

Four Decades of MIT Research on Aflatoxin

In 1960 a ship named the Rossetti delivered tons of a ground nut meal commonly called "peanut press cake" from Brazil to a port in the United Kingdom. Months later between one and two hundred thousand turkeys had perished from acute hepatic poisoning. Thus began the career of a young toxicologist at MIT, Gerald Wogan, who later went on to found the MIT Center for Environmental Health Sciences. Jerry and the scientists who collaborated with or trained with him have played central roles in identifying and characterizing one of the world's most interesting and important human toxins. They also translated their basic science into practical methods to mitigate the health risks associated with that agent. This article provides a view of this scientific journey as seen through the eyes of two people who directly participated in a few steps along this path of discovery.

The toxicosis 46 years ago in the British turkey poult was troubling not only for its obvious and huge economic impact on agriculture. More importantly, the high protein content of peanut meal made it a practical and inexpensive human food, making it fortunate that the Rossetti meal, as it later became known, had not found its way into the human food supply. A clue to the nature of the toxic substance was the presence of abundant fungal filaments in the meal (see Figure 1). When the filaments or spores from the meal were grown on fresh microbiological media, the potent hepatotoxic properties of the contaminated meal were reproduced. Moreover, feeding Rossetti meal or the toxin produced metabolically by the fungus to ducks and rats revealed that short and long term exposure to the toxin resulted in the efficient induction of toxicity and liver cancer. Liver disease represents a major pub-

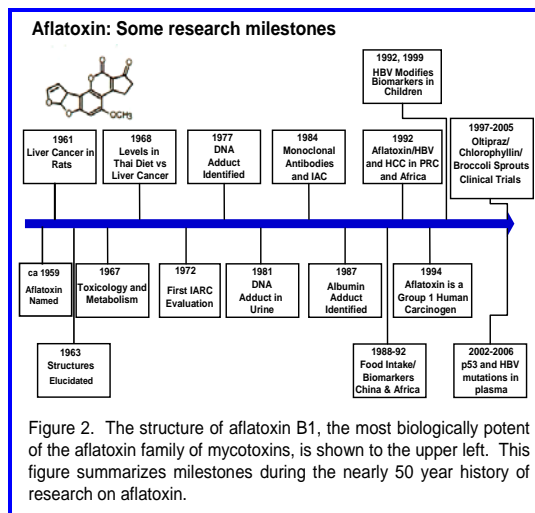


Figure 1. Rossetti meal. Peanuts are rich sources of both lipid and protein. Peanut oil is of high economic value as a commodity and is removed by pressing the peanuts, leaving behind a solid pressed peanut cake. The peanut cake, and flours derived from it, have high protein content and were viewed in the 1960s by agencies such as UNICEF as a possible source of protein nutrition, especially for children, in the economically developing world. It was also of value as a high protein feed for use in agriculture. This photo shows the ground nut meal from the ship, the Rossetti, labeled as "British Cake and Oil Mills, Ltd." of London, England. This is a sample of the meal that caused toxicity to turkeys owing to its contamination with aflatoxin.

lic health problem in sub-Saharan Africa and all of Asia where over 500,000 die each year of hepatocellular carcinoma. The fungus from Rossetti meal that pro-

duced the toxin was identified as *Aspergillus flavus* and the first four letters of the word aflatoxin link the chemical toxin with its biological parent.

The deep involvement of MIT in the aflatoxin story began with collaboration between Wogan and George Büchi, a synthetic natural products chemist in our Department of Chemistry. Jerry and his group had painstakingly produced quantities of purified toxin by growing the mold on peanuts, corn and other food materials. From extracts of these cultures, supplemented by a quantity of extract provided by the US FDA laboratories, they isolated a small amount of a blue fluorescent compound, named aflatoxin B₁ (B for its blue fluorescence under ultraviolet light and 1 for its chromatographic properties on TLC plates) that they found was the most potent of a family of low molecular weight toxins. Working with Büchi, the compound was identified in 1963 as molecule shown



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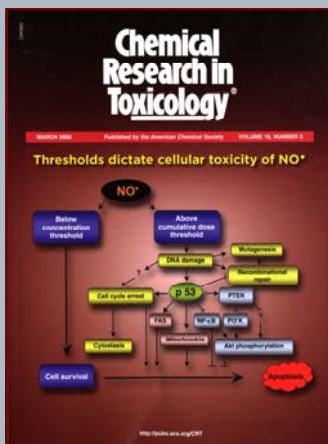
Dr. Gerald Wogan, Underwood-Prescott Professor of Toxicology, Emeritus, Professor of Chemistry, Emeritus

**2006
Annual
CEHS
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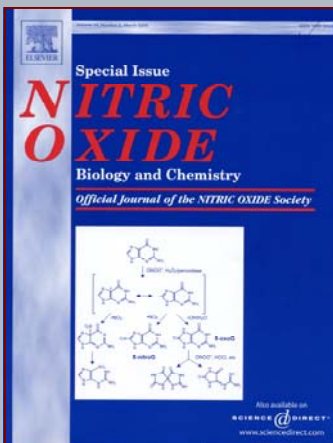
May 25, 2006
3:30-5:30pm

Walker
Memorial
Hall

CEHS GALLERY OF COVER ART



Li, C, Pang B, Kiziltepe, T, Trudel, L, Engelward, B, Dedon, P, and Wogan, G.(2006) Threshold Effects of Nitric Oxide-Induced Toxicity and Cellular Responses in Wild-Type and p53-Null Human Lymphoblastoid Cells, March 2006 Chem. Res. Toxicol. (19) 3, 399-406



Niles, J, Wishnok, J, and Tannenbaum, S (2006) Peroxynitrite-induced oxidation and nitration products of guanine and 8-oxoguanine: Structures and mechanisms of product information. March 2006 Nitric Oxide (14) 2, 109-121



Neeley, W. and Essigmann, J. Mechanisms of Formation, Genotoxicity, and Mutation of Guanine Oxidation Products. April 2006 Chem. Res.Toxicol. (19) 4, 491-505.



Garcia A, Bosques CJ, Wishnok JS, Feng Y, Karalius BJ, Butterton JR, Schauer DB, Rogers AB, Fox JG. Renal Injury Is a Consistent Finding in Dutch Belted Rabbits Experimentally Infected with Enterohemorrhagic Escherichia coli. J Infect Dis. 2006 Apr 15;193(8):1125-34. Epub 2006 Mar 6.

COMMUNITY OUTREACH AND EDUCATION PROGRAM (COEP)

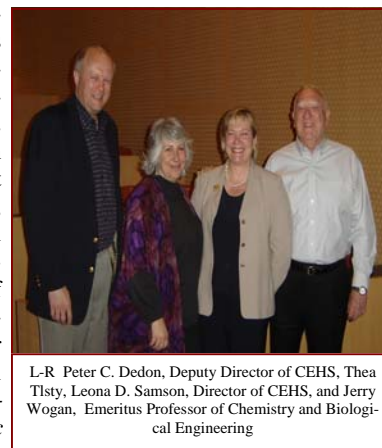
What's new at COEP? Quite a few things! We are currently creating the first-ever Learning Lab at the MIT Museum, having won a CEHS pilot project grant for this project with the museum. This Learning Lab on The Cell is designed to teach the basic concepts of genes and protein synthesis in order to help the public understand the connection between DNA damage and human health. The prototyping of the exhibit took place during the January '06 break, with six MIT UROPs (upper classmen from the Terrascope Program) creating the overall design. Already this spring, groups of middle and high school students have been transcribing and translating DNA codes into amino acid sequences at the museum. This isn't being done with mirrors-- but with the LEGO Life Science models that Kathy Vandiver designed for this purpose. The MIT Museum Learning Lab is scheduled to open in November 2006.

Another recent COEP splash occurred at the Boston Museum of Science. In late March, all three of COEP program leaders including Co-Director Prof. Bevin Engelward, Director Dr. Kathy Vandiver, and Coordinator Amy Fitzgerald were major contributors at the Museum's Biotech Educator's Symposium. Kathy Vandiver and Amy Fitzgerald ran two highly attended teacher workshops. One was called "Tails of the Fish: Connecting Meiotic Events with Mendelian Genetics" in which a LEGO fish and its LEGO chromosomes were used to illustrate how successive generations of fish could be affected an environmental pollutant that killed only the genetically susceptible individuals. These models teach the basic concepts in a very engaging way. In addition, to an auditorium of more than 320 teachers, Prof. Engelward gave the keynote lecture on factors that can cause mutations in cells, concluding with some examples of DNA repair mechanisms from her research. The talk was very instructive. The teachers appreciated learning about DNA repair and its connection to health, as it helps them make the topic of DNA more engaging and relevant to the students they teach.

Our COEP program continues to run the successful programs we have developed in the past: 1) the after school sessions on environmental health science for Summerbridge Cambridge, an excellent academic program for local middle school students; 2) the two-day summer workshop on environmental health science research for science teachers in July; and 3) the classroom instruction sessions at the Edgerton Center on environmental health topics such as cell division and groundwater for students who come to MIT on field-trips from all over New England. These and other COEP events will be presented at the poster session in May 2006.

"The great tragedy of Science -
 the slaying of a beautiful hypothesis by an ugly fact."
 Thomas H. Huxley *English biologist (1825 - 1895)*

The Center for Environmental Health Sciences and the Biological Engineering Division co-hosted the **Robert S. Harris Lecture** on April 27th. The guest lecturer Thea D. Tlsty, Ph.D., a Professor in the Pathology Department at University of California, San Francisco presented her research on "*Mechanisms of Genetic and Epigenetic (tumor/stem) Cell Plasticity*". Dr. Tlsty's lecture was very well received by all who attended.



L-R Peter C. Dedon, Deputy Director of CEHS, Thea Tlsty, Leona D. Samson, Director of CEHS, and Jerry Wogan, Emeritus Professor of Chemistry and Biological Engineering

Knowledge of the structure of aflatoxin revealed its molecular weight and, with the availability of homogeneous natural or synthetic material, it was possible for Wogan to characterize, quantitatively, just how potent the toxin is. The results were staggering – in most species it was, at the time, the most potent frequently occurring natural liver toxin and it caused cancer with equal facility in many animal species at parts per billion levels, which are easily achieved in the diets of people in many areas of the economically developing world.

The availability of analytical procedures for quantitative determination of the toxin made it possible for MIT to turn its attention to the possible link between the presence of the toxin in the food supply and prevalence of human liver cancer, and even acute hepatotoxicity. For these studies, international health partnerships were needed owing to the minimal nature of the aflatoxin contamination of the US food supply. The first such partnership was with the Kingdom of Thailand in the mid-1960s. Ronald Shank, the first graduate student to do his doctoral thesis research in the Wogan laboratory, later became an MIT faculty member and moved to Thailand to head the field-aspect of the “MIT-Thai Project,” an epidemiological study supported by the US Agency for International Development and by the Rockefeller Foundation to determine if associations exist between the level of ingested aflatoxin and cancer registry data on local liver cancer incidence in man. The MIT-Thai Project started in 1967 and continued for about five years and established the definitive epidemiological link associating aflatoxin with hepatocellular carcinoma. Other studies in Africa and in other parts of the world strengthened the association. Indeed, aflatoxin was among the very first compounds explored in Volume 1 of the International Agency for Research on Cancer monographs. In 1974, it was listed as a group 2A probable human carcinogen. Another 22 years would pass before it was reclassified as a Group 1 known human carcinogen. Wogan participated in the first review and some of his students and collaborators participated in the second.

The late 1970s temporarily returned the aflatoxin project back to MIT, where detailed studies of its mechanism of action were conducted. Both of us played roles on the project in this area. We suspected that the binding of the toxin to DNA could result in the formation of DNA adducts that could force replication errors, or mutations. One or, more likely, a series of such genetic changes ultimately could result in the conversion of normal liver cells into cancer cells, which would then outgrow into a tumor. The challenge we faced stemmed from the need to identify infinitesimal amounts of the putative aflatoxin-DNA adducts. The project pushed the analytical technology of the day to its limits but in the final analysis, the structures of the DNA adducts were revealed. Later work from Essigmann’s group, when he had become an independent investigator, defined the adducts likely to be responsible for the toxicity of aflatoxin, and the adducts likely to cause the mutations observed in end stage liver tumors. The structures of several of the aflatoxin-DNA adducts are shown in Figure 3. The stereochemistry of the major DNA adduct held an important bonus that much of the world missed at the time – it revealed a snapshot of the chemical intermediate that existed ephemerally an instant before the toxin reacted with DNA. This intermediate was a metabolically generated beta-epoxide in which an inserted atom of oxygen spanned the 8 and 9 carbons of aflatoxin. The relevance of this epoxide to protection of populations against aflatoxin in the environment will be discussed later in this article.

Robert Croy and Jerry Wogan showed in the early 1980s

Pathways of aflatoxin B₁ metabolism

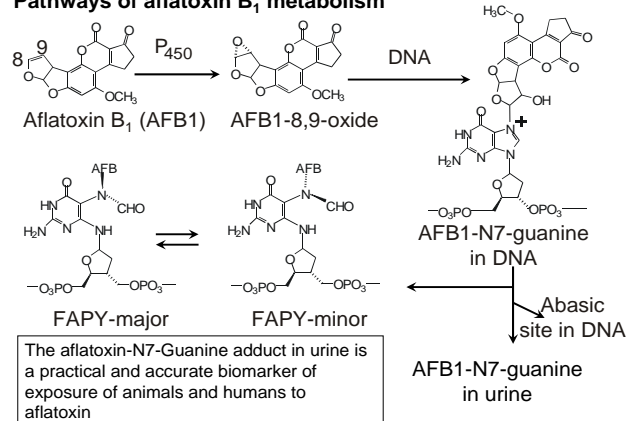


Figure 3. Metabolic activation and primary DNA adducts of aflatoxin. The parent toxin, aflatoxin B₁, is metabolized to the beta-epoxide, which reacts with DNA to form the AFB₁-N7-guanine adduct. This adduct is chemically unstable. It is released easily from DNA, leaving behind an abasic site, and about one third of the amount of adduct initially in liver later appears in urine. The level of urinary adduct is a useful biomarker of exposure to aflatoxin. The initial adduct also can be attacked by water in its imidazole ring, to form a carbinolamine, which opens to form several isomeric formamidopyrimidine (FAPY) adducts. These adducts are chemically stable in DNA and are hypothesized to be responsible for toxicity and mutagenesis. The FAPY major adduct shown is the principal lethal lesion and FAPY minor is the principal mutagenic lesion of aflatoxin. FAPY minor is an efficient inducer of G to T mutations, which are the principal mutations observed in aflatoxin induced liver tumors.

that animal species that are highly susceptible to the carcinogenicity and toxicity of aflatoxin form abundant adduct loads in their genomes, and the few species that are refractory to the toxin, such as the mouse, form diminished levels of adduct. The proportionality between adduct level in DNA and susceptibility led Wogan to conclude that the DNA adducts were likely to be excellent “biomarkers” or predictors of disease risk. In preparation for studies in which the Wogan team would once again take to the field in Asia, Richard Bennett, and the two of us developed sensitive methodology allowing the adduct to be detected in the urine of aflatoxin-dosed rats. Regardless of dose, one third of the adduct in DNA is excreted in urine, making measurement of the adduct in urine a method that would allow us to estimate the true biological body burden of the toxin. Other studies by Groopman and Steven Tannenbaum helped to establish protein-aflatoxin adducts as additional biomarkers. In addition to the elucidation of the mechanisms of action of aflatoxin, there was a rapid translation of the analytical strategies to cohort studies in China. This work established that aflatoxin synergized with hepatitis B virus infection, and possibly other infectious agents, as co-risk factors for liver cancer. In the early 1990s it further became evident that the magnitude of this synergy is very large. Relative risks of liver cancer are elevated by 60 to 100 fold in hepatitis B carriers who are additionally exposed to aflatoxin. The mechanistic basis of this powerful toxicological synergy remains a mystery.

A pivotal moment in aflatoxin research occurred in the mid 1980s when Thomas Kensler, a former Wogan student working at Johns Hopkins University, noticed that the anti-schistosomiasis drug oltipraz induces a specific glutathione-S-

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transferase that eliminates the beta-epoxide of aflatoxin discussed above. He and John Groopman, who was then also at Johns Hopkins, and another Wogan former student, Bill Roebuck of Dartmouth College, reasoned that oltipraz, given to rats, would lower and possibly eliminate the carcinogenic potential of aflatoxin. In other words, they hypothesized that feeding oltipraz to a rat would reduce the cancer susceptibility of that rat to a susceptibility approximating that of a mouse. Their alchemic studies on the pharmacological conversion of a rat into a mouse were successful and led quickly to clinical trials headed by Kensler and colleagues in the People's Republic of China. The objective of these studies was to assess the potential of agents such as oltipraz to prevent the formation of liver cancer in man. Their studies published in the late 1990s strikingly show that the aforementioned adduct biomarkers are reduced markedly in the Chinese subjects who took dietary oltipraz. It is anticipated that sustained use of this drug, or other agents that have similar biochemical activities, will mitigate the disease burden in the developing world. But there is a catch -- oltipraz is a drug approved for use over a short period of time, and it is prohibitively expensive, so more cost effective and practical methods to suppress biomarkers of aflatoxin exposure were needed. Current efforts are underway to find agents that will address the same biochemical pathways as oltipraz. Recently, Kensler and colleagues have found that market stage broccoli and, even better, broccoli sprouts, are very effective inducers of the biochemical pathways that either destroy or prevent formation of the adduct-forming aflatoxin beta epoxide.

Taken together, the MIT team headed for more than forty years by Jerry Wogan, and his posse of former students who remain close colleagues and friends, has helped to create a paradigm by which environmental agents responsible for human disease are identified, probed mechanistically, and addressed by intervention measures with the aim of reducing disease burden to man. The CEHS and its sister Center at Johns Hopkins have provided a framework (Figure 4) within which this program has embraced many disciplines from synthetic chemistry to mechanistic biochemistry to epidemiology to clinical studies and developmental pharmacology. Looking to the future, many exciting challenges remain. How does hepatitis B virus enhance the carcinogenicity of aflatoxin? Which biochemical sub-networks are affected by the toxin in sensitive and resistant species? How can we use modern toxicogenomic and informatics tools to predict which pathways are targets for pharmacological manipulation in order to suppress disease risk? How can we screen natural product and synthetic chemical libraries to identify single agents or mixtures that will prevent disease? What lifestyle variables, such as diet, pollution exposure, infectious agent exposure and tobacco use, impact disease risk from aflatoxin? As in the past, these challenges are larger and more complex than those that one research group or even one institution could reasonably address. Addressing the toxicological problems of today, as was the case in the past, will be best accomplished by the collaborative efforts of sister domestic institutions with valued collaborators on the ground in the areas of the world where the problems are felt first hand. The formation of a global network of collaborators established in the early days of toxicology research at MIT is now, as

Mechanistic basis for exposure and early effect biomarkers in hepatocellular carcinoma development

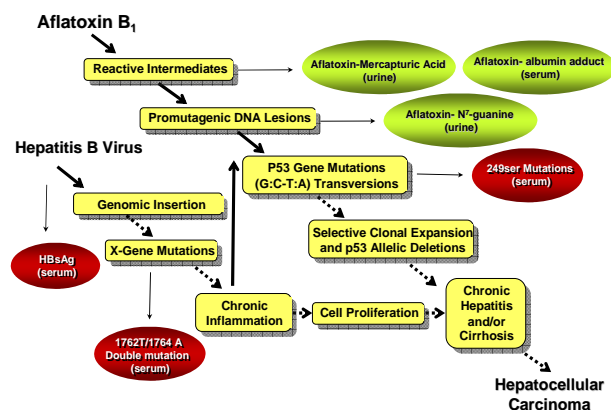


Figure 4. Mechanistic steps between exposure to aflatoxin and the appearance of hepatocellular carcinoma. Biomarkers that reflect specific stages in disease progression are indicated in ovals.

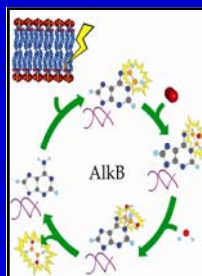
in the past, an excellent and proven model by which important public health problems can be efficiently discovered and solved.

John M. Essigmann and John D. Groopman

MIT Center for Environmental Health Sciences, Biological Engineering Division and Department of Chemistry, Massachusetts Institute of Technology (JME) and Center Director, Center for Urban Environmental Health, Department of Environmental and Health Sciences, Johns Hopkins University (JDG)

Feature Publication

AlkB reverses etheno DNA lesions caused by lipid oxidation in vitro and in vivo. (2005) Delaney JC, Smeester L, Wong C, Frick LE, Taghizadeh K, Wishnok JS, Drennan CL, Samson LD, Essigmann JM. Nat Struct Mol Biol., 12:855-60.



A collaborative research effort among the CEHS cores was published in Nature Structural and Molecular Biology, vol 12, 2005.

The Essigmann, Samson, and Drennan Groups have discovered that spontaneous DNA damage from oxidizing lipids is repaired by an unprecedented direct reversal mechanism by the enzyme AlkB. AlkB is a member of the alpha-ketoglutarate dioxygenases, which have been studied by JoAnne Stubbe, among others. Lipid-damaged DNA has been found in human cells at elevated levels in people suffering from the metal overload diseases of Primary Hemochromatosis and Wilson's Disease. There are 8 known human AlkB homologs, underscoring the importance of AlkB as a defense against cytotoxic agents. In vivo experiments show ethenoadenine, a lipid-derived base, to be very cytotoxic and mutagenic in cells lacking AlkB; however, the lesion is rendered non-toxic and non-mutagenic in cells containing the enzyme. In vitro repair experiments analyzed by ESI-TOF mass spectrometry show that AlkB epoxidizes the lesion; epoxidation is followed by hydrolysis of the epoxide to a diol, and ultimate dealkylation restoring the normal adenine base within DNA. GC mass spectrometry experiments reveal that the alkyl group is liberated as glyoxal, a dialdehyde.