

Dr. June Dunnick

Oral History Transcript

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Interviewer: Sara Shostak

Sara Shostak: The recorder is on and you were talking about how your first job at NIH was at NIAID.

June Dunnick: My first job was at the National Institute of Allergy and Infectious Diseases, where I served as program officer for the hepatitis vaccine development project. At Stanford, I developed lipid vesicles for drug delivery. I returned to NIAID as the antiviral substances program officer. This program used animal models to identify antivirals with therapeutic potential, and then examined the most promising in clinical trials with investigators at Stanford (Dr. Tom Merigan), University of Alabama (Dr. Rich Whitley), and others at Harvard. The NIAID still has many of the same contracts that conduct clinical trials for the Institute. We worked in collaboration with drug companies. For example, we studied the effectiveness of acyclovir for herpes, and interferon for hepatitis. Studies of amantadine and rimantidine for influenza were examined in collaboration with Dr. Ray Dolan (University of Vermont). Many of these

antivirals are now important antiviral compounds used in the clinic. It is now a common NIH practice to work with drug companies, to develop strategies for preventing or treating diseases.

Shostak: And at that time it was less common.

Dunnick: It was a groundbreaking approach for developing drugs.

Shostak: And then, when did you come down to NIEHS?

Dunnick: I came to NIEHS in 1981, to study how environmental chemical exposures affect health.

Shostak: So did you begin working at the National Toxicology Program as soon as you came?

Dunnick: Yes.

Shostak: And what was the focus of your research?

Dunnick: Well, at that time, NIEHS had recently become the center for the National Toxicology Program. NCI had started many 2-year studies to identify cancer, and this responsibility was transferred to NIEHS. In the 1980s, we had hundreds of chemicals we were working on to determine their cancer potentials. In the mid-1980s, the Doull Committee, organized by Dr. Rall, recommended increased emphasis on mechanisms. From that time on, we focused more on mechanisms, depending on the chemical situation or the relevance to humans.

Shostak: Okay. Dr. Doull [John Doull, M.D., Ph.D.] is a prominent toxicologist.

Dunnick: In the early '80s, we had many chemicals to manage and design studies

for, and then increasingly over the years, we focused more on mechanisms of disease and brought in more comprehensive information to understand biological functions. And, in fact, that was the impetus for the transgenic animal studies - to understand what genes are involved in cancer development. This included a study of a gene defect in the transgenic animals and how this defect would predispose to cancer. For example, how does the p53 gene deficiency predispose to cancer?

Shostak: You mentioned the Ames test when we were talking a couple of moments ago. Before transgenic models came along, what were the models or the tests available for looking at genetic mechanisms?

Dunnick: *In vitro* tests included the *Salmonella* test. Under the direction of Dr. Ray Tennant, NIEHS explored the usefulness of a whole series of *in vitro* tests to predict cancer. I'm sure you've talked to Ray.

Shostak: Yes.

Dunnick: NTP has been working to understand mechanisms, because in order to use animal data for predicting what will happen in human, you need to understand if disease mechanisms in animals are the same as in humans. And that's where the transgenics animals really seem to have a purpose – the ability to add to our knowledge of disease mechanisms. Cancer is an interaction between gene change, environmental exposures, and age. As cells proliferate, cancer gene changes are amplified. NIEHS focuses on how these factors contribute to the disease process.

Shostak: Was gene environment interaction a focus of research when you first came here in the early '80s?

Dunnick: In the '80s, we had so many studies to report that there was not as much focus on mechanisms. By the late '80s, and certainly in the '90s, there was a focus on mechanisms. But, of course, now, as new technologies have emerged it is possible to focus on how environmental exposures can cause gene change. Microarray technology has allowed us to use high throughput technologies to identify gene change.

Shostak: How has your research, or the focus of your research, changed over that period?

Dunnick: Well, here's a good example. I have initiated a heart toxicity project, and we're looking at how chemicals cause heart toxicity. This includes histopathology endpoints, and the identification of biomarkers. We are also looking at chemical-induced heart gene changes using microarray or serum protein changes using proteomics. We are dissecting the heart disease process in many different ways.

Shostak: And in that study, are you asking different questions than you would have asked 15 years ago?

Dunnick: Fifteen years ago, we wouldn't have been able to conduct a comprehensive study of gene change. Heart disease might be due in part to mitochondrial damage. Identifying gene changes will help to identify different signal pathways that take part in this disease process.

Shostak: Okay.

Dunnick: In the 1980s you might just have been able to identify the pathology of the heart lesion. Now we are looking for gene changes that cause the lesion. This is possible because new technologies.

Shostak: Now, when did you begin working with transgenic mouse models?

Dunnick: In the 1990s we used the transgenic mouse models in the phenolphthalein project to identify cancer mechanisms.

Shostak: Yes, I have the publications with me. I'm still going to ask you to tell me that whole story.

Dunnick: In this particular case, we started with a traditional 2-year study, and found that phenolphthalein caused cancer in rats and mice. Additional information was needed to determine how this finding related to humans. We screened the thymic lymphomas from the 2-year studies for gene change. A common site for chemical-induced tumor in 2-year studies might be liver or kidney, but phenolphthalein caused an unusual cancer, thymic lymphomas, a cancer also seen in humans. We found, using immuno-histochemical staining, that the phenolphthalein-induced thymic lymphomas had an alteration in the p53 protein. In fact, the tissues from this study are used as a positive control for p53 immuno-histochemical staining for other NIEHS studies. Phenolphthalein changed the p53 protein expression pattern in the thymic lymphomas. The p53 gene is commonly altered in human tumors. Probably more than 50 percent of

human cancers have an alteration in this gene. In fact, one of our scientists gave a talk last week calling p53 the master gene, because it's a common gene changed in cancer, and it controls cell cycle and many other cellular processes. We used the p53 deficient mouse model to follow-up on the mechanistic findings from the 2-year studies. We knew from the 2-year cancer study that the p53 gene was involved in the phenolphthalein-induced cancer. How would the p53 deficient mouse respond to phenolphthalein? Phenolphthalein was a common over-the-counter drug, and one of the concerns was a potential risk in women who might use this drug for weight control. They might consume doses in excess of what was recommended on the package insert. Phenolphthalein use could be abused. The question was, how were our findings relative to humans? We used a transgenic animal (p53 +/-) to see if the combination of an inherited gene alteration and an environmental exposure would cause cancer. We found that p53 deficiency combined with the phenolphthalein exposure caused thymic lymphomas within two or three months. A 2-year exposure time was not necessary to cause the phenolphthalein-induced cancer. The p53 model has one nonfunctional p53 gene, and one functional p53 gene. Phenolphthalein exposure completely deleted the normal p53 gene. This confirmed that the p53 gene was involved in development of phenolphthalein-induced lymphomas. In human lymphomas, p53 gene alterations are also involved in the multistep cancer

process. We also compared the toxico-kinetics of phenolphthalein in animals with that in humans. Industry provided information on blood levels of phenolphthalein in humans. The doses that produced cancer or genetic changes in animals were within a 35 times human exposure levels (based on mg/m^2 comparison). Of course, people using phenolphthalein in weight control, might take much larger quantities of phenolphthalein, and then the animals doses that caused genetic change, would be even closer to the human exposures.

Shostak: Is there a publication on this information?

Dunnick: Yes. The reference is in Toxicological Sciences 56:271-281, 2000.

Dunnick: Dr. Ron Mason, NIEHS, showed that phenolphthalein caused an increase in free radical formation. DNA damage may be caused by free-radical formation, and it's hypothesized that is how phenolphthalein caused p53 gene damage. This phenolphthalein project used transgenic mouse models, and other information to obtain a complete body of information for risk assessment. The project used data from the 2-year study, and the transgenic mouse model to understand cancer mechanisms. We were able to compare exposures in animals and humans and obtained other gene-tox data (micronuclei formation).

Shostak: Thank you so much for giving me these.

Dunnick: Many NIH projects involve a team effort. In order to do a comprehensive study, we need input from many scientific backgrounds, including

toxicologists, pathologists, chemists, and geneticists. So while I organized, designed, and led the phenolphthalein study, we worked as a team basis

Shostak: Other people talk about working collaboratively, and at the same time, when they talk about the phenolphthalein study, they all say I should talk to you about it.

Dunnick: It definitely was a team approach. I collaborated with Dr. John “Jef” French on the transgenic studies.

Shostak: Yes.

Dunnick: Dr. French helped develop information that showed that the p53 mouse model responded to genotoxic chemicals. We knew that phenolphthalein caused genetic damage, and hypothesized that it would cause cancer in the p53 deficient mouse. Phenolphthalein is not positive in the Salmonella test, but is genotoxic as measured by the formation of micronuclei. When you’re using *in vitro* tests to characterize a chemical’s gene-tox potential, there are many factors to consider. For example, o-nitrotoluene, is not positive in a Salmonella test because it goes through an entero-hepatic recirculation, as does phenolphthalein, and that can’t be mimicked in an *in vitro* test system.

Shostak: So, is that . . .

Dunnick: And so that’s one reason why Salmonella is not a 100 percent predictive of which chemicals will be genotoxic and cause cancer. *In vitro* tests can’t

completely mimic what happens *in vivo*.

Shostak: And is that part of why transgenic models are important?

Dunnick: Transgenic models are useful because you can look at how specific genes contribute to disease. Each transgenic model would be different: one might have a p53 gene deletion, another an activated *ras* gene.

Shostak: This is the Tg.AC.?

Dunnick: There is a Japanese model with an activated *ras* gene.

Shostak: Okay, I talked to Bob Maronpot yesterday.

Dunnick: Bob Maronpot's done a lot of work with the *h-ras* model, and that model is promising for detecting potential chemical carcinogens, particularly chemical liver and lung carcinogens, because in rodents, the *ras* gene may be involved with the development liver and lung tumors. The p53 gene is not always a major gene in rodent liver and lung tumors. The selection of what transgenic animal to use is based on an understanding of cancer mechanisms. If studies are designed to identify liver or lung carcinogenesis by non-genotoxic chemicals a good model might be the *ras* model, because this gene is usually found to be altered in rodent liver and lung tumors. NCI also has a program on the development of transgenic models for human disease.

Shostak: I have a scheduled conversation with Glen Merlino, NCI.

Dunnick: A study of how the environment impacts the development of human cancer includes an understanding of molecular pathogenesis and the genes

involved in the multistep cancer process. Perhaps the best known cancer model is that for human colon cancer, where there are three or four different gene alterations that are involved in the cancer pathway: p53 gene, APC gene pathways, *ras* genes, and DNA repair gene defects. Using mice with different gene changes in combination with chemical exposures, can help in the understanding of how genetics and the environment influence the cancer process.

Shostak: Right, right.

Dunnick: The spectrum of genes you're born with may predispose you to chemical effects, and eventually the potential for cancer. One initiative we are working on, along with Marc Jackson, ILS, is a knowledge system to evaluate chemical exposures associated with gene changes. In the 21st century, an understanding of how the environment contributes to gene change will be important in designing cancer prevention strategies. For example, this information can be used to identify which chemicals may cause gene change. Eventually such a knowledge system may be used in a doctor's office. A child might present to the doctor's office, and obtain a gene scan that shows he/she has gene alterations A and B. However, it may require changes in genes A, B, C, and D to complete the multistep process to cancer. The doctor could develop a strategy to prevent environmental exposure that might result in C and D gene change. The knowledge system is designed to allow examination of what chemicals

cause gene changes,

Shostak: This is a little bit of a tangent, and I want to get back to phenolphthalein in a second, but because I'm so interested in what you just said. What exposures would you want to avoid?

Dunnick: A major environmental exposure that can lead to cancer is tobacco smoke. Another condition to avoid, which predisposes to certain cancers, is being overweight.

Shostak: So in 10 years from now I may take my hypothetical child to the pediatrician, and obtain genetic information.

Dunnick: That would be part of a program to implement a genetic medicine examination. Knowledge on chemical exposure and gene change may help us develop a cancer prevention strategy.

Dunnick: When NIEHS talks about cancer prevention, they are often referring to preventing exposures to harmful environmental chemicals.

Shostak: There are some exposures where it is easier to control exposure than others?

Dunnick: Yes

Shostak: You were talking about the phenolphthalein research being a group effort. As a non-scientist, I'm very curious about how those groups get assembled. How does that work?

Dunnick: In order assemble a productive team, I work to create a win-win situation for everyone on the team, and that's what I did on the phenolphthalein

project. There was something in the project for people interested in genetic toxicity, chemistry, pharmacology, toxicology, or cancer. FDA was interested in evaluating how transgenic mouse models could provide information on cancer mechanisms for risk assessment decisions. An effective team is formed when everybody has the same goal and there is a winning situation for everybody on that team. The principles of team formation are the same whether for a science project or for a business project. Effective teams help groups make progress.

Shostak: And you were familiar with Institute scientists and their interests.

Dunnick: I've been at NIEHS for a long time - so, absolutely, I know the people.

Shostak: And from an outside perspective, that is part of what's interesting about this Institute, that people do really seem to know each other and what they're doing, and there's one cafeteria where you all have lunch. I see different people I've interviewed sitting together and talking, so there seems to be a lot of opportunity for interaction.

Dunnick: Yes, I would say that's true. I think people do want to help other people. This is a smaller institute, so that interaction might be a little bit easier than at a larger campus.

Shostak: There's that great quote in the *Wall Street Journal* article you sent me. You said when you first got here; you thought that perhaps plaid shirts were mandatory.

Dunnick: Yes. People were more relaxed and informal.

Shostak: So, the phenolphthalein team was assembled. Everyone had something to gain. And what was the quality of your interactions with FDA around this?

Dunnick: Well, I interacted with FDA on a daily basis, because the industry representatives reviewed our findings and raised many questions. In fact, I have two books of questions that industry groups raised. I worked with FDA to answer questions on cancer mechanisms. In risk assessment, you have to be prepared to answer questions from other people. The regulation process is a dialogue between many people. It's not just FDA alone. FDA has advisory committees, reviews of the data, and industry input. The NIEHS was the source of the scientific information. We acted as a team of three: FDA, NIEHS, and industry. I tried to be available to answer questions. The group worked together in this regulatory process. And so, as you can see from that article I sent you, that there were a lot of questions raised, and we tried to answer them.

Shostak: What would you say were the lessons learned from the phenolphthalein experience?

Dunnick: One of the lessons is that the NIEHS scientists are able to interact effectively. We showed how data from different model systems could be used to understand cancer mechanisms. We showed how NIEHS and industry can cooperate on a scientific project that provided information for risk assessment. Everybody worked together, including the industry, to

evaluate the significance of scientific data and its relevance to human health.

Shostak: Were there any specific implications for the understanding of transgenic models?

Dunnick: We showed how transgenic models can be used in conjunction with other tests to come to a conclusion and make scientific progress on understanding cancer mechanisms.

Shostak: Did that shape the further development of transgenic models?

Dunnick: Yes, we are using transgenic models to help understand potential long-term biological effects of AIDS drug therapy. We are working with FDA scientists at the National Center for Toxicologic Research, to study the effects of AIDS drugs in p53 deficient transgenic mouse models. Some of these drugs are genotoxic, and we want to know if there are any long-term biologic effects. We want to have a quicker way to identify biological effects. For example, with the phenolphthalein, tumors developed in two or three months in the transgenic model versus 2-years in the standard bioassay. We are exploring if transgenic models can be used to study toxic/cancer effects of AIDS drug combinations. We will not be able to do a 2-year study on every drug combination. In the pharmaceutical industry, drug companies conduct toxicity/cancer studies on their own drugs, but not the combination of drugs used in the clinic. Does one drug enhance the toxicity or cancer effect of another drug?

What's the biological effect of different combinations?

Dunnick: AIDS drugs save lives. However, because AIDS drugs have only been used for the past 10 – 15 years, we do not fully understand the potential for long term sequelae. Therefore, we are using animal models to study this issue. Use of the transgenic models should compress the time to identify potential adverse effects (e. g. cancer). This work is currently in progress.

Shostak: The phenolphthalein study you just described to me focused on an over-the-counter drug. How is that similar or different to studying environmental chemicals?

Dunnick: A broad definition of environmental exposures includes air, water, or drug exposures. Many older drugs, such as phenolphthalein, have been used for most of the 20th century. Before the NIEHS studies, there were no standardized toxicology studies for this drug. Many drugs were introduced before toxicology testing was required. One of the NTP objectives is to conduct toxicology studies of the “older drugs” such as phenolphthalein, penicillin, etc. Environment exposure doesn't mean just water or air exposures; it also includes exposures to drugs, hair dyes, lotions, creams etc.

Shostak: But I guess what I'm trying to understand is if there are ways in which studying more diffuse or less well-characterized exposures, something in the water or the air, makes that qualitatively different than studying

something that's purposefully used or purposefully ingested.

Dunnick: You would use the same toxicologic principals to study various environmental exposures. We design studies to understand the biological effects of environmental exposures. By looking at the chemical structure we might get clues to the biologic effect. For example some brominated flame retardants have a structure that is similar to thyroid hormone, and these chemicals alter thyroid hormone homeostasis.

Shostak: So transgenic models could have the same role no matter what?

Dunnick: Yes. Transgenic models may help us understand mechanisms in disease processes. In a toxicology study, the principles will be the same whether studying a drug or a water pollutant. Routes of exposure could differ (e.g. dermal, inhalation, or oral exposure).

Shostak: But transgenics are relevant in either case, and in fact what you learn in one case about transgenics can then be applied to other cases.

Dunnick: Toxicology is a broad area of science, and a diverse scientific team is needed to understand genetics, chemistry, and pathology.

Dunnick: NIEHS receives nominations for studies from sister agencies (e. g. NCI or NIOSH or FDA) and from the public. We then have a study design process, to determine the best study design to answer a particular question.

Shostak: So, let me kind of move us up to the present moment, then, and just, you mentioned the study on flame retardants. Are there other projects that you're working on currently that involve transgenic models in some

significant way?

Dunnick: We plan to study the AIDS drugs in transgenic models.

Shostak: And is it a fair assessment that transgenic models are being used in your research to explore mechanisms at this time?

Dunnick: Yes, transgenic models have been found to be particularly useful in studying cancer mechanisms.

Shostak: Is one of the strengths of the 2-year rodent bioassay, that there are so many endpoints that are possible?

Dunnick: Yes. For example, in an o-Nitrotoluene study, the chemical caused tumors at many sites including the colon, a common site for cancer in humans. In collaboration with Dr. R. Sills, we found that the molecular mechanisms involved in the o-Nitrotoluene colon cancers were similar to that seen in human colon cancer.

Shostak: The cross-species comparison seems like an important part of your studies.

Dunnick: That's correct. It is an important piece of information for risk assessment. IARC uses cross-species comparisons in their risk assessments.

Shostak: Long before I was doing any research on transgenics *per se*, when I was just interning here, it was actually Ken Olden who brought up this phenolphthalein example in a talk he was giving to a bunch of interns, and he mentioned it again, as paragon of the integration of different kinds of

science across the Institute for regulatory purposes. And I think I understand from our conversation some of how that happened, but is there any piece of that story you feel like we haven't touched on?

Dunnick: Well, I think I explained the team approach. We had some information on the cancer potential of phenolphthalein from the standard bioassays, and used information from the transgenic models to understand the cancer mechanisms. We used these studies, along with studies on metabolism and genetics to make a comprehensive story on the biologic effects of phenolphthalein. We integrated information from many scientific fields, to provide information for regulatory decisions.

Shostak: It's absolutely fascinating.

END OF INTERVIEW