

THE NUCLEUS

February 2007

Vol. LXXXV, No. 6

Monthly Meeting

Jeannette Elizabeth Brown Speaks on "African-American Women Chemists who Made a Difference"

Wallace J. Gleekman

*Master Teacher and Leader
1930-2006*

National Chemistry Week Report

By Christine Jaworek-Lopes

Summer Scholar Reports

*By Chayasith Uttamapinant and
By Todd C. Andrade and Dr. Donald W. Boerth*



National Chemistry Week 2006 Report

The Northeastern Section Celebrates Your Home – It's All Built on Chemistry

By Christine Jaworek-Lopes



L-R: Gordon College students and faculty at the Museum of Science: Erica Wetter, Autumn Brown, Rachel Shirron, Ted Monchamp, Sandra Gaston, Kara Raychard, Dr. Dwight Tshudy, and Tiffany Hurlbut. Photo by Irv Levy.

In anticipation of National Chemistry Week 2006, a volunteer preparation day was held at Emmanuel College on Saturday, September 30, 2006. Approximately 40 individuals representing eight different organizations attended this event which allowed volunteers to practice the hands-on activities and demonstrations in advance of

the October celebration. Members of the Boston Children's Museum and Museum of Science-Boston staff were on hand to choose which activities worked best for their respective audiences.



Tufts University students demonstrating soil erosion to visitors at the Museum of Science-Boston. Photo by Dave Sittenfeld

On Sunday, October 22, 2006, the Northeastern Section of the American Chemical Society sponsored a National Chemistry Week 2006 Kick-Off Event at the Wellesley College Science Center. More than 40 volunteers (from ACS, the Brauner Committee, Clark

University, Emmanuel College, Suffolk University, Wellesley College) ensured that the more than 400 visitors to the daylong event enjoyed a number of hands-on activities and demonstrations related to chemistry of the home.

Hands-on activities included: making a UV detector using UV sensitive beads and assessing the UV protectiveness of different types of windows; making play-putty using glue and borax; and studying polymer strength using spaghetti.

Demonstrations included: learning about soil, particularly erosion; types of boards and nails used to build your home; and the stability of skyscrapers.

Among the highlights of the day were the lecture demonstrations as part of the Phyllis A. Brauner Memorial lectures presented by Dr. Bassam Shkhashiri, Professor of Chemistry at the University of Wisconsin-Madison. These captivating lectures were enjoyed by children and adults alike.



Diane Perrito, Malden High School, discussing the chemistry of different types of nails with a visitor to the Museum of Science. Photo by Dave Sittenfeld.

In addition, NCW events were held at the Boston Children's Museum and the Museum of Science-Boston on Saturday, October 28, 2006. Approximately 2500 individuals experienced hands-on activities related to the NCW theme at these venues. A particular favorite at the Children's Museum was the making of the UV bracelets and then heading to the Blue Man Group room to observe the color changes. Children of all ages enjoyed preparing

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Cover: *Wallace J. Gleekman circa 1989*
(Photo courtesy of Myron S. Simon from the NESACS Archives)

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Wallace J. Gleekman

1930-2006



Wally Gleekman in 1998.

Photo from the NESACS Archive

Wallace J. Gleekman passed away on November 23, 2006. He was born in Boston. He received his undergraduate degree from Bridgewater State College and completed graduate work at Boston University, Union College, Harvard University, and Walden University and received his PhD. degree.

After completing his military service in the Far East, Gleekman began his teaching career at Wrentham (MA) High School and the King Philip Regional High School (MA) where he served as a science teacher and department head. In 1958 he moved to Brookline (MA) High School where he taught courses in chemistry, advanced chemistry, biology, physics, unified chemistry-physics, oceanography, environmental science, outdoor field experiences, general science and physical science.

Dr. Gleekman served as Housemaster (assistant principal) at Brookline High School as well as Director of the Brookline Summer School. In addition, he was an instructor of oceanography and an administrative assistant at

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Wally Gleekman

A Reminiscence,

by Myron S. Simon

A NESACS meeting was scheduled at U Mass Dartmouth. A long ride down and back? Not to fear. Wally has called, "Are you going to the meeting? Why don't you ride with me?" Agreed. At the meeting time, Wally was at the front door in his big car, and we are off. "We'll pick up Michaeline and Phyllis, and be on our way." And we did. Wally was prepared for the trip. After a couple minutes of greetings he asked if anyone wanted to hear a mystery novel. He had picked out an audio tape of a novel which, it turned out, required exactly the time he estimated to get us to Dartmouth, MA and back. In luxury we sat back and had an entertaining ride down and back, thanks to Wally's planning and love of driving.

A Reminiscence,

By Steve Lantos

Wally Gleekman passed away on Nov. 23, 2006. A tireless supporter and long-time active member of the NESACS, Wally could be found at most monthly meetings hosting, connecting fellow teachers and chemists, and promoting social events within the section. For years, many of us would show up in right field at Fenway Park only to realize that it was Wally that brought us all together by finding us tickets through NESACS. Wally reveled in bringing together fellow chemistry teachers, getting a good deal, and sharing important moments with colleagues and family.

For years Wally wrote and ran the Avery Ashdown Exam, the NESACS' annual high school chemistry exam competition and, qualifier for the US Chemistry Olympiad. His mission was simple: to promote and encourage chemistry secondary education and interest throughout the section. He was unflagging in encouraging participation across the Northeastern Section; often zealously reaching out to far away schools to connect with teachers

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unfamiliar with the annual exam or the ACS. When Wally set his mind to a task, he was dug in, determined (some might say stubborn), and laser-focused in his mission.

At Brookline High School for nearly 40 years, Wally served as dean, union president, member on numerous committees, and coach. But teaching chemistry was, literally, always his center stage. Wally's longtime classroom at BHS had raised rows where students faced him in a mini-amphitheater, so suited for his theatrical lectures. Ready to dress up as Amadeo Avogadro or Dimitri Mendeleyev to introduce the mole or the Periodic Table, Wally was at once showman and serious lecturer. His classes were always alive with the thrill and excitement of chemistry.

Demonstrations, student questions, and active learning were all a part of Wally's pallet. He believed in challeng-

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Monthly Meeting

The 876th Meeting of the Northeastern Section of the American Chemical Society

Thursday – February 8, 2007

Harvard Faculty Club,
20 Quincy St., Cambridge, MA 02138

2:30 pm Career Services Presentations and Resume Reviews, Mukund Chorghade, Chair, NESACS; Megan Driscoll and Jennifer Sass of PharmaLogics Recruiting.

4:30 pm Board Meeting

5:30 pm Social Hour

6:30 pm Dinner

8:00 pm Evening Lecture, Pfizer Lecture Hall, Mallinkrodt Building, 12 Oxford St, Dr. Mukund Chorghade, Chair, presiding.

Jeannette Elizabeth Brown, Educational Consultant, formerly New Jersey Institute of Technology, *African American Women Chemists Who Made a Difference*

Dinner reservations should be made no later than noon, Thursday, February 1, 2007. Please call or fax Marilou Cashman at 800-872-2054 or e-mail at Mcash0953(at)aol.com. Please specify vegetarian. Reservations not cancelled at least 24 hours in advance must be paid. Members, \$28; Non-members, \$30; Retirees, \$15; Students, \$10.

THE PUBLIC IS INVITED

Anyone who needs special services or transportation, please call Marilou Cashman a few days in advance so that suitable arrangements can be made. Free parking in the Broadway St. Garage (3rd level or higher). Enter the garage from Cambridge Street via Felton St. Directions to the Harvard Faculty Club can be found at <http://www.hfc.harvard.edu/>.

Next Meeting: March 8, Michael Wasielewski Speaking.

Biography



Ms. Jeannette Elizabeth Brown is a former Faculty Associate in the department of Pre-College Programs at the New Jersey Institute of Technology. She held the title of New Jersey Statewide Systemic Initiative (NJSSI) Regional Director having served as the NJIT NJ/SSI Coordinator previously. In this position she designed, developed and coordinated the NJIT NJSSI K-8 Professional Development Program. Ms Brown is a Fellow (Cohort 3) of the WestEd National Academy for Science and Mathematics Leadership. She is the Chemical Heritage Foundation 2004 Société Fellow.

She previously held the position of Research Chemist and worked at Merck & Co. Inc for twenty-five years in that capacity. She synthesized new compounds for testing as potential new drug candidates for human and animal health. She suggested new targets for development. At Merck she became co-author of 15 publications and 5 patents and has one patent in her name alone. She earned a Management Award for her work with the Merck Black University Liaison Committee in which she worked with Grambling University to try to improve the chemistry department. She started her industrial career at CIBA Pharmaceutical Co. as a junior chemist and worked there for eleven years. She has a research MS degree from the University of Minnesota and a BS degree in the Field of Chemistry from Hunter College. She was elected to the Hunter College Hall of Fame for her work as a mentor for young students.

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Abstract

African American Women Chemists Who Made a Difference

African American women in science have always labored under the “double bind” of being a woman and a minority in science. To date, limited knowledge exists on the educational experiences of African American Women Chemists. This was the motivation for the establishment of a project to extend the current knowledge base about African American women in chemistry. In this project we researched the biographies

of African American women chemists who went on to make a difference in the field of chemistry in spite of the roadblocks and opposition they faced in their chosen career. In this talk we will speak about Dr. Marie Daly, the first African American woman to receive a Ph.D. in chemistry. We will also include the lives of other African American women who chose to major in chemistry before and after Dr. Daly. All of the women chosen went on to make a difference in chemistry as educators or researchers, even though they may have been barred from working at major research institutions because of their race. ◇

Wallace J. Gleekman

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the Naval Reserve Officers School in Boston. He spent a sabbatical year at the American International School in Israel where he was science consultant and coordinator. While there he compiled a series of slide presentations for scientific, religious and citizen groups.

Gleekman was a speaker at local, regional and, national meetings of the American Chemical Society and, the National Science Teachers Association. He was Chair of the Northeastern Section of ACS in 1981 and served the Northeastern Section in a variety of other roles in the years after he was chair.

Among his honors, he was selected a General Electric Science Fellow. He received the Lyman C. Newell Award for Chemistry Teaching and the Northeastern United States Regional Award in High School Chemistry Teaching in 1972. He was presented with the James Bryant Conant National Award in Chemistry Teaching in 1974 as well as the John A. Timm

Award for outstanding contributions to chemistry education. Gleekman was elected to the *Aula Laudis* Society in 1989, the High School Chemistry Teachers "Hall of Fame" by the Northeastern Section. He was also the recipient of the Henry A. Hill Award for distinguished contributions to chemistry in 1989 and received the Theodore R. Williams Award for Excellence in High School Chemistry Teaching in 1994.

He was an avid sailor and was the varsity sailing coach at Brookline High School and later at the Winsor and Commonwealth Schools. He served as the presiding officer for the Massachusetts Bay League and the New England Schools Sailing Association and was on the Board of Directors of the Inter-scholastic Sailing Association.

He leaves his wife, Barbara A. (Zonis) Gleekman; his daughter, Dr. Hilary A. Gleekman-Greenberg and her husband, Dr. William J. Greenberg, of Scarsdale, NY, three grandsons, and many cousins. He was father of the late Nina R. Gleekman. ◇

NCW Contest Winners

Congratulations to our haiku winners. Prizes will be sent to each of our winners. In addition, their teachers will be receiving gift certificates from www.teachersource.com and coffee mugs.

Grades 1-3:

1st place: Nickel

by Jennifer Bindman (2nd grade)
Franklin Elementary School
West Newton, MA

*Silver color, shines.
It is made into coins, metal.
Solid, batteries.*

Grades 4-6:

1st place: Nickel

By Lucas DeAndrade (5th grade)
South Elementary School
Stoughton, MA

*Nickel is the best
Twenty-eight is green in H₂O.
It is worth five cents.*

2nd place: Calcium

By Daniel Santoro (6th grade)
McCall Middle School
Winchester, MA

*Calcium is cool.
Calcium is good for you.
Yay! Calcium rules.*

Special thanks to Dr. Christopher Morse, Olin College of Engineering, for designing the 2006 puzzles. ◇



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Wally Gleekman

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ing all of his students with both rigor and mirth. With colleagues, Wally shared a common dedication to educate all students. He shared curriculum and pedagogy, never with arrogance, and always with an openness and desire to bring other less-experienced chemistry teachers into the important world of chemical education, a world

about which, he cared so deeply. Wally was the consummate teacher.

About 20 years ago, Wally called me at home and asked if I'd like to go sailing with him the following Saturday. I met him across an inlet from one of Logan's taxiways at the Winthrop Yacht Club. We spent the afternoon puttering around in his pride and joy. It was a delightful afternoon with Wally at the helm. Wally was truly at home on the water; coaching the sailing team at Brookline for years and encouraging legions of students to take to the sails. I'm privileged to have known several sides of Wally over nearly 30 years of acquaintance as former student, colleague, and friend. I'll remember Wally for his wit, teaching, patience, and dedication to chemical education. As chemistry teachers and chemists, we owe so much to Wally and I know I will miss him a whole lot.

Sincerely, Steve Lantos

Steve Lantos is a chemistry teacher at Brookline High School. ◇

NESACS Ski Trip to Nashoba Valley

*February 28th
Save the Date!*

**Bus transportation
will be arranged from
the Boston area**

*For details visit the
NESACS website at
www.nesacs.org*



Two Day Training Course in Heterocyclic Chemistry

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[<http://euch6f.chem.emory.edu/heterocourse.html>]

Chemistry Week

Continued from page 2

play putty at the Museum of Science-Boston. More than 60 volunteers from Emmanuel College, Gordon College, Malden High School, Massachusetts General Hospital, Merck and Co., Inc., Northeastern University, Simmons College, Suffolk University, and Tufts University were available to assist visitors throughout the day.

Special thanks to:

- All of our volunteers
- Boston Children's Museum
- J.C. Adams Company
- Merck and Co., Inc. for providing partial financial funding for our

weeklong events as well as providing the materials necessary for running the chromatography activity at the Museum of Science – Boston.

- Museum of Science – Boston
- Northeastern Section of the American Chemical Society
- Phyllis A. Brauner Memorial Lecture Committee
- Stop and Shop, Hingham
- Thomas McHugh Custom Carpentry and Renovations
- True Value Hardware, Harwich Port
- Wellesley College

The theme for NCW 2007 is "The Many Faces of Chemistry," to be celebrated from October 21 -27, 2007. ◇

Biography

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She was appointed to the National Science Foundation Committee on Equal Opportunities for Women Minorities and Persons with Disabilities (CEOSE) and served on that committee for two terms, six years. She has been elected Councilor of the American Chemical Society from the North Jersey Section three times and is currently a Councilor. She was Chair of the Project SEED Committee and reorganized the committee to make it function efficiently. Project SEED is a program for economically disadvantaged high school students. She also acted as the chief fundraiser for the program until it was taken over by a professional fundraiser. She is a member of the National Organization for the Professional Advancement of Black Chemists and Chemical Engineers and the Association for Women in Science and the American Association for the Advancement of Science.

She is the 2004 Société de Chimie Industrielle (American Section) Fellow of the Chemical Heritage Foundation. She studied the History of African American women chemists and is currently lecturing and writing a book about her work.

Her awards include the ACS Women Chemists Committee Regional Award for Contributions to Diversity 2002. She is listed in Who's Who in America 2004, Vol. I., p 630. She is the 2005 recipient of the American Chemical Society Dreyfus Award for mentoring minorities in science. She received an Outstanding Alumni Achievement Award from the University of Minnesota in 2005. She also received an Alumni Award from the Hunter College Chemistry Department and her most recent award is the North Jersey Section of the ACS, Harvey Russell Award for service to High school teachers. ◇

November 2006 Meeting



Professor Brian P. Coppola, University of Michigan, with the Norris Award
(Photo by Richard Johnson)



Robert Lichter, Merrimack College, in discussion with Brian Coppola after Professor Coppola's evening talk on undergraduate education.
(Photo by Richard Johnson)



(L-R) Professors Arthur Greenberg, Howard Mayne, Richard Johnson, Christopher Bauer and Gary Weisman of the University of New Hampshire with Norris Award Winner Brian P. Coppola, a graduate of the University of New Hampshire (BS, 1978) and currently the Arthur F. Thurnau Professor of Chemistry, University of Michigan. (Photo by Morton Z. Hoffman)



(L-R) NESACS 2007 Chair Mukund Chorghade, Professor Brian Coppola, University of Michigan, and NESACS Chair-Elect Marietta Schwartz, UMASS Boston.
(Photo by Morton Z. Hoffman)

What exactly goes on at NESACS' monthly Board meetings?
www.nesacs.org/reports

Summer Research Scholar

Synthesis of Unnatural Nucleotides and Their Use as DNA Glycosylase Mechanistic Probes

Chayasith Uttamapinant, [uttamap\(at\)fas.harvard.edu](mailto:uttamap(at)fas.harvard.edu),
Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, MA 02138.

DNA in living cells is constantly subject to chemical modifications and damage, whether it be in the form of radiation, reactive radical species, or chemicals found in the environment, such as hydrocarbons from smoke. Failure to repair this DNA damage can lead to mutations and, subsequently cell death. DNA repair mechanisms after each round of replication and transcription are therefore indispensable in order for cells to survive. This makes DNA repair enzymes promising targets for cancer therapeutic agents, since rapidly dividing cells will be more affected by the loss of fidelity in cell division than normal cells.

Most damage to bases in DNA is repaired by the base excision pathway, which involves removal of a damaged nucleotide base, exclusion of a short piece of the polynucleotide around the abasic site created, and resynthesis with a DNA polymerase. DNA repair is initiated by DNA glycosylases, which are enzymes that recognize specific lesion bases in DNA and remove them. The resulting abasic lesion is then further processed to ultimately restore the original sequence. Mechanisms of many DNA glycosylases such as MutY, MutM, and hOGG1 have been extensively studied, notably in the lab of Professor Gregory Verdine. All three enzymes recognize mutagenic base pairs involving 8-oxoguanine, which is generated through ROS-induced oxidation of guanine. OxoG can mispair with adenine, leading to a G:C \rightarrow T:A mutation during subsequent rounds of replication. From previous structural studies, MutM and hOGG1 repair the damage by catalyzing the removal of 8-oxoguanine from the oxoG:C base pair, while MutY removes adenine from the oxoG:A.¹

The enzymatic mechanism of DNA glycosylases is similar. The nucleophilic attack at the 1'-carbon of deoxyribose ring, possibly aided by the formation of an oxocarbenium intermediate, creates an abasic site. MutY replaces the base lesion by the hydroxyl group from water, while MutM and hOGG1 use a catalytic basic residue (histidine and lysine, respectively) as a nucleophile.² In the cases of MutM and hOGG1, the enzymes can also act as β -lyases, which leads to DNA strand cleavage at the 3'-position.³

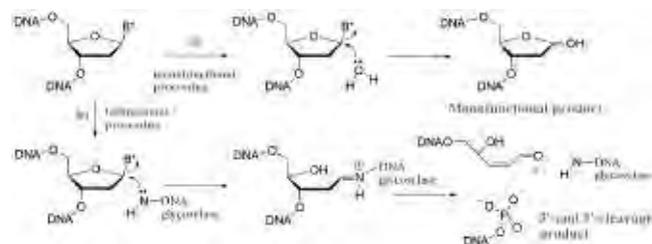


Figure 1. Enzymatic mechanisms for a) MutY (monofunctional glycosylase) and b) MutM and hOGG1 (bifunctional glycosylases).

Attempts have been made to synthesize inhibitors of these repair enzymes, both to serve as tools for structural studies and as potential drug candidates.⁴ Here, two strategies that employ the use of unnatural nucleotides are described, and initial results reported.

Part 1. Synthesis of 4'-fluorinated nucleotides

One novel candidate among DNA glycosylase inhibitors is 4'-fluorinated oligonucleotides. 4'-fluorinated oligonucleotides, with fluorine substituting hydrogen at the 4' position of the ribose ring, should exhibit similar binding affinities with the enzymes as the substrates as fluorine is isosteric to hydrogen. But since fluorine is very electronegative, the introduction of fluorine should disrupt the transition state formation via inductive effects and thereby inhibit the formation of the oxocarbenium intermediate. 4'-fluorinated oligonucleotides should therefore be able to act as a competitive DNA glycosylase inhibitor. Our goal is to create a library of aptamers in which one of the nucleotides is 4'-fluorinated. Since each repair enzyme scans for different base lesions, a synthesis strategy in which a variety of 4'-fluorinated nucleotides can be generated is essential. Once the nucleotides are synthesized and the oligonucleotides have been made, further structural and biochemical studies should determine the manner in which the repair enzymes bind to these modified oligonucleotides.



Figure 2. 4'-F DNA acts as DNA glycosylase inhibitor by disrupting the formation of oxocarbenium intermediate.

4'-fluorinated nucleotide synthesis has been previously studied⁵. However, the rather lengthy synthesis results in a low overall yield. In Figure 3, we describe a novel one-pot synthesis strategy in which 4'-fluorinated ribose is generated from 1'-acetylated ribose through a brominated intermediate. Subsequent N-glycosylation then yields the desired

¹ Bruner, S.D.; Norman, D.P.G.; Verdine, G.L.; *Nature*. 2000, **403**, 859.

² Bruner, S.D.; Norman, D.P.G.; Verdine, G.L.; *Nature*. 2000, **403**, 859.

³ Lindahl, T.; Wood, R.D. *Science*. 1999, **286**, 1897.

⁴ Schärer, O. D.; Verdine, G. L. *J. Am. Chem. Soc.* 1995, **117**, 10781

⁵ Guillermet et al. *Bioorganic and Medicinal Chemistry Letters*. 1995, **5**, 1455.

Call for Nominations

Philip L. Levins Memorial Prize

Nominations for the Philip L. Levins Memorial Prize for outstanding performance by a graduate student on the way to a career in chemical science should be sent to the Executive Secretary, NESACS, 23 Cottage St., Natick, MA 01760 by **March 1, 2007**.

The graduate student's research should be in the area of organic analytical chemistry and may include other areas of organic analytical chemistry such as environmental analysis, biochemical analysis, or polymer analysis.

Nominations may be made by a faculty member, or the student may submit an application. A biographical sketch, transcripts of graduate and undergraduate grades, a description of present research activity and three references must be included. The nomination should be specific concerning the contribution the student has made to the research and publications (if any) with multiple authors.

The award will be presented at the May 2007 Section Meeting. ◇

Summer Scholar

Continued from page 9

nucleosides. Alternatively, in case of 5-F-uridine, the nucleoside can be fluorinated directly to yield the desired compound. As previously described,⁶ the structures of 4'-F nucleosides are confirmed by the high vicinal coupling constant between 3'-proton and fluorine ($J = 14\text{--}18\text{ Hz}$).

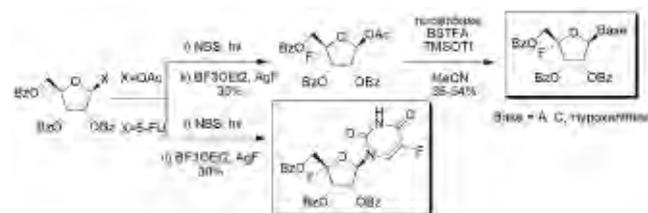


Figure 3. The synthetic scheme of 4'-fluorinated nucleosides.

One drawback to the strategy is that deoxyribose, unlike ribose, cannot be directly brominated. As such, an additional deoxygenation step on 4'-fluorinated ribonucleosides is required to complete the synthesis of 4'-fluorinated deoxynucleosides. An alternative synthetic scheme of 4'-fluorinated deoxyribonucleotides as described by Guillerm et al. is currently under investigation.

⁶ Guillerm et al. *Bioorganic and Medicinal Chemistry Letters*. 1995.5. 1455.

⁷ Phan, A.T.; Patel, D.J.; *J. Biomol. NMR*. 2002. 23. 257.

⁸ Phan, A.T.; Patel, D.J.; *J. Biomol. NMR*. 2002. 23. 257.

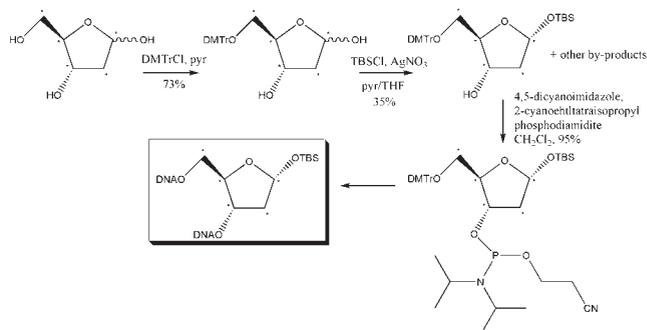


Figure 4. The synthetic scheme of ¹³C-labeled DNAs. Carbons marked by asterisks are ¹³C.

Part 2. Synthesis of ¹³C-labeled nucleotides

While the enzymatic mechanism of bifunctional glycosylases such as hOGG1 has been thoroughly elucidated, there are still some mysteries left to be explored. One is the structure of the Schiff base intermediate, as well as the 5'-end-product complex, especially the stereochemistry around the double bonds in both complexes. ¹H multidimensional NMR of the sugar ring region is often not very informative, as most sugar protons normally resonate in the same regions, rendering characterization of the protons extremely difficult.⁷ ¹³C NMR, if the natural sugar is used, would also not provide much information in correlation experiments since 99% of ¹³C neighbors are ¹²C.⁸ As such, a fully-¹³C labeled sugar is required in order to track the spatial orientations of each carbon and proton, e.g., through correlation experiments.

Using a sequence known for optimal binding to hOGG1, oxoG in oxoG:C base pair is replaced by an abasic site to mimic the initial cleavage product. The synthesis of the abasic nucleotide is illustrated in Figure 4. The overall yield is 24%. ¹H NMR shows the mixture of two phosphate stereoisomers. The presence of two stereoisomers does not impede DNA synthesis, and the abasic nucleotide is incorporated into the growing oligonucleotides almost as efficiently as normal nucleotides. Further NMR studies are currently under way to elucidate the manner in which hOGG1 cleaves this intermediate product.

Throughout the process, I have been greatly helped by Professor Gregory L. Verdine and Dr. Seongmin Lee, a post-doctoral fellow in Professor Verdine's group. They both have been sources of valuable knowledge, as well as sources of mental support. They congratulated me when the experimental results went well and encouraged me when I encountered obstacles. I would also like to express my gratitude to The Northeastern Section of the American Chemical Society and James Flack Norris and Theodore William Richards Research Scholarship selection committee for providing me with this invaluable summer research opportunity. This summer has provided me with a significant scientific research experience and will provide a strong foundation for my further studies on this subject, at both my under graduate institution and beyond. ◇

Summer Research Scholar

DNA Adduct Formation From Interaction With Pesticides In Plants

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Introduction

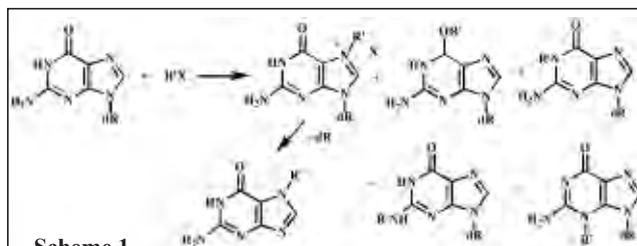
Pesticides are widely used for preventing, destroying, and repelling weeds, insects, fungi, and diseases that damage agricultural crops or harm humans. Typically life processes are interrupted by pesticide molecules or their metabolites at the biochemical level in the cell. Even though pest control agents degrade over time after application in the field, there is probably a sufficient duration of contact with the crop plant to allow attack on the biochemical systems of the plant, including genetic material. Pesticides used in crop production are known to pose risks to crop plants. These include elevated stress to the plant, resulting in inferior quality or yield of the agricultural product and inferior development of vine, leaf, or root systems. A number of studies point directly to plant stress caused by the genotoxic or oxidative stress involving DNA adduct formation with pesticides.(1-4) Adduct formation between DNA bases and various xenobiotic molecules has been established as a main source of mutation in biological organisms.(5;6) Many of these xenobiotic compounds, including numerous pesticides, are electrophilic and can react directly at various nucleophilic sites in the nucleic acid bases (Scheme 1). These xenobiotic agents may also induce formation of other reactive species via lipid peroxidation of arachidonic acid and other fatty acids (Scheme 2) via cyclooxygenases and lipoxygenases. Lipid peroxidation is known to produce various saturated and unsaturated carbonyls, such as the α,β -unsaturated aldehydes acrolein, crotonaldehyde, 4-hydroxynonenal (4-HNE), malondialdehyde (MDA), and hexenal (HXL).(7-14) Products of such oxidative stress leads to endogenous formation of cyclic DNA adducts with guanosine (Scheme 3).(7-9) These DNA modifications disable normal base pairing and disturb the replication process which can lead to strand breaks, lesions, deletions, and frameshift mutations.

In contrast to mammalian studies, little is known about the risk to plant DNA posed by adduct formation from pesticides or their metabolites.(15-21) The focus of this study was to examine the extent of DNA modification through direct interaction with nucleoside bases, such as guanosine, and to examine possible adduct formation through ^{32}P post-labeling studies by application to the plant. Guanosine was chosen because this DNA base is very reactive in formation of adducts with many drug molecules and contains several nucleophilic sites for possible binding with pesticides.

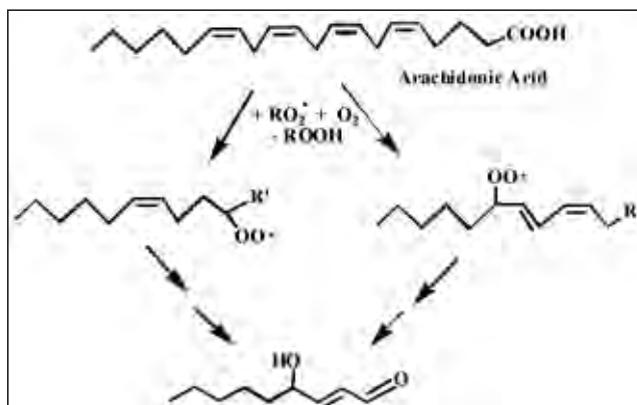
In this study we have chosen to investigate several herbicides, primarily representatives of the phenoxy and oxymimic classes. Included from these groups are: 2,4-dichlorophenoxyacetic acid (2,4-D), N,N-diethyl-2-(1-naphthoxy)propanamide (napropamide, Devrinol), 3,4,6-

trichloro-2-pyridinyloxyacetic acid (Triclopyr), and sethoxydim (Poast). Other structurally similar pesticides, 2,6-dichlorobenzonitrile (Dichlobenil) and 2,5-Dichloro-6-methoxybenzoic acid (Dicamba), were also considered.

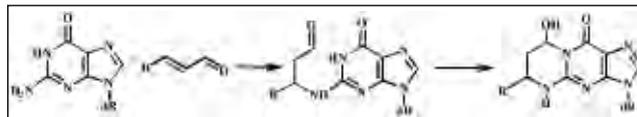
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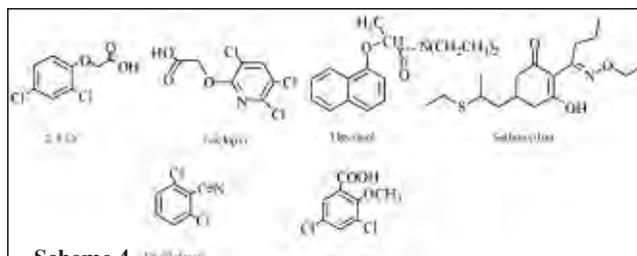
Scheme 1.
Direct Adduct Formation with Possible Putative Products



Scheme 2.
Lipid Peroxidation of Arachidonic Acid to 2-Hydroxynonenal



Scheme 3.
Cyclic Adduct Formation by Products of Oxidative Stress



Scheme 4.
Commercial Pesticides

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Experimental Procedures

In Vitro Reactions

Chemicals and Instrumentation. Guanosine, dimethyl sulfoxide (DMSO), and p-dioxane were obtained from Sigma Chemical Co. HPLC-grade water and methanol were purchased from Acros Organics and Pharmco-Aaper, respectively. The pesticides, Triclopyr, 2,6-dichlorobenzonitrile, Sethoxydim, and Napropamide were used as received from Supelco. Dicamba and 2,4-dichlorophenoxyacetic acid were purchased from Sigma-Aldrich Chemical Company. Analysis of reaction samples was performed on a Rainin-Varian Dynamax Model SD-200 HPLC system with model UV-1 UV/visible absorbance detector using a reverse-phase Varian microsorb-MV 100-5 C18, 250x4.6 column and gradient solvent mixtures of water and methanol. Ultraviolet spectra were recorded with a Shimadzu UV160U UV-visible recording spectrophotometer.

Reaction of 2,4-Dichlorophenoxyacetic acid with Guanosine. A 5.0 mL DMSO solution of 0.0362 mmol of 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.00306 mmol of guanosine, was stirred at 50° C for 48 hours. A parallel control reaction of 2,4-D (0.0362 mmol) was carried out in DMSO. The reaction solutions (initial and after reaction) were subjected to HPLC analysis by chromatography on a C18 reverse-phase column with a 35%-100% methanol-water gradient and a flow rate of 0.7 mL/min.

Reaction of 2,6-Dichlorobenzonitrile with Guanosine. A 20.0 mL DMSO solution of 0.988 mmol of 2,6-dichlorobenzonitrile and 0.0989 mmol of guanosine, was stirred at 51° C for 48 hours. The reaction solution and control were chromatographed on a C18 reverse-phase HPLC column with a 35%-100% methanol-water gradient and a flow rate of 0.7 mL/min.

Reaction of Dicamba with Guanosine. A 5.0 mL DMSO solution of 0.0543 mmol of Dicamba and 0.00306 mmol of guanosine, was stirred at 50° C for 48 hours. The reaction solution and control were chromatographed on a C18 reverse-phase HPLC column with a 35%-100% methanol-water gradient and a flow rate of 0.7 mL/min.

Reaction of Napropamide with Guanosine. 0.0306 mmol of Napropamide and 0.00612 mmol of guanosine in a mixture of 7.5 mL of 0.1 M K_2HPO_4 buffer and 3.5 mL of methanol, were stirred at 37° C for 48 hours. The reaction solution and control were chromatographed on a C18 reverse-phase HPLC column with a 35%-100% methanol-water gradient and a flow rate of 0.7 mL/min.

Reaction of Triclopyr with Guanosine. 0.0543 mmol of Triclopyr and 0.0107 mmol of guanosine in a mixture of 7.5 mL of 0.1 M K_2HPO_4 buffer and 3.5 mL of methanol, were stirred at 37° C for 48 hours. The reaction solution and control were chromatographed on a C18 reverse-phase HPLC

column with a 70%-100% methanol-water gradient and a flow rate of 0.7 mL/min.

Reaction of Sethoxydim with Guanosine. A 11.45 mL methanol solution of 0.0502 mmol of Sethoxydim and 0.0100 mmol of guanosine, was stirred at 37° C for 48 hours. The reaction solution and control were chromatographed on a C18 reverse-phase HPLC column with a 70%-100% methanol-water gradient and a flow rate of 0.7 mL/min.

Pesticide application/treatment

Corn, our model plant system, was grown under greenhouse conditions from seeds obtained from commercial sources. The pesticides were applied to groups consisting of 2-4 plants after growth for two to six weeks. Each group of plants was isolated and sprayed with commercially available pesticide formulations on four occasions over a period of two weeks.

DNA Isolation

The isolation of DNA from plant tissue was accomplished using Qiagen DNeasy® Plant Maxi Kits. One to two grams of plant material was ground to powder with the assistance of liquid nitrogen. Upon complete evaporation of the liquid nitrogen, 5.0 mL AP1 buffer (preheated to 65°C) and 10 μ L RNase A stock solution were added to the ground material. The cells were lysed by vortexing and heating for 10 minutes at 65°C. The samples are incubated with 1.8 mL AP2 buffer for another 10 minutes at 0°C and centrifuged for 5 min. at room temperature to remove non-DNA materials (proteins, polysaccharides, etc.). The liquid was vortexed with one and a half equal volume of AP3/E buffer and centrifuged on a spin column for 5 minutes. The eluant liquid was discarded and 12 mL of AW buffer was added to the spin column, and the procedure was repeated. The DNA product was eluted twice with 1 mL portions of AE buffer from the spin column. After standing for 5 minutes at room temperature, the eluant was centrifuged.

The purity of the isolated DNA in AE buffer was analyzed for purity and yield by UV spectrophotometry. The quantitative analysis of the DNA was evaluated at $\lambda=260$ nm where an absorbance of 1.00 is equivalent to 50 mg/mL of DNA. Absorbance ratios A_{260}/A_{280} and A_{230}/A_{260} were used to determine completeness of removal of RNA, plant proteins, and other plant materials.

³²P Post-Labeling

The ³²P post-labeling analyses were carried out in the laboratory of Prof. Erwin Eder at the Institute of Toxicology, University of Würzburg, Germany. The modified DNA was suspended and hydrolyzed to the nucleoside monophosphates with micrococcal nuclease and calf spleen phosphodiesterase. The resulting solution was treated with nuclease P1 following the protocol of Wacker et al(22), a modified procedure of Randerath, Reddy and Gupta.(23) The mononucleotides were then γ^{32} P-phosphorylated with [γ -³²P]ATP in the presence of T4 polynucleotide kinase. The adducted bases were analyzed by two-dimensional TLC separation on PEI cellulose. Elution was accomplished with a 1.7 M solu-

tion of ammonium formate at pH 3.5 in the first direction (D1), which was followed by elution with 2.7 M NaH₂PO₄ solution at pH ca. 3.8 in the second direction (D2). PEI Cellulose strips were then autoradiographed on a Packard Instant Imager with the intensity of the radioactivity quantified. Modified bases appear at different locations from the unmodified nucleotides on TLC, thus allowing for detection. Because this technique permits one modified base in 10⁹ to be detected, the presence of adducted bases and the extent of DNA modification can be clearly determined.

Results and Discussion

Chlorinated aliphatic and aromatic compounds, including a number of pesticides, have been shown to exhibit cytotoxicity and mutagenic properties.(24) Chlorinated pesticides particularly have the tendency to persist in the soil upon agricultural application and present an increased risk to the environment and to the crops themselves. They can be powerful inducers of microsomal enzymes, and with prolonged enzyme induction can result in taxed and damaged cells. The general high reactivity of pesticides coupled with their availability in cellular material in plants creates the opportunity for interactions with DNA leading to the formation of adducts. Therefore, *in vivo* experiments were carried out to detect adducts in pesticide-treated plants by ³²P post-labeling analysis.

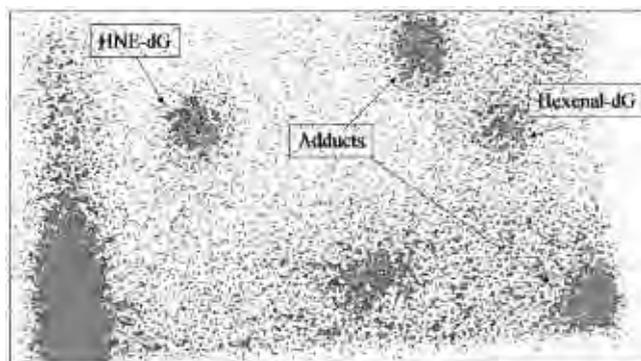


Figure 1. Radiochromatogram of Corn with 2,4-D(2,4-Dichlorophenoxyacetic Acid). The presence of HNE-dG and Hexenal-dG is comparatively evident with two additional unknown adduct spots.

The DNA isolated from corn, treated with the herbicide 2,4-D, produced multiple chromatographic spots upon ³²P post-labeling (Figure 1). Four adduct spots (not present in the control) are visible in the radiochromatogram of the corn. Both direct adducts from the pesticide or metabolites and indirect adducts from lipid peroxidation are present in the radiochromatogram. Although the adduct structures corresponding to two of the spots are unknown at this time, the other two spots on the autoradiogram are attributable to deoxyguanosine adducts formed with hexenal(HXL) and 4-hydroxynonenal(HNE). Both of these adducts have been previously identified in ³²P post-labeling studies.(22;25) Xenobiotic compounds, including many pesticides, also have the capacity to produce reactive oxygen species as

byproducts of their detoxification by cytochrome P450.(26;27) These reactive oxygen species generate peroxidized lipids via cyclooxygenases and lipoxygenases, leading ultimately to α,β -unsaturated aldehydes, hexenal and 4-hydroxynonenal, among others.(28) (29;30) HXL and HNE are well known to produce exocyclic adducts of guanosine.(7-9;11;13) (See Scheme 3.) The ³²P post-labeling analyses of the other pesticide-treated plant samples are pending and awaiting evaluation.

In order to further investigate the occurrence of direct adducts, guanosine was reacted with each of the pesticides *in vitro*. Guanosine was chosen as the first nucleoside to be studied because it is well known as the DNA nucleoside with the greatest tendency to form adducts. Possible products from attack of electrophilic pesticide molecules on the base guanine are revealed in Scheme 1. The presence (or absence) of adduct products was determined experimentally by reverse-phase HPLC chromatography. HPLC chromatograms of the reaction solutions appear in Figures 2-6. Control reactions of the pesticide alone under the same reaction concentrations and conditions were carried out for comparison. Guanosine under the same reaction conditions was stable and did not decompose or react. These *in vitro* reactions between guanosine and the pesticides considered in this survey show clear evidence of adduct formation in several cases and no reactivity in others.

2,4-Dichlorophenoxyacetic acid (2,4-D)

2,4-Dichlorophenoxyacetic acid is a common systemic herbicide in the phenoxy class which is used in the control of broadleaf weeds. It functions as a plant growth regulator that mimics the natural plant growth hormone auxin. Chromatograms of 2,4-dichlorophenoxyacetic acid reactions with guanosine (Figure 2) show complete disappearance of the 2,4-D and the appearance of an adduct product peak (ret. time 6.8 min.), which is not present in the control solution of 2,4-D alone. In light of the formation of 2,4-dichlorophenol as a metabolite of 2,4-D by cleavage of the carbon-oxygen bond at the C1-position on the aromatic ring, nucleophilic aromatic substitution by nucleophiles would be the likely reaction mechanism, since activation would take place by

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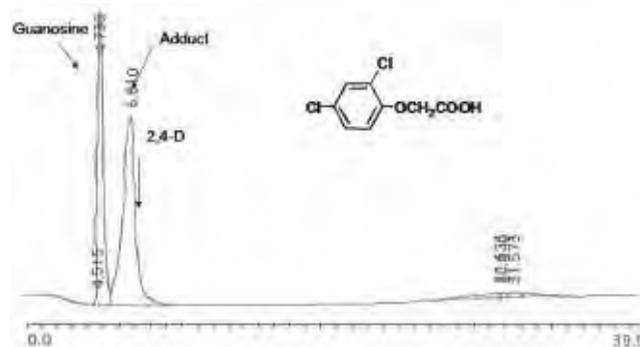


Figure 2. HPLC chromatogram indicating an adduct(6.81min) from 2,4-D and guanosine(4.73min) peaks. A trace amount of 2,4-D is present but the elution is similar to that of the adduct peak.

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chloro substituents at both *ortho* and *para* positions. Attack from the various nucleophilic sites in guanine (N7, O6, N1, N2, N3) on the aromatic C1-position in 2,4-D would provide several putative structures for the 2,4-D-Guo adduct.

Napropamide (Devrinol)

Napropamide, a phenoxy carboxylic acid similar to 2,4-D, is a selective systemic amide herbicide that is absorbed by the roots and works by inhibiting root development and growth. Napropamide contains an amide group, which is of limited reactivity, and has exhibited only slight toxicity in previous animal tests.(31) Napropamide began to degrade after 6 days and showed no reactivity prior to the degradation. The reactions with guanosine yielded no evidence of possible adduct formation under the experimental conditions. The HPLC chromatogram (Figure 3) of the reaction mixture appears unchanged from the initial solution. Apparently the naphthalene ring is not sufficiently activated toward nucleophilic aromatic substitution and the amide moiety is also sufficiently unreactive.

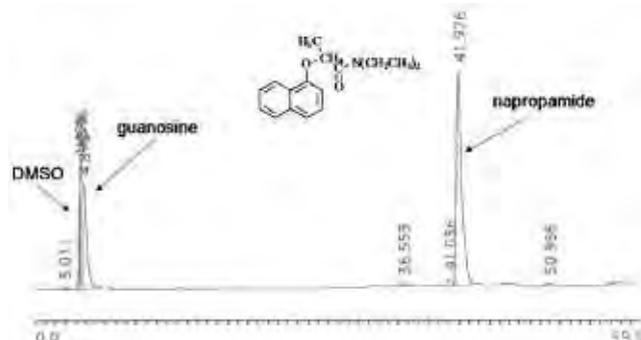


Figure 3. HPLC chromatogram indicating napropamide(42min), guanosine(4.8min) and DMSO(4.4min)peaks.

[(3,5,6-Trichloro-2-pyridinyl)oxy]-acetic Acid (Triclopyr)

Triclopyr is another aryloxyacetic acid with strong activation at C1 by both *ortho* and *para* activating groups. It exhibits acute toxicity and mutagenic effects in animals such as mice. After 48 hr the reaction solution of triclopyr and guanosine contained multiple product peaks, several of which are not present in the control. Two significant adduct peaks emerged with retention times at 20.95 and 34.22 minutes. (Figure 4) Considering the multiple leaving chloro groups, in addition to 2-oxyacetic acid group, the number of viable products from potential attack at the several possible nucleophilic sites on the guanosine molecule is large. Further analysis by LC/MS or LC/MS/MS is necessary for determination of the structure of the present adducts.

Dichlobenil (Casoron)

Dichlobenil is a benzonitrile herbicide used for the control of submerged weeds in cranberry bogs, fruit orchards, vineyards, etc. After incubation of Dichlobenil with guano-

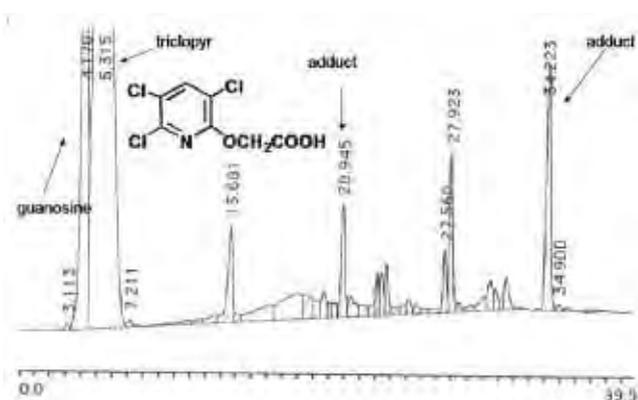


Figure 4. HPLC chromatogram indicating triclopyr(5.32min) and guanosine(4.17min) peaks. The identification of two adducts that appear to elute further down the chromatogram(20.95min,34.22min).

sine, followed by HPLC evaluation of the initial and 48 hr samples, an adduct emerged in the reacted sample with a retention time of 30.92 minutes.(Figure 5) The control solution, containing only the pesticide, retained the initial peak and displayed no decomposition except for hydrolysis to 2,6-dichlorobenzamide (ret. time 32.8 min.) under the reaction conditions. Again nucleophilic aromatic substitution is a likely mechanistic route to the adduct via activation of chloride displacement by the *ortho* nitrile substituent.

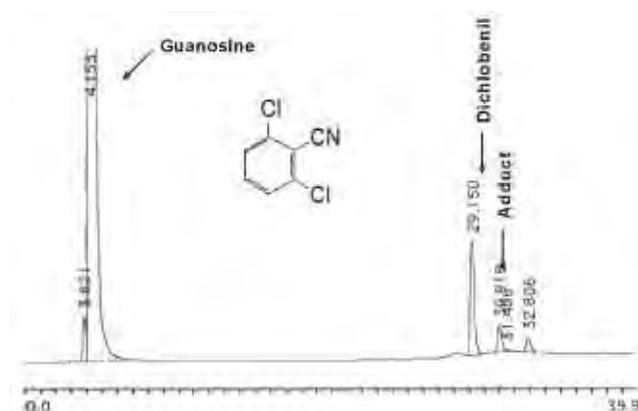


Figure 5. HPLC chromatogram indicating dichlobenil(29.15min) and guanosine(4.15min) peaks. The identification of an adduct is located near the dichlobenil peak at 30.92min.

3,6-Dichloro-2-methoxybenzoic Acid (Dicamba)

Dicamba is a benzoic acid herbicide and remains one of the most widely used products for controlling broadleaf weeds in corn. After 6 days, the samples began to degrade, but the 48 hr reaction sample with guanosine produced two products not present in the control solution. HPLC chromatograms of the reaction solution (Figure 6) revealed an adduct peak eluting at 28.23 min followed by a second peak arising at 35.84 min. In addition to small amounts of 3,6-dichlorosalicylic acid, the presence of 5-hydroxy-2-methoxy-3,6-dichlorobenzoic acid in soils and plants again is suggestive of nucleophilic aromatic substitution at C5 in the aromatic ring with displacement of chloride. Attack by

guanine nucleophilic centers with displacement of the chloro substituent at C5 in Dicamba is a plausible path to adduct formation due to its activation by ortho-carboxyl, para-chloro, and meta-methoxy groups.

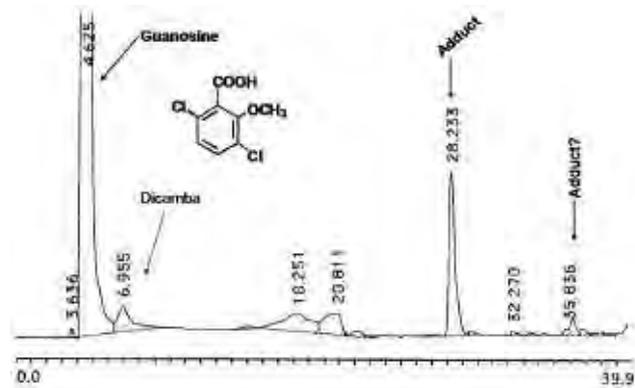


Figure 6. HPLC chromatogram indicating dicamba(6.96min) and guanosine(4.63min) peaks. The identification of an adduct at 28.23min is evident with a possibility of a second eluting at 35.84min.

Sethoxydim (Poast)

Sethoxydim is a selective herbicide associated with the cyclohexanedione family and is used to kill and suppress annual and perennial grasses. It inhibits acetyl CoA carboxylase which effectively prevents fatty acid production, leading to failure of cell membrane integrity and overall plant death. Using the standard methods, reaction of sethoxydim with guanosine yielded no evidence of potential direct adduct formation under the conditions, although considerable decomposition of sethoxydim is present (Figure 7). This was somewhat surprising because sethoxydim consists of an α,β -unsaturated carbonyl group which is known to undergo Michael addition of guanosine to produce exocyclic adducts in malondialdehyde and crotonaldehyde, as well as hexenal and 4-hydroxynonenal. The chromatograms were also free of adduct peaks under the same conditions in reaction solutions of sethoxydim with 2'-deoxyguanosine and 2'-deoxycytidine monohydrate. It is possible that sethoxydim with its α side-chain is too hindered for reaction with nucleosides.

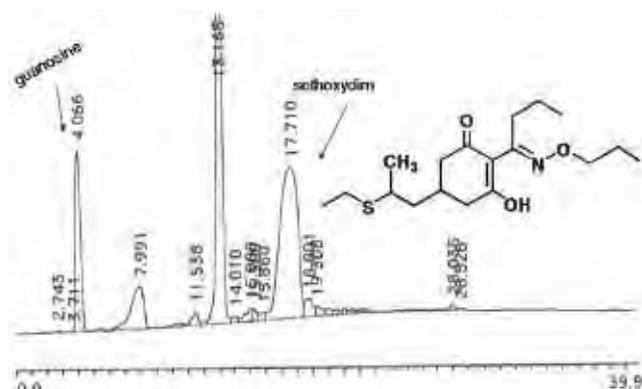


Figure 7. HPLC chromatogram indicating sethoxydim(17.7min) and guanosine(4.06min) peaks.

In conclusion, the reactivity of electrophilic pesticides molecules and DNA bases like guanosine appears to be a common phenomenon. This current research, combined with previous *in vivo* and *in vitro* investigations of the effects of pesticides in plants, demonstrates that pesticides are absorbed into crop plants, translocate relatively easily throughout the plant, and interfere with various biochemical systems, including plant genetic material. The continuing study of these interactions and structural details of these adducts will enable a better understanding of the mechanism of formation of these products, as well as insight into the risk posed by pesticides to crops, humans, and the environment.

Acknowledgments

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Feb 3

Boston Regional Inorganic Meeting
 Prof. Stephen Lippard (MIT)
 Prof. William Reiff (Northeastern Univ.)
 Prof. Roy Planalp (Univ. New Hampshire)
 Prof. Ann Valentine (Yale Univ.)
 Tufts Univ, Pearson building
 Hosted by Professor Elena Rybak-Akimova
 9:30 - 2:30.

Feb 6

Prof. Timothy M. Swager (MIT)
 TBA
 Boston College, Merkert 130
 4:00 PM

Feb 7

Prof. Silvia Cavagnero (Univ. Wisconsin – Madison)
 “How Do Proteins Fold at Birth? Mechanisms of in vitro and Cotranslational Protein Folding”
 Boston College, Merkert 130
 4:00 PM,

CDD Speaker
 TBA
 Northeastern University Hurtig Hall 12:00 PM

Dr. Ann M. Valentine (Yale Univ)
 “Bioinorganic Chemistry of Titanium in Medicine and the Environment.”
 UMass Dartmouth, Building Group II Rm 115
 4:00 PM

Feb 13

Prof. Michael D. Burkart (U.C. San Diego)
 TBA
 Boston College, Merkert 130
 4:00 PM

Feb 14

Dr. Dmitriy Alexeev (Harvard Medical School)
 “Pharmacological Sensitization of Vision”
 UMass Dartmouth, Building Group II Rm 115
 4:00 PM

Feb 15

Michael Fayer (Stanford University)
 TBA
 MIT, Room 6-120
 from 5:00 pm to 6:00 pm

Feb 20

Prof. John Montgomery (Univ. Michigan)
 2006/2007 Bristol-Myers Squibb Lecturer
 TBA
 Boston College, Merkert 130
 4:00 PM

Richard Hsung (U. of Wisconsin)
 TBA
 Univ. New Hampshire, Rm L103
 11:10AM

Feb 21

Dr. Michele Mandrioli (UMass Dartmouth)
 “Some Applications of WebCT Features in Face-to-Face Courses”
 UMass Dartmouth, Building Group II Rm 115
 4:00 PM

Feb 27

Prof. Brian M. Stoltz (Cal. Instit. Tech)
 TBA
 Boston College, Merkert 130
 4:00 PM,

Feb 28

Dr. Scott Calabrese Barton (Michigan State University)
 Northeastern University, Hurtig Hall 12:00 PM
 Dr. Linda H. Doerrer (Boston Univ.)
 “Metallophilic Interactions in Precious Metal Polyimine Double Salts”
 UMass Dartmouth, Building Group II Rm 115
 4:00 PM

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