a latent period related to the radiation. They did not go out far enough to pick up tumors.

A. BRODSKY: You didn't see any bone tumors in ^{233}U? You say the risk is high because of the expected dose?

G. TAYLOR: I say the risk is high based on the experience with radium and plutonium and the manner of deposition in the skeleton. We would place the toxicity of uranium somewhere between radium and plutonium. And we have seen tumors in the case of ^{226}Ra at a dose of 100 rads or less. I indicated that for two years, based on earlier figures, the dose from uranium would be on the order of 300 rads. So, we would expect a reasonably high incidence, at least in these particular animals.

A. BRODSKY: You're speaking of incidence from the ^{233}U alone, or also from the ^{238}U?

G. TAYLOR: We only have about 1/10,000th of a microcurie per kilogram injected of ^{238}U.

A. BRODSKY: So, with the ^{238}U you would still expect the chemical toxicity to the kidney to be acknowledged, even with the long half-life involved?

G. TAYLOR: But not in the skeleton, unless--I don't know. Maybe it's possible that there is an effect in the skeleton, but from the standpoint of radiation, we would not expect to see any tumor from ^{238}U in the skeleton. In the case of ^{233}U, based on the long retention time and the rad doses accumulated at least at 2.8 μCi, it would be somewhere on the order of 300 rads. And about two years. Based on our experience with plutonium or ^{226}Ra, we expect a high incidence of tumors with that dose.

R. ALEXANDER: Dr. Thun, may I ask you a question? Dr. Morrow, in his paper, gives us his belief that the dog kidney is more susceptible, more sensitive to uranium damage than that of man. I wonder hcw you would account for these very high kidney concentrations for tnese eight beagles. Dr. Taylor has given us an off-the-cuff estimate of 45 μg/g per gram that no clinical illness was observed in these dogs, even though they may be more susceptible than man.

R. THUN: As I understand it, these dogs had increased serum creatinine and BUN, the ones who would be exposed. Is that correct?

G. TAYLOR: Yes.

M. THUN: I think what you have to understand about renal disease is it's a relatively silent disease until you become pretty near terminal; until your renal function is about gone, you feel fine. Renal disease differs from more symptomatic diseases. I think that what you have to keep in mind for kidney

disease is that the purpose is to prevent the silent loss of nephrons. And the absence of symptoms should not be the marker of success.

Q: The BUN were normal in the ^{238}U. It was only the radiation, the ^{233}U, where the BUN were up.

G. DIAMOND: It went up, then down, didn't it?

M. THUN: I got a little confused about which dogs had which effects here. But the ones that I understood you to refer to appeared in no distress at elevated serum creatinines and BUN. And I found that entirely consistent with renal disease.

Q: Those were radiation doses, the ^{233}U. Not ^{238}U?

G. DIAMOND: In the first nine days, I thought they both had an increase in creatinine and in BUN. And the ^{233}U caused the persistent second phase. That's what I understood.

To add to your comment, I would just like to point out a study that was recently done in Cincinnati. These are not uranium workers, these are lead workers. They had no clinical signs of renal impairment, no protein urias detected in a routine clinical screening, et cetera. They subjected these individuals to a battery of tests to measure functional capacity. That is, they didn't measure what the urinary excretion of glucose was, but asked what the maximum secretion of organic ions was in these individuals. Many of them showed up with positive indices of reduced functional capacity, which in no way--they would have easily passed even a fairly extensive clinical urinalysis. So, again, we see, as Dr. Thun mentioned, you can lose a great deal of capacity without necessarily demonstrating impairment under normal physiological loads. These individuals maybe are susceptible to further stress and their health may be at risk.

R. ALEXANDER: Could you elaborate on the stresses you have in mind? Do you mean other types of kidney disease?

G. DIAMOND: Yes. For instance, if half or more of the functional nephrons are removed, if you were to specifically remove the kidney from an animal, the remaining kidney will hypertrophy, structurally and functionally, to take over the work that was carried out by two kidneys. Essentially, then, those fewer remaining nephrons are carrying out the workload of many more nephrons. And the functional reserve of that kidney has been substantially reduced. In fact, we have observed in our own laboratory that animals that have undergone nephronectomy actually are more sensitive to certain nephrotoxins. The dose-effect curve, as indexed by histopathological measurements and urinalysis measurement, has shifted to the left. So, it's conceivable that chemically induced damage may decrease the reserves sufficiently, as well.

R. ALEXANDER: If a highly exposed worker had lost a great deal of kidney function due to uranium exposure, and then was diagnosed as diabetic and lost his life rather soon, is there much chance at all that an association with

uranium exposure could ever be made? Never?

G. DIAMOND: Does "diabetic" mean glucosuria?

R. ALEXANDER: Whatever would involve the function of a kidney to keep the person alive.

G. DIAMOND: I don't know the answer to that question.

M. THUN: The physician would attribute his renal failure to his diabetes. An interesting example of how multiple stresses can make renal disease apparent following heavy metal nephrotoxicity exists for cadmium in Japan. There was heavy community exposure to cadmium through contaminated rice. All the people ate the rice. But post-menopausal women developed a disabling bone disease in which their bones would spontaneously fracture. It was called itai-itai disease, meaning ouch-ouch. The reason why they developed it and no one else is that with cadmium nephropathy, one of the problems of impaired reabsorption of the things that the kidney normally reabsorbs is that the people lose calcium and phosphate. These post-menopausal women who have demineralized bones and were malnourished had the additional straw of the cadmium to produce this severe clinical illness. So, insults are often additive.

R. ALEXANDER: Thank you.

J. FOULKE: Thank you very much, Dr. Taylor. We'll now continue with information on animal studies. Our next speaker comes to us from a foreign country. Dr. Bliss Tracy received a Ph.D. in Nuclear Physics from McMaster University in 1969 and spent four years teaching at the University of New Brunswick. For the last seven years, he has served as a research scientist with the Radiation Protection Bureau, Health and Welfare, Canada.

Dr. Tracy is going to discuss with us Uranium Animal Studies in Canada.

B. TRACY: Thank you very much for giving me the opportunity to present some of the data that we gathered at the Canadian Department of Health and Welfare. I hope it will complement some of the data that has already been presented today, and perhaps help to complete the story on uranium toxicity.

One of the functions of the Canadian Department of Health and Welfare is to set drinking water guidelines. That is really what led us into this uranium study. The drinking water limit for uranium in Canada is currently under review. The value is 20 μg/L. This value was set about six or seven years ago and was based on some early Soviet work. It was work that we felt ought to be repeated according to more strict protocols for doing this type of animal toxicity experiment.

In 1980, we began a study of the toxicity of uranium in two laboratory animals--rats and rabbits. The work that was done was a cooperative effort between two different bureaus of the Department of Health and Welfare. The Bureau of Chemical Hazards cared for the animals and conducted the pathology studies. The Radiation Protection Bureau became involved because we have the

capability of measuring low levels of uranium in tissues and water. This is done routinely in drinking water and urine. Secondly, we were interested because there was concern about the radiological effects of uranium.

To mention the individuals involved in the study with the Bureau of Chemical Hazards: there are Andy Gilman, Dave Villeneuve, Vic Secours, and Ken Mancuso; and at the Radiation Protection Bureau, besides myself, there are Jim Quinn and Joanne Lahey who were involved in the study.

The study actually took the form of 90-day subchronic studies in which the animals were dosed with uranium over an extended period of time (over 90 days). It was administered in the drinking water because it was the oral pathway that we wanted to evaluate.

The uranium was in the form of uranyl nitrate (natural uranium, not enriched or depleted). It was administered in drinking water to approximately 180 rats--Sprague Dawley, males and females--and 60 New Zealand white rabbits at six different concentration levels. The levels of uranium administered were 0, 0.46, 2.3, 11, 57, and 280 mg/L. In addition, a study was done on 40 female rabbits at four of those dose levels.

The animals were fed a normal diet and took water when required. In some cases they were put in metabolic cages, and the urine output and water input was measured. At the end of the study, the animals were sacrificed and subjected to an array of pathological, biochemical, and histological examinations. The uranium was measured in various organs. The technique used was just the standard fluorometry method.

Preconcentration of the uranium was carried out before the actual determination took place. The organs in question were dissolved, wet ashed, and the uranium was separated on an anion exchange column. It was then fused with sodium fluoride pellets and read with a conventional fluorometer.

The detection limit in the animal organs was in the neighborhood of about 0.05 μg/g, and that detection limit was really determined by the very small size of the organ or the material we had to work with. The following organs or tissues were studied: kidney, bone, liver, brain, spleen, in a few cases whole blood and urine. The only cases where we actually detected any uranium were in the kidney and bone. That is not surprising. And, of course, in the few urine samples that we did, we detected uranium.

I am presenting to you just the results for the male rabbits. The rat results were not very different from this, and the data for the female rabbits isn't all in yet. So, I don't have all the female data to compare it with.

The most important effects were kidney lesions, especially damage to the tubular epithelium--the type of damage that we have heard described today in other talks. We noted that in the three highest dose levels we were seeing damage, although it was slight in the fourth and fifth dose level and moderate in the highest dose level.

There were other effects observed, especially for the male animals. There was some evidence of kidney dysfunction, which showed up in the biochemical analysis of the urine in those animals with kidney lesions. Also, there was some damage noted in the liver and thyroid at these same dose levels, the three highest levels.

The data we have so far on female rats and female rabbits show much less kidney damage. There was very slight damage at the highest dose level. For some reason--I am not quite sure why--females seem to be more resistant than the males to kidney damage. This was true for both rats and rabbits.

Now, the other interesting thing to look at is the uranium concentrations. Obviously, one sees it in bone. The first two values are very close to our detection limit, so for all intents and purposes they represent background values. At the third level (2.3 mg/L) we began to detect uranium in the bone and in the kidney although we did not see any kidney lesions at this level. However, at the fourth level (11 mg/L) kidney lesions definitely began to appear. This would seem to indicate toxic threshold for rabbits of about 0.7 µg/g of kidney.

There are wide error limits on these numbers. In going from the 2.3-mg dose to the 11-mg dose, the average kidney concentration changes only from 0.5 to 0.7 µg/g. But it seems that the threshold or the no-effect threshold is in the neighborhood of 0.5 µg/g. This would be consistent with some of the numbers that were presented earlier today on dogs.

To finish off on the theme that I began, we originally did this experiment for the purpose of evaluating our drinking water standards. What implications would this have for the levels of uranium in drinking water?

The no-effect level corresponds to a uranium intake in rabbits of about 0.2 mg/kg per day. If we scale that up from a rabbit (1 kg) to an adult male human (70 kg), the dose becomes 14 mg per day. If we further assume a consumption of two liters of water per day, we obtain 7 mg/L. This is valid if humans react the same way rabbits do. So, if we divide that by a safety factor of 100, that would give us 70 µg/L, which would be still well above our current standards of 20 µg/L.

So, that terminates what I have to say. I will try to answer any questions that you might have.

Q: Are you talking about the current standard urinary output standards?

B. TRACY: No. I am sorry, this is the drinking water standard, the maximum acceptable concentration in the drinking water supplies. I am coming at it from a slightly different direction, I guess, than most of your people are.

Q: Do you assume that equilibrium is equal to the output?

B. TRACY: Yes, if everybody were consuming that much, I suppose. That is an interesting point. You mean, if you were consuming 20 µg per day, you

should be putting out 20 μg per day in urine?

Q: Yes, because you are talking about levels of the order of magnitude of what are permissible in urinary concentration.

B. TRACY: There is one strong difference. That is the absorption in the GI tract. That is maybe on the order of 1% or 5% for humans. So, if you are consuming 20 μg, you are probably only going to be absorbing 1 μg per day, which is about the same sort you get from food. So, that is getting down very close to what the background levels are of uranium in urine.

R. ALEXANDER: Do you know how the control kidney-uranium concentration for the rabbit compares with man in 0.06?

B. TRACY: I really don't know that.

R. ALEXANDER: Does anyone have that number for man?

Q: What is your question, Bob?

R. ALEXANDER: The uranium concentration in kidney tissues.

R. MOORE: Seven micrograms.

B. TRACY: 300 grams?

R. MOORE: 310.

R. ALEXANDER: So 7 over 300 would be the answer about.

B. TRACY: So, that is 0.02 or 0.03.

R. MOORE: Your 5 μg is about 20 times. Extrapolating that to a human may not be a reliable figure.

B. TRACY: I am not so sure about that figure. The error bounds were quite large.

Q: Do you have any way to account for the fact that the factors have increased in water concentration and are not followed by the factors in what you find in tissue? In other words, you want a factor of 5 in the water. You don't find a factor of 5 in the kidney.

B. TRACY: You don't seem to, that is true. You have a small sample effect there. These numbers are an average of six samples or six animals. So, there could be some fairly wide errors. Some of that may be just a sort of sampling error. Otherwise, I have no explanation of why it wouldn't fall.

G. DIAMOND: The water intakes of each group were sampled, though?

B. TRACY: Yes, that is right, and they were measured repeatedly. So, we

have a pretty good idea of what they were.

G. DIAMOND: One of the problems with the chronic cadmium studies of drinking water is that rats do not like something about cadmium in water.

B. TRACY: This is apparently not bothering the rabbits too much.

G. DIAMOND: It is not true for other metals--lead.

Q: Part of that effect, where you have to go way up in concentration increase, may be related to what they found at Argonne in Larson's group in regard to above certain quantities. You have got different GI absorption. The GI-absorption fraction is dependent on the quantity actually present in water.

B. TRACY: You are setting several levels off as you go up.

Q: You might get a 10^{-1} absorbed through the GI tract at the lower concentration but only 10^{-4} of the higher concentrations. The data scatter around those kinds of values, and also they are related to the pH. What is the pH of your water concentration?

B. TRACY: I am not sure what the exact pH was. It was pretty close to neutral, but I don't know exactly what it was.

Q: I would suggest that if that water is, as expected, exposed to ambient air, the pH might be closer to 5.5 or 6.

B. TRACY: That is something I will check out, see if they actually did pH measurements and whether there might be any variations on pH.

J. FOULKE: I guess there are no more questions. Thank you very much.

The next subject has already come up in the discussions. We have just been talking about GI-tract absorption. So, for that we will now invite Dr. Narayani Singh, from the University of Utah. I have known Narayani for several years, back when he was at New York University. He has done fine analytical work for NRC. Narayani?

N. SINGH: To really understand the toxicity of uranium, we have to know three parameters. One is the absorption of uranium through inhalation or ingestion. The second thing we have to know is the retention in bone. That will give us the radiotoxicity of uranium. The third thing we have to know is the retention in the kidney. That will give us the chemical toxicity of uranium.

These three we have to know. However, I am going to deal mostly with the GI absorption because that is what we are supposed to talk about, and that is what we have done some work on.

We see in the literature on GI absorption that uranium is absorbed 20% through the GI. ICRP assumed 5%. Then our colleague, Dr. Wrenn, and other

colleagues found that the best estimate would be 1 to 2% being absorbed through the GI. So, you can see a factor of 20 back there, and there could be a big error on the problem of the absorption of uranium.

Q: Are all those the same type of uranium?

N. SINGH: The same type of uranium. That's what exists in the literature. This is one controversy that should be solved. Let's look at a second one.

The equations given by ICRP for retention of uranium in bone and kidney are 20% to bone, with a biological half-life of 20 days, and 2.3% goes with the biological half-life of 500 days. The retention equation for uranium in kidney is 12% in the kidney, with a biological half-life of six days only, and about 0.0052% in bone with a longer half-life of 1500 days.

R. ALEXANDER: I hate to interrupt, but I feel certain that the retention function that you have there for the kidney, and it's covered in ICRP-30, is not intended to suggest that there is either a six-day half-life or a 1,500-day half-life. If you look at the report from which that equation is taken, it is clear that that is simply the sum of two exponentials that happen to fit the power function that he found. Those are not half-lives. Those are numbers for analytical convenience.

N. SINGH: Let's say that they are the best assumed numbers. All right?

R. ALEXANDER: Okay.

N. SINGH: And that's what we really have to find out, too, if those numbers are right or not right. If we don't know these equations are right, then we probably will make some errors.

Then, what is the radiation-induced bone sarcoma from uranium? Our colleague Chuck Mays says that there are 226 bone sarcomas per 10^6 person rads. And our colleague Ed Wrenn feels that 1 μg of uranium per gram kidney is the threshold for chemical toxicity. These are the numbers. They're the best accepted.

So, we proposed a study to EPA. We have proposed to find out, first, the GI absorption of uranium and also the equations for the uranium in the kidneys. We found that some drinking water in Ohio had about 130 pCi/L of ^{238}U and ^{234}U. I proposed we will have 10 volunteers at our lab. The persons will drink uranium, and we'll follow these people one week prior to the drinking of water and two weeks after taking the water. We'll take a urine specimen and also a fecal specimen. That will also give us the reabsorption.

The other part we have proposed is that, at the same time, the same volunteers would be injected with ^{237}U, with half-life of seven days, and with 208-KeV gamma, so we can follow by counting.

That would give us three very important answers: What is the retention of

uranium in bone? And the retention in kidney? Also, what is the effect of fasting versus nonfasting? There is data which suggests that if you take uranium in fasting, you absorb much more than when you are taking uranium when your stomach is full. So, that itself is an important parameter.

We have just started doing the work, so we don't have much to tell you.

What I'm going to do today is give some very interesting data on our dog studies. We wanted to find out what is their intake per liter and what is their retention in a dog which is chronically exposed, just like human beings, through its diet. Just like all human beings, they are exposed primarily through food or drinking water. So, they would give us a quite good estimate of the uranium.

For ^{238}U from food, we had three kinds of diet. Dog food, essentially meaning dry food, contained about 42 pCi/kg of ^{238}U. When this is multiplied, you had 126 μg/kg. The dog was given about 227 g of food per day. That means a total amount of ^{238}U coming from the dry food was 9.53 pCi. Similarly, for the canned food, the concentration of ^{238}U was very high--254 pCi/kg. And there's only 28.4 g of food per day. That gave about 7.21 pCi of ^{238}U. Then, they were drinking the water from our kennel. That was 0.5 pCi/L. This dog had been drinking 1,200 mg of water, and the total activity coming from water was 0.6 pCi. The total ^{238}U from dry food, canned food, and water was 17.34 pCi.

Then, we shall see the balance, whether everything is coming out or not. In the excretion model we measured the feces, which were about 133 pCi/kg, for a total fecal 36.7 g. In other words, the total amount of ^{238}U which came out through feces was 4.88 pCi. The next was urine, which was 0.34 pCi/L. The total is 590 mL. So the total amount that came out through the urine was 0.20 pCi. The total was only 5.08 pCi.

So what happened to the rest? Obviously, it did not get out of the body. Actually, what happened was this food had been mixed deliberately with some phosphorous and then uranium, so there were only some parts of this which did not get into the body. We estimated the total that did not go into the body, but was left in the ground.

How do we calculate the GI absorption? All we have to know is the urine specimen and the fecal specimen. That will give us the GI absorption of uranium. So, that number, the absorption in the situation, is the urine specimen divided by the total specimen in urine and feces, and that gives the very interesting number of 0.2 over 5.08, which equals 3.94%. So, we see ^{238}U GI absorption in food and water is 3.94%.

Now, the ^{234}U from the same dog, the same diet. The difference is very minimal, but there's a big difference in GI absorption. Why? I'll explain later on. On the dry food, the dog food, the same concentration was what we saw for ^{238}U, 42 pCi/kg. And it has the same amount, 9.53 pCi. Similarly, the canned food, same concentration, 254 pCi/g, and the same amount, 28.4 g. The total was 7.21 pCi. However, the drinking water had 1.3 pCi/L. If you

remember the level for ^{238}U, that was 0.5 pCi/L. And this was 1.3, almost three times higher. It's not very unusual because the lab water supplies had both ^{234}U and ^{238}U. That's not the point. The point is that there was a higher intake of ^{234}U than ^{238}U only through drinking water, which was not a great amount--only 1.56.

So, the total activity, was 18.3, but there is the excretion model. The feces had 1.45 pCi, and there was a total of 5.32 pCi urine. That's where the difference lies now. Urine was 0.97 pCi/L, with 590 mm and 0.57 pCi. So, the total was 5.89.

Compare the 5.89 to that of ^{238}U which was 5.08. The real difference came from drinking water, but how does that affect our GI absorption? If you look at the GI absorption, the percentage is 9.7%. That means the GI absorption of the ^{234}U is three times that of ^{238}U. What should be the reason for this? How do you explain it?

Well, I have two guesses. They're my guesses. You can say it's wrong. My guess is, since ^{234}U is much more labile in drinking water--in other words, there is more soluble form than coming through the diet--so, if uranium is going through the GI system in a very soluble form, obviously, the GI absorption will be much higher. In other words, we have to have two numbers--one for the diet (the food) and one for drinking water. Or there could be another possibility. The other possibility is you have to understand that when you fast and get uranium the absorption is much higher. Obviously, the dogs are drinking water the whole day when they're a little hungry. And, at that time, maybe the absorption of ^{234}U through water is a little bit higher because ^{234}U is there.

So, these two points I want to really emphasize: GI absorption of uranium may be very much affected depending upon if you are trying to measure it through water or food. The second thing, fasting and nonfasting could be an important factor. The numbers are looking very high, from 3.4% to 10%. So, I hope to bring you more data later on when I get a chance.

Thank you.

R. ALEXANDER: I guess what it amounts to is a ^{234}U enrichment in drinking water in Utah.

N. SINGH: Yes, that's not very common; 1.3 is not a great deal.

R. ALEXANDER: That hasn't been found every place else.

N. SINGH: One or two places, yes.

Q: Dr. Singh, I would like to call to your attention two NUREGs written by Maryka Bhattacharyya, in which she has shown that the base metal uptake in the fasting condition is 10 times that in the fed condition. So, you're simply demonstrating that.

N. SINGH: No, I'm not simply demonstrating that. That would be one of the factors. I am also suggesting that if uranium comes into your drinking water in highly soluble form, even that absorption would be different.

Q: That may be, but another question is how many samples of feces and how many dogs does this represent?

N. SINGH: Only one, but the complete study would be with two dogs for seven days each. And we have our second day, which was very much similar to this day. But we will have the complete data for two dogs for seven days.

G. DIAMOND: These numbers assumed that the animal is in a steady state with respect to intake? At least, some fecal and urinary excretion? Does that clearly reflect intake of uranium? That is, the sum of fecal and urinary output equal to the amount of uranium ingested?

N. SINGH: Intake, yes. And the GI absorption is affected by--that would be the total intake other than for the total absorbed.

G. DIAMOND: I guess my question is, does fecal excretion and urinary output equal the amount ingested?

N. SINGH: Ingested? No. The total ingested and the total coming out. The reason we don't have a balance is because the total amount did not go into the body.

G. DIAMOND: A lot left on the tray.

N. SINGH: A lot was left on the tray, yes. The total intake is suggested by the feces, plus urine.

R. ALEXANDER: To summarize what we've learned today and what has gone into the record, the public record on which the people at the Nuclear Regulatory Commission will arrive at a decision: I believe that on strictly scientific and biological grounds, the preponderance of the information we've heard today would point in the direction of prescribing a lower limit to protect the kidneys from soluble uranium.

I think that it's difficult for government people to turn their back on animal data in the absence of conflicting human data, and I think that the point has been made by a number of people today that the fact that we don't have on record anyone who has gotten sick from uranium exposure doesn't necessarily mean that there has been no kidney damage to people who have been highly exposed. I found the technical evidence presented for that case to be persuasive. It's difficult to turn your back on the thought that people who might have a considerably decreased kidney function, unknown, would be more vulnerable to kidney disease.

Of course, decisions like this are not always based entirely on the best science available. Other things that we have to consider at the NRC are the impact of lowering that nephrotoxic limit, the impact on the sister agencies,

such as the Department of Energy, and the Mine Safety and Health Administration, and the Department of Defense. Here in our own country, there are a number of Agreement States who license work with uranium, including soluble uranium compounds. We have to take into account the views of labor unions and, certainly, the views of the business people and the cost to them of lowering an intake level by a factor of 5. I think that lowering the limit by 30% or something like that might not work a hardship or have much of an impact outside of our own country, but if we lower it by a factor of 5, it would be very difficult, I think, for government officials in other countries to do nothing about this.

I find this to be a particularly serious decision to make.

I'd like for you to understand and for readers of the record to understand the role of the NRC staff in questions like this. We certainly don't make the final decision. But sometimes I think the decision we do make is more important than the final decision, because the decision we make is what recommendation to put in front of the final decision makers.

If we decide at our level, for example, to drop the question, then it really isn't very likely that someone else within the Agency will raise it. On the other hand, if we make a decision to recommend lower limits, then we will simply be called upon to justify our recommendation. If that recommendation would be approved at high levels in the NRC, including probably, in a case like this, the Commission itself, then our recommendation would be subjected to public scrutiny, and there would be a public comment period and public comments that develop a more persuasive rationale than ours might set aside our recommendation. So, we would seek a final decision that was made by the country as a whole, as best we can do that in a situation where the Commissioners are not limited by the legislation approved by Congress.

Now does anybody here have anything that they would like to say before we close the meeting?

This is Paul Stansbury, who I believe has probably been holding his guns for the last two days. Paul may be the first or one of the first people from the fuel-fabrication industry to speak at these meetings. Paul Stansbury.

P. STANSBURY: Yes. I'm an operational health physicist at the General Electric Nuclear Fuel Manufacturing Department in Wilmington, North Carolina.

My first reaction to Bob Alexander's comments is "ain't democracy wonderful," having listened to his interpretation of the data, and I realize the problem here of looking at data that are incomplete. I come to a different conclusion, but I come from a different perspective, and I would like just a second of your time, and also perhaps get in the record.

If we look at radiological limits as they're done today--and uranium is of interest to me and also to you--we suffer from two problems today, and I would briefly outline one single-intake mentality.

If you look at 10 CFR Part 20, it is concerned with limiting single intake

exposures, euphemistically referred to as breathing a certain concentration for a certain period of time, but that's French for single-intake. That's the way regulation is done, and we really can't gripe against the enforcement people for enforcing the regulations the way they're written. That's their job.

The second thing we have in internal dosimetry is an internal radiation, which seems to be built into the regulations ICRP 26 and 30, perhaps, bring this out best, when they point out that exposure at MPC is equivalent to 2-1/2 mrem/h of external exposure. Yet we see in our industry (not just the uranium industry but in other places where a worker might be in a significant external field) that this worker is required to go slower because he has to have protective clothing and respiratory protection to protect him against accruing external exposures, a commitment at the rate of 2-1/2 mrem/h.

Mr. Lowell has got some eloquent articles on this subject, and I won't use this as a forum to beat that wagon much more, except to say that the proposed changes to Part 20 will tend to cure the radiological problems because they're placed on the basis of doses and estimate for risk.

When I listened over the last couple of days, the first thing that comes out at me is not necessarily the need for a lower limit. But it is obvious that a single limit is not an acceptable way of protecting workers, either in government or an industrial business. The objective is to protect people from harmful effects of radioactive materials, and it would be uranium in this case that has the chemical component that we're concerned about. It's rather obvious from all the stuff that we've seen that a single limit won't do, yet that seems to be the easiest solution. "Let's have a lower single limit." We know it's the history of exposure, though, that makes the difference.

So, one of the things I hope will come out from this meeting is looking for better ways to do protection. It seems obvious to me that what we're looking for is a way of meaningfully measuring kidney damage. That's something that can probably be done noninvasively. We look at the new technology, the ability to synthesize specific protein probes, and it seems to me there's technology that could be used very well there to come up with a way of meaningfully measuring kidney damage.

I guess the problem here is... I'll use the analogy of participating in debates when I was a kid in high school. The tree falls in an isolated part of the forest. Does it make a noise? Theories can go on about that debate forever. The real question is, that if the tree falls in the forest, does it hurt anybody? That gets me back to the question of, if I measure the meaningful total of kidney damage, is the measurement of any change in kidney function? That may not be a problem. The death of a single nephron may not be a problem. We learn today that you lose 1% a year anyway, just from living. So the question is, is a small fraction added to that 15% that you're going to lose in the natural process of aging, significant enough to involve spending lots of regulatory and private industry and personal resources to prevent it?

Obviously, there's a level of loss of kidney function that nobody would want to undergo, because of the chance of running into a serious threat, where

you need all the kidneys that you have. But it seems to me that we don't want to be trapped into regulating absolutely-no-measurable-effects-on-the-kidney as a limit because if we do, we're not going to be putting the resources where we could do the most good.

Thank you.

R. ALEXANDER: Are you open to questions?

P. STANSBURY: Certainly.

R. ALEXANDER: Since we both know that the intake limits of the Commission for many years and for the foreseeable future are based on equilibrium conditions, what is the basis for your first statement about single intake control?

P. STANSURY: Can I get that question again?

R. ALEXANDER: Yes. Since the Commission's intake limits, even for chemical toxicity of uranium, are based on equilibrium conditions, where the intake is equal to the loss from biological elimination and physical decay--

P. STANSURY: Except for long-lived emitters.

R. ALEXANDER: There are 17 radionuclides that do not reach equilibrium in the body in a 50-year period, but in that case, it's the cumulative deposition in the organ. The dose-rate limits that the ICRP used in either of those cases, either in the equilibrium case or the nonequilibrium case, are not based on single intake considerations. So, I'm not sure what you mean.

P. STANSURY: The value of the limit is based on the ICRP-2 modeling that is based on equilibrium conditions; however, the enforcement of that limit is based on consideration of a single intake.

R. ALEXANDER: Enforcement?

P. STANSURY: Demonstration of compliance is the euphemism. If you can't demonstrate your compliance, you're in noncompliance. That is based, essentially, on a single intake.

R. ALEXANDER: I think we need to understand that more, Paul.

P. STANSURY: A 520 MPC-hour limit is considered an overexposure at that point. My thought is that when we started in the radiation-protection business with ICRP-2, we had to make this terrible organ assumption. We are a little smarter today, and we see that things are not quite that simple. So, my thought is that it's not obvious to me that conceding a set of regulations that depend on controlling single intakes is appropriate. That's the way we started; that's the way we're doing business today. But right now we're in the process of looking at much larger changes. For instance, the new Part 20 is not going to be tied so directly to a single intake as the means for evaluation of a situation, as a means for demonstrating compliance or noncompliance. The

new Part 20 brings in the radiological factors in various different ways.

My thought is that if we're going to look at chemical toxicity, perhaps some of the same wisdom that would go into looking at controlling it on the basis of dose for radiological concerns ought to be used for controlling chemical toxicity, in much the way that lung counting and urinalysis are used along with air sampling. It seems to me there needs to be some consideration given to the biologic equivalent of a lung count for a kidney, as far as measuring its function, something that you can do after the fact to assess what happened, besides just looking at the exposure conditions. And my thought is, with the new biological technology that's around, the stuff is probably out there to do it. It's just a matter of finding it.

Did that address your question? I know I didn't answer it.

R. ALEXANDER: I think you mean if an inspector finds a high bioassay result in your facility, he assumes that the exposure occurred the next day after the preceding bioassay specimen was taken. You call that a single exposure; you calculate the intake. Is that what you said?

P. STANSURY: I'm sure that has been done.

R. ALEXANDER: Do you want to shed any light on this?

D. COLLINS: I think I understand what you're saying. I think you're saying that even though our regulations are based on a 50-year exposure, in fact, our regulations have 13-week limits. If that is the case, we do have 13-week limits. The scenario that Bob just ran I don't think is what is normally done. We encourage licensees to do the analysis, not us. And we review their analysis to be sure that it is reasonable and appropriate. We don't expect every conservatism that one might apply to be applied. We expect reasonable measurements based on all considerations and circumstances, and at fuel facilities, most fuel facilities I'm aware of, you don't have to make those kind of assumptions. There's a great deal of air sampling and operation data to base any intake analysis on. I don't think the scenario you presented, Bob, is necessarily one we would find in the inspection force.

R. ALEXANDER: One of the things I wanted to ask Paul. An inspector observes a bioassay record of one of your workers, and say they get a monthly specimen. One result is 25, the next one 35, the next one is 20, the next one is 30, and so forth. Surely, he is not going to claim that a single exposure--that some single exposure could possibly be responsible for urinary uranium records like that?

P. STANSBURY: No, I am not so much criticizing what is being done but rather looking at the philosophy as it is reflected in the regulations. If you read Part 20, the teeth in the regulation are focused around a single intake above a given amount. That was a way that essentially the ICRP-2 started. They had some other models, continuous intake models, et cetera, and a lot of that is the way business is done. My thought is that is changing for the radiological half of things. As we look ahead to proposed revisions to

Part 20, the emphasis has shifted away from a single intake determination to a consideration of radiological estimates of hazard, i.e., to dose. My thought is that it would be nice that instead of looking at a single intake and controlling a program for soluble uranium on the basis of a single intake, we could measure and look at kidney damage compared against some sort of reasonable standard.

R. ALEXANDER: It is true that in our regulatory guides for bioassays--you can correct me if I am wrong, Allen Brodsky--we try to get our licensees to establish a urinalysis program such that a single exposure that we would consider to be significant in one place or another would not go undetected. In other words, if an exposure took place the day after the previous bioassay, we would still detect it with the next bioassay specimen. But there is nothing like that in the regulations, Paul. Those are just in the reg guides.

P. STANSBURY: Yes, and we know about reg guides. But the one that I like the most is where it says, look at the uranium and determine whether it is soluble or insoluble. This is done in a block diagram. It says determine the amount of the intake, and then it says--in the 2.70 regs it says--report the noncompliance. My thought is that is a single-intake mentality, and it may not be the best way to apportion our resources to protect the health of workers.

R. ALEXANDER: Bob Thomas?

R. THOMAS: Can I say something? There is one thing that disturbs me a little bit about this. I just recently had to give a talk on uranium and I went through all the history on this. There must be thousands of animals that were exposed at Rochester, where they all make the 3 μg for the kidney look okay. Now, we come along with one or two experiments. Paul Morrow writes a letter and says because he did an experiment that shows that we ought to divide things by 5 and have a new value, all of a sudden that is what we look at and ignore the other thousands of animals that have been done. That does bother me, and it is too bad Paul wasn't here because I would have liked to have talked to him.

The other thing that bothers me, Bob--and I am speaking for myself when I say this, and I will try not to be too derogatory--I recognize the job security that comes in getting together and lowering standards by a factor of 5 every 10 years. What bothers me is I do think that this group, not me, is going to be saying maybe 10 years from now we ought to divide by another factor of 5. That is all we seem to do is keep lowering things.

This says several things to me, one I just mentioned that I won't repeat. The other one is that it implies that we must not have known what we were talking about over the last 40 years because periodically we get together to reduce the limit. It doesn't matter whether it is uranium in the air or in drinking water or whatever it is. That seems to be what we are about.

This does bother me, and I will guarantee you that I can do an experiment of some kind that will show you that the 3 μg should not only be divided by 5, but probably it should be divided by 10. You are going to look at the one experiment that does that every time they come along. It just bothers me, the

whole philosophy.

Furthermore, you know, looking at any little damage that something does and call that toxic, that also bothers me. It goes along with the zero-risk society that we seem to be living in today. My lord, it seems to me there is a limit to just how low we have to have limits on everything that we deal with. I think sometimes we get carried away.

R. ALEXANDER: I imagine we do. However, I have also examined data that were published on the animal studies, the early animal studies at the University of Rochester, and came to, as I mentioned earlier, a different conclusion than you have. Significant kidney damage for all the species that they have looked at is reported for kidney concentrations of less than 1 μg/g going on down to 0.6 and 0.5.

If you look at Voegtlin, Hodge, and Stannard--I hope I have all the editions--if you look in there in their discussion of the establishment of the nephrotoxic limit, they report (in fact, they show a table that summarizes the early Rochester data) that there was significant renal damage at kidney concentrations considerably less than 1 μg/g, and they even have a pertinent statement. I can't quote it exactly, but there is a statement in their book that says apparently the ICRP was more persuaded by the absence of clinical symptoms in overexposed workers than by the animal data. So, I feel very comfortable in my concern about the animal studies. To my knowledge, in every case where those studies have been done, renal damage has been effected at less than 1 μg/g.

Other than that, I agree with everything you said. I think whatever we do about this has to be done in concert with a lot of other government agencies, as I pointed out, and I think that the time it would be done would be when the new Part 20 is published in final form. I can't see that happening sooner than two years. So, I think we have time to thresh this out. I think our public meeting here, the one we had today, starts us off in the search for the correct answer as to what to do.

Warren Keene?

W. KEENE: Perhaps I am simply displaying my ignorance, coming from an engineering background rather than a medical and biological background, but I have seen enough evidence today for changes in renal function with uranium exposure. However, what seems to be missing is a satisfactory definition of what is significant renal damage. Some of the changes in renal function certainly are only transitory, and we have seen that the function returns to normal shortly after administration. So, I think what is seriously needed is an effort to consider whether changes in the regulation are appropriate, and what is an adequate definition from the medical community of what is significant renal damage.

R. ALEXANDER: Oh, I agree with that. Of course, all I have been able to do so far is to speak to physicians who are knowledgeable about such matters. Everyone I have ever spoken to has had the same answer, which is essentially, don't toy with a man's kidneys. You don't want to damage a worker's kidneys.

That is pretty qualitative advice in nature. I think one of the biggest questions remains unanswered. We know that a lot of people have been overexposed to uranium, and we think we know that in every case the worker recovered because he was excluded from additional uranium deposition in the kidney until the kidney had a chance to eliminate the uranium. It may very well be that is why serious damage has not been reported, i.e., that the insult was terminated.

So, I think one of the biggest questions is in the bioassay program: how long can the kidney tolerate more than 3 μg/g before the damage becomes irreversible? We have signed a contract with the University of Rochester to investigate that question. So, the next time we meet we will have a little bit more information along that line.

There are just a lot of unanswered questions. There is also the bothersome points that Max Scott brought up today. Max Scott--and I wish he were still here to hear me say this--Max Scott and Hap West have done better than any of us have ever done to document and make available to others information about overexposure to uranium. Nobody that I know of has even come close. I don't know how they did it. He was pointing out today essentially when it comes to monitoring uranium in the workplace, you can't go by urinalysis, you can't go by air sampling--that is even worse--you can't go by in vivo counting, so what does that tell you if you are trying to protect workers?

It says to be conservative. That sticks in my mind, too. If you are going to make an error, which surely you are, then err on the conservative side. Do that hopefully without destroying the uranium business in the United States. As a matter of fact, do that hopefully without wasting a dime.

Q: You have obviously thought about what a new limit should be. You seem to indicate it might be 3 μg/L of urine. What do you think the impact of that will be on the industry?

R. ALEXANDER: 3 μg/L?

Q: You are talking about cutting the nephrotoxic limit by a factor of 5, and I believe the limit is 15 μg.

R. ALEXANDER: We use 15 μg as an investigation level.

Q: And you are thinking of cutting that presently to a factor of 3.

R. ALEXANDER: I don't think that we would go as low as 3 because the 15 from which the 3 would spring is not the correct number. It is 50% of 30 μg/g, which you can calculate on the back of an envelope as being associated with the standard man and equilibrium conditions with the MPC. That assumes that all uranium comes from the kidney tissues. We know that is not true. Another 20 or 30 μg comes from the bone, and the uranium deposited in other organs of the body.

So, if we were to publish a Reg Guide today, we would not use the 30 number, although I guess the way the final Reg Guide 8.22 is coming out--

Dr. Brodsky, you can correct me if I am wrong--you have to look at the fact that there is a Class-Y component, a Class-W component, a Class-D component in most cases. A really scientifically satisfactory control system would be incredibly complex. And remember that in the regulations, simplicity is extremely important. So, I think Allen Brodsky is as good as anybody at looking at complex data and coming up with something satisfactory and simple. If I am not mistaken, the number that we are going to have in the final version of the Reg Guide 8.22 is not very far from 30 or 35. But that is because the Class-Y material, based on the effective dose equivalent, is so much lower, where for someone just exposed to Class-D material, I think the number would probably be 60.

Q: There are several action levels. It has not completely simplified the one number, but they are all action levels.

R. MOORE: Bob, just for historical perspective, the cases that were involved in our study in the period of time in which they had the elevated urine levels, they were on an action level of 15 μg/L and were restricted until they returned to 1 to 5.

R. ALEXANDER: That is the usual practice, something like that. Restrict them till they get down to about half.

R. MOORE: This was half of the published data at that point. The published action levels at that point were 150, and they had operated at 50 and 25.

Q: The reg guide uses 50 now.

R. ALEXANDER: Would anybody else like to inject some reason into our deliberations?

(No response.)

R. ALEXANDER: If not, I want to thank you for coming. I would also like you to tell your employers that we very much appreciate their cooperation in providing for your attendance here today to help us with this problem, and we hope that the final decisions that our Commission makes will be soundly based and, while they might not be exactly the decisions that you would have come up with, ones that you can live with.

Thank you very much.

[At 4:45 p.m., December 4, 1985, the meeting was adjourned.]

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13. ABSTRACT (200 words or less)

Edited transcripts are provided of two public meetings sponsored by the Occupational Radiation Protection Branch of the Division of Radiation Programs and Earth Sciences, Nuclear Regulatory Commission. The first meeting, held on December 3, 1985, included nine presentations covering ultransensitive techniques for measurement of uranium in biological specimens. Topics include laser-spectrometric techniques for uranium bioassay, correlation of urinary uranium samples with air sampling results in industrial settings, delayed neutron counting, laser-kinetic phosphometry, isotope dilution mass spectrometry, resonance ionization spectroscopy, fission track analysis, laser-induced fluoresence, and costs of sampling and processing. The nine presentations of the second meeting, on December 4, 1985, dealt with the nephrotoxicity of uranium. Among the topics were the physiology of the kidney, the effects of heavy metals on the kidney, animal studies in uranium nephrotoxicity, comparisons of kidney histology in nine humans, renal effects in uranium mill workers, renal damage from different uranium isotopes, and Canadian studies on uranium toxicity. Discussions following the presentations are included in the edited transcripts.

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