Hollaender Award 2023: Adventures in applied genetic toxicology

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Abstract

I am honored to receive the 2023 Hollaender Award, for achievements in "application of the principles and techniques of environmental mutagenesis to the protection of human health". People may assume that a career in applied science might not be as exciting or impactful as basic research. I hope my career "adventures" into environmental science, carcinogen investigations and photobiology, as well as publications in Nature and Science, will counter this assumption. The narrative is described in terms of "mentors" whose advice had a lasting impact: "come early and work hard" (meanwhile, have fun); " think instead of/while screening" (i.e. performing mundane tasks); "avoid the big boo-boo"; "just go in the lab and do experiments"; "become an expert". Many of the most critical events in science and in life are "random", as demonstrated by accidental adventures that led to scientific as well as life-altering personal realizations. Adventures included forays into nitrosamine mutagenicity, nanomaterial assessment, germ cell mutagenic risk, bacterial mutagenicity assays, genotoxicity of cell phone radiation, personalized cancer prevention, and >25 years in regulatory safety assessment at FDA: review of genotoxicity data, experiments in the lab, and collaboration with others to foster better analyses of DNA damaging agents, generally in relation to cancer risk. Finally, with my work and that of my lifelong tripmate William Lijinsky as models, I suggest that a "non-hypothesis driven", open-ended approach to research can be path-breaking and forefront.

Disclaimer

This commentary discusses my work experience at or with several different institutions. The opinions expressed are my own and do not represent policy or practice in any official or unofficial context. Trade names mentioned do not represent endorsements.

ABSTRACT

I am honored to receive the 2023 Hollaender Award, for achievements in "application of the principles and techniques of environmental mutagenesis to the protection of human health". People may assume that a career in applied science might not be as exciting or impactful as basic research. I hope my career "adventures" into environmental science, carcinogen investigations and photobiology, as well as publications in Nature and Science, will counter this assumption. The narrative is described in terms of "mentors" whose advice had a lasting impact: "come early and work hard" (meanwhile, have fun); "think instead of/while screening" (i.e. performing mundane tasks); "avoid the big boo-boo"; "just go in the lab and do experiments"; "become an expert". Many of the most critical events in science and in life are "random", as demonstrated by accidental adventures that led to scientific as well as life-altering personal realizations. Adventures included forays into nitrosamine mutagenicity, nanomaterial assessment, germ cell mutagenic risk, bacterial mutagenicity assays, genotoxicity of cell phone radiation, personalized cancer prevention, and >25 years in regulatory safety assessment at FDA: review of genotoxicity data, experiments in the lab, and collaboration with others to foster better analyses of DNA damaging agents, generally in relation to cancer risk. Finally, with my work and that of my lifelong tripmate William Lijinsky as models, I suggest that a "non-hypothesis driven", open-ended approach to research can be path-breaking and forefront.

INTRODUCTION

I am honored to receive the 2023 Hollaender Award, for achievements in "application of the principles and techniques of environmental mutagenesis to the protection of human health". Thank you EMGS supporters, mentors and colleagues over many decades.

During my career I've been an awardee of EMS/EMGS travel and service awards and served as newsletter editor, secretary, journal editorial board member, program chair and president. I edited issues of the society's journal, initiated the special interest groups (SIGS) and created the EMS video for the 25^{th} anniversary. Thus, EMGS has been a long- standing part of my scientific life.

With this review of my personal adventures as a genetic toxicologist, I want to recognize the unsung heroes of genotoxicity and carcinogenicity safety assessments, those in the contract labs, regulatory agencies, and national laboratories. Especially I want to demonstrate the value and surprising outcomes of seemingly routine genetic toxicology work. While working, if you keep your mind open and your brain turned on, you can discover amazing things. This is only following on the oldest traditions of scientific inquiry and observation as practiced by early scientists but seems lost in contemporary science. Notably, this is *not* hypothesis-driven research. It's applied research and it's anything but dull. The most interesting discoveries were often *random* and unexpected.

This presentation is focused on the advice of mentors, followed by demonstration of how important these recommendations turned out to be for my career, a fact I realize only now.

COMMENTARY

First Job

My first mentor was Dr. Milton Weiner, a scientist with whom I worked at my first job after graduating from the University of Rochester with a degree in Biology. We were in the Fermentation Development lab at Bristol Labs in Syracuse NY, where a group mutagenized a culture of a microbe making penicillin every week using the same mutagen (I'm guessing MNNG) and the same method, then screening for strains making increased amounts of penicillin. I realized much later that this was not likely to yield many different results week after week and month after month using the same method. Dr. Weiner was dismissive of this. Our small group was investigating new methods. **"Think, don't screen"** , he said.

Oak Ridge National Labs Biomedical graduate school with Jane Setlow, Dick Kimball and Alexander Hollaender

My graduate career began at Oak Ridge National Labs, while Alexander Hollaender was the director of the Biology Division, building it into a scientific powerhouse of ideal conditions for scientists. The only goals and obligations were to produce as much good science as possible. Different groups were forging different paths without common mind-sets. He encouraged people, and was the one-minute manager walking around hearing about everyone's research and then saying, **"this is good, but what else do you have; I know you've got more"** . To encourage expeditious habits he said, **"if everybody came early, everybody would be able to find a parking place".** Dr. Hollaender obtained all the funding for the lab. Noone ever wrote for grants or stood before site visit committees. It was free-style research, most definitely *not hypothesis-driven research* . It's only because functioning as a scientist soon became so difficult and so straitjacketed into hypothesis-driven research (as long as the hypothesis met the common mind-set), that I realize what a great place it was. I once gave Alex a ride home from the airport, after we both arrived on the same plane from Washington, and he invited me in to his house, a very modest house, to see "the collection". All manner of modern art work was on the walls, but also lined up on the floor. My favorites were the Hundertwasser's hung in his office, though; they must have been his favorites too. His wife Henrietta used to travel with him and haunt art galleries around the world. The Toulouse-Lautrec hung in the bathroom. I surmised that their most valuable work was located where a thief wouldn't think to look. Hollaender moved to Washington after Oak Ridge, to please his wife, so it was said, and so did I (to start a post-doc at NCI-Frederick in Maryland). When Hollaender died, I attended his funeral service in a small chapel in Washington National Cathedral. Their art collection was donated, and some can be seen on a search at the University of Wisconsin, where

he obtained his PhD. I didn't see any Hundertwasser's though.

My next mentors were Dick and Jane Setlow, Jane being my thesis advisor (along with Dick Kimball) and Dick being a lecturer in the graduate school. Dick, a discoverer of DNA repair who should have won the Nobel prize but died before it was awarded to others, said **"don't worry about the third decimal point, beware of the big boo-boo;"** worry that you have made a big mistake, like doing the wrong experiment. One of his lectures was entitled "A random walk in science". I don't remember what he talked about, but I do remember not understanding it. However, the idea of "random" as an aspect of science has stayed with me.

In Jane's lab we had conversations via 20 numbers, such as #2.**"Get your crap out of here."** #9, "to break thermometer, tilt back lid (on the water bath)". Thus, you could just holler, #2. This taught me a lot about humor, organization and getting along in the lab.

One of the first things I did in the Jane Setlow lab was to demonstrate that something they had published was wrong *(the big boo-boo, yes).* My thesis project was thus derailed. I started out studying transforming DNA with MNNG in *hemophilus influenzae* – treat isolated DNA, transform mutagenized DNA into recipient bacteria, measure mutations. The treated DNA was diluted 100-fold into the bacteria prepared for transformation and mutations were seen in the recipients. However, the residual MNNG was sufficient to generate the mutations seen, and if the treated transforming data was dialyzed, the mutations were not seen. Thus, the whole premise was incorrect and the in vitro treatment of transforming DNA did not lead to mutations in transformed recipients.

This might have been the first time, but definitely was not the last time that I was "trouble" in the lab (overheard quote from an FDA office director).

Graduate research project: N-Nitroso compound mutagenicity mechanisms and comparison with carcinogenicity

Since the project with mutagen-treated transforming DNA wasn't going to work, I ended up testing some nitrosamines. [Willie was always recruiting people to work on nitrosamines. He gave Bill Russell ENU and that became the animal super mutagen]. Willie had said **Don't go to medical school, science is much more interesting";** and later**: "Just go into the lab and do experiments; things will happen!".**

It was fortuitous that Evelyn Witkin's graduate student, Donna George, provided some of Evelyn's DNA repair deficient *E. coli* strains when she joined Jane's lab as a new post-doc. Thus, Willie's nitrosamines and Evelyn's *E. coli* strains, along with Jane's*Haemophilus influenzae,* morphed naturally into my thesis project**[a sequence of random events]** .

I had a set of methylating agents, MNU, MNNG and Nitrosocarbaryl (nitrosated insecticide Sevin) and some ethyl homologs. Nitrosocarbaryl was mutagenic and carcinogenic. It was likely the first nitrosamine considered a possible human environmental risk (Elespuru et al. 1974). Why were the mutagenicities of diverse methylating agents so different? They broke down to the same intermediate, in principle, but mutagenicity and carcinogenicity varied a lot. Also, how did mutagenicity and carcinogenicity compare?

Differences greater than 3 orders of magnitude in the mutagenic potency of methylating agents (MNU, MNNG and N-nitrosocarbaryl) could be accounted for by differences in their uptake into *Haemophilus influenzae* (using ^{3}H-labeled nitroso compounds) (Elespuru, 1979). The results correlated roughly with the octanol/water coefficient, indicating the critical effect of lipophilicity on uptake. This result was surprising and likely important, but uninteresting from a mechanistic standpoint, as it was not based on a function of active intermediate generation. *There goes our hypothesis* . Moreover, the differences we found did not correlate with carcinogenicity (Lijinsky and Elespuru 1976; Lijinsky and Schmael 1978). Nitrosocarbaryl was the most mutagenic but not the most carcinogenic methylating agent. Was lipophilicity and distribution an issue with different effects in mammalian systems? We have no idea.

As a contribution to mechanistic studies, I performed mutagenicity experiments with deuterium replacing

the alpha carbon hydrogens of NDMA, for comparison with the carcinogenicity studies. Diminished mutagenicity matched diminished carcinogenicity for NDMA, and thus alpha carbon metabolism was key for both mutagenic and carcinogenic effects (Keefer et al. 1973; Elespuru RK 1978; Lijinsky 1986).

Post-doc Donna George brought *E. coli* WU3610 from Evelyn Witkin's lab. This was a UV repair-deficient strain with two mutational markers at 2 amino acid auxotrophic loci, amber and ochre, both with AT base-pair targets. Using this strain with methylating and ethylating nitrosamines, I noticed something really interesting: mutations induced by ethylating agents appeared in one day, while those induced by methylating agents took two days. Thus, the *E. coli* strain was able to differentiate what was happening at the molecular level. DNA targets of ethylating and methylating agents were different (most likely direct alteration of AT base pairs vs. alteration of slower growing GC-based suppressors). Thus, I could differentiate between methylating and ethylating intermediate-caused mutations. With this tool we could examine the effects of nitrosomethylethylamine (NMEA) as compared with nitroso dimethyl- and diethyl-amine. Would the mutagenicity results resemble that of NDMA or NDEA, neither or both? The mutagenicity results in *E. coli* at AT base pairs indicated a preponderance of ethylation, a result which it seems I didn't publish. However, alkylation of DNA in vitro or in vivo indicated an overwhelming bias toward methylation vs. ethylation of N7-alkylguanine by NMEA alkylation (von Hofe et al., 1986, 1991). [Although O6 alkylation was not always studied, differences between methylation and ethylation would not alter the conclusions]. Deuterium isotope effects for carcinogenicity of NMEA indicated complex results, including targeting of esophagus, an organ targeted by neither NDMA or NDEA (lijinsky and Reuber, 1980, Streeter et al. 1990). A later paper expanding on the idea of studying differential chemical interactions using mutational endpoints was probably the first use of the term "mutational fingerprints", a paper I thought novel and worthy of PNAS, but not accepted by Dick Albertini (Elespuru et al. 1991).

[Random result; Just go in the lab and do experiments; things will happen]

Post-doc with Yarmolinsky (NCI-Frederick)

My post-doctoral work was at NCI-Frederick Cancer Research Center with Michael Yarmolinsky, who, upon returning from France wanted to be at NIH, not boondocks Frederick in the Basic Research Program. This was a P1 lab. P1 bacteriophage was unique in packaging bacterial DNA and facilitating transfer to recipient strains. It provided a unique mechanism for facile genetic manipulations. The lab was full of stars, including a brilliant Nat Sternberg who developed the Lox-Cre system for bacterial and then mammalian genetic manipulations. It was a joke based on lox and cream cheese, but an acronym for locus of recombination, which is what it targeted genetically. My project was to create a recombinant SOS response assay based on lambda phage induction, using a reporter gene, and to demonstrate how "easy" it was to detect DNA damaging agents. It was designed to compete with Ames' new mutagenicity assay. This was among the first uses of *lacZ* as a reporter, it's expression dependent on lambda phage induction, an SOS response. The genetic strategy was laid out for me and I was expected to implement it. I succeeded after an unhappy 2 years. At the end, I had finally learned enough to converse somewhat on their level. Later I called it the BIA (Biochemical Induction Assay). It's published in the inaugural issue of the EMS journal, EMM Volume 1 (Elespuru and Yarmolinsky 1979). After Bruce Ames declined to accept it for PNAS, he suggested the new EMS journal. How naïve to expect that Ames would accept such a viable competitor. It had much to recommend it, including a single bacterial strain to detect multiple types of DNA damage, and an elapsed time of 4 hours between test initiation and final results.

I spent a lot of time with this assay in subsequent years, discussed briefly later. Quillardet and Hofnung copied our methods and published a nearly identical assay using a different SOS response endpoint, *sfi* , creating the SOS Chromotest, 3 years later. They even copied our method of calculating enzymes units as the endpoint. Errol Zeiger said he reviewed the Quillardet paper for Mutation Research and told Fritz Sobels they had to cite our work, but they didn't. They created a commercial assay and promoted it relentlessly. However, I knew from my own work that the SOS assay misses some types of DNA damage, especially missense mutagens. Also, by commericializing the assay they couldn't compete with Ames, who gave away his bacterial strains. Nevertheless, you might say I had some recompense in the end by publishing a paper

in Science using the assay. It was actually terrific for high school science projects. The results were seen the same day, color developing as from a polaroid film. This was exciting for students. I think such a rapid assay could still be useful in certain contexts.

Staff scientist, Chemotherapy-Fermentation Lab, NCI Frederick

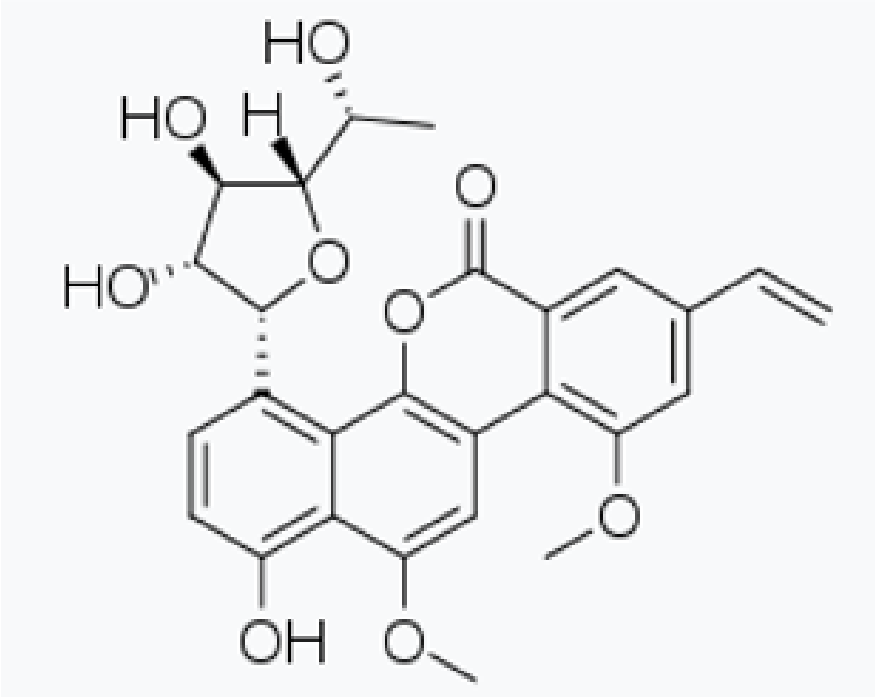
The BIA was used to screen fermentation broths for DNA damaging activity, as putative new cancer chemotherapeutic agents (Elespuru and White, 1983) supported by the Natural Products Branch of the NCI. I used 243 mm x243 mm bioassay plates - petri dishes with a layer of top agar containing BIA strain BR 513 - to spot 100 fermentation broths in a 10 by 10 grid. This might be considered one of the most boring, mundane lab jobs imaginable. Fermentation broths yielding colored spots, with DNA damaging agents present, were further characterized by chemists to isolate the active principle in the broth and determine if it was a new, undiscovered agent. There were usually many colored spots on the plate, with characteristics indicating a rough dose-response in each spot (a ring was a high dose with a toxic center).

One such spot was processed by the chemists and fraction samples provided in order to follow the active principle. But I found nothing active in any of the samples. The same result ensued with subsequent samples. As our project was closed down, I had moved to a different lab where these tests were performed. After a year had elapsed, it finally occurred to me that the lighting conditions were different in the new lab. Thus, I retested some samples under the yellow light conditions in the new lab, as well as under ordinary white lights. Eureka! That produced an all or nothing result with nothing under the yellow lights. We had discovered a photoactive DNA damaging agent. It turned out to have been discovered in multiple labs (with different names), but no one knew it was activated by ambient lighting.

[think while screening]

[Just go in the lab and do experiments; things will happen]

Gilvocarcin V (for vinyl) was 5 orders of magnitude more potent than 8-methoxypsoralen (8-MOP), a model UVA-activated DNA damaging agent used clinically for treatment of psoriasis that we immediately adopted as a positive control and comparator. 8-MOP is a structural analog with an identical coumarin core. Gilvocarcin V is likely the most potent DNA damaging agent ever discovered. I found out later that the chemists thought I was incompetent when I couldn't find any activity in the samples they provided, and they actually tested it in the old lab without me using the BIA (with positive results), and published it without my knowledge. However, when I discovered that it was light-activated, everyone wanted to be a co-author on our paper. But it was published in Science as Elespuru and Gonda (my technician), 1984.



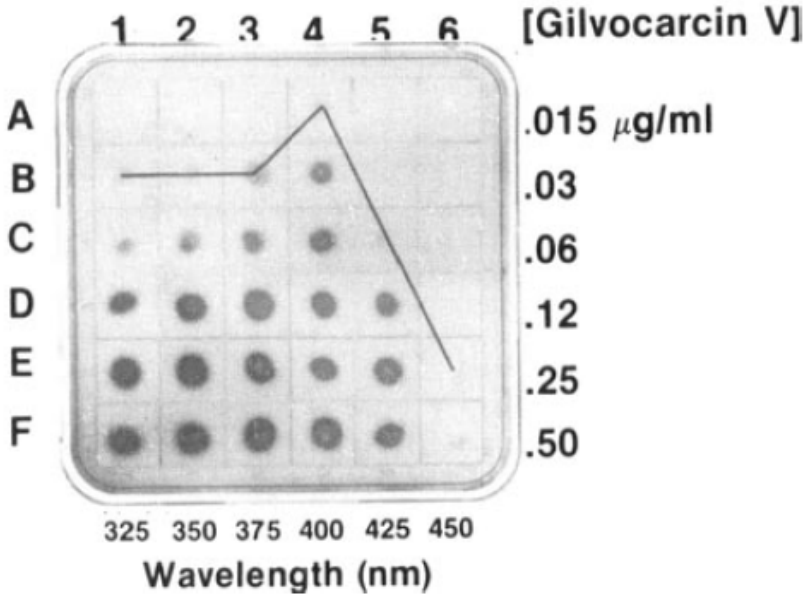


Figure 4a. Plate assay (spot test) for induction of lambda-*lacZ* prophage by photo-activated gilvocarcin V. Induction by limiting dilutions of gilvocarcin V spotted on a bacterial lawn. Fluence was 10 J m^{-2}.

[random event major career path]

not-yet-known not-yet-known

not-yet-known

unknown

Personal impact of photobiology science

Episode 1: The child

For several years my young daughter had stopped learning in school after the clocks changed. Then we spent a month in Australia in winter (their summer). The improvement in my daughter's school performance after we got back was astonishing. It wasn't the same child. When we took her to a photobiology meeting in Florida in June, she was bouncing off the walls, and the light of the situation hit me. She had Seasonal Affective Disorder (the SAD syndrome). The reaction in June was the manic phase. SAD had been recently reported by Rosenthal at NIH and was a subject at photobiology meetings. Thereafter my daughter's academic work was satisfactory in the winter after light therapy (a special lamp). Experiments showed that the light exposure to the eyes was the trigger affecting mental functioning. Light on the skin had no effect on the SAD syndrome.

[Random effects; Think about what is going on; Recognize the big picture]

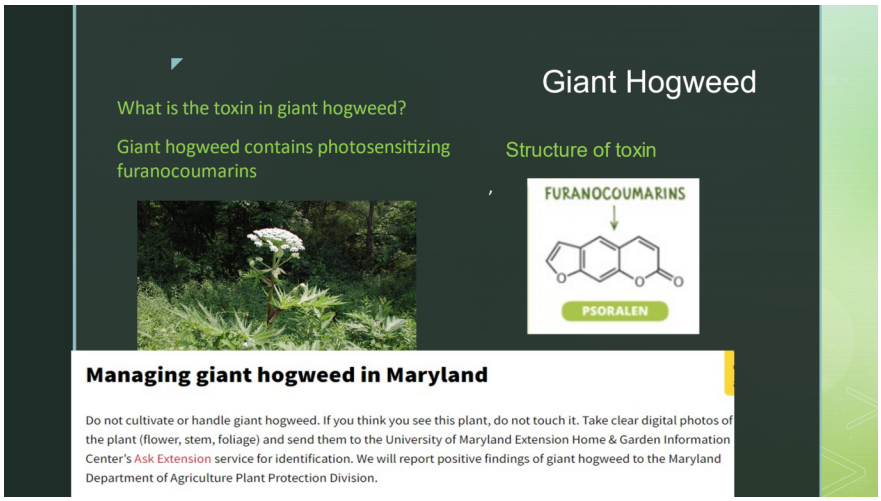


Figure 2. From the University of Maryland Extension web site

At NCI-Frederick with the Chemical Carcinogenesis Program

The Nitric Oxide story with Larry Keefer: Mutagenicity of Nitric Oxide (NO)

Working with Tom Cebula and Walter Koch at FDA/CFSAN, we determined the nature of the mutations induced by Larry Keefer's nitric-oxide donating agents in *Salmonella* bacteria, using Walter's method of oligonucleotide hybridization. NO donors were positive in strain TA 1535 (*HisG* 46 GGG triplet target). This was the first detection of NO, a newly discovered agent found as a major player in many biological processes, as a mutagen. Larry was creating NO donor agents as models for study and as potential cancer chemotherapeutics. TA1535 is also the target strain for N-Nitroso compounds like MNU and MNNG. Nitric oxide and nitroso compounds are not related chemically, but may seem formally similar with an "N-O" group in each. Targeting the same*Salmonella* genetic target led to the obvious question - whether NO mutagenesis was mechanistically similar to N-nitroso compound mutagenesis. Our experiments with oligonucleotide hybridization showed that the sequence changes resulting from NO and nitroso compound mutagenesis were not the same. One targeted the first G in the triplet, while the other targeted the second G in the triplet

(the third position, "wobble" is not selected when altered). Thus, the mechanisms were different. What a delightful result! This data led to another paper in Science (Wink et al.,1991) that I had spent a lot of time editing before submission, in order to get the context right. I had submitted the Gilvocarcin paper first to Nature and they returned it without review. I thought hard and re-wrote it, working on the context especially, before submitting to Science, where it was accepted without revision. [Batting average: Science: 1000, PNAS: 0]

At FDA Center for Devices and Radiological Health

Researcher/reviewer

I became a subject matter expert over the course of three decades of genotoxicity data review experience with mainly medical device and drug submissions, but also occasionally for veterinary and food contact products or food and cosmetic dyes. We were accustomed to working in teams, which I learned to value in relation to integrating diverse sets of expertise, mine being determination of genotoxicity. The FDA Center for Medical Devices and Radiological Health (CDRH) with engineers, physicists, chemists and software engineers is the most diverse FDA center. It was satisfying to provide a useful contribution to product safety assessment. Eventually I ran or participated in genotoxicity training sessions in specific centers or FDA-wide over many decades. Assessment of cancer risk (via surrogate genotoxicity) was a legal requirement for most FDA-regulated products (except biologicals).

Genotoxicity reviews

Because most products were developed absent of genotoxic risk, genotoxicity reviews of hundreds to thousands of medical device and drug submissions showed mostly negligible human safety concerns. Rare exceptions of notable human hazards included a known mutagen with direct exposure to human germ cells, encountered early on. This was not recognized as a hazard despite my efforts. Later on, my opinion might have had more impact. A different agent, methylene blue, was reviewed as a drug with multiple indications and was also a component of medical devices. This was the most interesting agent I ever encountered, considered non-hazardous by physicians because of its continuous use for by Larry Loeb and others and activated by enzymes or light. It has been cited as the world's oldest drug and seems to be treated as GRAS (generally recognized as safe), although that attribution is usually limited to food-based agents. You can buy it at Walmart for your fish tank. I made a presentation illustrating the dual non-overlapping pathways of methylene blue as a therapeutic and as a classical mutagen. And never the twain have met.

Animal testing

A more important contribution to safety assessment was probably my 10-year crusade to get rid of animal-based genotoxicity assays for assessment of medical devices, usually an in vivo micronucleus assay. This was well before the ground swell against animal testing, and might be seen as prescient, but the truth was that medical devices themselves or device extracts could not reach the OECD guideline dosimetry requirements for a valid assay. Thus, there were hundreds of invalid assays, usually using 90 animals per test. Of course they were always negative. I recall starting even earlier to eliminate the carcinogenicity tests, first their occurrence and then the option for performing them under any circumstances. As practiced, they were even farther removed from the requirements for any sort of scientifically reasonable carcinogenicity assessments, based not only on dosimetry but also on the numbers of animals used. I remember reviewing one test on a sum of 5 animals. The use of animals for medical devices was an incredible waste with no benefit.

[Think about the big picture]

Medical devices

In the case of medical devices, the most egregious submissions, ironically, failed for reasons other than genotoxic exposures. Thus, rarely if ever was a product denied approval or clearance solely as a result of a positive genotoxicity assay. But a positive result often led to investigations and further processing of the product to remove the genotoxin. Drugs with positive genotoxicity tests have this property added to the

label, but in my experience, that is not the case for medical devices, even though I requested it at least once. Some medical devices contain bovine or porcine collagen or other material preserved with glutaraldehyde. These invariably tested positive in the Mouse Lymphoma Assay (MLA) but not Ames bacterial assays, even in some cases where the device was rinsed to remove glutaraldehyde. In cases where we were not aware of the presence of an animal product, or such presence was denied, a positive MLA led me to query about this and generally I was right. Genotoxicity test results were thus sometimes informative in unexpected ways. I never could understand why an unstable and reactive glutaraldehyde could still be genotoxic after incubation at 37° for 3 days. Maybe it reacted and produced another genotoxic product or there was an artifact related to this specific assay. This issue is still outstanding.

The requirement for genotoxicity tests in product submissions FDA-wide aided in general safety assessment, since product development always focused on assuring a non-genotoxic effect if feasible. However, in the medical device center there is a new policy of assaying for genotoxins via chemical characterization of medical device extracts instead of in vitro biological testing. Besides requiring identification of hundreds of constituents, [at the time I left] the analytical method didn't generally include a step that would detect aldehydes like glutaraldehyde, formaldehyde, or nanoparticle effects. For this and other reasons, this seems problematic.

The FDA-wide harmonization of genotoxicity assessments no longer exists, as each center is operating independently and differently.

Cell phone radiation

FDA/CDRH regulates cell phone radiation (post-market, i.e. no regulatory action unless there are strong epidemiological signs of adverse events). Pre-market clearance is entirely different, where safety and efficacy must be demonstrated. When the Federal Communications Commission (FCC) requested a review of the genotoxicity data on cell phone radiation, I was the only person remotely qualified. Actually, CDRH had a long history of radiological assessments, including Extra Low Frequency radiation (ELF), but the difficulties involved in assessing and understanding it led the Center to give up on this area. I should have realized that no one else would agree to touch it. I reviewed some 900 papers on the genotoxicity assessment of cell phone radiation assembled by the FDA library. The review showed a combination of mostly inadequate or unremarkable studies, including a few that were fraudulent (as outed in other papers), along with several concerning epidemiological studies. Meanwhile the NIEHS had spent 10 years creating an experimental design that provided adequate exposure of mice without immobilizing them, to allow irradiation of their heads. This project was kept secret even from my supervisor. Kristine Witt and I communicated in code and secretly at EMGS meetings. Finally, the results became available. NIEHS found some comet assay positives. Kristine and I independently assessed the results as "not nothing". However, the Center reverted to the post-market lack of evidence of harm as the regulatory standard. After some difficulty, NIEHS published the results, but no one seemed to care.

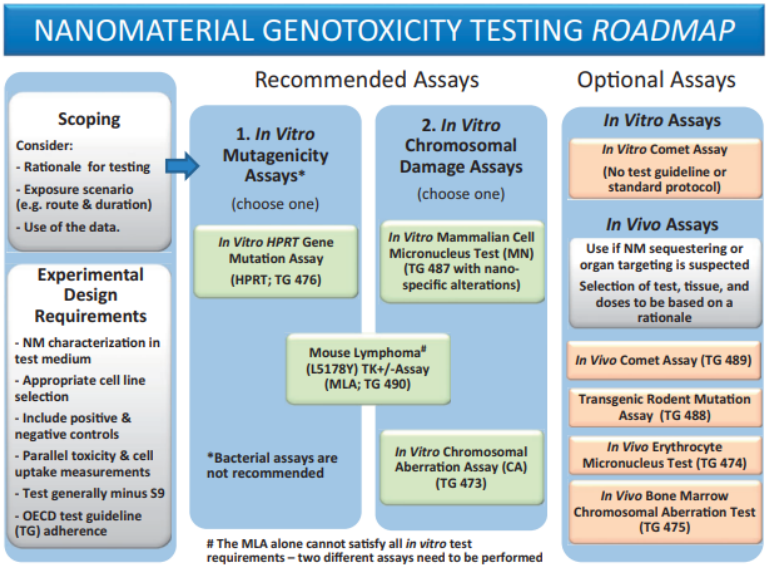


Figure 1. Nanomaterial genotoxicity testing roadmap.

The test battery recommended for NM assessment uses all in vitro mammalian systems, without bacterial mutagenicity, without animals unless there is clear evidence of animal-specific effects.

Figure 3. Nanomaterial roadmap: Genotoxicity test battery for nanomaterials (Toxicological Sciences, 164, 2018, p.393], with permission.

After describing what was wrong with the literature, we published 4 papers from HESI describing how genotoxicity assays should be conducted using OECD credentialed assays altered for assessment of NM (Figure 4).

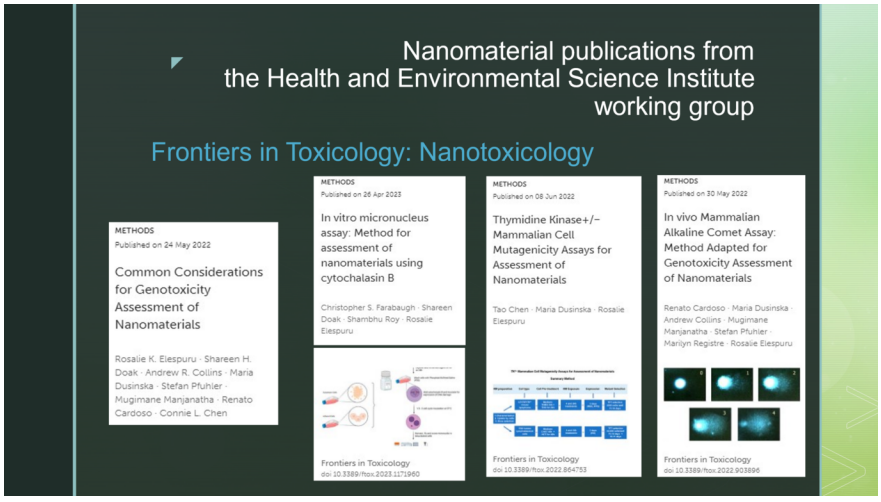


Figure 4. Summary of recommendations on individual genotoxicity tests for NM

FDA policy seems little changed, even concerning the requirement for accurate disclosure of the presence of NM. Most efforts to study NM ceased without notable differences in safety assessment approaches. Certainly,

the shift to chemical characterization, in lieu of biological testing, obviates the collection of information about the effects of reduced size and physical effects of NM particles on biological systems.

Covid-19 diagnostics

Regulation of diagnostic testing (apart from CLIA lab-based tests) is the purvue of CDRH. In 2020 CDRH approved ˜325 Covid diagnostic tests. I was recruited to the review of PCR-based tests and was pre-reviewer on 25 of them. This was a very difficult time during the pandemic, working at home and still doing genotoxicity safety reviews and trying to balance funded research projects as well. I did appreciate working with the diagnostics office teams, though. These were quite difficult reviews and the staff was very smart. All, from Office Director on down were impressive in their dedication, including in the transparency of weekly public phone conferences with the regulated industry creating the tests. I always tuned in to these to understand the technical nuances and to keep up with what was going on. Practically everyone suffered mentally from the work and the pressure. I only did this for 6 months. This was certainly one of the agency's finest hours. I was proud to be part of it.

Covid era non-lab projects .

Many groups embarked on collaborative projects via Zoom, including HESI and several Leadscope sponsored projects related to carcinogenic risk (Tice et al. 2023, Bassan et al. 2024). Three non-lab proposals for research funding were approved in 2020 but were slow to develop due to other priorities. One utilized, once again, my experience in photobiology (Vig et al., 2023). Among the most interesting was the analysis of >10,000,000 adverse event reports and sex-based differences in the CDRH MAUDE database, funded by the FDA Office of Women's Health. This was essentially a fishing expedition to find out what was being reported. We demonstrated the opportunities for novel analysis concerning sex-based effects in women, and other results benefitting the public health. An example of*non-hypothesis driven research* , I count it as one of my most important publications (Liao et al. 2024).

The real world of regulatory genotoxicity

It's generally the case that most products exhibiting overt genotoxicity are not submitted to FDA for approval at all, unless for life threatening conditions or those indications where there are no reasonable alternatives. Regulatory genotoxicity reviews may characterize weak or inconsistently mutagenic test articles. These tests are not easy to perform or to review. Thus, I want to call out and note the efforts of colleagues at FDA and in the regulated industry for this difficult work. In a sense, however, we are doing the same thing we have done for the last 40 years, presently just doing it a little better. Instead of detecting mutants in bacteria and mammalian cells, we can specify which mutations and where, via ecNGS and in different cells and systems. There is a focus on quantitative assessments, a linear extrapolation in a 4-dimensional world. This is useful in certain contexts, but current assessments are not likely to enlighten human risk of a specific FDA product under review. We need a new paradigm or new approaches to better address human risk. This may be more likely with the advent of big data analysis, but it won't happen unless we admit that the present offerings aren't sufficient.

Conclusion

Attention to the advice of mentors described may be beneficial. At the same time, it's best to focus on the science itself, thinking independently and ignoring bureaucratic issues, overly competitive colleagues and other distractions. The reward is the often-surprising outcome of scientific investigations themselves.

Acknowledgements: Collaborations

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