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CHEMISTRY
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AGRICULTURE

PICOGRAM V. 90
ABSTRACTS

AMERICAN CHEMICAL SOCIETY
252nd National Meeting and Exposition

Chemistry of the People, by the People, and for the People

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AGRO DIVISION

AGRO 1

Fundamentals of EPA good laboratory practices

Patricia M. Maldonado[1], *p_maldonado@hotmail.com, Carol Lee*[2]. *(1) Dow AgroSciences, Indianapolis, Indiana, United States (2) Lee Compliance Assessments, Phoenixville, Pennsylvania, United States*

Good laboratory practices (GLP) broadly refer to a system of quality controls established to ensure the consistency, reliability, reproducibility, and integrity of laboratory testing. However, not all GLPs are created equally. This talk will focus on the US Environmental Protection Agency (EPA) GLP Regulations set forth in 40 CFR Part 160 which prescribe the requirements for conducting studies that support, or are intended to support registration applications for pesticide products regulated by the EPA to assure the quality and integrity of the data submitted to the agency. The different topics within this presentation are intended to convey that following the GLPs is not only a requirement, but it also contributes to good science and to good business.

The first part of this two-part presentation will start with a history of the regulations in order to understand why they are necessary. The presentation will go on to cover the roles and responsibilities of the personnel involved in GLP studies with emphasis on the testing facility management, the study directors, and the quality assurance unit. The requirements for testing facilities and equipment, which includes provisions for preventing cross-contamination and ensuring proper equipment design, maintenance, and calibration, will also be discussed. Finally, the presenter will go into details regarding the operational aspects of the testing facility, such as developing standard operating procedures and elements of the test system.

AGRO 2

Fundamentals of EPA good laboratory practices

Carol Lee[2], *leecomplianceassessments@gmail.com, Patricia M. Maldonado*[1]. *(1) Dow AgroSciences, Indianapolis, Indiana, United States (2) Lee Compliance Assessments, Phoenixville, Pennsylvania, United States*

Good laboratory practices (GLP) broadly refer to a system of quality controls established to ensure the consistency, reliability, reproducibility, and integrity of laboratory testing. However, not all GLPs are created equally. This talk will focus on the US Environmental Protection Agency (EPA) GLP Regulations set forth in 40 CFR Part 160 which prescribe the requirements for conducting studies that support, or are intended to support registration applications for pesticide products regulated by the EPA to assure the quality and integrity of the data submitted to the agency. The different topics within this presentation are intended to convey that following the GLPs is not only a requirement, but it also contributes to good science and to good business.

The second part of this two-part presentation will delve into specific GLP requirements, including:
- The test, control, and reference substances used in a GLP study,
- The elements of a study protocol, the document governing the conduct of the study,
- The raw data and the final report, the document summarizing the outcome of the study, and
- Records retention.

The discussion will conclude with a summary of the overall process. A panel of quality professionals from the EPA GLP Specialty Section of the Society of Quality Assurance (SQA) will be available to answer questions from the audience.

AGRO 3

EPA GLP inspection program: Interpretation, enforcement, and case studies

Mark Lehr, *Lehr.Mark@epa.gov. US Environmental Protection Agency, Denver, Colorado, United States*

This talk will be an overview of the Environmental Protection Agency (EPA) Good Laboratory Practices (GLP) inspection program and provide an EPA inspector's perspective on how laboratories may assure the quality and integrity of test data submitted to the Agency under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA). Mr. Lehr will share what policies, practices and procedures laboratories must have in place to meet EPA GLP regulations and how to avoid potential EPA enforcement actions.

AGRO 4

Quality systems approach to implementing good laboratory practice in the analytical lab

Richard Wedlich, *rwedlich@nsf.org. NSF Pharmalytica, Bristol, Connecticut, United States*

The laboratory operation can be understood and mapped out in terms of the requirements to perform a single study and multiple simultaneous studies, all in compliance with Good Laboratory Practices. As the laboratory grows, taking on more and more studies, so does the risk of non-compliance and the task of managing the lab. Lab managers typically separate the lab operation into unit operations, some of which apply to studies, and others that apply to the platform that supports all studies. This is the quality systems approach. We use our own lab as an example. By walking through a typical study conducted in our lab, we introduce in this presentation many fundamental GLP ideas in a practical, hands-on setting and show how the lab minimizes the risk of non-compliance by establishing, monitoring, and maintaining the appropriate quality systems.

AGRO 5

Principals of data recording and best practices in documentation of good laboratory practices (GLPs) studies for the agrochemical professional

Kim B. Watson, *kwatson@stone-env.com. Stone Environmental, Inc., Montpelier, Vermont, United States*

Why do we have or care about good documentation practices? Many of the reasons for establishing GLPs and best practices in documentation came about due to careless study conduct, failure to follow Protocols, not reviewing the data, not reporting all the data, having untrained/unqualified personnel, using improper lab procedures, sponsors failing to monitor studies, and sponsors not validating the data or the report. Proper documentation practices were thus deemed to be critical elements in GLP compliance that help to ensure the validity and reconstructability of a study.

This session will focus on the Principals of Data Recording. The golden principal of data recording for GLP studies is: "Record, review, analyze, and store raw data as you would have others do for you". GLP studies must be traceable and reconstructible; therefore, if something is not written down

then it didn't happen. We will look at examples of bad data and then what makes raw data of excellent quality. We will look at what physical forms raw data can take and identify best data collection practices. As agrochemical professionals and GLP Quality Assurance Units, we will look at inspectional tips on reviewing and auditing raw data and documentation.

AGRO 6

Managing multi-site studies: An overview using a field residue trial example

Tammy W. Barkalow, *white@aesop.rutgers.edu. The Office of IR-4, Rutgers, The State University of New Jersey, Princeton, New Jersey, United States*

For over 25 years the US EPA has required that certain multi-site studies be performed. For residue studies, test systems (crops) have to be grown and dosed in many locations. Their crop samples (grain, seed, fruit, etc.) are collected and sent to analytical labs or to processing facilities that produce GLP compliant crop fractions. All samples ultimately go to an analytical lab for analysis. In order for all the "parts" of the study to be completed in compliance with the GLPs one study director (SD) has to be appointed by Testing Facility Management (TFM), and the remaining "study participants" have to be organized in such a way as to "feed" all the information and decision making to the SD.

How do you keep track of 10, 50, or more trials being performed at 5, 10, or more different locations during the growing season? What is expected of SDs in this situation? Is the Quality Assurance personnel (QA) at the test sites required to send their reports to the SD and TFM? Who makes sure all members of the QA have the protocol(s) they need? Who has to have access to the master schedule? Are there multiple master schedules? How is information communicated to assure the SD is kept informed of the study conduct? How is the master schedule updated to accurately express the current status of the studies and their associated trials and "parts"? How is data handled from the field sites? Who archives that data? Who writes the final report?

These and many other questions will be explored in this session. Multiple site studies are not as complex as imagined and have been successfully conducted for years. We will present a case study that will hopefully answer these questions and more.

AGRO 7

Regulatory submissions of pesticide data in the US and worldwide

Eva Haszcz, *eva.haszcz@fmc.com. Global Regulatory Sciences, FMC Corporation, Manalapan, New Jersey, United States*

The ultimate goal of a GLP study is to have it accepted by the Regulatory Agency in support of product registration. In the current environment of globalized economy, most companies are performing or contracting studies internationally and submitting them across the globe. The submissions of study reports are no longer limited to the regulatory authority in the country where the study was conducted, but reports are intended for international registrations. The purpose of this presentation is to discuss potential issues encountered when conducting pesticide studies intended for submission in many countries.

According to the Organization of Economic Collaboration and Development (OECD) under Mutual Acceptance of Data (MAD), a study conducted in a GLP facility of one MAD-adhering country should be accepted by the regulatory agencies of all other MAD members. However, the country-specific reporting requirements and some differences in interpretation of GLP create ambiguity in the generation of internationally acceptable data. The speaker will discuss nuances in designing a global study, from the selection of an appropriate testing facility to the formatting of the report suitable for global submission.

AGRO 8

Application of GLP principles to computerised systems (OECD Consensus Document 10)

Chris Wubbolt, *chris.wubbolt@qacvconsulting.com. QACV Consulting, LLC, Bethlehem, Pennsylvania, United States*

The draft OECD Consensus Document on Application of GLP Principles to Computerised Systems, published in September 2014, is intended to replace the 1995 GLP consensus document. The use of computerized systems and subsequent concerns related to data integrity continues to increase in all regulated industries. The new draft was updated to incorporate additional information related to risk assessment, validation life cycle expectations, and other topics related to the validation, use, and maintenance of computerized systems. This presentation will provide an overview of the document as well as examine specific details which will be relevant to the audience.
This presentation will discuss the following:
Significant changes from the 1995 document.
The impact of the changes reflected in the document.
Possible changes that may be incorporated into the final draft of the document.

AGRO 9

OECD guidance for conducting pesticide terrestrial field dissipation studies and Ecoregion Crosswalk

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Data on pesticide terrestrial field dissipation/accumulation are required through legislation in the European Union and by regulatory agencies worldwide. The acceptance and use of field studies conducted at foreign sites for national and global joint reviews would reduce economic and regulatory burdens for both registrants and regulators.

An *ad hoc* expert committee completed the development of an OECD guidance for conducting terrestrial field dissipation (TFD) studies and an ecoregion crosswalk between North America and Europe. The purpose of this project is to provide guidance for generating field data that are acceptable to both North American and European regulatory agencies.

The project has two components, TFD guidance and an ecoregion crosswalk tool. Guidance was developed based on a conceptual model/modular approach which is based on pesticide properties and laboratory fate studies. The modules covered in this guidance include a basic study, $DegT_{50}$, runoff, deep leaching, volatilization and plant uptake. The guidance provides full details on experimental layout, sampling, analysis, results and use of pesticide TFD data in exposure and risk characterization.

The second component is the ecoregion crosswalk. A GIS-based tool was developed to identify similar ecoregions between Europe and North America. Two levels are used to assess the similarity: an holistic approach where all the five parameters are considered, and a weights of evidence approach, where only parameters that are important for the identified concerns are considered. The model also includes a site selection tool by which a site is selected based on use areas and concerns, and that would be acceptable to both North American and European regulators.

AGRO 10

Terrestrial field dissipation studies under the new OECD guidance: An industry view from Europe

Dieter Schaefer, *dieter.schaefer@bayer.com. Bayer CropScience, Monheim, Germany*

The new OECD Guidance Document introduces a global framework for the conduct of Terrestrial Field Dissipation (TFD) studies for pesticide authorization. It establishes a harmonized study design that combines existing guidance from NAFTA and Europe. In addition, the Ecoregion Crosswalk concept allows assessing the representativity and relevance of any TFD study for any region around the world. It is now for the first time possible to combine TFD studies from different regions in one consistent global data package.

On the other hand, the harmonization was partly achieved by agreeing on the lowest common denominator, resulting in potentially increased efforts to conduct the studies. There was also no harmonization with regard to the interpretation of TFD data and their use in regulatory risk assessments. Finally, there are still open questions around the application of the Ecoregion Crosswalk concept.

This presentation provides a critical assessment of the new OECD Guidance Document from the point of view of the European agrochemical industry. It points out remaining issues and opportunities, and gives examples of how the new guidance may change the way regulatory TFD studies may be conducted in the future.

AGRO 11

Terrestrial field dissipation studies: Best practices and lessons learned from the field

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Terrestrial field dissipation studies can be complex and labor intensive to complete. Proper conduct of test substance application and sampling activities by the contract research organizations (CRO) will result in reliable samples being submitted to the analytical laboratory for analysis. This presentation will cover best practices, insights, and lessons learned from the perspective of a field CRO.

AGRO 12

Our experience with cropped plot field dissipation studies

Ashok K. Sharma, *ashok.k.sharma@dupont.com. DuPont Stine Haskell Research, Newark, Delaware, United States*

The Environmental Protection Agency [EPA] in the Unites States issued updated guidance [835.6100], which has largely been incorporated into the OECD guidance for these studies. The objectives defined by the EPA propose development of a conceptual model for field dissipation of a product and suggest that all significant dissipation routes be included in the conduct of field dissipation. In cases where a product has foliar applications to target crops, plant uptake, foliar interception and degradation in plants, and photo-degradation on soil surface, these can all be logical dissipation modules. Determination of whether these processes contribute to more than 20% of the overall dissipation in an outdoor environment would require the application of a plant protection product to a cropped plot and analysis for the resulting residues in multiple matrices. We have conducted several cropped plot studies recently, and our results from such studies will be the subject of this paper. This presentation will include results of foliar interception as well as similarity/differences in soil residues in cropped plots as compared with bare soil plots.

AGRO 13

Assessment of data generated from terrestrial field dissipation studies

Andrew Newcombe[1], *andy.newcombe@arcadis-us.com, Ralph L. Warren*[2], *Tianbo Xu*[3]. *(1) Arcadis, Wilmington, Delaware, United States (2) BASF, Research Triangle Park, North Carolina, United States (3) Bayer CropScience, Durham, North Carolina, United States*

Global regulatory guidance for the conduct of terrestrial field dissipation studies has evolved within the last 10 years. Study designs have become more complex and the need to generate fit-for-purpose studies has increased.

This paper will evaluate the analytical data generated from multiple terrestrial field dissipation studies, and an assessment of data trends observed will be presented. Specifically, different test substance application verification approaches, zero day recovery in soil using different sampling techniques, variability in soil residues by subplot, and transit stability vs. field fortification data will be evaluated.

AGRO 14

Analytical method and soil storage stability considerations for support of terrestrial field dissipation studies

Ralph L. Warren, *ralph.warren@basf.com. BASF Corporation, Research Triangle Park, North Carolina, United States*

As regulatory terrestrial field dissipation studies have increased in complexity and scope, much attention has been given to field design aspects. Just as important to the successful conduct of a terrestrial field dissipation study are the underlying analytical methods and generation of supporting soil storage stability data.

In this presentation key points of the analytical methodology used for soil residue data generation will be reviewed. Selection of analytes to include in the analytical method, setting of method limits (LOD/LOQ), validating the soil residue extraction method versus the soil metabolism extraction method, and method strategies to define depth of leaching will be addressed.

In addition, generation of soil storage stability data will be discussed. Regulatory agencies have placed renewed emphasis on having robust soil storage stability data available to support each terrestrial field dissipation study. Approaches for developing the necessary storage stability data and the scope of data needed will be addressed.

AGRO 15

Maximizing use of data from terrestrial field dissipation studies conducted in North America and Europe via the ENASGIPS Ecoregion Crosswalk tool

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Regulatory Agencies in Europe and North America are increasingly evaluating data from terrestrial field dissipation (TFD) studies conducted on foreign soils. The acceptability of studies conducted on foreign soils has largely increased as a result of the OECD Ecoregion Crosswalk project which established guidance and tools to assess the geographic representativeness of TFD studies conducted in North America and Europe. This acceptance of data from studies conducted on foreign soils can significantly reduce the number of TFD studies that are required globally, which reduces costs for both industry and regulatory agencies. To evaluate the acceptability of study data, a crosswalk must be performed to ensure the soils and climate of the TFD are representative of soil and climate conditions where the pesticide is intended to be used. As part of the OECD crosswalk project, the Pest Management Regulatory Agency - Health Canada and the US Environmental Protection Agency in collaboration with Agriculture and Agri-Food Canada and the EC Joint Research Centre, developed the "Europe – North America Soil Geographic Information for Pesticide Studies" (ENASGIPS) tool. ENASGIPS is a GIS-based model that includes a database of soil, climate, and crop information for North America and Europe. The tool identifies ecoregions in North America and Europe with similar climate and soils. In addition, ENASGIPS facilitates TFD site selection based on user defined soil, climate, and crop criteria. This presentation will provide an overview of key features of the tool and demonstrates two case-studies that crosswalk North American TFDs to Europe and vice-versa. The paper also discusses important considerations for appropriate use of the tool.

AGRO 16

ENASGIPS – Implications of user's choices

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OECD's ENASGIPS tool is a module in the new harmonized terrestrial field dissipation guidelines (as of January 2016). It is a GIS based application that enables users to quickly determine if test sites located in a particular ecoregion have similar ecoregions elsewhere in Europe or North America. By demonstrating similarity it may not be required to conduct additional studies to achieve registration. ENASGIPS uses long-term annual total rainfall, average precipitation, soil texture, soil pH, and soil organic matter to calculate a similarity index in a hollistic approach. As not all listed parameters may influence a pesticide's behavior in the soil, a weight-of-evidence approach in which the user selects just the parameters that affect their pesticide can be used as well. The selection of just a few variables has a significant impact on the results; therefore, defining the conceptual model of the pesticide's behavior is critical.

ENASGIPS can also be used by non-GIS experts to quickly select locations based on a given set of selection criteria. The site selection module in ENASGIPS will do this. Executing the site selection tool is easy; however, selecting the ranges to determine which areas adhere to is not. The input ranges change based on the question asked and how strict a user wants to be.

In this presentation we shall focus on the implication of choices users can make when using the ENASGIPS tool to determine similar ecoregions and to select sites.

AGRO 17

Innovative approaches in designing agrochemical metabolism studies

Jalees Afzal, *Jalees.afzal@basf.com. Department of Global Consumer Safety, BASF Corporation, Research Triangle Park, North Carolina, United States*

The primary objective of a typical metabolism study is to characterize and identify at least 90% of the total radioactive residue (TRR) for each commodity. In many cases, it is extremely challenging to identify a significant portion of the TRR specifically when the TRR is inherently low or incorporated into the natural carbon pool or when the test material itself is extensively metabolized

The analytical tools which are used to identify metabolites are constantly advancing, and it becomes imperative to use the state-of-the-art technique to achieve identification of metabolites. Normally, identification of metabolites is carried out by co-chromatographic retention time comparison with known reference compounds using two dissimilar chromatographic systems or unequivocal confirmation by LC/MS/MS and/or NMR.

In an effort to identify a significant portion of the TRR, innovative experiments are designed to ease or facilitate identification of major metabolites. These innovative approaches include use of stable isotopes as mass markers, enzyme-specific hydrolysis experiments, early-stage *in vitro* assays, and ruminant-specific rumen fluid hydrolytic procedures, which will be discussed.

AGRO 18

Study design for successful metabolite identification: Considerations for isotope labeling

Jason LaMar, *jlamar@ptrlwest.com, Thomas Fleischmann, Gary Quistad. Metabolism, PTRL West (a division of EAG, Inc.), Hercules, California, United States*

Radiolabeled-isotopes in metabolism studies are useful both as a tracer, and in many cases also as a marker for mass spectrometry and metabolite identification. Study designs which include test substances with significant radiolabel enrichment, or containing additional stable-isotopes, have exhibited advantages when trying to identify unknown metabolites by MS. A review of select metabolism studies will show various combinations of primary isotope, radiolabel isotope and stable secondary isotope with varying isotopic patterns. Examples will be presented while demonstrating some of the advantages and disadvantages of different labeling variations. Other cases will illustrate a comparison of the relative mass spectrum intensities that have been utilized to select and identify unknown metabolites in complex mixtures. Considerations of radiolabel location, the presence and positioning of halogens, and the benefits of stable-isotope labeling will be discussed. The isotopic content and the incorporation of stable and radiolabel-isotopes in metabolism studies should be considered in study design to improve the chance of success for unknown metabolite identification.

AGRO 19

Challenges encountered at critical stages of agrochemical metabolism studies and how to address them

Mahbubul A. Jalal, *maf.jalal@valent.com, Tasha T. Nguyen, John Whitby, Kirk Gohre, Richard Allen. Valent USA Corporation, Dublin, California, United States*

Agrochemical metabolism studies are conducted in plant, animal, soil and other matrices following defined guidelines published by the EPA, OECD, JMAFF and other regulatory agencies. Often, significant challenges are faced to understand and meet the requirements of these guidelines. Some of the less understood features of these guidelines and the difficulty in meeting them will be discussed. Each type of the metabolism study has unique operational phases; for example, the entire aerobic soil metabolism study is conducted in a laboratory, while a plant metabolism study is divided into two phases, a field phase to generate the RAC samples, and an analytical phase to analyze the samples. Each of these phases and various stages inside each phase may be quite challenging depending on the physicochemical properties of the test substance, methods of application or treatment, rate and pathways of metabolism, properties of the metabolites, their conjugates and bound residues, etc. Separation, Identification and quantitation of nanogram quantities of metabolites mixed with gram quantities of matrix impurities offer another unique challenge, especially in the case of plant and animal samples. The challenges encountered at critical stages of the plant metabolism and confined rotational crop studies will be presented with examples from actual studies, and the solution to these challenges will be discussed.

AGRO 20

Challenges encountered at critical stages of agrochemical environmental fate studies and how to address them

Kirk Gohre[1], *kgohr@valent.com, Jeane C. Aston*[1], *Joel J. Maurer*[1], *Mahbubul A. F. Jalal*[1], *Simon Kang*[2], *Richard Allen*[1]. *(1) Valent USA Corp, Dublin, California, United States (2) Smithers Viscient, Wareham, Massachusetts, United States*

Agrochemical environmental fate studies are conducted in soil, water and other matrices following defined guidelines published by the EPA, OECD, JMAFF and other regulatory agencies. Often significant challenges are faced to understand and meet the requirements of these guidelines. Some of the less understood features of these guidelines and the difficulty in meeting them will be discussed. The goal of these environmental fate studies is to assess the significant degradation processes (rate of degradation and degradate distribution) under standardized conditions. The rates of degradation and degradate distributions are used for modeling inputs and determination of significant residues. These studies are conducted under a set of constrained experimental conditions in the laboratory (temperatures, moistures, light, etc.). While the laboratory studies are intended to mimic outdoor degradation, their design limitations will be discussed. Each type of environmental fate study has unique operational challenges; for example, compounds with limited water solubility make the conduct of aqueous photolysis and adsorption/desorption studies particularly challenging. Aerobic/anaerobic studies have many challenges including physicochemical properties of the test substance, experimental setup, methods of application or treatment, rate and pathways of degradation, properties of degradates and bound residue bound residue extraction and identification, etc. The separation, identification and quantification of low concentration degradates has always been a challenge, although the advent of exact mass LC-MS has made identification easier. Additionally, results interpretation (mass balance, extraction sufficiency, kinetics etc.) is critical for determination of guideline acceptability and risk assessment inputs. Challenges encountered at critical stages of various environmental fate studies will be presented with examples from actual studies, and the solution to these challenges will be discussed.

AGRO 21

Application of capillary electrophoresis for the separation and analysis of C-14 labeled highly polar photolytic degradation products

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The application of CE for separation, detection, and quantification of chromatographically challenging highly polar photolytic degradation products will be discussed. The photolytic degradation products of 14C-labeled compound X were investigated in a phosphate buffer. The study was conducted in quartz vessels with labeling on two positions in sterile aqueous potassium phosphate buffer at pH 7. After loading the vessels with compound X, samples were irradiated with a xenon-arc lamp for up to 15 days at 22C. By Day-7, half of the parent molecule had degraded to one major metabolite eluting at the solvent front by HPLC and the remaining peaks were scattered throughout the 90-minutes run time. By Day-14, all parent and major metabolite had converted to one large metabolite eluted in the solvent front. The solvent front (the region of interest) was fraction collected by HPLC-Reversed phase, and this region was called ROI-1. The ROI-1 fraction was investigated by CE-PDA. Using CE the ROI-1 fraction was separated to more than 15 degradation products. The built-in fraction collection in CE-PDA was utilized to collect multiple small and large individual C-14 labeled degradation peaks based on their migration times. To determine mass balance the entire elution profile was sectioned to 5 regions: the anionic region (3 sub-regions), followed by cationic species and non-chromophorous products regions. The CE fractions were tested by LSC to distinguish related degradation products from possible interferences. It was concluded that all fractions were C-14 labeled residues of the compound X. The anionic fractions consisted of approximately 70%, the cationic 20% and less than 10% was recovered in non-chromophorous regions. Between 1-3% was recovered from the waste vial. This study with utility of CE proved that the largest degradation compound by HPLC was in fact multiple degradation products.

AGRO 22

Volatile organic compounds defend plants against insect herbivory

James H. Tumlinson, *jht2@psu.edu. Entomology, Penn State University, Julian, Pennsylvania, United States*

In response to insect herbivore feeding, damaged plants release a great variety of volatile organic compounds (VOCs). These herbivore-induced plant VOCs (HIVOCs) serve as host location cues for parasitoids and predators of the herbivores and are important in reducing herbivore damage. As would be predicted, based on the species diversity of plant biochemical processes, different plant species produce different blends of HIVOCs. In addition to species differences in HIVOCs, different plant varieties or ecotypes also produce quantitatively and qualitatively different blends. Further, the quality and quantity of the HIVOC blend changes with time of day and growth stage of the plant. Nocturnal VOCs may differ greatly from those released during the day. In one instance, while diurnal VOCs attract natural enemies of the feeding caterpillar, the same plant releases a nocturnal blend that repels conspecific moth females. One of the most surprising discoveries is that the blend of VOCs released by a plant will change depending on the species of herbivore feeding on it.

Parasitoid wasp females can distinguish HIVOCs from a plant fed on by a host, from those released by the same plant damaged by a non-host of the wasp. VOCs induced by root-feeding insects disperse through the soil and attract nematodes that attack the root herbivores. In addition to feeding damage, oviposition can induce plants to release VOCs that attract egg parasitoids. It is apparent that plants have evolved dynamic and effective indirect defenses against insect pests.

AGRO 23

Identification of an aggregation pheromone from the small hive beetle, *Aethina tumida* (Coleoptera: Nitidulidae)

***Charles Stuhl**, charles.stuhl@ars.usda.gov. Chemistry, US Department of Agriculture, Gainesville, Florida, United States*

Male-produced sex attractants are frequently referred to as aggregation pheromones because they signal to both sexes. Most of the insects that use male-produced sex attractant are beetles. The small hive beetle, *Aethina tumida* Murray is a European honey bee (*Apis mellifera* L, Hymenoptera: Apidae) pest that can be destructive to honey bee colonies, causing damage to comb, stored honey and pollen. Although not a direct cause of Colony Collapse Disorder (CCD), small hive beetle is an opportunistic pest that will take advantage of a stressed hive. When the beetle infestation is high, the bees will abandon their hive. The rapid spread of this pest and its stress on the honey bee population has warranted an effective trapping system to control its impact on honey production and honey bee survival. Currently, there is no effective trapping system employed to monitor or control beetle populations. This study utilizes an aggregation pheromone isolated from adult male small hive beetle along with a fruit odor attractant. The target strategy of this system is directed at attraction and capturing adult beetles upon emergence from the soil before they enter the hive. The synergistic effect of the synthetic pheromone blend in conjunction with a fruit volatile blend has been shown to be an effective control measure. The synthetic blends are highly attractive and extremely successful in trapping the small hive beetle. This discovery has the potential to control this invasive species that is effecting honey bee survival worldwide.

AGRO 24

Development of a kairomone-based monitoring tool for the invasive redbay ambrosia beetle

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The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff, is an exotic wood-borer that vectors the fungal agent responsible for laurel wilt. This disease has had severe impact on forest ecosystems, and has spread to eight states in the southeastern US since the first detection of the beetle in Georgia in 2002. The disease is killing a large proportion of native *Persea* trees and currently poses an economic threat to avocado production in Florida. To control the spread of this lethal disease, effective attractants are needed for early detection of the vector. By comparing beetle ecology and its host plant preferences to the volatile chemical profiles emitted from the plants, we were able to confirm the importance of sesquiterpene compounds for host location by dispersing females. Based on this information, we were able to develop a better kairomone-based lure, now available for more sensitive detection of redbay ambrosia beetle.

AGRO 25

Exposure to a putative insect pheromone enhances the anti-herbivore defenses of its host plant

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Insect feeding damage is known to induce plant defenses. More recent discoveries have found that plants can also perceive environmental cues associated with the presence of insect herbivores, allowing them to prepare their defenses for future attack. For example, plants may detect insect footsteps or oviposition, and some even use olfactory cues to sense the presence of nearby herbivores. Several studies have found that undamaged plants can eavesdrop on volatiles emitted by their insect-damaged neighbors and respond by enhancing their own anti-herbivore defenses. Adding to these findings, our work has shown that some plants can also perceive and respond to olfactory cues emitted directly by insect herbivores. More specifically, we discovered that tall goldenrod (*Solidago altissima*) plants exposed to the putative sex attractant of the specialist gall fly *Eurosta solidaginis* exhibited enhanced anti-herbivore defenses, including reduced palatability and stronger induction of the defense phytohormone jasmonic acid. We also investigated the ability of *S. altissima* plants to perceive and respond to individual compounds in the *E. solidaginis* emission. We found that goldenrod plants exposed to the most abundant compound in the fly emission exhibited an enhanced defense response similar to plants exposed to the entire emission blend. These results suggest that goldenrod plants eavesdrop on signals of their insect antagonists and exploit them as indicators of impending herbivory, thus documenting a new class of olfactory-mediated interactions with broad significance for the evolutionary ecology of plant-insect interactions.

AGRO 26

Below-ground chemical ecology

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The phasing out of methyl bromide as a soil fumigant has led to a need for new technologies to manage below-ground plant pests, and a sustainable approach would be to utilize semiochemicals comparable to above-ground IPM. As now is well known for insect damage above ground, also plant roots can release herbivory induced volatile organic compounds (VOCs), for example, roots of *Zea mays* in response to feeding by the beetle *Diabrotica virgifera virgifera* and citrus cultivars (*Citrus paradisi xPoncirus trifoliata*) when fed upon by the root weevil, *Diaprepes abbreviatus*. VOC released by corn roots (e-b-caryophyllene) and citrus roots (pregeijerene) have been shown to attract beneficial entomopathogenic nematodes (EPNs) such as *Steinernema* spp. and *Heterorhabditis* spp. In addition, these terpenoids can be used for selective nematode rearing and, as we recently found, for behavioral priming of EPNs for improved targeted pest control. Despite these initial successes, understanding the similarities as well as differences, compared with successful above-ground use of induced plant volatiles and tritrophic interactions for pest control, is necessary to make below-ground IPM more feasible. For example, a below ground root-herbivore-parasite system is considerably more static than a similar above ground system; therefore, standard methods for sampling of semiochemicals (removing large volumes of water or gas) can have a strong detrimental effect on gradual changes and concentration gradients within a studied system. Recent progress will be presented on the development of low volume sampling and a GC/MS technique with "solvent-less on-column injection" that, in addition to low sample degradation, can handle the small sampling volumes necessary for a low system impact. It will also be shown how the low volume sampling works for laboratory as well as field sampling.

AGRO 27

Re-investigation into the use of sesquiterpene lactones to limit damage caused by sunflower insect pests

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Previous research in wild sunflowers (*Helianthus* spp.) identified several sesquiterpene lactones (STL) contained in glandular trichomes that act as natural insect repellents or biocides. Sesquiterpene lactones also appear important to sunflower interactions with pathogens and weeds, but STL have not been used in sunflower breeding. To better understand how STL could mediate insect-plant interactions in sunflower, a series of ongoing experiments was initiated using both wild and cultivated sunflowers. Comparison of wild and cultivated *H. annuus* using HPLC showed that various groups of sunflower had qualitative and quantitative differences in STL composition. Tests with larvae of the sunflower moth, *Homoeosoma electellum*, indicate that both crude extracts and purified single STL can lead to developmental delays or mortality. However, efforts to examine dose-responses of single compounds or synergy of specific mixtures is hindered by a lack of availability of STL. Because the amount of various STL in a sunflower line is correlated with glandular trichrome number, additional research has been aimed at understanding the inheritance of glandular trichome number. Another project is directed at determining the basis for differences found between wild and cultivated *H. annuus*. Because of several possible limitations to real-world efficacy of STL as a plant defense, additional ongoing trials are aimed at assessing the value of the trait against a second floret-feeding sunflower pest.

AGRO 28

Phytotoxic and antifungal activity of a fungus isolated from *Brachiaria eruciformis* (signalgrass)

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Brachiaria eruciformis, commonly known as signalgrass, is a weed found in Mississippi. During late spring and early summer months, the leaves of *B. eruciformis* are infected with a fungus causing necrosis. The infected leaves ultimately turn brown and wither. From an infected leaf of *B. eruciformis*, a fungus was isolated and cultured in potato dextrose agar plates. This fungus was identified via molecular techniques as *Pyricularia grisea*. This fungus was grown on Czapek Dox broth culture medium for 21 days under continuous light at 24 ^{0}C. The culture filtrate was extracted with ethyl acetate. The ethyl acetate extract was phytotoxic in a seed germination bioassay and had antifungal activity against plant pathogen *Colletotrichum* species in TLC bioautography. The major phytotoxic compound was isolated and identified as pyrichalasin H by spectroscopic techniques. Pyrichalasin H was phytotoxic against both monocots and dicots with a ranking of 4 at 1mg/mL, where 5 is complete inhibition of germination and a 0 ranking is no difference between the control and the treated seed. This compound also had antifungal activity against *Colletotrichum fragariae* in TLC bioautography. This is the first report of antifungal activity of pyrichalasin H against phytopathogens. Isolation, structure elucidation, and biological activities will be discussed.

AGRO 29

Accurate mass applications in agricultural research and development

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Advances in high resolution LC/MS instrumentation have dramatically impacted many of the discovery, development, and registration activities in the agrochemical industry. Improvements in the sensitivity, speed, and mass accuracy of LC/MS systems have made them an essential tool in many areas of R&D including: the search for new lead compounds from natural sources, evaluating the suitability of lead structures for advancement, and determining the environmental fate of agrochemicals which is required for compound registration. This talk will discuss the impact LC/MS has made in several of these important areas of modern agrochemical research.

Nature continues to serve as a rich source of potential lead chemistries. However, the complexity of natural product extracts makes the search for novel leads in nature a challenging undertaking. LC/MS has many applications in natural product discovery including both 1) dereplication to rapidly screen large numbers of natural product extracts for known biologically active compounds, and 2) detailed structure characterization of unknowns using accurate MS and MS/MS. In this presentation, we will describe the use of several high-resolution tools for the discovery and identification of the complex biologically-active structures often encountered in nature.

Registration of a new agrochemical requires the determination of its fate in several environmental systems including: plant and animal metabolism, photolysis, soil degradation, hydrolysis, and water sediment. LC/MS plays a critical role in the structural elucidation of the complex trace level metabolites generated in these studies. Modern LC/MS instrumentation combining of high resolution mass spectrometry with tools including micro and nano-scale separations, supercritical fluid chromatography, and ion mobiliseparations have been applied to speed the identification of these challenging environmental degradates. In this presentation we will provide a brief introduction to several of these techniques and how they can be applied to the identification of impurities and environmental metabolites.

AGRO 30

Ambient mass spectrometry imaging with laser ablation electrospray ionization for agrochemical R&D

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An ongoing analytical research project at DuPont Crop Protection aims at improving laser ablation electrospray ionization mass spectrometry (LAESI-MS) and developing methods to elucidate the distribution and mobility of pesticide active ingredients in plants. The LAESI-MS technique is being applied to the elucidation of agrochemical systemic or non-systemic properties with the objective of optimizing the active ingredient delivery to the desired site (e.g., plant tissue, pathogen cells, insect organ) in pesticide discovery and development efforts. The delivery of agrochemical active ingredients to the target site and the active ingredient potency are essential for adequate agrochemical efficacy. Consequently, foliar penetration, translocation, and metabolism of active ingredients in plants are extensively studied by industry during the discovery and development of new pesticides. LAESI-MS is quickly emerging as a technique capable of profiling the three-dimensional (3-D) location of chemicals within complex living tissue. Therefore, this technique was selected for mass spectrometry imaging in agrochemical R&D at DuPont. A LAESI DP-1000 instrument (Protea Biosciences, Inc.) was coupled to a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific), which allowed high mass accuracy to be used as the main parameter to increase analytical selectivity. Scanning electron microscope (SEM) imaging of leaf cross sections, as well as optical microscopy after laser ablation sampling events on a leaf, allowed a qualitative assessment of the leaf depth sampled in the sequential laser ablation events. For example, it was estimated that each LAESI DP-1000 laser shot, with the laser energy set at 600 μJ, created craters of ~200 μm diameter and ~50 μm depth that sampled (ablated) few dozen cells from a 14-day old Consort wheat leaf. Overall, the study demonstrated that LAESI-MS can be used to image pesticides in plants as a function of time, allowing the assessment of systemic and non-systemic properties of agrochemical active ingredients.

AGRO 31

Making the most of the information in accurate mass spectrometric data

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The use of accurate mass instruments is becoming more prevalent in agricultural chemistry, especially for metabolite ID. It is important to understand both the power and the limitations of the data. It is also important to use the information in the data as fully as possible. This talk will go over the true, practical meaning of ppm and how it is critical to interpreting the data. Most accurate mass instrumentation actually produces data with accuracy that is more practically measured in mDa rather than ppm especially at low mass-to-charge ratios (smaller compounds). This will be illustrated with examples showing that an error of 10 ppm, while sounding quite far off, can actually be quite good data at some mass values while, on the other hand, an error of 1 ppm on its own may not be good enough at other masses. It will also show how the use of isotope pattern information and the accurate mass MS/MS fragmentation pattern of a compound supplement the information found in the accurate mass value alone and can help to confirm or to deny the assignment of a chemical formula to an accurate mass value. We will also show how isotopic labeling can help and can hinder formula assignment by practical examples.

AGRO 32

Revealing the chemical basis of organoleptic properties of a Cabernet Sauvignon wine using global LC and GC/QTOF workflows

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Introduction
While the use of proprietary EI flavor databases has been used in wineries for many years, few labs have applied metabolomic protocols to fully characterize secondary grape and yeast metabolites. To this aim we have applied metabolic workflows to processed Cabernet Sauvignon grapes. These approaches include recursive analysis as well as accurate mass and analytical retention time (AMRT) libraries to correctly identify known components and characterize unknown components. This rigor allows us to apply multivariate statistical software to evaluate the chemical basis of wine organoleptic properties.
The use of multivariate software is becoming more prevalent in food and flavor laboratories as scientists work to understand how individual compounds combine to create a specific odor or taste. It is important to correctly identify compounds to build accurate statistical models that are capable of differential analysis. To this effect, we have constructed the accurate mass GC/Q-TOF library that currently contains about 400 flavor compounds. The core of the library is comprised of the wine volatiles with added pyrazines, furanones, and lactones to give it broader appeal. We have also applied the METLIN metabolic workflows in order to properly characterize yeast and grape metabolites. We then used multivariate statistics to correlate secondary metabolites with their organoleptic properties and terroir.

Preliminary Results:
All the major volatile components are present and surprisingly consistent. The esters such as isoamyl acetate, ethyl hexanoate, ethyl octanoate that give wine sweet, fruity characteristics are abundantly present. Monoterpenes found at lower concentrations such as limonene, α-terpineol, citronellol, and nerol add citrus, green, woody and floral notes.

Although not in the targeted flavor library, several interesting components gave good NIST library matches and were annotated. For example, vitispirane is an expected norisoprenoid which lends a pleasant earthy flavor to a wine. On the other hand, expected organoleptics with low odor thresholds such as methoxypyrazines, γ-lactones, and thiols are typically found at concentrations below the detection limit of GC/TOF and GC/QTOF instruments.

The LC/QTOF data was searched against the METLIN and Vitis Vineferea databases. The more targeted Vitis Vinefera database clearly showed that flavonoids were differentially expressed in the costal and valley wines.

AGRO 33

Isolation and analysis of botryodiplodin in soybean plants by liquid chromatography coupled to mass spectroscopy

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Agricultural commodities are often negatively impacted by natural disease which includes Charcoal Rot. Charcoal Rot is a plant disease that is characterized by stem lesions, a black discoloration, and dry rot of internal tissues at certain locations on the plant. Charcoal Rot is caused by the fungus Macrophomina phaseoli which is particularly common in extreme environments (higher temperatures/water stress) during the growing season. The soybean plant becomes more vulnerable in this harsh environment and is susceptible to the growth of Macrophomina phaseoli, which can result in the production of the biological toxin botryodiplodin. Botryodiplodin, also referred to as a mycotoxin, is believed to be used by the fungus to inhibit cell metabolism at the level of replication, transcription and transduction of the infected plant. In order to learn more about botryodiplodin and its role in Charcoal Rot we plan to investigate botryodiplodin's exact mechanism of action. In this particular study the goal is to develop, validate, and utilize a Liquid Chromatography coupled to a Mass Spectrometer (LC-MS/MS) method for the identification and quantification of botryodiplodin. The developed LC-MS/MS method should be proven to be reliable, cost-effective, and accurate. The extraction of botryodiplodin from the soybean matrices will also be developed and validated. Although the specific goal in this study is the development of an analytical method, the overall goal is to better understand botryodiplodin and its relationship with the infection of soybean commodities.

AGRO 34

Acceptance criteria for confirmation of identity of chemical residues using exact mass data

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In 2002 Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) published Guidance for Industry (#118) titled “Mass Spectrometry for Confirmation of the Identity of Animal Drug residues”. In this document details of criteria for various types of unit-resolution MS were given, but specific guidelines were not provided for high resolution mass spectrometry (HRMS). The use of HRMS for identification of chemical residues has expanded rapidly due to improved instrumentation. However, a major impediment to the adoption of HRMS has been the lack of a standard to determine what constitutes unambiguous identification (confirmation) of the chemical present. There are only a few published guidelines available that provide information on confirmation of identity of chemical residues by HRMS. None of these documents provided sufficient information to adequately meet the needs of FDA scientists and outside scientific community using HRMS in residue analysis. The guidance was formulated after extensive review of published literature and our accumulated experience in using commercially available HRMS instruments for the analysis of contaminants in food. The purpose of these guidelines is to describe the specific criteria FDA Foods and Veterinary Medicine will use to evaluate HRMS data for a residue in a sample by comparing to a reference standard to confirm its identity. The primary focus is the use of HRMS for targeted analysis when a comparison standard is available, although aspects of non-targeted analysis are discussed. The internal guidance which is approved for FDA use will also be available on FDA internet site. According to our knowledge this document is the only guidance for the use of HRMS for the identity of chemical residues in the U.S.A. Guidance in criteria for confirmation such as signal requirement, mass accuracy, retention time, number of ions necessary for confirmation, etc. are documented in the guidance.

AGRO 35

Improvements in pollen/nectar sampling and analysis techniques to support regulatory submissions

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Trace level quantitative analytical methods are essential for product characterization, dietary exposure assessment and environmental risk assessment. The challenge of accurately and reproducibly quantifying insecticide residues in relevant bee matrices hinges upon critical field phases such as agronomic practices, sample collection, storage and shipment and extends to the analytical laboratory where sample extraction, clean-up and detection techniques must be further optimized. With smaller sample sizes and the expectation of lower detection limits, this talk will explore practical improvements in sampling of flowers for nectar and pollen, as well as development of robust, reproducible analytical methods for the determination of trace level insecticide residues, using case studies. In addition, regulatory requirements for GLP method validation and residue data acceptance criteria will be discussed.

AGRO 36

2013/2014 Washington State assessment for neonicotinoid insecticide residues in/on bee bread and wax

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A comparative assessment of apiaries in urban, rural and agricultural areas was conducted in 2013 and 2014 in Washington State to examine pesticide residues from off-the-shelf neonicotinoid and commercial-scale treatments. This study specifically evaluated residues in/on wax and bee bread (stored pollen in the hive) for the nitroguanidine-substituted neonicotinoid insecticides clothianidin, thiamethoxam, dinotefuran, imidacloprid and its olefin metabolite. Apiaries ranged in size from one to hundreds of honey bee colonies, and included commercial, sideline (semi-commercial), and hobbyist beekeepers. Although most analytical methods cited for wax and bee bread have been a variation of the QuEChERS, we found sample clean-up problematic at needed levels of quantification by LC/MS/MS and instead employed an EPA enforcement method specific and sensitive for imidacloprid and its associated metabolites. This method was extended for quantifying clothianidin, thiamethoxam, and dinotefuran. For our 2013 colony assessments, the method limit of quantitation (LOQ) was determined at 5 parts per billion (ppb) for parent neonicotinoids and 10ppb for imidacloprid olefin. In 2014 improvements in method cleanup and instrument sensitivity lowered the LOQ from 5 to 2 ppb for all compounds except the olefin which remained at 10 ppb. Averaged recoveries for concurrent fortified neonicotinoid quality control wax and bee bread samples ran with each analytical set usually fell within 70-120% ± 20% SD. Residues were stabile over the maximum interval of storage before analysis. Overall, this survey of apiaries indicates that non-commercial colonies are not likely to be exposed to detectable residues of neonicotinoid residues from homeowner products. Observations at commercial colonies, however, indicated a greater possibility of exposure to thiamethoxam or its insecticidal metabolite clothianidin, but the residue levels were arguably low (< 5 ppb) for making any conclusions in regards to the likelihood of adverse sublethal effects at the colony level.

AGRO 37

Measurement process of pesticides in beeswax matrix: evaluation of the different contributes to global error

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Contamination of bee products with pesticides has been widely documented in the literature. It has two principal sources, environmental and apicultural practices. Most of the time, acaricide treatments lead to residues in hives and subsequently in beeswax. Studying beeswax is particularly interesting because most of pesticides and acaricides are fat soluble, non-volatile and persistent, and so accumulate easily herein. Moreover, these chemicals resist the wax melting temperature. Besides, it is a common beekeeping practice to recycle wax almost continuously. As a consequence, pesticides can be accumulated for decades. The aim of this paper is to evaluate the uncertainty related to the determination of pesticide's residues in beeswax with GC MS method. The global uncertainty associated to a measurement has two different components, uncertainty of sampling and uncertainty of the method. This can be expressed, as reported by W. J. Youden (J. AOAC 1981, 50, 1007), as a function of standard deviation: $s_0^2=s_s^2+s_m^2$ where s_0 is the global standard deviation, s_s sampling standard deviation, and s_m method standard deviation. Sampling uncertainty has often been found to be of considerable magnitude, sometimes much greater than the pure measurement uncertainty, and it is also difficult to evaluate.

AGRO 38

Analysis of pesticide residues in pollens and nectars from plants at ornamental nurseries and bee-collected pollen at those nurseries

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Protection of pollinators from pesticides used by the ornamental horticulture industry requires an understanding of time course of exposure. How does the timing and mode of application of systemic pesticides translate to concentrations of those pesticides in pollen and nectar? Furthermore, which of the many plants grown are being visited by pollinators? To answer these questions we have begun to study pesticide residues in pollen and nectar from plants being grown by nurseries in Connecticut. Collection of these materials can be quite laborious, and concentrations can be in the parts per billion range, so it is important to have analytical methods that are sensitive and specific even with the use of small sample sizes. We have collected pollen and nectar from two different rhododendron cultivars treated with imidacloprid and analyzed them with a modified version of QuECHERs followed by LC/MS-MS. One cultivar had relatively low concentrations in both the pollen and nectar (1-7 ppb); the other had concentrations that ranged from 1-21 ppb for nectar and 14-35 ppb for pollen. Given the differences between cultivars it will become important to analyze more samples than we can do by the LC/MS-MS methods alone, so we are developing an analytical protocol that will allow samples to be analyzed by either LC/MS-MS or ELISA. The ELISA techniques can be used for larger numbers of samples but can have some possible interferences - using LC/MS-MS on a subset of the same analytical extractions will allow calibration of the ELISA data. At the same time we are also examining bee collected pollen from hives located within ornamental nurseries to look at actual pesticide exposure. Details on sample collection methods, the analytical protocols and preliminary data will be presented in the presentation.

AGRO 39

Poor versus good in nectar and pollen sampling techniques

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A new tiered risk assessment process for pollinators was proposed by the United States Environmental Protection Agency, in collaboration with Health Canada's Pest Management Regulatory Agency and the California Department of Pesticide Regulation in 2014. In order to assess real world dietary exposure to pollinators, magnitude residue studies are needed to measure pesticide concentrations in nectar and pollen of treated crop (Guidance for Assessing Pesticide Risks to Bees, USEPA 2014). Poor sample quality of collected nectar and pollen may lead to overestimation of pesticide risk to pollinators. Understanding of flower structure, yield of nectar and pollen, timing and length of crop bloom, collection techniques, effect of weather conditions, quality of nectar and pollen samples, and the resources required to generate one sample are crucial elements in success of such studies. Direct collection of nectar and pollen from plants is typically done using a "hand collection" technique. Alternatively, honeybee foragers can be used to collect samples of nectar and pollen in confined conditions (e.g., in cages or tunnels). Experience and challenges learned from sampling of nectar and pollen from different crops will be discussed.

AGRO 40

Residue method for the determination of neonicotinoid insecticides and their metabolites in nectar, pollen, flower and leaves by LC-ESI-MS/MS

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In past years, the consequent colony collapse disorders (CCD) have been of critical concern in agriculture and plant pollination productions. Neonicotinoids have been suspected as a source of sub-lethal effects on honey bees (*Apis mellifera* L.). To provide a simple, prompt, effective, and accurate analytical method for screening and support of the EPA risk assement process for pollinator studies, we investigated and developed an analytical method using QuEChERS procedures to determine residue levels of commercially available neonicotinoid insecticides in nectar, pollen, flower and leaf matrices. Neonicotinoid residues were quantitated by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Details of the development and validation will be presented.

AGRO 41

Determination of neo-nicotinoid insecticide residues in bee-feeding matrixes of soybean, a low-pollen producing crop

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Crop residue analytical methods are routinely employed to determine neonicotinoid insecticides levels at parts-per-billion (ppb) concentrations. More recently, crop residue methods have been modified for use with semi-micro sample sizes (50-100 mg), which are suitable for extraction of bee-relevant matrices such as pollen and nectar. Due to small sample sizes, the resulting extract volumes are reduced accordingly to maintain desired limits of quantitation at sub-ppb levels. Reduction of observed matrix effects may not be amenable to extract dilution due to sensitivity limits, even with modern liquid chromatography tandem mass spectrometry (LC-MS/MS) instruments. In recent work, matrix effects have been mitigated through the use of matrix-matched calibration standards and addition of deuterium cold-labeled internal standards.

The described analytical procedures were verified in our laboratory for use to determine residues of thiamethoxam and metabolite CGA322704 in varieties of soybean (*Glycine max* (L.) Merr.) leaves, flowers, and nectar. Due to the physiology of soybean plants, both pollen and nectar production are very low making it difficult to collect enough material for analysis. Therefore, anthers of the soybean flowers were sampled and analyzed as a surrogate for pollen; nectar was sampled by collecting foraging honey bees and removing their honey stomachs containing nectar. An overview of the sampling procedures and analytical method performance results of thiamethoxam and CGA322704 in low pollen producing soybean plant matrices will be presented and discussed.

AGRO 42

High-throughput determination of neonicotinoid insecticides in pollen and nectar using liquid chromatography with tandem mass spectrometry detection

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The unexplained death of a honey bee population is referred to as Colony Collapse Disorder. Several possible factors including viruses, parasites, poor nutrition, and exposure to neonicotinoid insecticides are being studied. The increase in study related samples has facilitated the need to create a high throughput workflow for sample analysis. Pollen and nectar are collected from agricultural crops in very small quantities, usually less than 100 mg. Study requirements for limit of quantitation and detection are also very low (<1 ppb), making extraction of the entire sample necessary. Liquid chromatography with tandem mass spectrometry detection is the technique of choice for analysis of neonicotinoids. The small sample sizes are well suited for extraction and clean up to be performed in a 96 well plate format, making processing large numbers of samples possible. This research provides bridging data for a representative neonicotinoid insecticide, thiamethoxam, and its major metabolite clothianidin. Fortification recovery using high-throughput techniques at the LOQ and 10x LOQ levels were in excess of 70% with a relative standard deviation of <20%. Implementation of these techniques can help laboratories meet the throughput requirements for the ever increasing number of pollinator health studies to be conducted in the near future.

AGRO 43

Consideration of pesticide monitoring data in environmental exposure assessments

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The United States Environmental Protection Agency's (USEPA) Office of Pesticide Programs (OPP) is responsible for regulating pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). OPP conducts environmental exposure assessments that are integrated into human health and ecological risk assessments. A key component of OPP's exposure assessments is the evaluation available monitoring data. Monitoring data are available for various media including water, sediment, air, precipitation and biota. These data are collected by federal, state, and local agencies, universities, registrants, and volunteers. The utility of the data for risk assessment purposes depends on many factors including sample media, study design (*e.g.*, objective, location, sample frequency), and ancillary data. This presentation will focus on describing how monitoring data are evaluated and used in pesticide exposure assessments.

AGRO 44

Long-term trends in agricultural pesticides from tributaries to Lake Erie and the Ohio River

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Pesticides used for crop protection and improved agricultural production are frequently detected in water bodies in agricultural regions. The National Center for Water Quality Research has been monitoring pesticide concentrations in 10 rivers in the Lake Erie and Ohio River basins continuously since 1980. Since then we have measured 20 pesticides that are widely used in agriculture in nearly 20,000 samples using chromatographic techniques. The main objectives of the program are to accurately quantify spatial and seasonal pesticide trends from watersheds of varying land uses and to assess the long-term effects of changing agricultural management practices on the prevalence of pesticides. All pesticides we measured were mostly below detection during the non-growing season, which ranged from mid-October to mid-March. More intensive sampling was conducted during the growing season with up to 3 samples per day, 7 days a week. Herbicide concentrations were consistently higher in watersheds draining high proportions of row-crop agriculture (>70%). Atrazine had the highest concentration among all the measured pesticides, ranging from 0.01 – 121.46 µg/L followed by metolachlor ranging from 0.01 – 98.76 µg/L. Detection of alachlor and most of the carbamates, organophosphates and dinitroanilines decreased after the early 2000's to quantifiable levels <1 µg/L because the pesticides were unregistered or replaced by more effective substitutes. Peak atrazine, simazine and acetochlor concentrations were variable over time and showed no distinct decreasing trends. Monthly time weighted mean concentrations of atrazine were highest in May and June, corresponding to peak concentrations. Generally, peak concentrations of pesticides were found after the first few runoff events following application. Intense sampling enabled us to get detailed concentration trends. The chemographs rise rapidly, peak during peak discharge and decline thereafter not exhibiting any substantial tailing. Scale effects were prominent; smaller streams had higher concentrations for shorter periods of time with more temporal variability than more consistent and less variable concentrations of larger streams. Long term monitoring for pesticides in natural waters is important because concentrations vary each year depending on factors such as pesticide application rates and usage, length of the growing season, precipitation intensity and other spatial, temporal and anthropogenic factors.

AGRO 45

Development of a liquid chromatography-tandem mass spectrometry method for determination of 229 pesticide compounds in water samples for National water monitoring studies

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A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for determination of 229 pesticide compounds (113 pesticides and 116 pesticide degradates) in water samples from stream and groundwater sites. The method was developed in response to the need to add many new pesticide compounds and their potentially bioactive degradates to the analytical methods for National monitoring studies conducted by the U.S. Geological Survey.

The method involves direct injection of a 100-µL sample onto the LC-MS/MS without any sample preparation other than filtration. Samples are analyzed with two injections, one in electrospray ionization positive (ESI+) mode and one in ESI negative (ESI-) mode, using dynamic multiple reaction monitoring (MRM) conditions, with two MRM transitions for each analyte. The detection levels in reagent water range from 1 to 250 ng/L, with a median of 3 ng/L for pesticide compounds determined in ESI+ mode, and from 2 to 250 ng/L, with a median of 40 ng/L for pesticide compounds determined in the ESI- mode.

The method has been successfully implemented in the laboratory for routine operation for determination of pesticide compounds in more than 3,000 samples per year. Challenges encountered during implementation included matrix effects for some pesticide compounds that caused biased results or required raised reporting levels, and lack of stability of compounds in water samples and in solvents due to high sample pH or exposure to warm temperatures. In addition, analytical batches containing over 100 samples -- a typical analytical batch consists of about 109 analytical vials with 75 environmental samples, 2 laboratory quality-control (QC) samples, and 32 instrument QC samples (blanks, calibration standards, verification standards) -- were required to analyze the large numbers of samples submitted for National monitoring studies within holding-time limitations. Over the three-days required to these large batches, performance of the LC-MS/MS can decrease from sorption of sample matrix components to the LC-MS/MS components, resulting in raised reporting levels for some pesticide compounds. Despite these challenges with implementation in routine operation, mean recoveries of most analytes in the method were within data-quality objectives of 100 ±30 percent and detection levels were suitable for National monitoring studies.

AGRO 46

Use of complementary sampling methods to assess pesticides in Midwestern streams: water, bed sediment, and passive samplers

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In an intensive synoptic study of Midwestern streams, pesticides were analyzed in water, sediment, and passive samplers from 100 wadeable streams by the U.S. Geological Survey National Water-Quality Assessment (NAWQA), in collaboration with the U.S. Environmental Protection Agency. During May–August, 2013, 227 pesticide compounds were analyzed in 12 weekly water samples and in extracts of Passive Polar Organic Chemical Integrative Samplers (POCIS) deployed over a 5-week period. At the end of the water-sampling period, bed sediment was collected and analyzed for 118 pesticide compounds, and invertebrate communities were surveyed. Pesticide mixtures in water were complex, with medians of 25 compounds detected in water and 62 detected in POCIS extracts. Herbicide concentrations were higher in agricultural watersheds, and fungicide and insecticide concentrations typically were higher in urban watersheds. At all sites, the Pesticide Toxicity Index (PTI), which reflects both concentrations and invertebrate toxicity of pesticides within a mixture, was dominated by insecticides—usually a single insecticide—most often by the neonicotinoid imidacloprid in water and by the pyrethroid bifenthrin in sediment. Pesticide occurrence in POCIS was consistent with mean concentrations in weekly water samples collected during POCIS deployment, with a few exceptions. For example, insecticides flubendiamide, methoxyfenozide, and two pyrethroid degradates (cis-cyhalothric acid and 3-phenoxybenzoic acid) were detected more often in POCIS than in water samples, and fungicides pyraclostrobin and trifloxystrobin were detected more often in water than in POCIS. Bifenthrin occurrence in bed sediment was only weakly correlated with occurrence of the two pyrethroid degradates in POCIS, suggesting that POCIS may provide an indicator of total pyrethroid occurrence that is not always captured by bed sediment or water sampling. In boosted-regression models, the pyrethroid degradates in POCIS were significant explanatory variables of invertebrate community metrics. Similarly, pyrethoids in sediment showed strong (significance level <10%) multivariate correlations with invertebrate communities.

AGRO 47

Bifenthrin causes trophic cascades in aquatic food webs and alters subsidies to terrestrial food webs

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Direct and indirect effects of bifenthrin, a widely used insecticide, in stream ecosystems have not been well studied beyond standard toxicity testing. To investigate such effects, a 30-day experimental stream test was conducted after exposure to bifenthrin-contaminated sediment, and results were interpreted in the context of a regional-scale assessment of Midwestern US streams. The experimental stream test provided ecologically relevant effect benchmarks at levels of bifenthrin previously thought safe for aquatic life. At concentrations greater than 213 ng bifenthrin/g OC, direct effects included reduced larval macroinvertebrate abundance, richness, and biomass. Indirect effects at bifenthrin concentrations greater than 252 ng bifenthin/g OC included increased abundance of *Baetis tricaudatus* after competitive release and a trophic cascade in which periphyton abundance increased after scraper biomass decreased. Adult emergence was stimulated at 57.7 and 102.2 ng bifenthrin/g OC and suppressed at concentrations > 134 ng bifenthrin/g OC. Bifenthrin was detected in 48% of Midwestern streams at concentrations as high as 774.2 ng bifenthrin/g OC. Aquatic insect larvae responses in Midwestern streams were consistent with those observed in the experimental streams. Extrapolating results from the experimental streams to the regional stream assessment suggests pervasive ecological effects. Emergence dynamics likely are altered in 39% of Midwestern streams sampled while 11% of the stream sediments likely are toxic to aquatic insect larvae. We provide new evidence that a common pyrethroid, bifenthrin, can alter aquatic (trophic cascade) and terrestrial (emergence dynamics) ecosystem function at the regional-scale.

AGRO 48

Development of passive samplers for measuring pyrethroids in surface water

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Pyrethroid insecticides have been detected in surface water aquatic systems. This study aims to develop inexpensive, durable, and reproducible passive samplers for *in situ* monitoring of pyrethroids in surface water. Different types of thin films, including polyethylene (PE), polyurethane, polyoxymethylene, and polymethylmethacrylate, were tested for their absorption capacity for 8 pyrethroids in water. The PE film displayed the greatest enrichment factor for pyrethroids with $K_{\text{sampler-water}}$ values ranging from 0.8×10^5 to 1.5×10^5. The absorption kinetics of 8 pyrethroids on PE film were then evaluated under simulated conditions, and the concentration on film did not reach a steady state by 30 d after treatment. To circumvent the long equilibration time requirement, stable isotope labeled analogs were used to preload the sampler and used as performance reference compounds (PRCs). The validity of PRCs was confirmed by testing the symmetry of desorption of PRCs from the film and absorption of analytes onto the film. The PRC-preloaded samplers were deployed in large fish tanks spiked with a mixture of 8 pyrethroids under simulated flow conditions and at three field sites in southern California in the winter of 2015 and spring of 2016. The derived C_{free} values after 2, 4, and 7 d were statistically similar in the fish tank test, validating the usefulness of PRCs for non-equilibrium sampling. At the field sites, fenpropathrin, lambda-cyhalothrin, bifenthrin, permethrin, and esfenvalerate were detected after 7 days of exposure. Results to date demonstrate the feasibility of coupling PE film with PRC for *in situ* measurement of pyrethroids in surface water.

AGRO 49

Temporal analysis of high resolution spatial datasets in the refinement of pesticide exposure risk assessments

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High resolution spatial/temporal environmental datasets have enabled the development of new approaches to ecological risk assessments. These high resolution datasets, including terrain maps, hydrography, and temporally variable crop and satellite imagery provide a wealth of site-specific information that can be used to derive more refined estimates of pesticide exposure needed for ecological and endangered species risk assessments. An approach utilizing multiple high resolution, temporally variable spatial datasets was applied in modeling aquatic pesticide exposure for the California tiger salamander (CTS). During the winter rainy season, CTS migrate to a breeding habitat which consists of vernal pools approximately 0.15 to 1 m in depth. The first step in identifying vernal pools within the three CTS distinct population segments was to review the 1:24,000 scale National Hydrography Dataset (NHD) High Resolution waterbody dataset, the best available nationwide hydrography data. The NHD annual water body features were then compared against satellite imagery time-series available in Google Earth. NHD features were evaluated to determine if they were seasonal or perennial based on their representation in the imagery over time. Additional vernal pools, not depicted in the NHD, were added based on the Google Earth time series analysis. This imagery analysis resulted in 4,297 vernal pools within the CTS habitat range that were then assessed for aquatic exposure. Detailed watershed boundaries for each pool were delineated from 10-m resolution digital elevation models (DEMs) available from the USGS National Elevation Dataset. These watersheds were used to determine the drainage area affecting each vernal pool and potentially contributing to pesticide exposure. A 5-year time series of 30-meter resolution crop data from the Cropland Data Layer was used to characterize the likelihood of pesticide use sites within each watershed based on recent cropping history. The spatial-temporal analysis of landscape datasets allowed for more accurate and site specific probabilistic exposure analysis and subsequent risk assessment.

AGRO 50

Radiovalidation of Oryzalin and Bensulfuron-methyl analytical methods using QuEChERS in various matrices

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Analytical methods for determination of agrochemicals must undergo successful validations before being approved by EPA for use in registration and enforcement. Residue methods should also be rugged and accurate enough to satisfy the rigorous recovery guidelines for metabolism studies using radiolabelled analytes, as well as residue methods for trace quantities of an analyte in raw agricultural commodities and environmental samples to prove their validity. To that end, methods for quantitative determination of [^{14}C-phenyl] Oryzalin, a dinitroaniline herbicide, and [^{14}C-phenyl] Bensulfuron-methyl (BSM), a sulfonylurea herbicide, were radiovalidated in various matrices. Soil and animal tissues analyses utilizing Quick Easy Cheap Effective Rugged and Safe (QuEChERS) clean-up were tested for their specificity, linear dynamic range, limit of detection (LOD), limit of quantification (LOQ) and precision. Additional aqueous matrices, drinking and surface water, also underwent analysis. These methods were slightly modified from previous analytical methods for Oryzalin and BSM in the aforementioned matrices to improve sensitivity and efficiency. Analyses were performed on both freshly fortified and aged residue samples using liquid chromatography-mass spectrometry (LC-MS/MS) and liquid scintillation counting (LSC). The LOQ was established at 10 ng/g for solid matrices and 10 ng/ml for aqueous. The QuEChERS methods for soil and animal tissue matrices were successfully radiovalidated, with mean total toxic residue (TTR) recoveries for [^{14}C-phenyl] Oryzalin and [^{14}C-phenyl] BSM falling within acceptable limits of 90-110% with relative standard deviation (RSD) <20%. Aqueous methods for the analyte were also effective, with mean TTR recoveries also within 90-110% and RSD <20%. This research aids in radiovalidating methods using QuEChERS as a tool bridging metabolism analytes to trace residue methods.

AGRO 51 - Withdrawn

AGRO 52

Development of a multiplexed crop residue method and Its radio-validation with samples from metabolism studies

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The challenges associated with developing a multiplexed crop residue method, intended to be universally applicable to all crop matrices and include as analytes the active ingredient and a number of its significant metabolites (with a wide range of chemical properties), are quite complex. To develop a versatile residue method, a single combined extract is desirable and, as such, the extraction must be robust enough to capture all analytes of interest across a broad spectrum of matrices. In addition, oxidative metabolic reactions create metabolites with moieties more inclined to conjugate with soluble endogenous plant materials (e.g., organic acids, amino acids, sugars, etc.) that alter the solubility properties of the metabolites, making them more polar and adding another layer of complexity to the extractability of bioavailable residues. Following extraction, several additional challenges present themselves: Quantitation of free unbound analytes and of conjugated residues, while avoiding double accounting, in the data generation method is imperative. The development of a vigorous hydrolysis step capable of freeing conjugated metabolites in the presence of a wide range of matrices, without degrading the target analytes, can be a particularly tricky balancing act as well. The use of ^{14}C labeled metabolism samples in the method development process can be an invaluable tool in overcoming these obstacles to develop and validate a streamlined universal crop residue method. Examples of these challenges encountered in the development of an actual multiplexed crop residue method for a novel pesticide will be presented, and the solutions to these challenges will be discussed.

AGRO 53

Confirmation of pesticide exposure in wild birds

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The avicide 3-chloro-4-methylanaline hydrochloride (chloro-p-toluidine hydrochloride, CPTH, DRC-1339) is used to control pest bird species which damage agricultural crops. Traditional detection techniques have been employed to look for residues in exposed birds. While these techniques have significantly low detection limits (20-24 ng/g) detection in birds exposed during field applications of CPTH have not been reliable. Studies conducted with radio-labeled CPTH revealed that over 90% of the dose received is excreted in less than 6 hours while time to death can be as long as 3 days. Investigations into the metabolic pathway for CPTH have revealed two significant metabolites as well as significant tissue binding in the kidney of exposed birds. The current data indicate that the metabolism of CPTH is rapid and produces a reactive metabolite *in-vivo.* These results will be used to focus development of a sensitive method to detect adducts formed in the kidneys of exposed birds.

AGRO 54

Development of robust analytical methods for determination of glyphosate residues

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As global guidelines require more sensitive and selective residue analytical methods with lower limits of quantitation, developing a robust and reliable analytical method has become more challenging. Innovation in sample extraction and clean-up paired with cutting edge analytical instrumentation must be leveraged together to meet analytical performance needs. This presentation will focus on residue analytical method development and validation for glyphosate in matrices ranging from commodities of plant and animal origin to human body fluids. When working across such a large range of matrices, extraction and sample clean up procedures are vital to produce robust analytical methods. Practical examples of how these challenges were met will be discussed.

AGRO 55

Removal of foliar-applied pesticide residues on wheat leaf surfaces

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Foliar uptake of compounds is often assessed by dipping or washing plant leaves in the various solutions and measuring recovery of compounds in the solutions, based on the assumption that washing with certain solutions only removes compounds on the plant surface. We previously demonstrated that the epicuticular wax layer of wheat leaves is not altered physically or chemically by several water:organic solvent mixtures with organic solvents up to 40% by volume and further suggested the use of solvent washing procedures to assess unabsorbed compounds on wheat leaf surfaces. In the present study, solubility of 15 commercial pesticides in water:acetonitrile mixture (60:40, v/v) was determined and thirteen ^{14}C-labeled pesticides were evaluated for removal from wheat leaf surface by the acetonitrile mixture up to 48 hr after foliar application. Our results show that separation of a wide range of pesticide residues remaining on the leaf surfaces from those penetrating into the tissues can be successfully achieved using a simple solvent washing procedure. This presentation will provide a new insight on foliar uptake process of pesticides, enabling us to better assess uptake and metabolism of compounds in plants.

AGRO 56

Regulatory perspectives on multi-residue methods used for enforcement

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Extraction efficiency of residue analytical methods used for risk assessment and enforcement must be appropriately characterized and demonstrated to effectively bridge the analytical procedure to the methodology implemented in the respective metabolism studies. The challenges of newer global regulatory expectations have driven the need for more integrated extraction efficiency evaluation procedures, including the assessment of QuEChERS methods. In the case where a multi-residue method is deemed acceptable, the method may be considered to be the enforcement method and therefore extraction efficiency must be thoroughly evaluated. Regulatory requirements and acceptance expectations for implementation of multi-residue methods for quantitative use as an enforcement method will be discussed, using case studies.

AGRO 57

Extraction efficiency for residue analytical method: Trends, requirements and challenges

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With the continuous changes in the global regulatory environments for agro chemicals, the development of the analytical methods for residue analysis is becoming more and more challenging. The guidelines and the requirements for analytical methods are also changing globally. Consequently, the evaluation of extraction efficiency became one of the key requrements in plant, animal and environmental residue analytical methods. Data must be provided as proof of adequate extraction of residues that are included in the residue definition.

The evaluation of the suitability of the extraction procedures is mainly based on available information from existing metabolism studies performed with radiolabelled active ingredients to generate incurred residues. However, metabolism procedures are usually longer due to multiple extraction steps that are required for material balance. As there are tremendous demands for cost effective analytical methods for high throughput analysis, this becomes extremely challenging for residue chemists to find a way to bridge the metabolism and residue procedures. The samples from radiolabeled metabolism studies may not be available at the time when residue analytical methods are developed. The extraction efficiency for soil, plant, and animal should also consider the presence of isomeric analytes, existence of the metabolites at low level and suitability of enforcement methods (i.e., QuECHERS type multi-residue method). This presentation will focus on extraction techniques to match the extraction from metabolism studies for challenging analytical methods with multiple analytes and different matrices, including extractability data generation using cold reference substances. The evaluation of extraction efficiency, based on the global requirements with case studies will be presented and discussed.

AGRO 58

Exploitation of fungal volatile organic compounds (VOCs) in agriculture

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Volatile organic compounds (VOCs) are a small portion of the total metabolites produced by fungi; however, their unique properties enable them to mediate important biological functions in aerial and terrestrial environments. In agriculture, the potential uses of VOCs include volatile-mediated inhibition of pathogen growth, increased plant systemic resistance, and inducing plant growth promotion. Our lab has studied the effects of fungal VOCs on several biological systems including *C. elegans*, *Drosophila*, filamentous fungi, and plants. We found dose and time dependent effects of fungal VOCs and several compounds caused significant stimulatory and/or toxic responses.

Filamentous fungi in the genus *Trichoderma* are widely used as biocontrol agents that serve as plant growth promoters and for protection against pathogens. Previously, we demonstrated the ability of *Trichoderma*-derived VOCs to stimulate *Arabidopsis* growth. In this study, *Trichoderma* emission profiles, concentrations, quantities, and VOC-mediated effects on plants were measured in order to develop a mechanistic understanding of the volatile-mediated *Trichoderma*-to-plant interactions. Volatile-induced growth promotion was isolate-specific and dependent on the age of the fungal culture, developmental stage of the plant, duration of the exposure, and characteristic of the compounds. We identified individual compounds that mimic the effects of *Trichoderma* volatile mixtures on plant growth leading to significant increases in seed germination, plant fresh shoot weight, chlorophyll, and expression of genes involved in responses to hormones and cell wall modification induced by auxin. In addition, we identified several individual compounds to have strong toxic effects on plants and other filamentous fungi including *Aspergillus*, *Penicillium*, and *Pseudogymnoascus*. Our data demonstrates that exploitation of fungal volatiles as plant growth promoters and/or disease suppressors has the potential to become a powerful tool in agriculture.

AGRO 59

Drosophila suzukii-yeast interactions: Applications for pest management

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Insects and budding yeasts often have close and complex associations. For example, insects disperse yeast and, in turn, some of the yeasts are consumed as a source of amino acids and nutrients. Many *Drosophila* spp. are understood to have strong mutualistic associations with the yeast communities that best support their growth and survival. Spotted wing drosophila, *Drosophila suzukii,* is a globally important insect pest of soft-skinned fruit crops. *D. suzukii* larvae and adults feed upon yeasts, among which *Hanseniaspora uvarum* is the most frequently isolated from larval frass and adult midguts. *H. uvarum* is a relatively ubiquitous yeast that is commonly isolated from mature fruits, early stages of wine fermentation, fermentative spoilage, and *Drosophila* spp. Given that it ferments glucose and some strains produce yeast-killer toxins that provide a competitive advantage, *H. uvarum* is well adapted for early colonization of fruit. *D. suzukii* adult females are attracted to volatile organic compounds derived from yeasts and exhibit high attraction to *H. uvarum*. Therefore, *D. suzukii*-yeast interactions may be used to improve pest monitoring and develop novel attractants. Additionally, *D. suzukii*-yeast interactions provide innovative targets for developing sustainable pest management tactics including development of behavioral manipulation, phagostimulants, and RNAi biotechnology.

AGRO 60

Effects of exogenous application of methyl jasmonate on foliar volatile emission in citrus and it effect on aggregation behavior of Asian citrus psyllid (*Diaphorina citri*), vector of Huanglongbing pathogens

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Methyl jasmonate (MeJA) is a well-known activator of chemical defenses in plants. We tested the ability of MeJA to alter the odor of young citrus foliage, the site of Asian citrus psyllid (ACP) reproduction and development. ACP vectors *Candidatus* Liberibacter asiaticus (Las), the pathogen responsible for Huanglongbing, a devastating citrus disease. A solution of MeJA mixed in 0.1% aqueous Tween solution was sprayed onto potted infected (Las+) and healthy (Las-) Valencia orange trees. Control trees received 0.1% Tween solution. A purge and trap headspace system was used to collect volatiles for two days from the following treatments: Las-/control; Las-/MeJA; Las+/control; Las+/MeJA. Las+ trees had Ct values in the low-20's. Response to MeJA was dose dependent; the volatile profile of trees treated with 10mM MeJA was significantly altered: 1) The total amounts of volatiles increased, with Las+ trees emitting the highest amounts; 2) The ratios of limonene to E- β-ocimene and alkyl aldehydes (C8-C12) to monoterpenes were reversed relative to control trees; 3) natural enemies attractants, such as indole and E-jasmone, were emitted; and, 4) higher amounts and a greater variety of sesquiterpenes were emitted. The Las+/control trees emitted the highest amounts of methyl salicylate (MeSA), suggesting that MeJA may antagonize MeSA biosynthesis. Las induces emission of MeSA, which is a potent ACP attractant. Application of MeJA may reduce the attractiveness of the Las-infected foliage to ACP while, at the same time, enhance the abundance of ACP natural enemies. In laboratory assays, similar numbers of psyllids settled on untreated control and MeJA-treated citrus sprigs. However, the numbers of psyllid aggregations ($\geq$ 10 psyllids) were significantly higher on MeJA-treated than on control sprigs. Identification of the volatiles responsible for eliciting aggregation behavior could be used to develop highly effective scent attractants for ACP detection and monitoring.

AGRO 61

Detailing the diverse response profiles and biological activity of acidic terpenoid phytoalexins in maize-microbe interactions

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Kauralexins (KA) and zealexins (ZA) are newly described secondary metabolites in maize that serve as inducible chemical defenses. In contrast to the abundance of terpene volatiles in leaves, herbivory leads to only a mild production of these non-volatile terpenoid phytoalexins; however, they are strongly induced by pathogen infection. To understand the role of acidic terpenoid phytoalexins in diverse maize-microbe interactions, we profiled phytoalexins and other common defense metabolites in commercial hybrid and publicly available inbred lines infected with varied pathogenic fungi. The necrotroph *Cochliobolus heterostrophus* induced a typical response with heightened levels of jasmonic acid, 12-oxo phytodienoic acid, salicylic acid, and phytoalexins including a novel terpenoid zealexin, designated zealexin A4. Infection with *Aspergillus flavus* generated a diverse phytoalexin profile derived from regioselective oxygen moites, which were individually inactive, yet mixtures with less oxygenated phytoalexins showed synergistic antimicrobial activity. The hemi-biotroph *Colletotrichum graminicola* caused broad suppression of all maize phytoalexins and experienced reduced growth when exposed to endogenously occurring levels of kauralexin A2/B2. Similarly, *Fusarium graminearum* infection repressed zealexin A4 production, but growth was highly inhibited by this metabolite *in vitro*. These data demonstrate the complex nature of maize-microbe interactions in phytoalexin-based agrochemical innate immunity.

AGRO 62

Environmentally safe alternative biopesticides for controlling sea lice

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Salmon louse (Lepeoptheirus salmonis) infestations are currently one of the most significant threats faced by aquaculture industries. The elevated feeding activity of sea lice in areas of high salmon density can cause physical harm to the fish and serve as a disease vector between farmed and wild salmon. Current methods of controlling sea lice involve the application of synthetic pesticides, namely emamectin benzoate, through in-feed medication or bath treatments. These commercially available ingredients have shown to be harmful to non-targeted species, while targeted parasites have become more tolerant over time. Herein we present the development of a biopesticide extracted from a natural source, *Azadirachta indica*, as an environmentally safer alternative for controlling sea lice infestations. Analytical techniques, namely high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS), have been developed to extract, characterize, and monitor the fate of these potential drug candidates with high selectivity and sensitivity, limit of detection of < 1 pM. Incorporation of these active ingredients through in-feed medication was closely monitored and resulted in the successful delivery and distribution of these compounds in the outer tissue of salmon. Azadirachtin A was shown to have high efficacy towards preventing sea lice attachment, as increasing concentrations of this compound in tissue resulted in a decrease in sea lice attachment.

AGRO 63

Preparation and characterization of degradable nanocapsules that release pesticides for an extended period of time

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Pesticide efficacy is limited by evaporation and precipitation. These processes can result in the need for costly pesticide re-application. By using a nanocapsule to contain the pesticide, these two problems can be greatly reduced. Produced nanocapsules adsorb on the surface of the plant and are not washed away by rain. In this study, menthol and peppermint oils were chosen as model systems because they are considered to be minimum-risk pesticides. Pesticides were encapsulated into the shell prepared from protein and degradable polymer, poly(ethyl cyanoacrylate). The size of prepared nanocapsules was smaller than 200 nm and readily adsorbed onto the hydrophobic surfaces. The protocol of this technique can readily be modified to accommodate various types of pesticides.

AGRO 64

Plant-microbe relationship that influences an insect pest of Califronia tree nuts

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California produces a large portion of the worldwide supply of pistachios. The navel orangeworm is considered a major insect pest of California pistachios, and causes significant damage to pistachio kernels in addition to introducing aflatoxigenic fungi. Despite the development of semiochemical-based attractants for efficient monitoring of navel orangeworm in almond orchards, this success has not translated well for monitoring of navel orangeworm in pistachios orchards. Because navel orangeworm are known to have a mutualistic relationship with aflatoxigenic fungi as well as being attracted to fungal volatiles, our laboratory has investigated the volatile profiles of pistachio kernels contaminated with naturally occurring fungi. Discussed will be results from this investigation and their application to the development of attractants for navel orangeworm in pistachio orchards.

AGRO 65

History and current status of glyphosate

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Glyphosate has been called a once-in-a-century herbicide. Since it was commercialized in 1974, its use has grown to dominate and shape the herbicide marketplace. In 2008, half of the U.S. herbicide market, as measured by the amount applied, was glyphosate. Much of this use was on transgenic, glyphosate-resistant (GR) crops, which have been the dominant transgenic crops worldwide. The combination of GR crops with glyphosate provided the most effective and inexpensive weed management technology in history for a decade or more. However, the dominance of glyphosate and GR crops are eroding due to the rapid increase in weed species that have evolved resistance to glyphosate (*ca*. 35 since 1996), by a variety of fascinating genetic, physiological and biochemical mechanisms. Glyphosate is the only herbicide to target the enzyme 5-enolpyruvyl-3-shikimate phosphate synthase (EPSPS). It is a high use rate, non-selective herbicide. After application, it translocates primarily to metabolic sinks, killing meristematic tissues far from the application site. Thus, it kills subterranean tissue, making it an effective herbicide for perennial weeds. Its slow action, allows the herbicide to move throughout the plant. Although glyphosate chelates divalent metal cations, the complete resistance to glyphosate of transgenic plants with transgenes encoding GR EPSPS indicates that this property does not contribute to the herbicidal activity or cause any significant changes in plant mineral nutrition. After EPSPS is inhibited, deregulation of the carbon and nitrogen metabolism eventually leads to plant death. Glyphosate is toxic to some plant pathogens by inhibiting their EPSPS. Shikimic acid pathway product-based defenses to plant pathogens are compromised in non-GR plants by glyphosate. Ultra low doses of glyphosate stimulate plant growth by unknown mechanisms. Glyphosate and its changing role in agriculture continue to be fascinating topics.

AGRO 66

Rise and future of glyphosate and glyphosate-resistant crops

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The epidemic of glyphosate-resistant (GR) weeds is rapidly decreasing the value of using glyphosate and glyphosate resistance traits alone. In areas where glyphosate and GR crops have been totally adopted, GR weeds evolved and now glyphosate and glyphosate traits need to be combined with other technologies. The chemical company solution is to combine glyphosate with other chemicals, while the seed company solution is to combine glyphosate traits with other herbicide traits. Unfortunately, no new commercial herbicide mode-of-action has been discovered for over 30 years, and herbicide traits have been commercialized or are near commercialization for all existing herbicide types with high utility. The foreseeable new combinations are not going to be enough to keep up with the demonstrated evolutionary capacity of so-called super weeds. Glufosinate, auxin and HPPD-inhibiting herbicide traits, even when combined with glyphosate traits, are temporary solutions. Even if all growers implement best weed management practices, the chemical and seed business will not be able to adequately support what critics call the chemical and transgenic treadmills for at least the next decade. Because of the lack of new weed management alternatives, the use of glyphosate and glyphosate traits will increase despite the rapid spread of GR weeds. Growers will continue to use glyphosate and glyphosate traits alone and in combinations where GR crops are fully adopted and their use will increase substantially in new geographies and crops.

AGRO 67

Economics of HT crops and glyphosate resistance

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U.S. corn and soybean farmers employ a range of weed control practices. That said, most rely heavily on chemical herbicides for weed control. Herbicides were applied to more than 95 percent of U.S. corn acres planted in 2010 and U.S. soybean acres planted in 2012.

Over the course of the last two decades, U.S. corn and soybean farmers have dramatically increased their use of glyphosate (the active ingredient in popular herbicide products such as Roundup) and decreased their use of herbicide products containing other active ingredients. In 2015, economists at the USDA's Economic Research Service (ERS) found that the percentage of soybean acres treated with glyphosate, by itself or in combination with other herbicides, increased from approximately 25 percent in 1996 to 95 percent in 2006. The percentage of corn acres treated with glyphosate rose from about 4 percent in 1996 to 35 percent in 2005. This shift in herbicide usage contributed to the development of over 14 glyphosate-resistant weed species in U.S. crop production areas.

This presentation will discuss the findings of recent and ongoing research by ERS economists. The focus will be on the economic impacts of glyphosate resistance in corn and soybean production.

AGRO 68

Impact of glyphosate-resistant sugar beet

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Glyphosate-resistant sugar beet became commercially available to sugar beet growers in 2008. Grower acceptance to glyphosate-resistant sugar beet was almost universal in the US and Canada soon after they became available because weed control was the most challenging pest management aspect of sugar beet production. This was because there were no herbicides that could control weeds in sugar beet as effectively as glyphosate. Prior to the development of glyphosate-resistant sugar beet, growers would commonly apply a preplant or pre-emergent herbicide followed by two to three postemergence herbicide applications. Or, if a grower was not using a preplant or pre-emergent herbicide, they would often make four to five herbicide applications beginning when the weeds and the crop were just beginning to emerge. This often resulted in some level of crop injury, but was accepted in order to reduce the impact of weeds. In addition to multiple herbicide applications, conventional sugar beet was cultivated one to three times during the early half of the growing season. And if available, hoeing crews were hired to manually weed the fields. Sugar beet is a minor crop compared to corn, soybean, cotton and alfalfa in terms of acres grown. However, it is arguably one of the most suitable crops to be genetically engineered for glyphosate-resistance. Some of the reasons why it was such a good candidate for developing glyphosate-resistance include: 1) sugar beet cannot be grown in the same field year after year due to disease concerns, thus, growers have to grow other crops in a three or four year rotation, which means glyphosate isn't used year after year in the same field; 2) the sugar beet plant is a biennial so when grown as a crop, there is no pollen produced that can possibly cross with wild relatives and create glyphosate-resistant weeds from pollen-mediated gene flow; and 3) the processing of the harvested roots to extract the sucrose rapidly degrades the DNA in the extracted raw juice and subsequent refining so that no DNA and hence no GMO evidence is present in the refined sugar.

AGRO 69

Interactions of glyphosate use with farm characteristics and cropping patterns in central Europe

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In Europe, glyphosate is used as a non-selective herbicide in non-transgenic crops. Thus, patterns of use are different from situations in other parts of the world, where genetic modified crops are grown in herbicide resistant cultivars. Recently, in Europe, glyphosate is under review for re-approval, and the discussion is controversial. As a study case, Germany was chosen, where farmers use glyphosate on roughly 40% of arable area preferentially after harvest and as a pre-seeding application. A public and scientific debate emerged whether this extended use is agronomically necessary and sustainable regarding the resistance selection pressure on weeds. Therefore, an inventory was carried out to investigate farmers' glyphosate use patterns and their motivations for use. The survey was carried out during winter 2014/15. Online and paper-based questionnaires were sent to professional farmers. 2,026 farmers responded and were included in the analyses. Regression models and clustering was used to identify variables that could explain influence of farm type, soil cultivation regime and labor requirement on glyphosate use patterns. Furthermore, the growing of cover crops, crop rotation structure, dominating weed species and management of resistant weeds were included in the analyses. It was found that farmers use glyphosate in extremely diverse patterns. A strong proportion of farmers are able to carry out land use without glyphosate. On the other hand, some farmers use glyphosate on large proportions of their arable land. Non-users typically run farms with a higher share of plowed land and spend more labor for farmwork than intensive users. Farm scale of non-users is typically below average. Further analyses were carried out to identify why glyphosate users behave in different ways.

AGRO 70

Glyphosate hormesis

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As all herbicides act on pathways or processes crucial to plants, in an inhibitory or stimulatory way, low doses of any herbicide might be used to beneficially modulate plant growth, development, or composition. Glyphosate, the most used herbicide in the world, is widely applied at low rates to ripen sugarcane. Low rates of glyphosate also can stimulate plant growth (this effect is called hormesis). Several studies have shown that glyphosate applied at low rates can increase plant growth; induce shikimic and quinic acid accumulation; increase photosynthesis and stomatal opening; increase seed production and shorten plant lifecycle. Low rates of glyphosate applied to the leaves have caused one or more of the mentioned effects in an expanding group of species, including soybean, corn, sorghum, sugarcane, eucalyptus, *Urochloa decumbens*, *Conyza canadensis* and *Arabdopsis thaliana*. Under field conditions pesticide rates are not uniform, causing some target organisms to receive rates low enough to cause hormesis. It is consensus that glyphosate is not significantly available for root absorption as it has fast decomposition and strong bonding to the soil. Nevertheless, in a recent study, glyphosate applied at commercial rates to six kinds of soils, had no effect on germination, but stimulated initial growth of sorghum plants in two of the soils. Glyphosate was detected in soil solution at very low concentrations (the maximum value was 3.53ng/L) and stimuli were observed when sorghum plants grew up in the two soils showing the highest concentrations of the herbicide in soil solution. Up to now, low doses of glyphosate have not been recommended as a growth stimulant for crops, as the hormetic dose can vary considerably, depending on many factors, including climate, plant variety, plant developmental stage, and glyphosate formulation.

AGRO 71

Ligand-gated chloride channels and phenolamine GPCRs as important targets of pest control chemicals

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We focus on two types of invertebrate neurotransmitter receptors, ionotropic and metabotropic receptors, as targets of pest control chemicals. Of these receptors, we have mainly been studying insect γ-aminobutyrate (GABA)-gated chloride channels (GABACls) and octopamine G-protein-coupled receptors (GPCRs). GABACls are receptors for the inhibitory neurotransmitter GABA. Our GABACl studies began with the finding that bicyclophosphates inhibit miniature inhibitory junction potentials generated by the presynaptic release of GABA in the earthworm longitudinal muscle. Structure-activity relationship analysis of bicyclophosphates and other GABACl antagonists revealed structural differences in their binding site between mammals and insects, providing information about designing safe insecticides. Isoxazoline and benzamide insecticides inhibit insect GABACls by interacting with distinct site(s) from that for phenylpyrazole insecticides. Studies into the actions of competitive antagonists on the orthosteric site would also provide an opportunity to develop novel insecticides. Aside from GABACls, insects have glutamate-gated chloride channels (GluCls). GABACls and GluCls exhibited distinct spatial immunolocalization patterns when examined using houseflies. We compared the pharmacological characteristics of GABACls with those of GluCls by expressing their cDNAs in *Xenopus* oocytes or using native cockroach neurons expressing these channels. All antagonists tested to date showed selectivity for GABACls, whereas avermectins had selectivity for GluCls. Another main focus in our studies is on the phenolamine octopamine, which is widely distributed in the insect body and regulates or modulates various physiological processes of insects. We cloned cDNAs encoding four phenolamine GPCRs from silkworms and examined the signaling pathways and pharmacological properties of octopamine and tyramine receptors expressed in HEK-293 cells. Of these receptors, β-adrenergic-like octopamine receptors, which elevate intracellular cAMP levels upon activation, are most abundantly expressed in every tissue and are most sensitive to agonists such as formamidine metabolites. Our studies indicate that ligand-gated chloride channels and phenolamine GPCRs remain potential targets of pest control chemicals.

AGRO 72

Pharmacology, signaling and physiology of insect biogenic amine receptors

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In vertebrates, biogenic amines such as dopamine, serotonin, epinephrine and norepinephrine act physiologically as neurotransmitters, neuromodulators and neurohormones to regulate many important processes. In contrast, norepinephrine and epinephrine do not appear to be present in insects, as they lack dopamine β-hydroxylase that converts DA to norepinephrine. In fact, their role is fulfilled by their invertebrate counterparts, the monoamines tyramine and octopamine. The insect biogenic amines carry out many of the physiological roles such as reproduction, development, growth, circadian rhythms, endocrine secretion, and behaviors. They exert their effects by binding to specific receptor proteins that belong to the superfamily of G-protein coupled receptors, many of which have been characterized not only from Drosophila but also from several other insect species. Thus, blocking or over stimulating these GPCRs in pests may either result in the death or reduce fitness to control pest populations. On the other hand, many insect biogenic amine GPCRs have been found to show different pharmacological properties with their vertebrate counterparts. Therefore, they can be potential targets for the development of next generation of insecticides. We used a combination of approaches from pharmacology, cell biology, molecular biology, neurobiology, immunology and genetics to study the pharmacology, signaling and physiology of biogenic amine receptors in lepidopteran insects (*Chilo suppressalis* and *Pieris rapae*) and model animal *Drosophila melanogaster.* In the meantime, we also discovered several novel families of insect biogenic amine receptors.

AGRO 73

New mode-of-action chemistries for vector control: Small molecule inhibitors of arthropod GPCRs

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Transformative solutions are required to control diseases transmitted by arthropod vectors and protect human health and biosecurity on a global scale. Vector control relies heavily on insecticides that are neurotoxic to many organisms, including humans, and ineffective against insecticide-resistant pest populations. We are pursuing rational drug design to discover small molecule inhibitors and modulators of arthropod G protein-coupled receptors (GPCRs) as new mode-of-action chemistries for vector control. GPCRs mediate essential neurological processes in arthropods and are attractive targets for insecticide discovery. Using high-throughput chemical library screening and hit-to-lead optimization, we have identified potent antagonists of the D_1-like dopamine receptors (DARs) from the mosquitoes *Aedes aegypti, Anopheles gambiae* and *Culex quinquefasciatus* and the tick, *Ixodes scapularis*. These chemistries exhibit greater than 100-fold selectivity for mosquito and tick DARs versus orthologous human and honeybee receptors *in vitro*, and are highly toxic to mosquito larvae and adults *in vivo*. Transcriptome analyses indicated that antagonists operate via modes-of-action that are distinct from existing insecticides and could act as synergists to extend the utility of permethrin products. Structure activity relationship studies are ongoing to develop a pharmacophore and identify leads for development. These studies demonstrate proof of concept for a target-based approach to deliver novel classes of vector-selective insecticides. Strategies to enhance the selectivity of chemistries, including pharmacological exploration of other GPCR targets and translate insecticide discovery efforts to the agrochemical industry will be presented.

AGRO 74

Aminothiazolines: Novel foliar insecticides for the control of piercing-sucking pests

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Newly emerging pests and increasing resistance put constant pressure on existing insecticidal classes, thus increasing the demand for effective alternatives with new modes of action. As a result of our continuous efforts to explore novel chemistries, a new class of piercing-sucking insecticides was discovered. Starting from an early screening hit, a benzylamine moiety was found as a common structural motive for *in vivo* activity in subsequently synthesized compounds and became the focus of further structure-activity relationship work. Mass screening along with several rounds of optimization finally led to the discovery of the aminothiazolines. The compounds show excellent activity against aphids and whiteflies on a broad variety of crops and appear to act as octopamine receptor agonists in the central nervous system. An overview of the synthesis, SAR, and *in vivo* activity of insecticidal aminothiazolines will be presented together with insights into the putative mode of action.

AGRO 75

G protein-coupled receptors involved in vitellogenin uptake into the oocytes

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To identify G protein-coupled receptors involved in juvenile hormone-regulated Vg uptake, we performed RNA interference (RNAi) screen targeting genes coding for GPCRs in the red flour beetle. Out of 112 GPCRs tested, knockdown of 41 GPCRs resulted in a reduction in fecundity. Interestingly, RNAi against only two GPCRs (a Rhodopsin-like receptor and a Dopamine D2-like receptor) led to a significant reduction in Vg accumulation in developing oocytes, without much effect on circulating Vg levels. Functional assays on these two GPCRs showed that JH triggers a dose-dependent inhibition of intracellular cAMP levels in HEK293 cells expressing *Tribolium* Dopamine D2-like receptor. These data suggest that Dopamine D2-like receptor may be involved in regulating Vg uptake in the red flour beetle.

AGRO 76

Octopamine and tyramine receptors as targets for naturally occurring terpenoids

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The biogenic monoamines octopamine and tyramine perform diverse physiological functions in arthropods by activating their respective G-Protein-Coupled receptors (GPCRs). Octopamine and tyramine receptors can be found within the arthropod's central nervous system, but are also found in peripheral tissues. A limited number of insecticides have been developed to target GPCRs despite their importance in different physiological systems in arthropods. Furthermore, GPCRs are an underutilized target compared to other nervous system proteins. The need to identify new insecticide targets, like GPCRs, is highlighted by the increasing incidence of insecticide resistance to currently used chemistry and mechanisms of action. Furthermore, there is a growing interest in identifying biorational chemical pesticides that have a reduced impact on non-target organisms and the environment. This interest is partially motivated by public consternation over the impact of conventional pesticides, and the increase in governmental restrictions of conventional compounds. This presentation will give an overview on our recent work in developing heterologous octopamine and tyramine screening systems to investigate the ability of plant essential oil terpenoids to interact with an octopamine and tyramine receptors. Additionally, we have performed preliminary studies to examine the physiological relevance of a tyramine receptor in the southern cattle tick.

AGRO 77

Leveraging ambient and focused monitoring data to refine regulatory modeling exposure estimates

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Re-registration of crop protection products under USEPA or Health Canada's PMRA regulatory frameworks lead to a revision of exposure estimates based on updating of regulatory tools and guidance for use of environmental fate data. Particularly with revised groundwater tools, risks are being identified that are substantially different than previous assessments or as demonstrated in the monitoring data. For products with a long use history, extensive monitoring data is often available. In this case study, the authors will present a comparison of the extensive monitoring data and new exposure estimates. Learnings from detailed studies like Prospective Groundwater (PGW) Studies along with the use of monitoring data points with demonstrated applicability to labeled use patterns are used to improve the modeling predictions by presenting scenarios and parameterization refinement options. The authors will highlight areas where monitoring can be used to augment the modeling efforts leading to protective, yet more realistic exposure estimates for use in risk assessments.

AGRO 78

Interpretation of residue data from a groundwater monitoring study in Europe to define environmental safe use

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Current regulations governing the registration of plant protection products in the European Union (1107/2009) mandate that an assessment for the potential to reach groundwater is made. A recent Sanco opinion (Sanco/13144/2010 rev. 3) has provided guidance on demonstrating "safe use" through monitoring.

In 2012, a groundwater monitoring study was initiated with the objective of deriving a statistically defensible under conservative assumptions 90th and 95th percentile concentration for the non-relevant S-metolachlor metabolites CGA354743 (ESA) and CGA51202 (OXA). From 796 quarterly samples collected from 124 locations across 11 European countries over a time period of 12-24 months , for OXA, 40% (321 samples) were <LOQ and >99% (789 samples) were <10 µg/L.

For ESA, 16% (126 samples) were <LOQ and 97% were <10 µg/L (774 samples). Of the samples >10 µg/L (22 samples; 2.7%), only 5 sites were found to have a long term average >10 µg/L with no sites demonstrating analytical residues consistently >10 µg/L. The 90th and 95th percentile of ESA concentration was derived using sampling survey statistics and these were determined to be significantly below the EU trigger. Even using a worst-case assessment of the maximum site average in each relevant FOCUS zone, Châteaudun, Piacenza and Thiva residues were < 10 µg/L. Of the 5 of 124 sites with averages greater than 10 µg/L, these were linked to the FOCUS Hamburg and Kremsmünster climate zones, but since a simple 90th percentile tolerance interval of ESA concentration for Hamburg scenario was <10 µg/L and for Kremsmünster scenario only slightly >10 µg/L, this indicated that exceedances are not widespread. The relative vulnerability of the sites was determined by linking the actual field properties back to the original European cumulative distribution of ESA mass flux, placing 35% of the sites above the 90th centile of vulnerability.

AGRO 79

Use of pesticide monitoring data in spatial aquatic model (SAM) development

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The Spatial Aquatic Model (SAM), under development by the U.S. EPA's Office of Pesticide Programs, generates daily estimates of pesticide concentrations in U.S. streams, rivers, lakes, and reservoirs based on spatial inputs of soil, land cover, weather, hydrology, and crop conditions/ practices. After initial development focused on runoff transport in corn-soy-wheat agriculture, U.S. EPA is expanding SAM to include sediment transport and a broader diversity of agricultural crops. A key part of SAM development is evaluating model outputs against pesticide surface water monitoring data. Model outputs are currently being evaluated with monitoring data. The extent to which monitoring adequately reflects day-to-day, seasonal, and yearly variations in pesticide concentrations in surface water depends on how frequently samples are collected, and whether sampling is targeted to pesticide use areas and application events. This presentation discusses available monitoring data and associated uncertainties, such as availability of pesticide use information, and provides preliminary comparisons of model outputs from SAM with pesticide surface water monitoring data.

AGRO 80

Estimating pesticide concentrations in U.S. streams from watershed characteristics and pesticide properties

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Empirical regression models have been developed by the U.S. Geological Survey (USGS) for estimating the concentration of atrazine in streams; the models are based on pesticide-use intensity and watershed characteristics. Watershed Regressions for Pesticides (WARP) models were developed to estimate time-weighted annual mean and selected maximum moving-average concentrations. The models were developed from USGS National Water-Quality Program monitoring data collected from more than 100 streams throughout the United States. Pesticide use in a watershed was the most-significant explanatory variable, but several hydrologic and soil parameters were useful in explaining the variability in observed pesticide concentrations. The atrazine models have been extended to other pesticides by an adjustment factor that incorporates pesticide properties, and they are referred to as WARP-MP models. Predictions from these models for 2012 are accessible through the WARP Web application website. Future updates of this Web application will include predictions for previous years and selected national summaries.

AGRO 81

SWAT model predictions of annual maximum pesticide concentrations in flowing water bodies

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The need for models and tools to predict pesticide exposure in flowing water bodies has become increasingly important for pesticide exposure assessments. This has been driven by the continued need to protect surface water sources for human drinking water consumption, as well as ecological risk assessments including requirements of the Endangered Species Act (ESA). This paper will evaluate the ability of the Soil and Water Assessment Tool (SWAT) to predict annual maximum pesticide concentrations in the flowing water bodies of high vulnerability watersheds. SWAT was applied to 27 watersheds, largely within the Midwest corn belt of the United States, ranging in area from 20 to 386 km^2. Twenty-five of these watersheds were selected from the Atrazine Ecological Exposure Monitoring Program (AEEMP) dataset; a dataset that contains high temporal resolution atrazine sampling data from watersheds identified as having exceptionally high vulnerability to atrazine exposure. The remaining watersheds were selected from the Heidelberg Tributary Loading Program (HTLP) monitoring dataset. The SWAT model was tested based on an un-calibrated parameterization using consistent inputs and parameter estimation approaches across all watersheds. The model performance was assessed based upon its predictions of annual maximum moving averages of atrazine concentrations in 1-day and 60-day durations. The predictions of the annual maximum moving averages of pesticide concentrations are critical in pesticide ecological risk assessments when evaluating the potential for acute and chronic risk. The simulation results showed that for the majority of watersheds simulated, the un-calibrated SWAT model was able to predict annual maximum pesticide concentrations within a narrow range when accounting for uncertainty in atrazine application timing patterns. These results suggest that the SWAT model is well-suited for use in regulatory modeling of pesticide concentrations in flowing water bodies draining from agricultural watersheds; an application for which an operational model does not currently exist in the regulatory toolbox.

AGRO 82

Evaluating the effectiveness of streamside vegetation as a mitigation technique to reduce aerially applied pesticide loading to streams

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The National Marine Fisheries Service January 2015 Biological Opinion on the registration of diflubenzuron, fenbutatin oxide, and propargite discussed opportunities for agricultural producers to receive reduced no-spray buffers where vegetative filter strips or riparian areas are in place to reduce pesticide loading to surface water.

In 2015 WSDA conducted an edge of field monitoring study in Whatcom County, WA, to assess the effectiveness of riparian vegetation to reduce insecticide loading to surface water following aerial malathion applications on blueberries. Sites without dense woody vegetation were used as controls and compared to sites with established riparian vegetation communities. Riparian vegetation and channel geometry were characterized at two control and three vegetated sites. Riparian areas at vegetated sites varied in average width from 5 to 9 meters with higher species diversity than control sites, and were dominated by dense woody vegetation. Eight aerial applications were monitored (four at control sites and four at vegetated sites). Pesticide movement was assessed using depositional samplers placed on 6 transects running perpendicular to the stream at each site. From each transect, depositional samples were collected one hour after the pesticide application was complete at the crop edge, edge of the riparian vegetation facing the field, and in the center of the stream. At sites with flowing water, composite water samples were collected both upstream and downstream of the application area. Four composite water samples were collected at each upstream and downstream location, throughout the duration of the application and after application completion. At sites with standing water only, grab samples were collected before and after the application event. Field data regarding pesticide application methodology, and weather conditions were also collected at all application events.

Analysis confirmed that instream malathion deposition was significantly lower at vegetated sites than at control sites. In addition, increasing distance from edge of crop (both to vegetation and to water) and increasing canopy angle and canopy cover all resulted in statistically significant reductions in malathion deposition. This study provides evidence supporting the effectiveness of riparian vegetation at reducing drift into streams from aerial pesticide applications and therefore supports reduced no-spray buffers.

AGRO 83

Novel aquatic passive sampler technology for time-weighted-average continuous measurement of neonicotinoid and other current-use insecticides in environmental waters

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Many monitoring studies for pesticides collect water for samples by grab or spot sampling. While valuable, such sampling can result in significant disadvantages, as measurements can only be made at discrete intervals of time with no information gained in between sampling periods. This can result in misleading chemical levels in waters, particularly if concentrations are changing over time as is often the case for pesticides which have varying input rates, e.g., over the growing season. Passive samplers sequester chemicals of interest continuously to provide time-weighted-average concentrations while deployed, resulting in more extensive evaluation of chemical concentrations over the course of a monitoring study. In this research, two passive samplers are evaluated in the lower Red River valley of southern Manitoba, Canada: the Polar Organic Chemical Integrated Sampler (POCIS) and the organic Diffusive Gradients in Thin Films sampler (o-DGT). While both samplers sequester polar organic chemicals, the o-DGT is largely unaffected by environmental factors such as variable water flow rate. This allows for prediction of o-DGT sampling rates by molecular diffusivity and more accurate results. Concentrations of the corn herbicide atrazine ranged from 5-1000 ng/L in the lower Red River and 2-20 ng/L further downstream in Lake Winnipeg. Fluxes of atrazine at the US border were up to 15 kg/d, drawrfing the g/d inputs from sub-watersheds within Manitoba. This indicates that the main source of atrazine to the lower Red River was due to transboundary transport. Neonicotinoid insecticides (e.g., thiamethoxam, clothianidin) had multiple agricultural inputs from both the U.S. and localized sources with concentrations ranging from 5-65 ng/L.

This study demonstrates both the utility and the limitations of different types of passive samplers for pesticide monitoring studies.

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GCxGC-TOFMS comparison of PDMS stir bar sorptive extraction and liquid-liquid extraction for the determination of emerging contaminants in wastewater

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A topic of growing importance to environmental scientists is the presence of contaminants of emerging concern (CECs) in municipal wastewater treatment facilities and the possibility of them remaining post water treatment. Many of the current treatment technologies are not effective at selectively removing these CECs. One example is a class of compounds, commonly used as corrosion inhibitors, called benzotriazoles. They are resistant to microbial degradation, thus are only slightly removed during traditional treatments, such as activated sludge. CECs from personal care products, industrial processes, and pharmaceuticals were analyzed in this study. Pre-treatment influent and post-treatment effluent water samples from the Penn State Wastewater Treatment Facility were extracted and then analyzed by multidimensional gas chromatography (GCxGC) coupled to time of flight mass spectrometry (TOFMS). Liquid-liquid extraction (LLE) and polydimethylsiloxane stir bar sorptive extraction (SBSE) were compared for their extraction efficiency, "greenness", and facility of process. The preliminary data from this project finds CECs present in both the influent and effluent, signifying that many are not being removed during the treatment process. Both extraction methods were effective, LLE extracts a more broad range of compounds but SBSE is more sensitive. SBSE is a much "greener" and more facile approach than LLE. GCxGC TOFMS allows for excellent separation of these complex mixtures and is especially useful in the identification of the CECs in wastewater samples.

AGRO 85

Analysis of ionophore antimicrobials and their transformation products in poultry litter and dairy manure

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Ionophores are the second top selling class of antibiotics used in food-producing animals in the United States. In chickens, ionophores are used as feed additives to control coccidiosis, while in dairy cattle they are approved to prevent subclinical ketosis among other things. In this presentation, the development of a sample preparation procedure and analysis of ionophores in poultry litter, and in the solid and liquid fractions of dairy manure will be discussed. This study aimed to optimize a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method to quantify ionophores, and identify their transformation products (TPs) in animal wastes after on-farm composting, and during an advanced treatment process using a livestock water recycling system. The validation parameters of the optimized method showed good recovery (7o to 120% recovery) and relative standard deviations at ≤19% using three different spiking concentrations (10, 50 and 100 µg/kg). Monensin, salinomycin and narasin, were detected in the poultry litter samples prior to composting at 290.0±40, 426±46, and 3113±318 µg kg^{-1}, respectively. Results revealed a 13-68% reduction in ionophore concentrations after 150 d of composting, depending on whether the compost was aerated, turned, or subjected to a combination of both aeration and turning. Three transformation products and one metabolite of ionophores were identified in the composted litter using high-resolution liquid chromatography with quadrupole time-of-flight mass spectrometry (LC-Q-ToF/MS). In dairy manure, monensin and lasolacid are commonly detected, and they are mostly in the solid fraction of the manure with little amounts found in the liquid fraction.

AGRO 86

Comparison of sample preparation techniques and screening for >120 veterinary drugs in animal meat

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Veterinary drug (VD) use, as a means of controlling diseases in livestock bred for food is widely employed. However, excessive VD administration or mismanagement can lead to residues being present in processed animal meat prepared for human consumption. Hence, monitoring VD residues for regulatory enforcement and risk assessment is essential. Several hundred VDs spanning vastly different structures, solubilities and polarities are regulated or banned in animal meat and need to be analyzed. A critical step before analysis is the extraction of these VDs from the meat into an extract that can be measured on a mass spectrometer or similar sensitive analytical instrument. In this study, two sample preparation and extraction techniques were evaluated in a cross-lab validation. The traditional and widely used QuEChERS with dispersive-SPE extraction procedure used at the USDA-ARS was compared against a new lipid removal material, Enhanced Matrix Removal (EMR)-Lipid method. The study involved the comparison of both methods for extracting >120 VDs spiked at the maximum tolerance levels in bovine kidney, muscle and liver. Sample extracts were then evaluated using a veterinary drug library with accurate mass, MS/MS spectra and retention time confirmation on a liquid chromatograph coupled to a quadruple time-of-flight mass spectrometer.

The results of this study indicated that both methods are well suited for extraction of a wide variety of VDs from bovine meat. While some specific compound classes had differences in recoveries between the methods, overall recoveries were acceptable. Other parameters evaluated in this validation study included co-extractive amount, absolute recoveries and matrix effects for the VDs tested. Further, the ability to do sensitive quantification at the ng/g level using the LC-QTOF will also be presented. The results from this study indicate that there are simple, robust and viable methods available for the extraction of veterinary drugs in meat. Finally, the ability to perform screening and quantification of VDs along with identification of transformation products and unknown contaminants in meat using the LC-QTOF will also be discussed.

AGRO 87

Recent advances in sample preparation and GC&LC-MS/MS analysis of organic emerging contaminants and pesticides in food of animal origin

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Food of animal origin is estimated to contribute >95% of the human exposure to lipophilic anthropogenic contaminants, such as pesticides and environmental contaminants. Many emerging contaminants (for example, flame retardants) possess lipophilic properties, high log K_{ow} values, suggesting their ability to bioaccumulate in animal and human tissues and warranting their monitoring in foods. Fast, simple, efficient, and cost-effective analytical methods for contaminants are needed in food safety monitoring programs to provide high sample throughput and accurate results. We developed and validated a simple and fast method for simultaneous determination of 243 diverse environmental contaminants (PCBs, PAHs, PBDEs and other flame retardants) and pesticides in meats (swine, cattle, and poultry muscle). The extraction was based on quick, easy, cheap, effective, rugged and safe (QuEChERS) approach with acetonitrile, and salting out with ammonium formate. An aliquot of the extract was subjected to an ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis after filtration. For low pressure gas chromatography (LPGC)-MS/MS analysis, the extracts were cleaned with filter-vial dispersive solid phase extraction (d-SPE) cleanup, and analyte protectants were added to improve peak shapes, sensitivities, and reduce relative matrix effects. The method was validated at three spiking levels (10, 25, and 100 ng/g), and satisfactory recoveries (70-120%) and precision (RSDs ≤ 20%) were demonstrated for 200 (82%) of the targeted analytes. Lowest calibrated levels (LCLs) were ≤ 5 ng/g for 90% of the analytes allowing their determination at or below established tolerance levels (10-100 ng/g). The final method was simple, fast, and inexpensive, and the overlapping (10 min each) UHPLC-MS/MS and LPGC-MS/MS coverage of 55 pesticides allowed confirmation of the analytes in the extracts using orthogonal techniques. The applicability of the developed method was demonstrated on the analysis of incurred meat samples from the market.

AGRO 88

Automated instrument-top sample preparation for high-throughput analysis of chemical residues in foods

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High-throughput analysis of pesticides, veterinary drugs, and environmental and other contaminants in foods is needed in a variety of applications, including regulatory monitoring to improve food safety. The simultaneous performance in parallel of sample preparation by a robotic liquid handler (including autosampler) and instrumental analysis not only increases sample throughput, but also reduces labor costs, improves precision of results, and minimizes possible analyte degradation in the final extracts prior to analysis. In this study, mini-cartridge solid-phase extraction (SPE) for cleanup in instrument-top sample preparation (ITSP) of initial QuEChERS extracts of food commodities was demonstrated to yield high-quality results in high-throughput residue monitoring applications. Different cleanup sorbents were evaluated, and tested commodities included avocado, black olives, salmon, pork loin, apples, kiwi, bovine kidney, orange, carrot, kale, dried basil, and wheat grain. Cleanup was superior to dispersive-SPE commonly used in QuEChERS. ITSP results showed mostly 100% recoveries and <10% RSDs for hundreds of multi-application analytes in samples spiked as low as 10 ng/g followed by 10 min analyses by low-pressure gas chromatography and/or ultrahigh performance liquid chromatography each coupled to tandem mass spectrometry.

AGRO 89

Sources of imidacloprid in urban aquatic environments

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Currently used pesticides have been found in urban aquatic environments and urban runoff throughout the USA, sometimes at concentrations that exceed aquatic life protection benchmarks. Imidacloprid has among the highest detection rates for insecticides in California urban waterways with concentrations reaching—and in some cases exceeding—levels known to cause toxicity to sensitive aquatic organisms. A conceptual model of imidacloprid use and transport from urban areas to surface waters has been developed, based on a literature review, pesticide product labels, California pesticide sales and reported use data, pesticide retail shelf surveys, and unpublished data from municipal urban runoff programs. Imidacloprid can be washed into storm drains during or after outdoor pesticide applications, outdoor use of consumer products with pesticide protectants (e.g., preservatives), or as a consequence of pesticide handling activities. Since it is a systemic insecticide, imidacloprid is present in debris from treated vegetation, like leaf litter, pollen, and lawn clippings, which provide a potential pathway for transport into storm drains. Pet flea treatments – which can wash off during pet bathing and washing of surfaces like owner’s hands and pet bedding post-treatment – are more likely to reach indoor (sewer) drains than storm drains. The conceptual model provides a framework to guide further investigations of urban imidacloprid sources, and to develop appropriate strategies for preventing and mitigating urban imidacloprid water pollution.

AGRO 90

Neonicotinoid insecticides in agricultural and urban impacted U.S. streams

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To better understand the fate and transport of neonicotinoid insecticides, water samples were collected from streams across the United States. In a nationwide study of 38 streams, at least one neonicotinoid was detected in 53% of the samples collected, with imidacloprid detected most frequently (37%), followed by clothianidin (24%), thiamethoxam (21%), dinotefuran (13%), and acetamiprid (3%). Clothianidin and thiamethoxam concentrations were positively correlated to the percentage of the land use in cultivated crop production, and imidacloprid concentrations were positively related to the percentage of urban area within the basin. Additional sampling for neonicotinoid insecticides was also conducted in localized research areas to complement the national-scale results, including: (1) basins which have both agricultural and urban land uses (California and Georgia), and (2) predominantly agricultural basins in two nationally important ecosystems (Chesapeake Bay and San Francisco Bay-Delta). These environmental data are important in determining the potential risk of neonicotinoids to non-target aquatic and terrestrial organisms.

AGRO 91

Assessing groundwater vulnerability following a neonicotinoid use on turf: Optimized GIS site selection, results from a prospective groundwater study, and comparison to model predictions

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In June 2014, a prospective groundwater (PGW) study was initiated at a test site in Michigan. The PGW study was conducted to assess the potential for residues of clothianidin, an insecticide, to move to shallow groundwater, following a labeled application to turf. The PGW study is being conducted to fulfill requests from the U.S. Environmental Protection Agency (USEPA) and the Health Canada Pest Management Regulatory Agency (PMRA).

An initial GIS site-identification refinement phase was conducted, where geospatial data were compiled in order to identify potential areas of interest to assist and refine subsequent field reconnaissance efforts. Potential PGW study sites were required to meet certain site-selection criteria, including soil texture, organic matter, flat slope, common ecoregion, and depth to groundwater. Following identification and regulatory approval of the test site a national-scale geospatial leaching vulnerability assessment was conducted using an enhanced golf course turf layer containing over 17,000 locations for the US and Canada. Results of the vulnerability assessment based on site-specific characterization data indicated that the PGW site was well within upper 90th percentile leaching conditions for potential clothianidin use areas.

The PGW test site consisted of a predominately loamy sand surface soil with sandy loam, loamy sand and coarse-textured sand in the subsoil. The depth to groundwater at the test site was determined to be shallow and varied between approximately 13 to 16 feet below ground. Despite these vulnerable soil and depth to groundwater characteristics no detections of clothianidin were observed in any groundwater samples collected through two years following clothianidin application. The results from the PGW study will be compared to the predicted groundwater concentrations outputs from PRZM-GW using USEPA model inputs under similar conditions.

AGRO 92

Adsorption of thiamethoxam on natural soil and its influencing factors

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Thiamethoxam is a broad-spectrum, systemic insecticide which has been widely used on numerous crops around the world in recent years. The presence of thiamethoxam in soil may threaten the health of humans and ecosystems, for the Food and Agriculture Organization (FAO) of the U.N. assessed thiamethoxam as moderately hazardous to humans, mildly toxic for birds, highly toxic to midges, and acutely toxic for bees. Adsorption is one important process controlling the fate of organic pollutants in soil, but currently little information about the adsorption of thiamethoxam is available. This study aims to characterize the adsorption of thiamethoxam on natural soil and to determine its influencing factors. Soil samples with different organic matter fractions (f_{oc}) were collected from agriculture lands. The adsorption kinetics and isotherms of thiamethoxam on the tested soils were investigated by static adsorption experiments. The results showed that a two (fast and slow)-compartment first-order model was more appropriate for describing the sorption kinetic data, compared to a one-compartment first-order model or second-order model, especially in the initial sorption stage within 15 h. In the range of equilibrium concentrations studied (0.01 mg/L, 0.05 mg/L, 0.1 mg/L, 0.25 mg/L and 0.5 mg/L), the adsorption isotherms of thiamethoxam on the tested soils were better fitted by the Freundlich model than by the liner model, particularly for the low f_{oc} soil. The soil adsorption coefficient of thiamethoxam was significantly positively correlated with the soil f_{oc} (R=0.975, $P<0.05$). For a specific soil, the soil adsorption coefficient of thiamethoxam was significantly positively correlated with the soil temperature (R=0.969, $P<0.0$). This indicated that the adsorption of thiamethoxam on soil was an endothermic reaction. Calculation of the adsorption enthalpy change further revealed that the adsorption might be mainly due to hydrogen bond force, Van der Waals' force, and Dipole force. These results provided scientific basis for managing the risk of thiamethoxam on natural soils.

AGRO 93

Reduction of neonicotinoid insecticide residues in Prairie wetlands by common wetland plants

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Neonicotinoid concentrations are frequently detected in wetlands during the Canadian Prairie cropping season. These detections also overlap with the emergence of aquatic plant species (e.g., *Typha latifolia*) that commonly surround agricultural wetlands. We hypothesized that wetland plants may accumulate and/or slow neonicotinoid transport into wetlands from surrounding agricultural fields. We sampled 20 agricultural wetlands in central Saskatchewan, Canada to investigate whether wetland plants were capable of reducing lateral flows of neonicotinoids from cultivated fields and/or reducing concentrations in surface water by accumulating insecticide residues into their tissues. Study wetlands were surrounded by canola fields (clothianidin-treated seed) and chosen based on the presence (n=10) or absence (n=10) of a zonal plant community. From pre-seeding to the mid-growing season (8 weeks), wetlands were sampled for water, plant tissues, or both. Neonicotinoid concentrations were detected in 8% of all plant samples: *Alisma triviale* (imidacloprid, range: <LOD – 2.51 µg/kg), *Typha latifolia* (imidacloprid, range: <LOD – 2.61 µg/kg, thiamethoxam, range: <LOD – 8.44 µg/kg), and *Equisetum arvense* (clothianidin, range: <LOD – 2.01 µg/kg). Overall, total summed neonicotinoid concentrations in water were significantly higher in unvegetated than vegetated wetlands (β ± S.E.: -0.83 ± 0.26, P = 0.002). We assessed the importance of vegetative measures (e.g., zone width, emergent plant height) on neonicotinoid concentrations in Prairie wetlands using linear mixed-effects models. Neonicotinoid concentration was negatively correlated with emergent plant height ($\beta = -0.47 \pm 0.14$, $z = 3.31$, $P = 0.0009$). Concentrations were lower in wetlands surrounded by taller plants regardless of vegetation zone width (i.e., buffer). The results of this study suggest that some wetland plants have the ability to accumulate neonicotinoids and/or slow neonicotinoid-laced runoff during the growing season. Consequently, maintaining wetland plant structure may be important for mitigating contamination of wetlands.

AGRO 94

Fate and transformation of neonicotinoid insecticides during water and wastewater treatment

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Despite their widespread detection in surface waters, little is known about the fate of neonicotinoid insecticides during engineered water and wastewater treatment. This work explores the transformation and removal of common neonicotinoids via physical, chemical and biological processes used in both conventional and more advanced treatment systems. The goals of this work are to establish the rates and extent of neonicotinoid transformation and/or removal during simulated treatment operations in the laboratory, while also identifying major transformation products generated during chemical and biological treatment processes. In ongoing work, occurrence studies are seeking to identify transformation products from laboratory experiments in finished drinking water and wastewater effluent. The results of this work will help to better identify potential risks posed to humans and other non-target organisms by neonicotinoids and their transformation products generated during treatment.

AGRO 95

Overview of glyphosate resistance worldwide

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Glyphosate-resistant weeds are rapidly increasing in the number of species identified (now 35 globally) and the area they infest. The most common glyphosate-resistant weeds belong to 4 genera - *Conyza* sp. (3 species, 16 countries), *Lolium* sp (3 species, 13 countries), *Amaranthus* sp. (4 species, 4 countries), and *Ambrosia* sp. (2 species, 2 countries). They are primarily found in Roundup Ready cropping systems, orchards, chemical fallow, and various non-crop sites (roadsides, fence lines etc.). Fifteen glyphosate-resistant weeds are found in the USA, nine selected in Roundup Ready Cropping Systems, four in orchards, and two selected in chemical fallow. Glyphosate-resistant *Conyza canadensis* is the most widespread; however, glyphosate-resistant *Amaranthus palmeri* in the southern USA is the most economically damaging glyphosate-resistant weed worldwide, now infesting 25 states. Glyphosate resistant *Amaranthus tuberculatus* is rapidly increasing, infesting 17 US states and the province of Ontario in Canada. Glyphosate-resistant *Kochia scoparia* has rapidly become a major economic threat, infesting 10 US states and 3 Canadian provinces. While Australia has 12 documented glyphosate-resistant weeds, their economic impact is so far minimal in comparison to the USA because they are primarily found in chemical fallow, and along fence lines and roadsides. In South America, glyphosate-resistant *Sorghum halepense* (Argentina) and *Digitaria insularis* (Brazil and Paraquay) found in Roundup Ready soybeans are widespread and cause serious economic damage. The impact of glyphosate-resistant weeds has been exacerbated by a sharp increase in weeds that have multiple herbicide resistance (glyphosate + numerous other sites of action) and the fact that no new herbicide sites of action have been brought to market in over 35 years. New herbicide resistance crop traits (synthetic auxins) will enable older herbicides to be used in new ways and will buy some time to find new weed control methods if used appropriately. The era of relying on herbicides alone for weed control is over; sustainable weed control needs to rely upon timely use of a variety of chemical and non-chemical control methods aimed at destabilizing the evolution of weeds to any weed control practice.

AGRO 96

Mechanisms of glyphosate resistance

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There are 35 species resistant to glyphosate today (3/10/2016, www.weedscience.org) on the 6 continents in agricultural production. Six genera have multiple species found and several of these are on multiple continents suggesting their "genomes" have a trait(s) that can be selected for resistance. The most potent mechanisms of glyphosate resistance have been exclusion type systems with vacuole sequestration (*Conyza canadensis*, *Lolium rigidum*) or cellular exclusion (restricted cellular uptake, *Sorghum halepense*, *Amaranthus rudis, A. palmeri*) where a transporter has reduced the ability to enter the cell. Exclusion mechanisms are established for xenobiotics in bacteria where export via multidrug resistant pumps occurs, but these are so far not found for glyphosate. The novel gene duplication of EPSPS contributing resistance to *A. palmeri*, *A. rudis*, *L. rigidum* and *Kochia scoparia* may also be a "sequestration" type mechanism. The EPSPS enzyme can together with S3P form a "dead-end" complex with glyphosate and lower the concentration available to inhibit the shikimate pathway. However, gene duplication in some Palmer plants, inspite of high expression levels, does not sponsor resistance at all. Target site resistance until recently had been found only at the Proline106 position in 7 species, and this offered very low levels of resistance magnitude. The recent discovery of the double mutant Thr102Ile-Pro106Ser in *Eleucine indica* in Malaysia makes it clear that even very rare sets of mutations are attainable. This particular double mutant was used to make the first glyphosate tolerant corn when over-expressed. This double mutant enzyme however carries a serious fitness penalty observable in the untreated plants. A summary of these mechanisms will be presented.

AGRO 97

BioDirect™ and herbicide resistance

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The topical application of oligonucleotides targeting pest critical genes is the basis of Monsanto's BioDirect™ platform. BioDirect holds promise as a novel tool against the wave of resistant weeds to protect crop production. Weed resistance is dominated by the *Amaranthus sp.* complex in the USA in all the major row crops impacting the use of 6 different sites of action with some particular accessions of Palmer amaranth or waterhemp with resistance to 4 and 5 sites of action together that now in some locales leave very few herbicide options. Attacking the resistance head-on is possible using BioDirect to reverse and/or augment the activity of well established herbicides whose use is now limited due to resistance. Examples of controlling glyphosate resistance as well as improving herbicides targeting *A. palmeri* and *A. tuberculatus* demonstrate the opportunity. Explaining how siRNA silencing of the small subunit of ALS results in reversing resistance to Classic® and Staple® is detailed.

AGRO 98

Effects of glyphosate on plant disease

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Plants synthesize phenolic compounds and other shikimate pathway derived compounds as an active response to pathogen infection. These compounds aid in defense as antimicrobials, antioxidants and cell wall structural components. As a result of its mode of action, glyphosate reduces the ability of plants to synthesize phenylpropanoids and other shikimate pathway-based defenses and thus increases the susceptibility of plants to pathogens. For example, reduced amounts of the pterocarpan phytoalexin glyceollin and increased susceptibility is observed in *Phytophthora*-resistant soybean treated with glyphosate prior to inoculation with that pathogen. Because of these types of observations, there has been concern that glyphosate resistant (GR) crops are more susceptible to disease as a result of glyphosate treatment. However, research has demonstrated that susceptibility of GR crops is based in the inherent susceptibility of the cultivar used for transformation. For example, GR soybean is no more susceptible to the white mold pathogen, *Sclerotinia sclerotiorum*, than are lines that are near isogenic and non-GR. Both field and greenhouse-based research has confirmed the lack of effect of the GR trait on disease expression in several other crops. Glyphosate may, however, impact disease in GR crops indirectly through a phenomenon known as 'green bridging" where killed sensitive weeds may serve as a habitat for increased amounts of pathogen inoculum. Glyphosate may also help reduce certain diseases by fungicidal action, and examples of where this has been examined will be described. Although glyphosate can enhance disease severity in non-GR crops, research in several plant-pathogen systems indicates that use of glyphosate does not directly increase disease severity in GR crops.

AGRO 99

Glyphosate and effects on soil biology and function

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Widespread studies have shown that glyphosate applied at standard application rates (PEC = 3 mg/kg) has little impact on microbial biomass, and stimulation rather than inhibition of microbial activity is frequently observed at higher rates (Nguyen et al., 2016). *In vitro* studies in multiwell microtitre plates in our laboratory on a range of aquatic species of algae *(Anabaena, Chlamydomonas, Chlorella, Euglena, and Thalassiosira)* as well as yeast and nitrogen-fixing organisms, *Klebsiella* and *Rhizobium,* had no deleterious effects at realistic concentrations. The propensity of glyphosate to bind to soil and sediments rich in iron leads to strong binding of the phosphate, which is difficult to displace. As a result, attempts to set up passive samplers for glyphosate to monitor dissipation have universally failed. Freitas (MSc thesis, 2005) concluded that "the very reason glyphosate is difficult to recover for analysis by classical instrumental techniques may be those that make it a safe herbicide". Studies on nucleic acid composition of soil reflecting community structural changes in soil microbial populations have also failed to discover impacts other than transient alterations of profiles (see summary in Rose et al., 2016). Other herbicides, such as the sulfonylureas, are more frequently reported as having negative effects on soil functions, such as nitrogen cycling, but these effects usually do not persist for more than 28 days (Rose et al., 2016). Effects of glyphosate on mesofauna such as weight-loss in earthworms have been reported, though usually at concentrations [e.g. 8 mg/kg, Yasmin and D'Souza (2007)] greater than the PEC value of 3mg/kg. Perhaps the only cause for concern is that continued use of glyphosate could cause some herbicidal effects on crop plants by plant residues.

AGRO 100

Effects of glyphosate on mineral nutrition of glyphosate-resistant soybean and maize

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Glyphosate is the most widely used herbicide in the world, primarily because of its use in transgenic, glyphosate-resistant (GR) crops. There have been claims that glyphosate reduces the levels of certain minerals, especially Mn, in GR crops. The published literature related to this topic is conflicting. Most of the greenhouse studies that have reported reductions in divalent cation-forming minerals have examined only the foliage of glyphosate-treated, GR soybean. One greenhouse study that analyzed mature seed from such plants found no effects on the content of the fourteen (Ca, Mg, K, Mn, Zn, Fe, Cu, Sr, Ba, Al, Cd, Cr, Co, or Ni) minerals analyzed. Most of the field studies have found no effects of glyphosate on mineral content of the foliage of GR soybean. The two studies which examined the mineral content of mature seeds from treated plants in the field found no effects on mineral content. A 2-yr field study was conducted in Stoneville, MS, to rigorously examine the effects of glyphosate and glyphosate trait (GR transgene) on Mg, Mn, Zn, Fe, Cu, or Ni in GR and non-GR soybean and maize seeds. Glyphosate at recommended rates for weed control had no effects on content of these minerals in seeds of GR soybean or maize compared to non-treated plants. Similarly, the GR transgene had no effect on these metals in GR soybean or maize compared to non-GR plants. Most literature so far indicates that glyphosate and the GR transgene have no effects on mineral nutrition in GR crops. The generally linear increase in soybean and maize yields in the US since GR soybean and GR maize were introduced 1996 and 1998, respectively, also support that neither glyphosate nor the GR transgene had any substantial effects on mineral nutrition in GR crops.

AGRO 101

Hormonal convergence in regulation of *Drosophila* courtship memories

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Memory of prior experiences is essential for appropriate context-dependent decision-making. In *Drosophila*, courting males experiencing repeated rejection by mated females subsequently court less avidly when paired with virgin females due to formation of "courtship memories". We have identified critical roles for hormonal state and ecdysis triggering hormone receptor (ETHR) signaling in both short-term and long-term courtship memories. Ecdysis triggering hormone (ETH) is essential for male courtship memories via two signaling pathways: one is indirect through promotion of juvenile hormone (JH) synthesis, while the other is direct, via actions on mushroom body neurons in the brain. With regard to the indirect pathway, suppression of JH levels by RNAi silencing of ETH receptors (ETHR) in male corpora allata leads to both short-term and long-term courtship memory deficiencies; methoprene rescues all such deficiencies. Our evidence shows that JH is essential for 1) sensing aversive male-specific chemical cues from mated females and 2) recognition of behavioral cues provided by the mated female. Conditional gene expression and age-dependent rescue experiments demonstrate a critical period for JH action in memory formation. JH-dependent long-term courtship memory requires expression of the JH receptors MET and GCE in mushroom body neurons. Evidence for a direct role of ETH signaling in long-term memory formation comes its suppression by silencing ETHR in mushroom body neurons. Block of ETH release from Inka cells also negatively affects memory performance, confirming that hormonal state is crucial for memory formation. Our findings indicate that ETH promotes convergent signaling pathways contributing to hormonal "state-dependent" learning and memory in a social context

AGRO 102

Diamide insecticides: Understanding the basis for insect selectivity and target-site resistance

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Chlorantraniliprole (Rynaxypyr®), cyantraniliprole (Cyazypyr™) and flubendiamide belong to the diamide class of insecticides with sales of chlorantraniliprole alone exceeding $1billion in 2014. Diamide insecticides control pests through activation of the ryanodine receptor (RyR), an intracellular calcium channel critical to muscle contraction. Chlorantraniliprole is highly efficacious against chewing insects while cyantraniliprole exhibits broad insect control. Studies conducted using recombinant insect RyRs show that the improved hemipteran efficacy of cyantraniliprole is related to physical properties as opposed to intrinsic receptor activity. Single channel recordings show that diamides increase channel openings rather than modifying the RyR conductance state. Diamide insecticides bind to a site on the RyR distinct from that of plant alkaloid, ryanodine. Although the actual binding domain has yet to be defined, several regions that confer sensitivity to diamides have been identified and may play a key role in selectivity and target-site resistance.

AGRO 103

Investigation into the use of neurolemma-injected oocytes in determining age-related difference in the action of insecticides on native ion channels

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Microtransplantation of rat brain neurolemma into plasma membrane of *Xenopus laevis* oocytes is used to study ion channels in their native state *ex vivo*. This approach is advantageous for various reasons: tissue can be obtained from various sources and at different developmental stages; channels and receptors are present in their native configuration in their proper lipid environment along with appropriate auxiliary subunits; allowing the *ex vivo* evaluation of numerous neurotoxicants. Here we show that oocytes injected with post-natal day 90 (PND90) and 15 (PND15) neurolemma expressed functional ion channels, including voltage-sensitive sodium channels (VSSC), a major target site for neurotoxic insecticides such as DDT and the pyrethroids. All VSSC isoforms ($Na_v1.1$ – $Na_v1.9$) were found and VSSC were detected in oocyte plasma membrane microtransplanted with neurolemma. Using two electrode voltage clamp (TEVC) electrophysiology and in the combined presence of tetrodotoxin and niflumic acid, a TTX-sensitive inward Na^+ current was detected that mimicked the current seen when VSSC are heterologously expressed in oocytes. As a "proof-of-principle" experiment, the well-established structure-activity relationship between the neurotoxicant DDT and its non-neurotoxic metabolite DDE was examined. DDT elicited a concentration-dependent increase in TTX-sensitive inward Na^+ current upon pulse-depolarization and resulted in a slowing of sodium channel inactivation whereas DDE caused no significant effects. These results are consistent with findings obtained using many other electrophysiological approaches, validating the use of the microtransplantation procedure as a toxicologically-relevant *ex vivo* assay. A type I (permethrin) and II (deltamethrin) pyrethroid were similarly examined in neurolemma from PND15 and 90 rat brain. VSSC from PND15 neurolemma are more sensitive than channels from PND90 neurolemma, but differences appear small.

AGRO 104

Voltage-gated chloride channel blockers for varroa mites

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The varroa mite is a primary driver for the periodical losses of honey bee colonies. The mite requires bees for food and reproduction and, in turn, elicits physiological deficiencies and vectors viruses that compromise bee health. The most common and effective approach to mite management is the use of in-hive chemical interventions such as pyrethroid-, organophosphate-, or formamidine-based varroacides alone or in combination with organic acids and essential oils. These varroacides not only have adverse health effects on bees, but widespread metabolic and target-site resistance limits their use to reduce the risk of mite infestations and vectored viruses in bee colonies. The voltage-gated chloride channels (VGCC) are involved in numerous physiological functions, including the maintenance of electrical excitability, and are reported as an exploitable target site for chemistries with novel modes of action for pest management. The VGCC blocker 4,4'-diisothiocyano-2, 2'-stilbenedisulfonic acid (DIDS) has documented stomach poison and paralytic activity against insects and nematodes, respectively. Recent studies report the increased toxicity of DIDS to mites compared to in-hive varroacides. Here, we will report the (*i*) laboraotory toxicity and field efficacy of in-hive varroacides to susceptible and resistant mites, (*ii*) metabolic and target-site mechanisms in varroacide-resistant mites, and (*iii*) laboratory toxicity and field efficacy of DIDS and related VGCC blockers to varroacide-resistant mites. These data will be discussed not only for the improvement of existing in-hive chemical interventions, but for guiding the development of novel modes of action for mite management.

AGRO 105

Glutamate receptor-cation channel complex as an unexploited target for insecticide design

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Glutamate receptor-chloride channel complex is a proven insecticide target for avermectins and fiproles. However, glutamate receptors having an intrinsic cation channel and responsible for excitatory neurotransmission (GluRD) remain unexploited for chemical insect control. These receptors have been extensively studied at the insect neuromuscular junction, where activation of the receptor underlies the excitatory junction potential and triggers muscle contraction. Desensitization to a non-conducting, persistent ligand-bound state is a prominent characteristic of this receptor. By using a new headless larval assay to enhance compound penetration and electrophysiological methods, we found that agonists of muscle GluRD induce paralysis in larvae of the yellow fever mosquito, *Aedes aegypti*, by blocking synaptic transmission, and aspartate proved to be a strong paralytic agent in the headless larvae assay (PC_{50}=7 ppm) more so than glutamate (PC_{50}=311 ppm). We also performed feeding assays and intra-thoracic injection of adult female *Aedes aegypti*. In the feeding assay, agents were suspended in 5% sugar water to feed mosquitoes for 48 hours. The results suggest that the toxicity occurred in the following rank order of effectiveness: feeding < headless larvae < injection assays, which suggests that the agents might be readily metabolized or not be able to penetrate into the hemocoel. To see if other insects besides mosquitoes were sensitive to these compounds, a sugar water feeding assay was performed with adult female *Drosophila melanogaster*. Interestingly, there was little or no toxicity to adult *Drosophila*, indicating selectivity for mosquitoes via this route of exposure. Our results confirm that glutamate agonists are potential candidates for further development as new mosquitocides, but new lead compounds are required.

AGRO 106

Inhibitory chloride channels as targets for γ-BHC and its analogs

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GABA receptor Cl- ion channel complex (GABA-gated chloride channel) is a scientifically and practically important target site for various pharmaceutical and animal health drugs, and insecticides. Lindane (γ-BHC) is well known to be one of noncompetitive antagonists of this GABA-gated chloride channel. However the question, "why is γ-BHC among BHC isomers only insecticidal?" remains to be unsolved and a mystery. In the early 1970s a number of γ-BHC analogs were synthesized, in which some chlorine atoms were replaced by other substitutes such as hydrogen, halogens other than chlorine and alkoxy groups, etc. The analogs which replaced chlorine with some substituent such as OCH_3 show a similar level of insecticidal activity to γ-BHC against the housefly and German cockroach. Using newly developed techniques, we have recently started a new project to address the question and mystery by the above.

AGRO 107

Identification and physiological characterization of inward rectifying potassium channels in the arthropod salivary gland

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The tick salivary gland (SG) is the organ responsible for bloodmeal acquisition, pathogen dissemination, and osmoregulation and is therefore critical to their biological successes. Although there have been significant amounts of research performed on saliva specific proteins in ticks, little success has been made to develop vaccines that prevent the spread of tick-borne diseases. Furthermore, an understanding of the primary machinery required for proper SG function within arthropods is very limited. Studies have shown that mammalian SG function is dependent upon an immediate increase in membrane potassium conductance. Due to this, our research aims to characterize a 'superfamily' of potassium channels, known as inward-rectifying potassium (Kir) channels, and investigate the physiological role these channels have in the function of the arthropod SG. Proof-of-concept feeding studies were performed in *Drosophila melanogaster* to determine the influence Kir channel inhibition has on sucrose consumption. Preliminary data suggest that pharmacological inhibition of Kir channels significantly ($P<0.0001$) reduces the total volume of sucrose solution ingested by individual flies from 1.4 uL/fly to 0.3 uL/fly. Furthermore, genetic knockdown of SG specific Kir channels increased the time required to obtain a complete meal by approximately 3-fold, suggesting a reduced SG function in adult *Drosophila*. Due to the success of these studies, we are cloning the open reading frame of SG-specific Kir channel constructs for two tick species, *Ixodes scapularis* and *Dermacentor variabilis*. Preliminary studies on the isolated tick SG show exposure to barium, a specific Kir channel blocker, significantly ($P<0.01$) decreased transepithelial fluid secretion from 270 nl/5 min to 141 nl/5 min, suggesting Kir channels may constitute a critical K^+ conductance pathway required for proper SG function in ticks. Additional studies on the subcellular expression patterns of SG Kir channels, the role of these channels on the transepithelial voltage, and transepithelial ion secretion rates will be presented.

AGRO 108

Malathion residues in flowing waterbodies resulting from aerial drift in a high use intensity watershed: monitoring

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Malathion, a broad spectrum organophosphate insecticide, has been widely used since the 1950s and has been the focus of numerous environmental fate and transport studies. Prior studies conducted on malathion have primarily focused on runoff driven modes of transport, with little research focused on drift driven transport into aquatic systems. To understand this processes better, and improve watershed scale model predictions of environmental malathion concentrations, a targeted monitoring study was designed to gather data on aquatic concentrations of malathion, and its degradate malaoxon, in watersheds dominated by spray drift contributions to surface waters. Two watersheds located in The Dalles, Oregon, were identified from a nationwide assessment of watersheds with high malathion use and little precipitation during the growing season. In this region, ultra-low volume formulations (ULV) of malathion are applied aerially to cherry orchards with applications typically occurring from mid-May through the end of June. Applications were made by a single company for the entire region, simplifying the collection of watershed application data. During the 2015 growing season, monitoring stations were established in each watershed below areas receiving malathion applications. Stream samples were taken on a sub-daily (6-hour and hourly) basis during the entire application period using ISCO autosamplers. Stream flow and water quality were also measured continuously and at sample retrieval, respectively. Monitoring revealed the maximum concentration of malathion in surface waters downstream of high use areas was relatively low. The highest recovered residues in either watershed were 1.03 ppb for malathion and 0.048 ppb for malaoxon. Due to a scarcity of rainfall during the growing season in the study area, the residues observed in surface waters were drift-driven. The detailed dataset obtained in this study was used to parameterize the Soil and Water Assessment Tool (SWAT) drift algorithms in malathion modeling, discussed in Part II Modeling.

AGRO 109

Malathion residues in flowing waterbodies resulting from aerial drift in a high use intensity watershed: modeling

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The soil and water assessment tool (SWAT) is a watershed-scale flowing waterbody model increasingly being used to assess pesticide transport and fate. SWAT applications have largely focused on runoff dominated pesticide transport, with very few studies addressing the simulation of pesticide spray drift. A novel approach was developed for simulating spray drift contributions to flowing water bodies at the watershed scale. The objectives of this study were to utilize high temporal resolution monitoring data to evaluate: (a) if this new approach to simulating spray drift contributions is adequately conservative for use in regulatory pesticide exposure modeling, and (b) if the model simulation accuracy can be improved when site-specific data such as application timings/locations, wind speed/direction, and stream geomorphology are applied. A high temporal resolution sampling monitoring study in a drift-dominated watershed in The Dalles, Oregon, formed the foundation for this model validation study. Four simulation experiments were designed to incorporate an increasing amount of site-specific data within the SWAT model for this watershed. Results showed that the model simulations using the newly developed approach were highly conservative when compared to monitoring data under standard assumptions for spatial and temporal distribution of applications, wind speed and direction, and stream characteristics. When site-specific data were incorporated model results improved with each data addition, with greatest improvements after incorporating actual wind data. There are three major findings from this study: (a) the newly developed watershed scale drift modeling approach using the SWAT model provides conservative estimates of pesticide concentrations in flowing waterbodies at the watershed scale resulting from spray drift, (b) SWAT simulations can be improved greatly when more realistic wind speed and directions are incorporated into spray drift loading algorithms, and (c) the value of pesticide monitoring data is augmented by collecting spatially-explicit application characteristics and meteorology during the same time period.

AGRO 110

Trends observed from a long term collaborative surface water monitoring program for thiobencarb to manage water quality in the Sacramento River

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Water monitoring for thiobencarb in California, related to its use on rice, has been in place for over 20 years. Water samples have been collected from agricultural drains that flow into the Sacramento River and from the Sacramento River. The primary focus for this effort was driven by concerns about water taste, as both the cities of Sacramento and West Sacramento abstract drinking water directly from the Sacramento River.

Thiobencarb use on rice is restricted to counties near and above Sacramento, and a majority of the rice is grown in flooded fields. Thiobencarb is effective as an herbicide during the early development of the rice, and it requires warm temperatures during application. This use pattern narrows the window for sample collection to late spring/early summer. The connectivity of these waterways simplifies the monitoring, allowing for grab samples to be collected once or twice per week at 5 sample locations.

The water monitoring program is part of a stewardship program for pesticide use on rice, which includes the California Rice Commission, growers, and applicators. The analytical results from this program are shared with the Sacramento River water utilities. The cities of Sacramento and West Sacramento also provide results for river water samples that they collect and analyze. The consistency between these results supports the design of this monitoring program.

In 2012, the National Marine Fisheries Service used the water monitoring data and the management practices in an assessment for potential impact of thiobencarb on salmon. The determination that use of thiobencarb did not jeopardize salmon was due in part to the low level of exposure that was documented through the water monitoring program.

AGRO 111

Modeling and monitoring to characterize pesticide fate in the Zollner Creek Watershed, Willamette Basin, Oregon

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For more than 20 years pesticides have consistently been detected in the Zollner Creek watershed, Willamette Basin, Oregon, more frequently, and at higher levels, than in other surface waters of the Pacific Northwest. To address this concern the watershed scale ecohydrologic model – Soil and Water Assessment Tool (SWAT) – was used to better understand pesticide loading pathways that may direct monitoring efforts and the development of mitigation strategies. Using a mechanistic approach, SWAT ecohydrology was calibrated for the Zollner Creek watershed. To evaluate model performance in estimating pesticide fate, probabilistic methods were used to evaluate the spatial and temporal distribution of selected pesticide applications and resulting surface water concentration profiles. Model estimates – mean pesticide surface water concentration and 95% confidence interval as a result of the spatial and temporal probability distributions of pesticide applications – for 2010 and 2011 are compared to US Geological Survey and Oregon Department of Environmental Quality pesticide grab sample surface water concentrations. This modeling approach shows promise in estimating pesticide surface water concentration exceedance probabilities for alternate cropping practices, mitigation measures, and pest management strategies, as well as informing monitoring strategies and enhancing the value of monitoring data.

AGRO 112

Integration of SEAWAVEQ model predictions into bias factor development

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The concept of bias factors has been developed to address underestimation of peak and time-weighted pesticide concentrations from monitoring data with low sampling frequency. A critical aspect of bias factor development is the availability of spatially and temporally robust daily monitoring data. Unfortunately, daily monitoring data for pesticides are rarely available due to the cost in sampling and analytical procedures. This is important because bias factors are

dependent on watershed properties, including hydrology, pesticide use, and precipitation. To address the lack of temporally and spatially robust daily monitoring data, the U.S. Geological Survey (USGS) model for analyzing trends in chemical concentrations in streams with a seasonal wave (seawave) and adjustment for stream flow (Q) or seawaveQ is being used to generate daily time-series for bias factor development. USGS data, used in pesticides trend analysis, will be used in seawaveQ modeling to generate daily time series for bias factors development. This modeling exercise will consider pesticides with different fate properties and occurrence patterns. A major factor in the analysis will be the consideration of model error in bias factor development.

AGRO 113

Kriging prediction of pesticide concentrations in surface water draining agricultural watersheds

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The use of pesticide water quality monitoring data in regulatory exposure assessment is often limited by concerns that potential peak concentrations in annual site time series may be missed by infrequent (i.e., non-daily) sampling. Most water quality monitoring programs have a sampling frequency at every 7-day, every 14-day, or longer intervals. As a result, regulatory assessments often rely on deterministic hydrological field-scale models which use theoretical label use patterns and worst-case exposure scenarios without model evaluation by measured monitoring data. An alternative approach is to develop statistical models which can be fit directly to the data, leading to identification of a final model that is most compatible with the observed data, given model assumptions. Predictions on non-sampled days can be further improved by use of readily available covariates with daily sampled data such as stream flow or other watershed variables.

To this end, we present an introduction to the use of kriging methods to predict pesticide concentrations on days not sampled, and based on those predictions we are able to estimate exposure maxima. We provide an overview of results obtained in our development of kriging predictive models using near-daily sampled atrazine data and covariates. Drawing from a total of 151 site-years of daily/near-daily sampled measurements, we evaluate prediction of maximum m-day (m=1, 7, 14, 30) rolling averages from water monitoring data, applying every 7-day and 14-day sampling. This extensive dataset allows precise estimation of model prediction error and evaluation of covariate predictive performance. We describe the kriging method, challenges faced, and results obtained.

AGRO 114

Simple approach for assessing the potential implications of high fractions of samples with non-detectable residues from surface water monitoring programs

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The Lower American River flows through Sacramento, California, and was sampled between 2012 and 2014 in a detailed monitoring program designed to spatially and temporally characterize pyrethroid residues resulting from outdoor residential uses. Water samples were collected during 9 temporal sampling events at 8 separate locations, during runoff events and wet and dry seasonal flow conditions. Analytical results presented a challenge for characterizing the final dataset due to ta large number of samples below the already low Method Detection Limits (MDLs: typically 0.1 – 0.2 ng/L for 7 of the AIs in the water column). Consequently, conventional approaches for handling residues <MDL become problematic since summing "half the MDL" for each of several pyrethroids develops a cumulative concentration which implies potential toxicity.

In order to address non-detectable residues, several statistical approaches for properly characterizing residues have been examined. This showed that a simple but effective approach can provide bounds for potential total Pyrethroid exposures in the American River by substituting either zero or LOD in samples where the percent of detections was too low (i.e., 30% or less). Using the adopted method, it was determined that water samples collected in dry seasons, no cumulative pyrethroid residues exceeded the HC05 with either assumption, but for samples collected under wet season event conditions, there were a few exceedances using the lower bound, and their occurrence increased slightly using the upper bound.

AGRO 115

Comprehensive two-dimensional gas chromatography (GCxGC) – time-of-flight mass spectrometry: A powerful tool for finding and quantifying historical and emerging environmental contaminants in water

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GCxGC is a powerful multidimensional approach to sample analysis that yields superior separation power versus what can be achieved with one-dimensional GC. This is valuable when searching for trace level contaminants that can be easily compromised by matrix interferences. When paired with a fast acquiring time-of-flight mass spectrometer (TOFMS), it is unmatched in its potential for mega multi-analyte methods for target, non-target, and unknown compounds. The features of GCxGC-TOFMS include: monitoring and quantifying an unlimited number of target analytes because of the high speed capability of TOFMS, full mass spectra for target compound confirmation purposes, sub- to low- pg sensitivity, mass spectra/identification/semi-quantification of non-target and unknown compounds including emerging compounds of concern, historical records of mass spectra for future data mining, and the minimization of potential matrix interferences through multidimensional separation.

The above features for GCxGC-TOFMS will be demonstrated by characterization of a Las Vegas Wash water extract. The Las Vegas Wash is an urban river subject to many sources of environmental pollutant input from a population base of approximately two million people. The Wash flows into Lake Mead, which is where Las Vegas gets its drinking water. Various types of contaminants including but not limited to pesticides, polycyclic aromatic hydrocarbons, disinfection byproducts, flame retardants, personal care product compounds and pharmaceuticals were determined in Las Vegas Wash samples.

AGRO 116

Implementation of gas chromatography with atmospheric pressure gas ionization mass spectrometry (APGC) for the determination of known and unknown fatty acid esters and pesticides in avocado

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Atmospheric pressure ionization sources for mass spectrometry (MS) systems historically have been implemented with LC systems. Recently, this type of ionization source has successfully been interfaced with GC. A significant advantage in the use of atmospheric ionization is the preservation of parent molecule structure during the ionization process, information that is often lost in typical electron ionization (EI) processes. This preservation, followed by controlled fragmentation in an MS collision cell, affords more specificity and selectivity for typical GC analyzed compounds. Here we demonstrate the use of atmospheric pressure chemical ionization following GC separation (APGC) for the identification and determination of fatty acid methyl esters (FAMEs) and pesticides (as well as other food contaminants) in an avocado matrix. Because the parent structure is preserved, FAMEs and pesticides with common fragments were easily differentiated and useful fragmentation was achieved in the MS collision cell for confirmation and quantification. Another advantage with atmospheric pressure MS is that much higher carrier gas flow rates can be used compared with EI sources operated at high vacuum. In this study, fast carrier gas flow rates on the GC column were implemented in order to rapidly elute problematic compounds prone to thermal degradation. MS analysis was performed on both tandem quadrupole and quadrupole time-of-flight systems, the latter affording a better ability to structurally elucidate unknown prominent peaks of interest observed during the sample analysis

AGRO 117

Reusing wastewater in agriculture: Groundwater quality, plant uptake, and antibiotic resistance?

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With rising demands on water supplies, wastewater treatment plant (WWTP) effluent is often reused to irrigate agricultural lands. Emerging contaminants, like antibiotics, are frequently found in effluent due to limited removal during WWTP processes. Concern has arisen about their environmental fate, particularly the possible contamination of groundwater, plant uptake, and health effects, like antibiotic resistance in environmental bacteria. The aim of this study was to examine the environmental impacts of spray irrigating WWTP effluent at The Living Filter, a wastewater reuse site, by quantifying three antibiotics, sulfamethoxazole (SMX), trimethoprim (TMP), and ofloxacin (OFL), in WWTP effluent, groundwater and wheat plants (*Triticum aestivum*) and providing preliminary analysis of antibiotic resistance in environmental bacteria. Water samples were collected three times annually, while wheat and soil were collected once in the summer. Throughout the year, the three antibiotics were present in WWTP effluent and groundwater. Effluent concentrations ranged from 2 ng/L to 22 ug/L and varied based on the time of the year. In groundwater, SMX and OFL were quantifiable with a concentration range of 0.14 – 67 ng/L, and TMP was typically only detectable, but had a high concentration of 22 ng/L. Considering the concentrations associated with wheat plants, OFL was found in straw (10.2 ± 7.05 ng/g) and grain (2.28 ± 0.89 ng/g); TMP was only found on the surfaces of grain and straw; and SMZ was concentrated within the grain (0.64 ± 0.37 ng/g). For the analysis of antibiotic resistance in soil bacteria, antibiotic resistance to OFL was absent, while resistance to SMX and TMP was elevated compared to a control site not receiving WWTP irrigation nor manure applications. Based on these findings, antibiotics are present in groundwater and wheat plants following irrigation with WWTP effluent. Additionally, the introduction of human antibiotics appears to impact antibiotic resistance in soil bacteria. Overall, this study suggests that antibiotics introduced into the environment due to WWTP reuse may pose potential risks to the long-term health of ecosystems, with consequences for human and animal health due to antibiotic resistance. However, additional research is necessary to determine the full environmental and health implications of reusing WWTP effluent for the purpose of irrigating agricultural lands.

AGRO 118

Characterizing pharmaceutical sources and vulnerable aquifers in karst areas using scraped websites and measured water quality data

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Karst areas exist worldwide and are a drinking water source for 25% of the global population. They are characterized by unique hydrologic formations, which facilitate quick movement into the karst aquifer of nearly all surface water in the catchment basin. Thus karst aquifers are highly susceptible to contamination by chemicals from animal and human activities, potentially degrading drinking water and sensitive cave habitat. However, despite their vulnerability, very little research has investigated the presence and fate of emerging contaminants in karst aquifers. Twelve pharmaceuticals, fecal and total coliform bacteria, ions, and other water quality parameters were monitored for 2 years in 12 karst waters of southern Illinois, USA. Of the 58 water samples, pharmaceuticals were measured in 52. The most frequently detected were gemfibrozil and triclocarban, which were identified 33 and 47 samples, respectively. Computer code was written to scrape information on local weather, including precipitation and temperature, and local stream flow from government websites. Generalized linear modeling of measured water quality data, and parsed weather, stream flow, and land use information, was performed using Akaike’s second-order information criterion and stepwise-analysis to characterize the presence of pharmaceuticals in the aquifer. Sites generally had similar pharmaceutical contamination for each sample period, and stream flow and several ions were significantly related to total pharmaceutical contamination. However 2 sites exhibited overall higher pharmaceutical concentrations and a different relationship to water quality parameters. The identification of water quality markers is valuable to understand input sources and particularly vulnerable aquifers, in order to protect drinking water sources and cave habitats.

AGRO 119

Application of urban metabolism metrology to monitor chemical consumption, exposures, and population health in U.S. communities

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Urban Metabolism Metrology (UMM) is an exciting new methodology enabling the economical, reliable acquisition of near-real time data for epidemiological assessments. This tool includes sewage epidemiology, which has been successfully applied in the past primarily to estimate illicit drug consumption in communities by measuring drug and drug metabolite levels in raw wastewater entering municipal sewage treatment plants. UMM and sewage epidemiology rely on the fact that influent wastewater (raw sewage) is essentially a pooled sample of urine, feces and gray water from a large population of known size, where the concentration or mass of compounds of interest is proportional to the amount of these substances consumed, metabolized or produced. This approach makes possible the reliable, yet inexpensive comparison of the health status in different communities, as well as the monitoring of sustainability parameters, such as the stress level of populations, diet, and the consumption of medications, recreational drugs, and consumer products. In the present study, we employed the UMM approach to three small U.S. communities located in the Midwestern and Northeastern region, with population size ranging between 40,000 and 100,000 individuals, to monitor the progress in community health, life style and diet as a function of ongoing interventions. Composite samples collected over 24-h periods of raw influent wastewater were collected once a month and shipped overnight to our laboratory for analysis. Samples were processed using an automated solid-phase extraction unit, and the resulting extracts were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Initial analysis included tracking the consumption of alcohol, nicotine and various pharmaceuticals and personal care products (PPCPs) to estimate exposures in communities. Caffeine was used as a human marker to assess the dilution of domestic wastewater by other wastewater sources. Results of this ongoing, multi-year study already yielded a unique set of data on chemical consumption, exposures and health status of the participating communities, information that is difficult or impossible to obtain economically and rapidly with conventional approaches to public health assessment.

AGRO 120

Non-extractable residues (NER) from xenobiotic in soil and sediments: a new classification and relevance in the risk assessment

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Pesticides as anthropogenic chemicals are of high interest since they are deliberately applied in high amounts to soils to support food crop production. Chemicals entering this complex soil system may undergo various turnover processes. They can be degraded chemically, biologically by microorganisms, immobilised in form of non-extractable residues (NER), volatilized, or taken up by living organisms. Due to the difficulties in NER analytics in complex soil environments, their determination is limited mainly to quantitative analyses in ^{14}C turnover studies. This approach, however, does not provide any information about their chemical composition. Therefore, it is speculated that these compounds of unknown structure immobilised as NER may be released from organic matter (OM) of soil or sediment and transported to all compartments of the environment posing a potential risk for environmental and human health. However, it is generally known that microorganisms can use carbon from an organic contaminant to synthesise their biomass compounds, for instance fatty acids (FA) and amino acids (AA). After death, these biomass compounds can be incorporated into OM, forming ultimately hardly extractable biogenic residues. As the biogenic residues are composed of non-toxic natural compounds, they do not pose any risk to human and environment.

We investigated biodegradation of different classes of stable isotope labelled tracer compounds (e.g., $^{13}C_6$-2,4-D, $^{13}C_6$-metamitron, $^{13}C_3{}^{15}N$-glyphosate and $^{13}C_{10}{}^{15}N$-bentazone), in particular the metabolic incorporation of the $^{13}C(^{15}N)$-label into FA and AA and their fate in soil and sediment over time.

Low or no CO_2 indicates mainly the formation of xenobiotic NER, whereas the major formation of biogenic residues is expected for xenobiotics displaying high mineralisation rate in a short time course. However, in cases of more recalcitrant xenobiotics, but non-toxic to microorganisms like bentazone, the formation of bioNER cannot be completely excluded.

AGRO 121

New models to study nanoparticle interaction with biological membranes

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The interaction of engineered nanomaterials with biological interfaces must be understood to minimize potential adverse effects of unintended exposures to nanoparticles and to effectively design of nanomaterials for biological applications (e.g., therapeutics, diagnostics, pesticide delivery). Cell surfaces are chemically complex, and the so-called nano-bio interface is notoriously difficult to study. We have developed several new models for cell surfaces that allow their interaction with nanoparticles to be investigated using a variety of surface-sensitive techniques (e.g., quartz crystal microbalance with dissipation monitoring, optical waveguide lightmode spectroscopy, non-linear optics, super-resolution fluorescence microscopy, atomic force microscopy). These models allow detailed study of the processes and membrane properties perturbed by interaction with specific ligand-nanoparticle combinations. We will discuss recently developed models for cell surfaces and discuss examples of insights gained about the interaction of nanomaterials with specific cell surface components.

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Neonicotinoids viewed from a computational chemistry perspective: Conformations, interaction sites and binding to a 3D model of insect nAChR

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Neonicotinoids remain one of the most widely used classes of insecticides in the world. However, their adverse effects have contributed to the withdrawal of three of them (imidacloprid, clothianidin and thiamethoxam) from the European market in 2013. High resolution crystal structures of the soluble ACh binding proteins (AChBPs) have allowed deciphering the nAChR Ligand Binding Domain−neonicotinoid interactions for several members of this family. However, data are still lacking for some of these compounds, and a 3D model allowing the description and analysis of the interactions for all members of the neonicotinoid family would be a significant step towards the understanding of their selective toxicity at the atomic level.

In this contribution, we will present the results we have obtained using a multiscale molecular modeling approach (**Figure 1**).

Our results first allow to highlight the structural features of the various neonicotinoids. Secondly, they provide a comprehensive description of the key neonicotinoid-*Ac*-AChBP interactions. Finally, they allow a comparison between the interactions features of the different neonicotinoids with *Ac*-AChBP. More precisely, they throw light on the key role played by *water mediated interactions* in the case of neonicotinoids bearing a nitrile group on the push-pull fragment of the pharmacophore, in contrast to neonicotinoids carrying at the corresponding site a nitro group, which allows *direct interactions* with aminoacid residues of *Ac*-AChBP.
On the whole, the present work provides relevant information on important features of neonicotinoids and an accurate description of their interactions with *Ac*-AChBP, the established model for the ligand binding domain of insect nAChR. As such, it paves the way toward the understanding, at the atomic level, of the toxic effects of neonicotinoids.

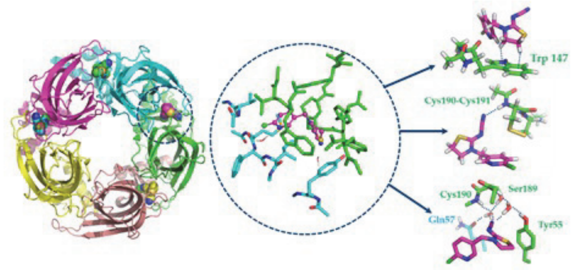

Figure 1. Schematic view of the 3D model considered for the QM/QM′ calculations in the case of thiacloprid.

AGRO 123

Review of crop pests targeted by neonicotinoid seed treatment

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Seed treatment with neonicotinoid insecticides is an increasingly popular crop protection practice, intended to reduce damage due to early season pests. A large proportion of major US crops are planted with neonicotinoid-treated seed. Use of the three most popular neonicotinoids (imidacloprid, thiamethoxam, and clothianidin) now exceeds 1.5 M pounds per year per compound in the US. Studies evaluating the usefulness of neonicotinoid seed treatments in reducing pest pressures and increasing crop yields suggest that response varies by growing conditions, pest populations, and other factors. To determine when and under what circumstances seed treatments would be most effective in specific crops, we reviewed the distribution, ecology, and historical management of target pests in US production of corn, soybean, cotton, and wheat. We summarized the life history, typical damage, historical threat and management, and risk factors associated with crop pests targeted by neonicotinoid seed treatments. This presentation will discuss a selection of the reviewed target pests, which include for corn: wireworms, white grubs, seedcorn maggot, seedcorn beetles, black cutworm, flea beetle, the rootworm complex, thrips, billbugs, stink bugs, sugarcane beetle, chinch bug, and aphids; for soybean: bean leaf beetle, soybean aphid, white grub, wireworms, seed maggots, threecornered alfalfa hoppers, pea weevil, Mexican bean beetle, grape colaspis, potato leafhopper, and thrips; for cotton: wireworms, seedcorn maggot, cutworms, aphids, plant bugs, fleahoppers, and stink bugs; and for wheat: cereal aphids, wireworms, and Hessian fly. This information provides a backdrop against which we will plan and conduct additional research to provide crucial region-and crop-specific information on pest incidence and impact on crop yield, and to assess the current state of knowledge on the utility of neonicotinoid seed treatments to crop production.

AGRO 124

Honeybee health monitoring study in Ontario and Quebec

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A field study was done to search for residues of neonicotinoids in 15 honeybee colonies in 5 apiaries in the corn and soybean growing area of Ontario and Quebec to determine if any bee loss or symptoms of stress were associated with such residues. Increased bee mortality and hive loss have been reported in these areas. The hives were mounted on Beewatch® electronic scales with in-hive probes for temperature and relative humidity. The hives were inspected 5 times during the 2014 season and in the spring of 2015. Live forager bees at the hive entrance, bees inside the hive, older larvae, pollen, honey and wax were sampled. Bees with symptoms attributed to pesticide poisoning were collected when present: dead bees and impaired bees in front of the hive, and black bees inside the hive. Samples were analysed for acetamiprid, clothianidin, imidacloprid, thiamethoxam and the metabolite TZNG. Live bee and larvae samples were assayed for virus diseases using the Quantigene® assay. Hive bees were also assayed for Varroa mites and other diseases. Pollen from foragers was used to identify the food sources of each colony.
Neonicotinoids were found in less than 10% of live bee samples, and the levels were below the NOELs for acute toxicity to honeybees. The aggregate toxicity calculated using the toxic units (TU) method was less than 1.0. The detections of neonicotinoids were not associated with any evidence of stress or bee loss. Black bees and impaired bees had viruses, but pesticides were absent or insignificant. The average TU in brood area bees or foragers during the spring build-up of colonies was not correlated to the weight gain recorded during this time, Pearson’s R 0.4959, 0.4919 respectively, p=0.07, n=14).

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Biological response of earthworm, *Eisenia fetida*, to five neonicotinoid insecticides

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Earthworms (*Eisenia fetida)* are one of the most abundant terrestrial species, and play an important role in maintaining the ecological function of soil. Neonicotinoids are some of the most widely used insecticides applied to crops. Studies on the effect of neonicotinoides on *E. fetida* are limited. In the present work, we evaluated the effects of five neonicotinoid insecticides on reproduction, cellulase activity and the tissues of *E. fetida*. The results showed that, the LC_{50} of imidacloprid, acetamiprid, nitenpyram, clothianidin and thiacloprid was 3.05, 2.69, 4.34, 0.93 and 2.68 mg kg^{-1}, respectively. They also could seriously affect the reproduction of *E. fetida*, reducing the fecundity by 84.0%, 39.5%, 54.3%, 45.7% and 39.5% at the sub-lethal concentrations of 2.0, 1.5, 0.80, 2.0 and 1.5 mg kg^{-1}, respectively. The cellulase activity of *E. fetida* was most sensitive to clothianidin. Significant disruption of the epidermal and midgut tissue was observed after 14 days exposure. In summary, we demonstrate that imidacloprid, acetamiprid, nitenpyram, clothianidin and thiacloprid have high toxicity to earthworm, and can significantly inhibit fecundity and cellulase activity of *E. fetida*, and they also damage the epidermal and midgut cells of earthworm.

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Ecological risk assessment for aquatic invertebrates exposed to imidacloprid due to labeled agricultural and non-agricultural uses in the United States

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A probabilistic ecological risk assessment (ERA) was conducted to determine the potential effects of acute and chronic exposure of aquatic invertebrates to imidacloprid arising from labeled agricultural and non-agricultural uses in the United States. Aquatic exposure estimates were derived using a higher tier refined modeling approach that accounts for realistic variability in environmental and agronomic factors. Toxicity was assessed using refined acute and chronic community-level effect metrics for aquatic invertebrates (i.e., species or taxon sensitivity distributions), developed using the best available data. Acute and chronic probabilistic risk estimates were derived by integrating the exposure distributions for different use patterns with the applicable species or taxon sensitivity distributions to generate risk curves, which plot cumulative probability of exceedance versus the magnitude of effect. Overall, the results of this assessment indicated that the aquatic invertebrate community is unlikely to be adversely affected by acute or chronic exposure to imidacloprid resulting from currently registered uses of imidacloprid in the United States.

AGRO 127

Toxicokinetics of imidacloprid in rainbow trout

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In vivo time-course studies were conducted with rainbow trout to characterize the disposition and elimination of imidacloprid, a neonicotinoid insecticide. Animals confined to respirometer-metabolism chambers were injected with a low (47.6 µg/kg), medium (117.5 µg/kg), or high (232.7 µg/kg) dose of imidacloprid and then allowed to depurate for 36 to 48 h. Plasma and water expired over the gills were sampled from all 3 groups at multiple intervals throughout the depuration period. Urine was collected at these same intervals from the medium dose group. The brain, kidney, liver, white muscle and bile were sampled from each animal at the end of the test. Biotransformation of imidacloprid was evaluated in liver S9 fractions using an *in vitro* substrate depletion approach. The plasma time-course data indicated a brief (4–6 h) distributional phase followed by a log-linear terminal elimination phase. These data were subsequently evaluated by non-compartmental kinetic analysis. Mean fitted values for the steady-state volume of distribution (V_D) were 1.72, 1.81 and 2.23 L/kg, and mean whole-body clearance (CL_{WB}) values were 0.0217, 0.0195 and 0.0270 L/h/kg for the low, medium and high dose groups, respectively. Estimated whole-body half-lives for the same groups were 67.0, 68.4 and 68.1 h, respectively. The two-fold greater V_D over that predicted for relatively polar compounds indicated some distribution of imidacloprid outside of total body water. Tissue:plasma concentration ratios reflected the relative affinity of imidacloprid for individual tissues under near-equilibrium conditions and were consistent with modeled V_D values. A mass balance analysis of expired water data revealed that branchial clearance accounted for approximately 50% of CL_{WB}, while an initial analysis of urine data suggested that renal clearance could account for 25–30% of CL_{WB}. A lack of measurable substrate depletion from the S9 fractions suggested that hepatic metabolism did not contribute to overall clearance. These data provide information required to model uptake, accumulation, and clearance of imidacloprid by fish in known or hypothetical environmental exposures.

AGRO 128

Methods of glyphosate and AMPA analysis

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Increased annual loading of glyphosate to fields has raised concerns of potential nontarget effects and offsite transport of both glyphosate and its principal degradate, aminomethylphosphonic acid (AMPA). To facilitate the environmental research needed to address these concerns, there is a need for simple, fast, efficient, and sensitive methods for their analyses in diverse matrices. While numerous reliable and sensitive analytical techniques have been published, in general they are complex and time consuming on account of glyphosate's physical and chemical properties. Furthermore, these methods were developed primarily for a single matrix, and may not be suitable for other matrices. Following extraction, many methods require extensive sample preparation, including derivatization and clean up, prior to analysis by a variety of detection techniques. In the past 15 years, numerous 'new' or 'revised' methods have been published on the analysis of glyphosate and AMPA in water, plant materials, and soils. These methods often involve modifying details of previously published methods, which can be as minor as changing the pH of the water in the extraction and/or cleanup procedures, changing volumes and/or concentrations of the sample or reagents in the derivatization step, and substituting different separation techniques, or detection systems. Also, the application of a previously published method to a new matrix has served as the basis for some recent publications. Very few articles have been published on multi-matrix methods. This presentation summarizes methods used during the past 15 years for analysis of glyphosate and AMPA in diverse matrices. The simplest methods use aqueous extraction of glyphosate and AMPA from plant materials and soil, no derivatization, solid phase extraction columns for clean up, guard columns for separation, and the analytes are confirmed by mass spectrometry. Anaylites are quantified using isotope-labeled internal standards, with levels of detection below regulatory limits in North America.

AGRO 129

Exposures to glyphosate in bystanders and applicators: A critical assessment

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The recent classification of glyphosate as a probable human carcinogen by the International Agency for Research on Cancer (IARC) was arrived at without a detailed assessment of risk to applicators and bystanders. Glyphosate is widely used as an herbicide, which might result in exposures of the general public and applicators. Exposures were estimated from information in the open literature and unpublished reports provided by Monsanto Company. Based on the maximum measured concentration in air, an exposure dose of 1.04×10^{-6} mg/kg body mass (b.m.)/d was estimated. Assuming consumption of surface water without treatment, the 90th centile measured concentration from samples of surface waters of the USA would result in a consumed dose of 2.25×10^{-5} mg/kg b.m./d. To estimate exposures of applicators, data from published papers and unpublished reports were used. Values from dosimeter studies were normalized for surface area, body mass, penetration through clothing and skin. For biomonitoring studies, measured concentrations in urine were normalized for the level of detection, the duration of measurements, and co-use of dosimeters and/or gloves. For applicators, 90th centiles for systemic exposures based on biomonitoring and dosimetry were 0.0014 and 0.021 mg/kg b.m./d, respectively. All of the measured and estimated exposures were less than the US EPA's normalized systemic RfD of 0.35 mg/kg b.m./d. The exposures were also less than the acceptable daily intakes from the FAO and EFSA, normalized for uptake in the gut (0.2, and 0.1 mg/kg b.m./d, respectively), supporting a conclusion of no unacceptable risk. Dietary exposures and concentrations in water are discussed in presentations by Bleeke et al. and Negley et al. in the session.

AGRO 130

Glyphosate residues in food and feed: Dietary exposure and risk assessment

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Glyphosate is a widely used herbicide that is used in agricultural applications in both conventional and herbicide-tolerant crops. There has been extensive testing done to define and quantitate the glyphosate residues in both crop and animal commodities resulting from these uses, as well as effects of processing on the residues. This presentation will provide an overview of the uses of glyphosate in crops, the potential for residues in food commodities, and the effects of processing on residues. The results of dietary exposure assessments based on food residues will be presented. Using established glyphosate tolerances (Maximum Residue Limits) across all crop commodities, the resulting highly conservative dietary exposure estimate is still well below the allowable daily intake established by regulatory agencies such as the US EPA for the human health risk assessment of glyphosate. By refining the dietary exposure assessment using realistic expected residue levels, amount of use, and reduction in residues as a result of processing, the expected exposure can be shown to be significantly lower than the amount used by regulatory agencies in their risk assessments.

AGRO 131

Glyphosate and AMPA long-term monitoring data trends for surface water and groundwater in the USA

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Long-term and spatially distributed monitoring data can significantly increase the confidence in exposure/risk assessments for crop protection products (CPPs). The US Environmental Protection Agency (USEPA) estimates exposure potential in aquatic environments and drinking water sources using deterministic models for surface water and groundwater. Monitoring data are essential for characterizing the conservatism inherent in modeled estimates compared to measured observations under real-world use of CPPs. This paper provides an assessment of long-term data trends for glyphosate and its primary metabolite, AMPA, within surface waters and groundwater of the USA. We present the results of an extensive literature search to identify surface water and groundwater monitoring data, as well as trends from over 150,000 records obtained from numerous federal and state level data sources, including drinking water systems. Data were compiled in a user-friendly data management system, reviewed, cleaned, and statistically analyzed to assess the overall status of glyphosate and AMPA in or surface water and groundwater of the USA. In addition, the paper covers important data quality considerations when analyzing monitoring data from multiple sources. The results demonstrate that while glyphosate is the most used CPP in the USA and the most used herbicide globally, detection in groundwater is rare across the US, and detections in surface water are well below published surface water standards.

AGRO 132

Glyphosate in the public eye: Science communication, risk perception, transparency and trust

Denneal Jamison-McClung, *dsjamison@ucdavis.edu. Biotechnology Program, UC Davis, Davis, California, United States*

Introduced to the marketplace in 1974, glyphosate has been widely used across the globe in landscaping and agriculture for over forty years. With the advent of glyphosate-tolerant biotech crops in 1996, use of the herbicide increased significantly and glyphosate was inextricably linked to the suite of agricultural technologies defined as "GMOs" in the popular press. Twenty years later, opposition to the use of herbicides in agriculture is an oft-cited reason for general opposition to GMOs. In early 2015, a new global conversation on the use of glyphosate was ignited with the International Agency for Research on Cancer (IARC) categorization of the substance as a "probable human carcinogen". The designation of glyphosate as a category 2a carcinogen has caused a furor in the scientific community and has led to sensationalized reports in the popular press that do not include a balanced discussion of environmental risks vs. benefits. A robust science communication effort is needed to tackle societal chemophobia and misinformation on glyphosate, as well as other modern technologies reliant on complex chemistries. Most consumers are unaware that all agriculture production systems (organic, conventional and biotech) rely on various chemical inputs, whether naturally or synthetically derived. In this session, we will look at science communication strategies to ensure that policy makers, consumers and other members of the general public have a scientifically sound frame of reference for evaluation of the risks and benefits of herbicides, like glyphosate, as well as other molecular and chemically-based agricultural technologies.

AGRO 133

Analysis of the nitrogen stabilizer compound, Nitrapyrin, and its degradate in agriculturally-impacted surface water

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Nitrogen stabilizers are registered in the US for use on corn, wheat, sorghum and strawberries and are co-applied with fertilizers onto agricultural soils. Their purpose is two-fold: (1) keep applied nitrogen fertilizer in the ammonium form to enhance plant uptake and (2) limit the bacterial conversion of ammonium to leachable nitrogen forms such as nitrate. The major active organic compound in nitrogen stabilizers is nitrapyrin, a bactericide that inhibits the activity of soil bacteria *Nitrosomonas*. Studies on nitrapyrin presence, persistence, transport, and effects in agricultural soils and nearby surface waters are limited. If nitrapyrin is persisting in agricultural soils and in corresponding drainage waters, there is concern that it could affect both aquatic and terrestrial organisms and natural bacteria populations. Nitrapyrin has been classified as "moderately to highly toxic" to aquatic organisms and as "suggestive evidence of carcinogenic potential" to humans by the US EPA. In this study, methods were developed to quantify nitrapyrin and its degradate, 6-chloropicolic acid (6-CPA) in water. These methods were used to analyze water samples collected from streams in the Midwestern US. All water samples were processed via solid-phase extraction (SPE) and analyzed using GC/MS. Results from this pilot study will be used to conduct more detailed field studies encompassing multiple surface water and soil sampling events and assessing the effects these compounds might have on natural bacteria populations.

AGRO 134

Improving continuous monitoring of VOC's emissions from alternative fertilizers

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Application of alternative fertilizers, such as biosolids, to agricultural fields is an environmentally-beneficial practice. Concerns regarding nuisance odors caused by specific volatile organic compounds (VOC) have lead to public opposition and may ultimately lead to lack of acceptance of biosolids management programs. Electronic nose sensors are designed to detect differences in complex air sample matrices and have been used in the food industry to monitor process performance and quality control. However, no information is available on the application of sensor arrays to monitor process performance of biosolids treatment processes. The objective of this work, therefore, was to examine the feasibility of an electronic nose to discriminate between treatment conditions of alkaline stabilized biosolids and to explore its performance by comparison with quantitative analysis of key odorants. Seven quick lime treatment rates from 0 to 30% (w/w) were prepared, and the off gas was monitored by with an electronic nose with ten metal oxide sensors. Pattern recognition models were created from the electronic nose data using linear discriminant analysis (LDA) and principal component analysis (PCA). LDA performed better than PCA and showed clear discrimination when single tests were evaluated, although when the full data set was included in the pattern creation, the discrimination of classes (lime dosages) was not as clear as with single test data. These limitations are found to be due to the intrinsic variability of the wastewater treatment process and the low specificity of the sensor evaluated. The electronic nose was able to discriminate from 0%, 15% and 30% lime and, to some extent 25% lime. These data suggest that under-dosed and over-dosed classes may be created to support the alkaline stabilization process of biosolids, assuming that 15% lime is the dosage needed to safely achieve a pH of 12 required for 99% pathogen reduction.

AGRO 135

Assessing the effectiveness of vegetative environmental buffers in mitigating air pollutant emissions from poultry houses

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Over 560 million broilers are produced on the Delmarva Peninsula each year. However, emissions from poultry houses have come under intense scrutiny due to the potential human and environmental effects of the released particulate matter (PM), ammonia, and volatile organic compounds (VOCs). Ammonia and some VOCs are known $PM_{2.5}$ (PM < 2.5 μm) precursors, and odorous VOCs and ammonia put people and animals in an unpleasant environment, and in extreme cases the compounds can cause health issues. Thus, producers need mitigation strategies to control or eliminate the release of these atmospheric pollutants. Vegetative Environmental Buffers (VEBs) planted next to the tunnel fans have been introduced as migration technology to control these emissions. VEBs are vegetation designed as a visual screen, which usually consist of trees, shrubs, grass and other potential plants. Early studies indicate that VEBs are able to reduce air pollutant emissions, but quantitative studies are needed to improve the overall design and assess their effectiveness. In this project, field experiments were conducted at several poultry houses to quantify the efficacy of VEBs in migrating air pollutants. Time-integrated PM, ammonia, and VOC samples were collected at multiple locations and heights. Concentrations in front of and behind the VEBs were compared to evaluate the removal performance. Results showed significant PM and ammonia concentration decreases with VEBs. Ammonia concentrations increased inside the buffer in some of the experiments, suggesting that ammonia might be trapped in between the rows of vegetation. Some volatile organic compounds show decreasing concentration gradients with VEBs present. These results will be used improve VEB design and will used in validating the effectiveness of National Conservative Practice Standard #380 and #420.

AGRO 136

Pesticide volatilization from plant surfaces

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Volatilization flux from the plant surface following application is a potentially important pathway for active ingredient dissipation into the atmosphere. While there is a great deal of

information regarding the process of chemical volatilization and transport from soil surfaces, currently there are limited models and empirical data to estimate volatilization flux of pesticides from plant surfaces. This information is important to fully assess the potential impact of pesticides on residential and bystander exposure, and regulatory agencies have expressed an interest in estimating pesticide evaporation and volatilization losses from plant foliage. Currently, prediction of volatility from leaf surfaces is based on the Woodrow's model which relates pesticide flux to vapor pressure. This presentation describes a novel experimental setup in which two dynamic flux chambers were utilized to estimate the volatilization rate of a semi- volatile pesticide from different plant surfaces. The experimental design is capable of capturing the temporal volatilization flux after pesticide applications to plants. This experimental setup provides controlled conditions to explore the impact of plant surface, temperature and humidity on leaf surface volatilization. The empirical data will be used for parameterization of a pesticide emission model (PEM), currently being developed, to reduce the uncertainty in the predicted volatilization rates. The utilization of empirical flux dataset will improve predictive flux models and ultimately reduce the uncertainties in the prediction of human inhalation exposure.

AGRO 137

Influence of EPA's newer groundwater model (PRZM-GW) on drinking water exposure assessment

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Traditionally, drinking water exposures assessed using the approach developed by the US Environmental Protection Agency (EPA) have shown higher drinking water concentrations from surface water exposures, which were estimated using the screening level FIRST model or the higher tier PRZM-EXAMS model. Ground water exposure estimates by the SCI-GROW model generally showed lower drinking water concentrations. However, there has been a shift toward ground water driving the risk with the introduction of the EPA's newer ground water model, PRZM-GW for drinking water assessment. Moreover, this ground water model generated much higher exposure concentrations under typical environmental conditions for most pesticides than any other EPA drinking water exposure model, including the Surface Water Concentration Calculator (SWCC) that EPA recently developed. This presentation will discuss the impacts of the increased water concentration estimates by PRZM-GW on risk assessment.

AGRO 138

Development of conceptual models for estimating aquatic exposure from the use of pesticides on rice using the pesticide flooded application model

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The Pesticide Flooded Application Model (PFAM) is used by the USEPA to estimate pesticide concentrations in surface water from the use of pesticides in flooded fields, such as rice paddies. PFAM simulates water and pest management practices, pesticide degradation in soil and aquatic environments, as well as discharge of paddy waters to lotic or lentic user defined waterbodies. Here, we present the progress in developing conceptual models and scenarios to use with PFAM for estimating pesticide exposure to human health (drinking water) and aquatic organisms. Conceptual models for drinking water were developed for rice grown in California and Arkansas. Monitoring data were used in the evaluation of the conceptual models. Concentration-adjustment "bias factors" for estimating a true peak concentration were applied to monitoring results with a less than daily sampling frequency.

AGRO 139

Photodegradation of 2,6-dichloro-4-nitroaniline (DCNA) in freshwater and saltwater

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The salinity of seawater can influence both the overall rate of degradation of chemicals and impact the distribution and types of photoproducts from the photodegradation of a pesticide. The fungicide 2,6-dichloro-4-nitroaniline (DCNA) is applied to crops grown in areas near both freshwater and saltwater bodies, and it can enter the surface waters where it is susceptible to photolysis; limited information is published on the photodegradation of DCNA. The photodegradation of DCNA was measured in distilled water, artificial seawater, estuarine water, and phosphate buffer to determine the degree of differences in the degradation rate and product distribution of the compound in various matrices. Solutions of DCNA at a concentration of 1 ppm were prepared and irradiated for 24 hours in an Atlas SUNTEST XXL+ photochamber that mimics the wavelength distribution and intensity of sunlight. Samples were withdrawn at 0, 2, 4, 6, 12, and 24 hours and analyzed for residual DCNA using an Agilent 1260 Infinity High Performance Liquid Chromatograph. The formation of ions such as nitrate, nitrite, bromide, and chloride were measured using a Thermo Dionex ICS-5000+ Ion Chromatograph. The half-life of DCNA in distilled water was calculated to be 7.62 ± 0.094 hours and 7.37 ± 0.279 hours in artificial seawater; statistically there was no significant difference in the degradation rate. Analysis of the quick formation of nitrite and chloride ions, and later formation of nitrate ions, suggests photonucleophilic substitution processes are occurring as the compound is degrading, followed by further degradation of nitrite to nitrate likely also due to photolysis processes. Measurable intermediate photoproducts were analyzed for formation rate; a difference in the concentration of product was noted between distilled water and artificial seawater suggesting salinity affects the rate of formation of this photoproduct.

AGRO 140

Monitoring approaches to provide temporal and spatial context to residential pesticide occurrence in the American river

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Extensive urban stream and river monitoring for residential use pyrethroids has been conducted in California over the last few years, and detectable residues of potential concern have been measured in some samples. Unfortunately, most of these monitoring programs have been based on simple grab sampling and have reported whole water residues; as a result, the temporal and spatial context of biologically relevant pyrethroid residues remains unknown. Consequently, a program was designed to address these questions by employing a spatially robust monitoring design at 8 locations down the length of the lower American River. The design specified multiple replicate sample collection across a variety of time scales (hourly to multi-day) during several dry weather and rainfall driven monitoring events over a 3-year period. Both discrete and depth-integrated water column samples were collected at each location with associated measurements of flow rate, depth and suspended solids concentrations. Both cross-sectional and Lagrangian sampling techniques were utilized, providing spatial characterization both within a river cross-section as well as longitudinally up and down a river reach. Analyses quantified pyrethroid residues and TOC co-occurrence. This rich long-term monitoring dataset, combined with GIS approaches allows a detailed analysis of residential pesticide residue occurrence in space and time and provides context for the program results which show that while higher pyrethroid residues do occasionally occur during storm events, they are spatially confined, transient (on a time-scale of a few hours), rapidly diluted and heavily modified by the presence of dissolved and particulate organic matter. This work also allows data from other monitoring programs and publications to be put into context.

AGRO 141

Theoretical prediction for plant uptake of pesticide from soil

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It is likely that pesticides persisted in soils are subjected to the root uptake by plant. One of ways for assessing this plant uptake is to predict theoretically using a mathematical model equation. In the present study, a plant uptake model was developed to predict the plant uptake of soil persistent pesticides with various parameters such as the pesticide mobility in soil, plant transpiration stream, root-soil transfer rate, plant growth, and pesticide dissipation either in soil or plant. An accuracy of developed model was evaluated by comparing the concentrations calculated by the developed model with the measured uptake concentrations of chlorpyrifos (CP) in lettuce, grown on the treated soil with it. The measured concentrations of CP in lettuce at 21, 30, and 40 d after its transplanting were between 5th and 95th percentiles of model variation. The high correlations between modeled and measured concentrations were found, showing correlation coefficients of > 0.97. In addition, the variation coefficients of mean factors to residual errors were acceptable with a mean value of 17.4 %. Therefore, our results show that the plant uptake model developed in this study could be used as an assessment tool for predicting the plant uptake extent of pesticides persisted in arable soils.

AGRO 142

Mitigating the off-site transport of plant protection products with runoff from golf course turf: Evaluation of management practices and turfgrass variety

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Strategies used to maintain managed biological systems, including golf course turf, often involve application of fertilizer and pesticides to optimize plant health and protection. The transport of applied chemicals with runoff to surrounding surface waters has been shown to result in enhanced algal blooms, promotion of eutrophication or negative impacts on sensitive aquatic organisms or ecosystems. Pesticides associated with the turfgrass industry have been detected in storm runoff and surface waters of urban watersheds; inferring contaminant contributions from residential, urban, and recreational sources. Golf course turf often requires multiple applications of pesticides at rates that exceed those typically found in agricultural or home environments. We will discuss the influence of management practices and turf variety on the volume of runoff and the quantity of nutrients and pesticides transported with runoff. This information is useful to grounds superintendents when selecting best management practices and to scientists seeking data relating runoff to land management for watershed-scale modeling.

AGRO 143

Effects of pesticide application methods on urban runoff of fipronil and its degradation products

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The phenylpyrazole insecticide fipronil and its primary environmental degradation products (collectively, *fipronils*)—fipronil desulfinyl, fipronil sulfone, and fipronil sulfide—are frequently detected in southern California surface water samples as a result of runoff from urban environments. The presence of fipronils in surface aquatic systems has been linked to non-target aquatic invertebrate toxicity. The purpose of this study was to evaluate the effects of three different pesticide application methods on the urban runoff of fipronils, as determined through analysis of runoff water samples, from a wall constructed on a concrete pad at the University of California Riverside and from 21 treated homes in Riverside, California. The standard method for pest management professionals involves applying the pesticide formulation around the perimeter of homes one foot up the wall and one foot out from the wall (1 foot * 1 foot treatment) and applying a 1 inch * 1 inch pinstream on the driveway. This standard method was tested against a 6 inch * 6 inch treatment and a 1 inch * 1 inch (pinstream) treatment. For the home applications, a pinstream was applied to the driveway regardless of the method used on the remainder of the home. The pesticide formulation utilized for all application methods was Termidor™, a fipronil spray. For the constructed wall-concrete pad system (containing 24 total wall segments), runoff of fipronils 1 day after application was significantly lower ($p<0.05$) for the pinstream treatment compared to the 6 inch * 6 inch and 1 foot * 1 foot treatments. No significant differences were observed at 30 days after application. For the treated homes, no significant differences in runoff of fipronils were observed at 1 day or 30 days after application. These results highlight the fact that altering pesticide application methods can impact runoff of fipronils.

AGRO 144

Environmental fate of ^{14}C-niclosamide in laboratory sediment-water systems under aerobic and anaerobic conditions

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Niclosamide [5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide] is a pesticide employed in an integrated program to control the sea lamprey, primarily in the Great Lakes Basin. The environmental fate of ^{14}C-niclosamide (using chlorosalicyclic acid ring- and aniline ring-labeled material) was studied in two laboratory sediment-water systems (“Big Creek,” Michigan; “Goose River,” North Dakota), under aerobic and anaerobic conditions, at an application rate of 0.5 µg/mL, for 100 days. Mean mass balance for each of the study conditions was >90% of applied radioactivity (AR). The amount of radioactivity in the water layer decreased from 49-102% AR at Day 0 to <u><</u>25% at day 100. Sediment-extractable (acidic acetonitrile) radioactivity generally increased over the course of the studies, but reached a plateau of <u><</u>32% AR at day 100. Non-extractable residues increased over the course of the studies, reaching a maximum of 48-86% AR at day 100. A significant portion of the radioactivity remained bound even after exhaustive extractions. Total volatiles were <6% AR. Levels of ^{14}C-niclosamide (total, water and sediment extract) decreased from 96-100% AR at day 0 to <1% AR at day 100. Two significant (>5% AR) metabolites – niclosamide amine and niclosamide formamide – were observed, with the amine at levels as high as 35-38% AR at days 7-14, before decreasing to <16% AR at day 100. Amine levels were higher in the aerobic systems than in the anaerobic systems. The metabolites were confirmed by comparison of HPLC retention time and mass spectra with authentic reference standards, which were synthesized via zinc reduction of niclosamide and characterized in the laboratory for use.

AGRO 145

Comparison of detection techniques for distribution of [14C] residues by HPLC

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Chromatographic techniques are commonly used in conjunction with [^{14}C]radiolabeled test substances for elucidation of metabolic pathways of active ingredients in various environmental, plant and animal studies. A comparison of techniques for detection of radioactive chromatographic components by fraction collection and radioisotope flow through methods are evaluated for the strengths and weaknesses in relation to sensitivity, resolution, and total analysis time per chromatographic run. Traditional technique of fraction collection and liquid scintillation counter (LSC) equipped with a single detector controls peak resolution by the fraction collection time and sensitivity by counting time. However, LSC of collected fractions is time consuming. HPLC equipped with a radioisotope flow detector has the benefit of providing real time monitoring of the radioactive components. Resolution of chromatographic peaks and sensitivity in radioisotope flow monitors are functions of the flow cell volume, flow rate through the counting cell and counting efficiency (liquid cell or solid cell). Detection using radioisotope flow monitors often requires the researcher to compromise between speed of analysis with lower sensitivity than the traditional fraction collection/LSC technique. Introduction of LSC instruments with capabilities to count 96-well plates with multiple detectors provides researchers improvements in counting time over the traditional fraction collection technique. HPLC chromatographic data with [^{14}C] detection will be presented that compares resolution, sensitivity, and time of analysis of the techniques.

AGRO 146

Evaluation of counting efficiency and matrix effects from crop and animal tissues on C_{14} using ultra performance liquid chromatography and microplate solid scintillation counting

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There are many ways to quantify the chromatographic profile of a radioactive sample. By coupling UPLC separation, fraction collection, and microplate solid scintillation counting (SSC), the sensitivity of the method significantly increases. As a result, SSC has gained increasing attention for radiolabeled agrochemical fate and metabolism studies. However, the quench parameters in the SSC analysis cannot be accurately determined at low levels of radioactivity to calculate the counting efficiency, and counting rates (counts per minute, cpm) cannot be readily converted to decay rates (decompositions per minute, dpm) for each UPLC fraction. As a result, the counting efficiency must be consistent or varied within an acceptable range across the entire UPLC run to ensure accurate quantitation of metabolite profiles. However, counting efficiency can be affected by a variety of factors, especially tissue matrix. In this work, the impact of crop and animal tissue matrix on the SSC quantification of two compounds with different polarities will be investigated. The results will be compared between the two different compounds and between the different matrices. Factors affecting counting efficiencies and metabolite quantification will be discussed.

AGRO 147

Investigating the mechanism of picolinic acids sorption to soils

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Picolinic acid has been well studied for its chelation effects on metal adsorption in animals and human. However, the knowledge of its sorption behavior to soils is not well established. In this study, picolinic acid, its isomers and a group of compounds with a picolinic acid functional group will be studied for their sorption to a variety of agricultural soils using the batch equilibrium sorption method. Liquid chromatography with mass spectrometry (LC-MS) will be utilized for quantifying aqueous concentrations. The effect of compound structure, solution ionic strength, pH, and soil characteristics, such as soil texture, pH, cation exchange capacity, etc., will be evaluated to investigate the sorption mechanism of picolinic acid and its structural analogs.

AGRO 148

Transcriptome profiles of *Tropilaelaps mercedesae* parasitizing honey bees

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Tropilaelaps mercedesae (Acari: Laelapidae) is an ectoparasite of the Western honey bee Apis mellifera and has become a major threat to the apiculture in Asia, including Korea. To establish gene resources of T. mercedesae, the whole transcriptome of mixed developmental stages of mites was analyzed by RNA sequencing. An mRNA-focused library was generated from total RNA extracted from the mixed stages (adults, nymphs and larvae) of T. mercedesae using the TruSeq RNA Library Preparation kit and sequenced using the HiSeq 2000 platform. A total of 59,041,198 reads were obtained with 85% Q30 value. Trimmed sequence data was de novo assembled into 64,868 contigs with an average sequence length of 878 bp. Transcriptome annotation to the NCBI non-redundant database resulted in a total of 14,336 Blast hits (22.1%). Functional analysis demonstrated that 11,919 contigs were grouped into 45 Gene Ontology (GO) terms, and 1,226 contigs were assigned to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Based on the transcriptome dataset, a large number of contigs associated with binding, cellular process and catalytic activity were identified. In order to provide basic information for efficient management of potential resistance to fluvalinate, a pyrethroid acaricide most widely used for the control of T. mercedesae, the gene encoding the voltage-sensitive sodium channel (VSSC), which is the molecular target of fluvalinate, was annotated and characterized.

AGRO 149

Behavioral actions of heterocyclic amines on honey bees

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The decline of honey bee (*Apis m.)* colonies presents a challenge to crop pollination services. The unintended exposure to pesticides is an outstanding threat to these pollinators. Current pollinator protection practices necessitate an alternative approach to minimize the contact of bees with harmful pesticides. The use of repellents is one approach to protect bees from pesticide-treated crops. Here, we examine the effect of heterocyclic amines (HCAs) on the locomotor activity of bees as a prerequisite for the discovery of pesticide protection products for these pollinators. The locomotor activity of bees was examined using an EthoVision XT video tracking arena and software platform. Individual bees were transferred to the video tracking arena and allowed to acclimatize at an ambient temperature of 28°C. A 50-µl aliquot of control (DI H_2O) or HCA (100% solution) was placed into the bottom of a petri dish arena (diameter = 9 cm depth = 1.5 cm). Individual bees were then placed into the top of the petri dish arena (diameter = 9 cm depth = 1.5 cm). Exposures to control and HCA compounds lasted 2 min each. The difference in locomotor activity was calculated as the velocity (cm/sec) of the bee crossing the video-tracking arena. Locomotor activity of bees exposed to the HCAs *N*-methylpyrrolidine, 1-ethylpyrroldine, piperidine, and 1-methylpiperidine was significantly reduced by *ca*. 61-68% compared to the locomotor activity of untreated bees. An exposure to HCA *N*-methylpyrroldine also resulted in spastic paralysis and uncoordinated locomotor activity in the bees that was not seen in the other HCA compounds. A multivariate analysis will be discussed as a predictive model of chemical structures, physiochemical properties, and efficacy for the testing of HCA analogs as spatial repellents for manipulating bee behavior.

AGRO 150

In-hive herbicide exposure elicits oxidative stress response in honey bees

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The decline of honey bee (*Apis mellifera L.)* colony numbers in recent years presents an economic and ecological threat to agriculture. One outstanding threat to bees is the unintended exposure to agricultural pesticides. Previous studies report that acute exposures to the common-use herbicide atrazine elicit oxidative stress in non-target insects; however, little information is currently available on the exposure risk of atrazine to bees. This project examined the oxidative stress response of bees following laboratory and field treatments of atrazine. Laboratory experiments were conducted with bees exposed to three sublethal concentrations of atrazine for 24 h whereas field experiments were conducted with bees exposed to one sublethal concentration of atrazine for 28 d. Biochemical and molecular markers of oxidative stress responses were examined in the atrazine-treated bees following the laboratory and field exposure periods. The antioxidant activities of atrazine-treated bees were significantly different compared to the untreated bees in the laboratory and field experiments. A semi-quantitative RT-PCR analysis of antioxidant-encoding genes reveals the differential expression of genes in atrazine-treated bees that are important for oxidative stress tolerance in the laboratory and field experiments. Here, we provide evidence that the laboratory and field exposure of bees to the common-use herbicide atrazine results in oxidative stress responses that can compromise the health of bee colonies. The data will be discussed with regard to the protection of these pollinators against the untended exposure of agricultural pesticides.

AGRO 151

Comparative study of the detoxification of the pesticide inert *n*-methyl-2-pyrrolidone in *Apis mellifera* adults and larvae

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Recent declines in honey bee populations have been linked to several factors including exposure to agrochemicals. This exposure can occur across all castes and stages of development and is not limited to active ingredients alone. Recent work exploring the effect of an agrochemical formulant *N*-methyl-2-pyrrolidone (NMP) has shown that while adult honey bees are tolerant to NMP under chronic oral exposure up to 2,000 ppm, honey bee larvae are sensitive to doses as low as 100 ppm. NMP, which is detoxified via a cytochrome P450 mediated pathway in humans, may be similarly detoxified by honeybees. Using LC-MS, this work will examine and quantify the metabolites generated by honey bee detoxification of NMP, and compare the efficiency of detoxification in adults and larvae. In addition, a fluorometric assay will be performed to measure P450 activity in all experimental groups. The results of this study will inform regulators and the producers of agrochemical products of the risks associated with the use of NMP, and more broadly, it will examine differences between larvae and adults and their capacity to tolerate exposure to this formulation solvent.

AGRO 152

Toxicological risks of agrochemical spray adjuvants and other inactive ingredients to bees

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Agrochemical risk assessment that takes into account only pesticide active ingredients without the other formulation ingredients and spray adjuvants commonly used in their application will miss important toxicity outcomes detrimental to non-target species including pollinators and humans. Lack of disclosure of adjuvant and formulation ingredients coupled with a lack of adequate analytical methods constrains the assessment of total chemical load on beneficial organisms and the environment. Adjuvants and co-formulants generally greatly enhance the pesticidal efficacy and inadvertently the non-target effects of the active ingredient. Spray tank adjuvants are largely assumed to be biologically inert and are not registered by the US-EPA, leaving their regulation and monitoring to individual states. We have found adjuvants like organosiloxane, nonylphenol and octylphenol polyethoxylate surfactants and the co-solvent N-methyl-2-pyrrolidone at ppm levels in beehive samples. These co-formulants at environmental-exposure levels significantly enhance the acute toxicities of the active ingredient residues. Adjuvants like organosilicone surfactants alone are orally and topically toxic to bees, but greater impacts are found on combination with single pesticides or their blends. Organosilicone surfactants are the most potent tank adjuvants and super-penetrants available to growers. Based on the California Department of Pesticide Regulation data for agrochemical applications to almonds, there has been increasing use of adjuvants, particularly organosilicone surfactants, during bloom when two-thirds of USA honey bee colonies are present. Increased

tank-mixing of these with fungicides and insect growth regulators may be associated with recent USA honey bee declines. Organosilicone surfactants are good stand alone pesticides, toxic to bees, and also present in drug and personal care products, and thus represent an important component of the chemical landscape to which pollinators as well as humans are exposed.

AGRO 153

Establishment of pre-harvest residue limit (PHRL) of fungicide pyraclostrobin and insecticide thiacloprid on mandarin during cultivation

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This study was carried out to investigate the residual characteristics of fungicides pyraclostrobin and insecticide thiacloprid in mandarin, and establish pre-harvest residue limits (PHRL) based on dissipation and the biological half-life of pesticide residue. The dissipation of pyraclostrobin and thiacloprid in mandarin was studied under rain sheltering greenhouse condition, in Uigwi-ri (field 1) and Taeheung-ri (field 2), respectively. The pesticides were sprayed onto the crop at the recommended dosage. The samples of mandarin were collected at 0, 1, 3, 5, 7, 10 and 14 days after application. Residual pyraclostrobin and thiacloprid on mandarin were analyzed by validated method with HPLC-DAD. Limit of Quantitation (LOQ) were both 0.02 mg/kg, average recoveries of pyraclostrobin at two fortification levels of 0.2 and 1.0 mg/kg were determined 92.1 ± 0.3% and 96.6 ± 1.8%, and thiacloprid were 90.3 ± 4.2% and 90.7 ± 3.3%, respectively. The residue levesl of pyraclostrobin were 0.23, 0.20 mg/kg at 0 DAT (day after treatment) and decreased to 0.077, 0.066 mg/kg at 14 DAT on field 1 and 2, respectively. The residue levels thiacloprid were 0.17, 0.16 mg/kg 0 DAT (day after treatment) and decreased to 0.042, 0.026 mg/kg at 14 DAT on field 1 and 2, respectively. The biological half-life of pyraclostrobin was about 8.4 days in Uigwi-ri (field 1), and 8.4 days in Taeheung-ri (field 2). The biological half-life of thiacloprid was about 7.3 days in Uigwi-ri (field 1), and 5.2 days in Taeheung-ri (field 2). In the case of application of pyraclostrobin and thiacloprid following guidelines on safe use of pesticides, the final residue level was predicted to be lower than Maximum Residue Limit (MRL, 1.0, 0.3 mg/kg, respectively).

AGRO 154

Agrochemical formulation development: design for sustainability, a paradigm shift in toxicology testing

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In order to meet the increasing expectations for sustainable agrochemicals, the design of new agricultural crop protection products requires consideration of sustainability parameters such as reduced toxicity and improved environmental profiles for the active ingredient and formulated products, while also aligning with the objective to reduce animal use in toxicology testing during the design and development stages.

A case study in formulation development, utilizing a reliable toolbox of animal-free methods for screening acute toxicity potential, is presented to exemplify how recent advancements in computational and *in vitro* approaches provide the opportunity to move beyond animal testing to consistently ensure that new products meet required high standards in bio-efficacy while also delivering added value attributes like safer handling and label classification by design.

The extensive data generated by the authors on a wide range of multi-component complex mixtures (agrochemical formulations) supports that ultimately these tests could be incorporated into an overall weight-of-evidence approach aimed to minimize or even replace the use of animals needed to support the registration dossiers of agrochemical formulations.

AGRO 155

Use of colorants in pesticide formulations

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Due to the toxicological nature of pesticides, their use is highly regulated by the US EPA. While pesticide formulation is a complex one, there are ingredients present in the pesticide formulations that serve functional purposes other than controlling pests. This paper focuses on one of the most important inert ingredients - colorants. This paper consists of five main sections:
(1) US EPA's definition of "pesticide"
(2) Classifications of colorants
(3) Colorants' roles in pesticide formulations
(4) US EPA's requirements on colorants; relevant 40 CFR sections will be examined
(5) Examples of the use of colorant in pesticide formulations

AGRO 156

Discovery and optimization of 1,3-diaryl-substituted heterocycles as novel insecticides

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The control of invertebrate pests is extremely important in achieving high crop efficiency to meet an ever growing agricultural need. This poster presentation will cover the discovery and optimization of 1,3-diaryl-substituted heterocycles as a novel and effective insecticide for crop protection. The chemistry, biology, and structure activity relationship will be presented.

AGRO 157

Cloning and functional characterization of inward rectifying potassium (Kir) channels from arthropod salivary glands

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Ticks are potentially deadly pests to both, humans and animals, as they are primary vectors for a multitude of diseases. Control of tick populations has become increasingly difficult due to increasing levels of acaricide resistance, which has highlighted the need for an increased understanding of the physiological systems within tick tissues that may serve as a novel target site of control. Ticks are hematoghagous arthropods and therefore rely on the multifunctional and morphologically complex salivary gland to ensure adequate blood meal acquisition. Studies have shown that salivary gland function is dependent upon an immediate increase in membrane potassium conductance. Due to this, our research is intended to characterize a ‘superfamily’ of potassium channels, known as inward-rectifying potassium (Kir) channels, by investigating the physiological role these channels have in arthropod salivary glands. Proof-of-concept feeding studies were performed in *Drosophila melanogaster* to determine the influence Kir channel inhibition has on sucrose

consumption. Preliminary data suggest that pharmacological inhibition of Kir channels significantly ($P<0.0001$) reduces the total volume of sucrose solution ingested by individual flies. Furthermore, genetic knockdown of salivary gland specific Kir channels increased the time required to obtain a complete meal by approximately 3-fold, suggesting a reduced salivary gland function in adult *Drosophila*. Due to the success of these studies, we have identified candidate genes encoding Kir channel subunits for two tick species, *Ixodes scapularis* and *Dermacentor variabilis*. Primers were designed to detect the tissue-specific expression of these genes that will enable cloning of the open reading frame and subsequent development of species-specific antibodies. The results of the ongoing investigation into the sub-cellular expression patterns and physiological role Kir channels play in the primary function of tick salivary glands will be presented.

AGRO 158

Cardiac regulation of viral infection in a model social insect

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Viral infections are thought to be a significant factor contributing to declines in the number of managed honey bee, *Apis mellifera* L., colonies. Recent studies have demonstrated an important role for inwardly-rectifying ATP-sensitive potassium (K_{ATP}) channels in *Drosophila melanogaster* antiviral immunity, but no information is available on their role in the heart-specific regulation of bee immunity. Consequently, it is important to understand the homeostatic mechanisms that permit bees to tolerate persistent viral infections. This research is intended to identify and characterize K_{ATP} channel subunits in bees and to investigate the role that K_{ATP} channel regulation plays in attenuating bee viral infections. Candidate genes encoding inwardly-rectifying potassium channel subunits and the sulfonylurea receptor subunit that likely comprise bee K_{ATP} channels have been identified. Primers and probes were designed to detect the tissue-specific expression of these genes. Effects of the direct application of K_{ATP} channel agonists and antagonists on bee heart rate were assessed by exposure and observation of the dorsal vessel. A protocol will also be described for assessing effects of K_{ATP} channel agonists and antagonists on viral infection rates in bees. The tissue-specific expression levels of these identified bee K_{ATP} channels will be reported, as will results of the pharmacological characterization of bee cardiac regulation by K_{ATP} channel modulators. Finally, the results of the ongoing investigation into the role that K_{ATP} channels play in the cardiac regulation of viral infection in bees will also be presented. This work provides needed insight into the biology of a valuable model insect and a powerful tool to screen ion channel-targeting chemistries, including pesticides, for negative effects on bee cardiac function.

AGRO 159

Monoterpenoid derivatives as biorational mosquito repellents

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Various plant essential oils have long been known to possess fumigant and repellent activities against insects, an effect that has often been shown to be attributable to monoterpenoids, phenylpropanoids, and other secondary plant metabolites. However, these compounds, particularly when isolated from the whole oil, tend to be relatively volatile, and may quickly dissipate when applied to a surface, resulting in reduced spatial and contact repellency within hours.

Simple functional group transformations provide a facile way to modify monoterpenoids to increase repellent activity while keeping much of the parent structure intact and maintaining or improving upon the original desired properties of monoterpenoids. A series of derivatives and analogues were synthesized using a single functional group transformation of readily available monoterpenoids, and these compounds were then tested on the Northern House Mosquito (*Culex pipiens*) for both spatial and contact repellency.

Spatial repellency was determined using a static air chamber, in which a filter paper treated with 1 ml of 0.5% repellent is placed at one end of a sealed tube containing mosquitos, and repellency was determined as the ratio of the difference between the number of mosquitos on the untreated side and treated side, and the total number of mosquitos in the tube. Long-term repellency was determined by allowing the treated paper to be exposed to open air for six hours before beginning the static air test. A contact irritancy high-throughput bioassay was used to determine efficacy of a compound's contact repellency. While the derivatized compounds showed a broad range of efficacy, many were competitive with *p*-menthane-3,8-diol, a commercially-used botanical mosquito repellent, in both spatial and contact repellency, and several derivatives showed efficacy similar to DEET

AGRO 160

Exploring the relationship between PaOA$_1$ receptor modulation and the insecticidal character of monoterpenoids

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Octopamine is a biogenic amine that has been implicated in the regulation of numerous physiological roles, such as reproduction, learning-and-memory, and nerve stimulation, to name a few. Previous studies have demonstrated that octopamine has a limited role in mammalian systems and thus octopamine receptors represent a promising target for the development of safer insecticides. Compounds identified to act upon these receptors would thus have little to no effect in mammalian and other non-target organisms. To date, we have developed a line of Chinese Hamster Ovary cells (CHO) that are stably expressing an α-adrenergic like octopamine receptor that has been isolated from the American cockroach (*Periplaneta americana*). Functionality of this receptor was screened using a calcium liberation detection assay using Fluo-4 NW dye (Invitrogen Tech.) Functional screening determined that octopamine is the preferred ligand with an EC50 of 89.6nM and tyramine, a closely related biogenic amine, binds with a lower affinity with an EC50 of 463nM. To date, we have made significant progress screening monoterpenoids against this receptor for positive and negative modulation. Our goal is to further characterize the biological significance of positive and negative modulation in this receptor by comparing this data set to American cockroach injection assays. This will allow us to assess to what degree octopamine receptor binding relates to insecticidal character. So far, we have characterized the toxicity of more than 15 monoterpenoids that have also been screened for positive and negative modulation of the octopamine receptor. This project will better elucidate which monoterpenoid characteristics modulate receptor binding, and whether or not this bioactivity is related to insecticidal potential.

AGRO 162

Molecular mechanisms of action of DDT and pyrethroid insecticides

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DDT and pyrethroids affect the function of voltage-gated sodium channels (Na_v), which are critical for electrical signaling in excitable cells. Numerous studies have shown that DDT and pyrethroids prolong the opening of Na_v channels by inhibiting channel deactivation and inactivation and stabilize the open state of sodium channels. To date, more than 50 Na_v mutations have been identified that are associated with resistance to pyrethroids in various arthropod pests and disease vectors, and some of them are also responsible for DDT resistance. Elucidation of mechanisms of pyrethroid resistance led to the identification of two DDT/pyrethroid receptor sites on the Na_v channel, and provided the molecular basis of the action of DDT and pyrethroids on Na_v channels. Besides the canonical Na_v channel, there is a sodium channel-like cation channel called DSC1 (*Drosophila* sodium channel 1 in *Drosophila melanogaster*). Functional analysis of the DSC1 channel and a DSC1 ortholog from the German cockroach, BSC1, in *Xenopus* oocytes revealed that BSC1/DSC1 channels represent a novel family of voltage-gated cation channels with high permeability to Ca^{2+}. DSC1 knockout flies confer resistance to DDT, but exhibit enhanced sensitivity to pyrethroids, suggesting distinct roles of the $DmNa_v$ and DSC1 channels in the neurotoxicology of DDT vs. pyrethroids and implicate the exciting possibility of using DSC1 channel blockers or modifiers in the management of pyrethroid resistance.

AGRO 163

Novel *Musca domestica Vssc* mutations and their role in insecticide resistance

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Mutations in *Vssc* (*voltage sensitive sodium channel*) can cause resistance to pyrethroid insecticides. While many different mutations have been described from multiple insect species, how many different resistance mutations are found in a single species is not usually known. For example, in *Musca domestica* the only known mutations are L1014F/H and M918T+L1014F. We selected field collected house flies with pyrethroids, genotyped individuals, discovered new *Vssc* mutations, and isolated strains having only these alleles. Bioassays with a range of structurally diverse pyrethroids reveal unique patterns of cross-resistance associated with each mutation.

AGRO 164

Targeting voltage-gated sodium channels for insect control: Past, present and future

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Voltage-gated sodium channels play a fundamental role in neuronal signaling. The crucial importance of sodium channels in nerve function is evident in the great structural and pharmacological variety of naturally-occuring neurotoxins produced by plants, animals and microorganisms that disrupt channel function, thereby contributing to the chemical warfare of predation and defense. The importance of voltage-gated sodium channels as insecticide targets was first established by the discovery of the natural insecticide pyrethrum more than two centuries ago. Since then, the empirical search for new insecticidal agents has "rediscovered" the sodium channel as a target many times, exploiting not only the receptor site for pyrethrum constituents and their synthetic analogs, the pyrethroids, but also other receptor sites on the sodium channel protein. This presentation reviews the history of sodium channel exploitation for insect control, the current status of and challenges for sodium channel-directed insecticides, and the likely durability of sodium channels as targets for the continued development of insect control agents.

AGRO 165

Modulators of insect nicotinic acetylcholine receptors with special reference to flupyradifurone

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Insect nicotinic acetylcholine receptors (nAChR) belong to the larger family of cys-loop ligand-gated ion channels. These receptors are composed of five subunits, four trans-membrane and an extracellular domain known to comprise binding sites for competitive modulators. Insects encode a number of different nAChR subunits, but the exact subunit composition of individually described receptor subtypes remains largely unknown. The receptor plays a central role in the mediation of fast excitatory synaptic transmission in the insect central nervous system (CNS). The discovery and development of modulators selectively addressing the insect nAChRs for the use as insecticides was a major milestone in applied crop protection research, as numerous products based on such compounds revolutionised efficient crop protection measures in a vast range of agricultural settings to guarantee sustainable yields. These compounds opened a new innovative era in the control of some of the world’s most devastating pest insects due to their high target-specificity and versatility in application methods. In addition to their importance as insect control agents in agriculture, these insecticides also contributed massively in extending our knowledge in insect nAChR biochemistry. The global economic success of competitive modulators of insect nAChRs as insecticides renders these ion channels as one of the most attractive target-sites for exploration in insecticide discovery. One of the latest competitive modulator of insect nAChRs discovered and globally registered for pest insect control is the butenolide insecticide flupyradifurone. The principal idea to explore butenolide chemistry was inspired by the naturally occurring stemofoline alkaloids, isolated from the Asian medicinal plant *Stemona japonica*, and described as nicotinic acetylcholine receptor agonists. Based on the lactone “head group” as a pharmacophore of plant-derived stemofoline and in combination with molecular modelling studies using structural motifs of other competitive modulators, a new bioactive butenolide scaffold was identified, which finally resulted in the synthesis of flupyradifurone.

AGRO 166

RNA A-to-I editing: A mechanism that broadens the pharmacological properties of the mosquito GABA receptor

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The insect RDL receptor (resistance to dieldrin) is a γ-amino butyric acid (GABA)-gated chloride channel and a target for insecticides. The nervous system is highly RNA edited and RDL from the dipteran *Drosophila melanogaster* is known to undergo adenosine-inosine editing, leading to changes in receptor sensitivity to GABA and insecticides. However, it is not known how RNA editing affects RDL receptor sensitivity in a disease vector.

Rdl was amplified from *Culex pipiens*, *Aedes aegypti* and *Anopheles gambiae* genomic and cDNA to identify RNA editing sites. *C. pipiens*, *Ae. aegypti* and *An. gambiae* were found to contain four, six and seven RNA editing sites respectively, recoding the genome to generate species-specific isoforms. To determine the impact of RNA editing on the pharmacological properties of a mosquito GABA receptor, we analysed over 100 cDNA clones of *An. gambiae Rdl*. The seven RNA editing sites were detected in 22 different edit combinations. Edited and non-edited isoforms of *An. gambiae* RDL were expressed in *Xenopus laevis* oocytes and the effects of editing on GABA, fipronil and ivermectin potency were assessed using two-electrode voltage clamp electrophysiology.

Electrophysiology results showed that the editing profile generates a spectrum of sensitivities to GABA, highlighting the functional diversity that is created by RNA editing. Furthermore, whilst no influence on fipronil potency was observed, editing at all sites significantly reduced the sensitivity of RDL to the antagonistic actions of ivermectin. These results highlight how RNA editing can generate functional diversity in a receptor between species even when they appear identical at the genomic level. Altering the editing profiles of RDL may also be a mechanism for resistance to ivermectin, an antiparasitic drug that is of interest in reducing malaria transmission through vector control.

AGRO 167

Insect ligand-gated ion channels as targets for insecticides

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The Cys-loop ligand-gated ion channels (LGICs) are membrane-bound glycoproteins that are comprised of five subunits possessing four transmembrane segments. LGICs open their built-in ion channel in response to binding of neurotransmitters to the orthosteric site. They play pivotal roles in neural transmission at neuronal and neuromuscular junctions of insects and vertebrates. Hence, they are important targets of insecticides. Here the author presents mode of actions and mechanism of selectivity of synthetic and natural compounds acting selectively on insect LGICs, focusing on some results obtained in collaboration with Prof. Yoshihisa Ozoe, the award winner in 2016.

AGRO 168

Mechanisms of resistance to insecticides targeting RDL GABA receptors

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Lindane and cyclodienes, such as dieldrin and α-endosulfan, represent the first generation of noncompetitive antagonists (NCAs) against insect RDL GABA receptor. It has been reported that A2'S and A2'G mutations in the membrane-spanning region M2 of the RDL GABA receptor confers resistance to lindane and cyclodienes. Fipronil is a second generation NCA, but A2'S and A2'G mutations provide a low level of cross-resistance to fipronil.

A novel A2'N mutation that confers fipronil resistance was found in M2 of the RDL GABA receptors from fipronil-resistant planthoppers, *Sogatella furcifera* and *Laodelphax striatellus* in the heterozygous state in Japan. Thus, problems are being caused by a worldwide outbreak of cyclodiene-resistant pests. Attention must be paid to development of fipronil-resistant pests of rice, such as *Sogatella furcifera*, *Laodelphax striatellus*, in Japan.

AGRO 169

Mechanisms of pyrethroid degradation on urban surfaces

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Intensive pesticide use occurs not just in agriculture, but also in urban areas to control the populations of pests such as ants and termites. However, urban areas contain a high percentage of impervious surfaces which facilitate the offsite transport of organic pollutants via runoff. Previous research has documented the presence of pyrethroids in urban surface waters, determined that the levels of pyrethroids in urban streams are large enough to elicit toxic effects, and traced the source of pyrethroids to runoff from residential neighborhoods. Recent research has shown that degradation products of the pyrethroids may have toxic effects as well, but few studies have been conducted regarding the occurrence of the main degradation product 3-phenoxybenzoic acid (3-PBA) in the environment. Furthermore, little is known about pyrethroid degradation mechanisms on urban impervious surfaces. In this study, the occurrence of pyrethroids and 3-PBA in two urban streams was monitored monthly for one year and the role of pyrethroid photodegradation on concrete was examined. Seven pyrethroids were spiked onto concrete cubes or glass at a rate of 1 μg cm^2 and then placed either in the dark, outside receiving natural sunlight, or in a UV chamber. The dissipation of the parent pyrethroids and corresponding production of the degradation product 3-PBA was then measured over time. Results showed that the production of 3-PBA was greater under light conditions and was also greater on the concrete cubes than on glass, which indicates that the high pH of concrete may induce base-catalyzed hydrolysis of the ester bond present in pyrethroids. Similarly, 3-PBA was commonly detected in urban streams that receive storm water runoff from residential neighborhoods. These results indicate that a potential source of toxicity is currently being overlooked and that further monitoring and toxicological studies should be conducted to assess the environmental risk of 3-PBA.

AGRO 170

Assessing the effects of urbanization on the environment with soil legacy and current-use insecticides: A case study in the Pearl River Delta, China

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To evaluate the impacts of anthropogenic events on the rapid urbanized environment, the levels of legacy organochlorine pesticides (OCPs) and current-use insecticides (CUPs), i.e., dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorocyclohexanes (HCHs), pyrethroids and organophosphates in soil of the Pearl River Delta (PRD) and surrounding areas were examined. Spatial concentration distributions of legacy OCPs and CUPs shared similar patterns, with higher concentrations occurring in the central PRD with more urbanization level than that in the PRD's surrounding areas. Furthermore, relatively higher concentrations of OCPs and CUPs were found in the residency land than in other land-use types, which may be attributed to land-use change under rapid urbanization. Moderate correlations between gross domestic production or population density and insecticide levels in fifteen administrative districts indicated that insecticide spatial distributions may be driven by economic prosperity. The soil-air diffusive exchanges of DDTs and HCHs demonstrated that soil was a sink of atmospheric *o*,*p*'-DDE, *o*,*p*'-DDD, *p*,*p*'-DDD and *o*,*p*'-DDT, and was a secondary source of HCHs and *p*,*p*'-DDT to atmosphere. The soil inventories of DDTs and HCHs (100 ± 134 and 83 ± 70 tons) were expected to decrease to half of their current values after 18 and 13 years, respectively, whereas the amounts of pyrethroids and organophosphates (39 and 6.2 tons) in soil were estimated to decrease after 4 and 2 years and then increase to 87 and 1.0 tons after 100 years. In this scenario, local residents in the PRD and surrounding areas will expose to the high health risk for pyrethroids by 2109. A strict ban on the use of technical DDTs and HCHs and proper training of famers to use insecticides may be the most effective ways to alleviate the health effect of soil contamination.

AGRO 171

Environmental degradation of imazosulfuron

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Assessing the degradation processes that remove agrochemical residues from the environment is important in order to avoid potential adverse effects. For the herbicide imazosulfuron (IMZ), aqueous photolysis and biotic soil degradation seem to be the most important degradation routes. These transformation processes were evaluated and compared in simulated California rice field conditions. Degradation of IMZ via simulated sunlight was investigated. Modern approaches for exploring photolytic rates were employed to elucidate reaction pathways and the relative contribution of indirect photolysis to the overall photolytic rate. In CA, a new commercial product for control of weeds has been introduced, containing IMZ and thiobencarb. Thiobencarb has a long history of use in CA rice fields, while IMZ was more recently registered and has not yet been widely applied. Pesticide application can alter the community of soil microorganisms and may therefore also change their ability to degrade a contaminant. Degradation of IMZ and thiobencarb in rice field soil was compared between mixed applications (reflective of the commercial product's formulation) and single-chemical applications.

AGRO 172

Transformation of atrazine, 2,4-D, and 2,4,5-T on simulated leaf surfaces

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Atrazine, 2,4-D, and 2,4,5-T are commonly used herbicides in agriculture and home and garden sectors. Previous literature has been focusing on the transformation of these compounds in aqueous environment; the transformation on plant surfaces, however, has not been thoroughly investigated as a component of the fate models, despite the common foliar application mode. This study explores the transformation of atrazine, 2,4-D, and 2,4,5-T on leaf surfaces via sunlight photolysis, oxidation by airborne oxidants ozone or nitrogen oxides, or the combination of photolysis and oxidation. Experiments were conducted in 1) a fritted gas-washing reactor containing aqueous herbicide solutions, and 2) a gas tight reactor with a quartz irradiation window and herbicide spread on a paraffin film placed in the reactor. Direct photolysis and oxidation by ozone and nitrogen oxides all contributed to atrazine decay, but nitrogen oxides alone lead to the formation of *N*-nitroso derivatives at levels relevant to ambient air. 2,4-D and 2,4,5-T experienced faster decay via direct photolysis than atrazine, but slower oxidation by ozone. Attempts were made to quantify light exposure on the paraffin surface.

AGRO 173

Assessing exposure to semi-volatile pesticides from treated agricultural fields

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The United States Environmental Protection Agency's (USEPA) Office of Pesticide Programs (OPP) is responsible for regulating pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). OPP conducts environmental exposure assessments for pesticides, some of which are volatile or semi-volatile. Ambient air monitoring data suggest pesticide volatilization may be a major route of dissipation even when predictions based on physical-chemical properties alone suggest otherwise. This presentation will provide a case study for estimating exposure to semi-volatile pesticides based on available air monitoring data, including field volatility studies.

AGRO 174

Community multi-scale air quality (CMAQ) modeling effort for pesticide emissions

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The objective of this project is to simulate ambient gas phase and aerosol phase concentrations of a pesticide active ingredient, resulting from post-application emission flux, chemical and physical processing, and transport using photochemical air quality modeling. The concentrations were modeled using the U.S. Environmental Protection Agency's Community Multi-scale Air Quality (CMAQ) Model. A base-case CMAQ model was developed using a 12-km grid covering the contiguous United States for the 2011 emission year. The Sparse Matrix Operator Kernel Emissions (SMOKE) modeling system was used to generate the emissions during a 5-month period over the summer (May-September). Emissions were estimated for a variety of crops based on pesticide emission flux data from application on crops (tomato, potato, peanut, etc.) over a 10-week period beginning in May. The flux data were generated using the pesticide emission model (PEM). Sensitivity analyses were conducted to investigate secondary aerosol formation from the active ingredient and to investigate partitioning directly from the gas phase to the aerosol phase. Modeled gas phase and aerosol phase levels from the active ingredient for May through September 2011 will be discussed. Overall, the gas phase concentrations decreased by ~25-33% when the atmospheric oxidation reactions were included in the model. Depending on the properties of the oxidation products, there may be more semi-volatile compounds that would partition into the aerosol phase than were represented in the model. Without knowing the oxidation product parameters, there is uncertainty associated with the predicted aerosol concentrations. However, the dominant removal pathway of the gas phase concentrations is through the oxidation reactions, rather than direct partitioning to the aerosol phase.

AGRO 175

Regulatory perspectives on protein detection for agricultural biotechnology

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Characterization and quantitative detection of novel proteins expressed in genetically engineered (GE) crops are essential for product characterization and risk assessment. The level of GE protein expressed in plants informs the exposure assessment for environmental and food and feed risk assessments as well as being a reference for dose selection for toxicological studies. Accurate and timely measurement of a GE protein in plant matrices can be challenging due to the nature of proteins. Multiple advanced analytical technologies have been recently developed and applied for GE protein quantitation. In this talk, the basic principles and challenges of selecting a suitable method for regulatory studies will be presented using case studies. In addition, regulatory requirement and considerations for protein detection methods will be discussed.

AGRO 176

Multiplex approach for the analysis of peanut allergens using liquid chromatography-tandem mass spectrometry

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In the US, up to 80% of allergic reactions are caused by proteins from peanuts (*Arachis hypogaea*) or tree nuts, affecting ~1% of the population, with prevalence steadily rising. To date, at least 12 unique protein allergens have been identified from peanuts, ranging from 10-70 kDa in molecular weight. Enzyme-linked immunosorbent assays (ELISA) are commonly used to screen for peanut protein allergen contaminants, but are susceptible to false negatives/positives in complex food matrices and are limited in specificity. The detection limits are typically 1-2 ppm, which is appropriate for this application, but the data obtained are usually qualitative or semi-quantitative. Confirmatory methods are therefore needed, and liquid chromatography with tandem mass spectrometry (LC-MS/MS) has been demonstrated as a highly specific and sensitive method for the detection and identification of peptides from trypsin digests of proteins. Here we demonstrate a simple and effective method for isolating and detecting peptides from peanut proteins using LC-MS/MS with multiplexing analysis. Method development included evaluation of different approaches for extracting and digesting proteins in food matrices, either sequentially or simultaneously. Isolation of the tryptic digest peptides was accomplished using molecular weight cut-off filters (FASP^{TM}) and appropriate marker peptides were then identified using LC with high resolution mass spectrometry. Finally, LC/MS/MS was used to evaluate limits of detection and the overall quantitative performance of the method. Results will be compared with those previously reported from ELISA and recommendations will be made as to when confirmatory methods should be used to support ELISA screening results.

AGRO 177

Quantification of membrane proteins in genetically engineered crops by liquid chromatography coupled with tandem mass spectrometry

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The detection and quantification of target proteins in genetically modified crops has been accomplished primarily by immunoassays such as enzyme-linked immunosorbent assays (ELISA). However, ELISAs require high quality antibodies that may be difficult and time-consuming to produce, and ELISAs may not work for membrane proteins. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has been used for many years to quantify pharmaceuticals and pesticides, among other small molecules. In recent years, LC-MS/MS has also been applied to quantify proteins in transgenic crops in multiplexed and high throughput assays. We developed and validated LC-MS/MS assays to quantify two membrane proteins, zmARGOS8 and gmDGAT1, expressed in maize leaf and soybean seed, respectively. gmDGAT1 has nine transmembrane domains making solubilization and the production of antibodies challenging. With LC-MS/MS, target proteins are quantified via peptide surrogates and therefore denaturing conditions may be used to extract the proteins. An extraction buffer containing CHAPS and Triton X-100 was determined to be suitable for both zmARGOS8 and gmDGAT1. Both methods exhibited high recovery and good reproducibility. Recombinant proteins that had been expressed and purified from baculovirus or bush bean for zmARGOS8 and gmDGAT1, respectively, were used to prepare standard curves with good linearity and accuracy. This study demonstrates the suitability of LC-MS/MS for quantification of membrane proteins in transgenic crops.

AGRO 178

ELISA validation and correlation to mass spectrometry

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Protein content in early stage research samples is now often measured by mass spectrometry. This technique allows for shorter assay development time and reduced assay development cost since there is no need to generate validated antibodies required for immuno-based detection. However, ELISA has a long tradition of use in agricultural biotechnology. The method offers specificity to the protein of interest and is easily scalable to meet the demands of large scale research studies necessary for product approvals. Once a project advances in the product pipeline, significant work goes into developing and validating each ELISA method prior to running the first sample. Experiments are designed to address assay accuracy, stability, sensitivity and robustness. This talk will illustrate the validation criteria, specifically around ELISA methods, that are used for regulatory studies. Assay accuracy will be demonstrated by presenting the correlation of results between ELISA and mass spectrometry assays.

AGRO 179

Targeted protein quantification by LC-MS/MS: Applications in the agricultural biotechnology

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Immunoassays like Western blot and enzyme-linked immunosorbent assays (ELISA) are widely used for transgene protein detection and quantification, and they have played a very important role in the agricultural biotechnology traits research, development, and safety characterization in the

1980s. However immunoassays come with many limitations including uniplexing, the need of antigens and high quality antibodies, and long assay development/validation time (turnaround time 10-20 months). It is possible but very challenging to differentiate closely related mutant proteins. More importantly, no one is sure whether an assay works or not until tested with real transgenic samples. Additional protein detection/quantification challenges are presented by the product pipeline changes in our agricultural biotechnology industry as we are moving from mainly foreign, soluble, relatively high expression proteins (such as Cry and EPSPS) to a broad range of proteins including endogenous and/or membrane proteins with low expression. Recent technology development makes it possible to develop a liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) based protein detection/quantification method in 10-20 days if not 10-20 hours. This is achieved without the need of antibodies or even protein standards. Many other advantages of this new technology including predictable/superior selectivity at molecular (protein sequence) level, multiplexing, being able to quantify intractable membrane proteins and endogenous proteins will be discussed.

AGRO 180

Development of multiplex LC-MS/MS strategies for the quantitation of plant-expressed proteins

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Since the early commercialization of genetically modified (GM) crops in the mid-1990's, there has been a need in the field of agriculture to quantify transgenic proteins in plants. The quantitation of proteins in complex plant matrices requires robust analytical platforms with high sensitivity and specificity. The measurement of protein levels in GM crops has been achieved almost exclusively by immunoassays, such as enzyme-linked immunosorbent assays (ELISA); however, immunoassays rely upon the use of antibodies which are both highly specific and sensitive to the protein of interest, but may not be readily available in the early stages of the discovery process. In addition, many of the latest GM crops provide multiple modes of protection to the plant through the use of 'stacked traits' resulting in the simultaneous expression of several transgenic proteins. For product development, the expression level of each protein must be measured. There has been recent interest in developing LC-MS/MS based methods to quantify several plant-expressed proteins in a single analysis. These 'signature peptide' analyses involve tracking protein expression levels by quantifying several highly specific digest fragments of the proteins of interest. This is typically accomplished using liquid chromatography coupled with selected reaction monitoring (SRM) tandem mass spectrometry. Several multiplexed LC-MS/MS platforms have been developed and utilized to quantify plant-expressed proteins in matrix with minimal sample manipulation. These methods have been evaluated for analytical figures of merit including accuracy, precision, linearity, limits of detection and quantitation; and for other considerations including sample throughput, transferability, and ease of use. Subtle differences in protein expression levels among various tissue types and growth stages in several crops were readily detected using the described LC-MS/MS technology. Parallel analysis of samples using ELISA demonstrates that LC-MS/MS can be used to complement or replace existing strategies to quantify multiple proteins in GM crops.

AGRO 181

Assessing risks of pesticides to bees: Challenges and opportunities

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Pesticides have been identified as one of multiple factors associated with declines in pollinator health in both North America and in Europe. The US Environmental Protection Agency has been working with its international regulatory counterparts to identify and implement tests for measuring exposure to and effects from pesticides on both honey bees (*Apis mellifera*) and non-Apis bees such as bumble bees (*Bombus* spp), mason bees (*Osmia* spp), and stingless bees (*Meliponini* spp). These efforts compliment goals identified in President Obama's 2015 National Strategy for protecting pollinator health. The National Strategy and the 2012 EPA White Paper on a conceptual framework for assessing risks of pesticides to bees, discusses multiple uncertainties related to assessing exposure and effects to individual bees (representing solitary bees) and bee colonies (representing social bees) not only from individual pesticide use, but also from pesticides that may be applied in combinations through co-formulations or tank mixes. This presentation will discuss the current battery of tests, efforts to advance new methodologies and predictive tools, and potential partnerships that may accelerate the process and achieve a greater economy of effort. **The information contained in this presentation may not necessarily reflect the position of the US Environmental Protection Agency or the US Government.

AGRO 182

Current advancements for evaluating the risk of agrochemicals to developing bees

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Pollinators are essential for agriculture. Honey bees, as well as many species of wild bees, may be exposed to environmental toxins during foraging. Laboratory bioassays are conducted to determine the toxicity of a compound for use in a risk assessment. The larval toxicity, repeated exposure test is designed to measure the rate of survival of bee brood as they develop from larvae to pupae to enclosed adult workers when exposed to a toxin during the larval stage of development. This test design has historically proven to be challenging to meet validity criteria and produce consistent results. Recent modifications to the larval toxicity, repeated exposure methodology, were developed at the University of Florida and were adopted for a 2016 ring test with the purpose of evaluating pupal survival through adult eclosion. While substantial advances in this methodology have improved survival and repeatability, there exist differences in how measurement endpoints are generated and interpreted. Here I present an overview on the larval toxicity, repeated exposure methodology and how to appropriately generate and interpret endpoints for use in risk assessment.

AGRO 183

Consideration of increased tolerance of eusocial bees to toxins for risk assessment

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The ability of honeybees to forage for nectar and pollen that contains natural toxins has been recognized for many years and appears to be an evolutionary advantage for eusocial behavior. More recently, it was reported that honeybees have fewer genes for detoxification than other insects, with a suggestion that this makes them more susceptibile to pesticides. However there is an alternate interpretation of this. In a field study done to monitor hive health and pesticides in honeybee colonies, live forager bees at the hive entrance, bees inside the hive, older larvae, pollen, honey and wax were sampled. The samples were analysed for acetamiprid, clothianidin, imidacloprid, thiamethoxam and the metabolite TZNG. Pollen from foragers was used to identify the food sources of each colony. A clearly defined pattern of exposure and concentrations was observed.

While the main route of entry of toxic materials into the hive is in pollen carried by foragers, the movement and fate of residues inside the colony is determined by the social structure of the colony and the ability of worker bees to rapidly metabolize toxic materials. Thus, young larvae and queen bees are fed exclusively with royal jelly, a glandular secretion, by nurse bees that do not leave the hive. This presents a barrier to transfer of toxins to the reproductive castes in the colony. Similar results have been shown for chlorpyrifos. The large decrease in the concentration of pesticides between the pollen arriving at the hive and the larvae represents process of innate tolerance that reduces the need for detoxification genes. Implications for risk assessment will be discussed.

AGRO 184

Regulatory framework for assessing pesticide risks to bees: A case study with the neonicotinoid insecticide imidacloprid

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Pesticides are among several stressors that have been implicated in contributing to the decline of honey bees and native bee populations in North America over the last few decades. Among those garnering the most attention and research are the neonicotinoid insecticides, which target the nicotinic acetylcholine receptor (nAChR) in insects. Recently, the Office of Pesticide Programs (OPP) within the Environmental Protection Agency (EPA) published its preliminary Pollinator Risk Assessment for imidacloprid, one of the most widely used neonicotinoid insecticides in the United States. Neonicotinoids are characterized as being highly mobile and persistent in the environment, with high water solubility. These attributes contribute to their systemic properties whereby residues are taken up by roots, leaves and stems of plants and subsequently translocated to other plant tissues, including pollen and nectar. Residues in pollen and nectar are considered primary exposure routes for honey bees. Using a tiered assessment framework process that was collaboratively developed by the EPA, the State of California's Department of Pesticide Regulation (CDPR) and Canada's Pest Management Regulatory Agency (PMRA), risk at the individual organism and colony levels was assessed for a diverse set of agricultural use patterns registered for imidacloprid. Through the tiered risk assessment process utilizing multiple lines of evidence, risk determinations were made for each use pattern of imidacloprid and associated application method. This presentation will discuss the tiered assessment framework for bees and its application to imidacloprid.

AGRO 185

Industry involvement in the pollinator risk assessment process in North America

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On May 19, 2015, the US government released the *National Strategy to Promote the Health of Honey Bees and Other Pollinators,* which described the manner in which the government would establish new processes and procedures and modify existing ones to better protect pollinators. Previously the US EPA had presented to its Scientific Advisory Panel its Pollinator Risk Assessment Framework (PRAF). For pesticides, the key process is the generation of pollinator risk assessments (PRAs) by the US EPA. On January 4, 2016, the US EPA released its *Preliminary Pollinator Assessment to Support the Registration Review of Imidacloprid,* the first of four PRAs to be developed, one for each of the four neonicotinoid insecticides registered in the US – clothianidin, dinotefuran, imidacloprid, and thiamethoxam. PRAs for other compounds will be issued in the future. Each registrant of a pesticide product is responsible for developing the product-specific data required by the EPA to address the risks associated with the registered uses of their products. Nine registrants of products with potential pollinator risks have recently formed a task force to address more generic aspects of the PRAF. Possible ways to facilitate the use of the PRAF and information that may be useful to regulators as they generate PRAs will be described.

AGRO 186

EPA perspectives on pesticides and cannabis

***Nicole Zinn**, zinn.nicole@epa.gov. Office of Pesticide Programs, U.S. Environmental Protection Agency, Arlington, Virginia, United States*

While marijuana remains illegal federally, a growing number of states and tribes are allowing the medicinal and/or recreational use of marijuana, with nearly half the states now allowing medicinal use marijuana. As cannabis cultivation increases in these states and tribes, EPA is aware that pesticides are being used to control pests. However, there are no federally registered pesticides for use on marijuana and the use of unregistered pesticides on marijuana may have unknown health consequences, as no pesticides have undergone risk assessments for use on marijuana at this time. EPA has been working with the states to address this issue, including providing guidance on the Federal Insecticide Fungicide and Rodenticide Act section 24(c) process, which allows states to register pesticides for special local needs, as well as on other emerging issues related to pesticide use on cannabis. This presentation will discuss the EPA's position and the ways EPA is working with the states and tribes on pesticide issues related to cannabis.

AGRO 187

Responsible cultivation policy: Preserving personal cultivation rights while regulating commercial cultivation as agriculture

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This presentation will cover the following topics: Overview of Cultivation policy nationally, Cultivation Techniques or Methods used Nationally, Plant Count vs. Square Footage

Regulatory Models, Environmental Considerations, Cultivation Bans, and Responsible Solutions.

Within the thirty-five states referenced in the States Report, various types of cultivation techniques, or methods, are allowed depending on the State and possibly the locality in which the cultivation is taking place. While the vast majority of States with medical cannabis programs require that cannabis be cultivated indoors, a few states also allow for open air, greenhouse and row cover methods of cultivation. All cannabis cultivation poses risk to the environment relating to the use of mediums, nutrients, water, and pesticides.

Indoor cultivation poses additional environmental concerns due to the necessary and intensive electricity use associated with the high intensity discharge lighting systems, ventilation systems, heating and cooling systems, etc. In 2011, Dr. Evan Mills released a report on the carbon footprint of indoor cannabis cultivation entitled, *Energy Up in Smoke*. Dr. Mills estimated that cannabis cultivated using national average grid electricity results in the emission of approximately two pounds of greenhouse gas per cannabis cigarette produced.

Throughout the United States two different methods are used for regulating cannabis cultivation. The most popular method is to restrict the number of plants allowed to be cultivated, referred to as a plant count based regulatory system. The most responsible regulatory method, however, is to regulate based on the square footage of mature plant canopy. Square footage based regulatory systems are used in some States and within California depending on local regulatory ordinances. It is important to note that the Department of Food and Agriculture utilizes a square footage based model to regulate crops produced for human consumption.

Based on the information presented in this workshop, the conclusion can be drawn that utilizing a square footage based model for regulating cannabis cultivation provides not only the most accurate means of estimating yields but also allows farmers to utilize cultivation techniques that vastly reduce the potential for adverse environmental impacts. Therefore regulating cannabis cultivation in a manner that is analogous to other crops produced for human consumption provides the most responsible means of regulation.

AGRO 188

Regulation of agrochemicals use on medical marijuana in Nevada

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The legalization of medical marijuana in Nevada during 2015 has resulted in an active discussion and implementation of various regulations regarding the growing, harvesting and processing of this crop. Effectively all of this crop is grown indoors under artificial light, and requires a variety of techniques for management of insect pests and plant diseases. As is the case in each state in the US, there are no registered pesticides for marijuana, and no tolerances established for any of the pesticides in current use. However, as is the case in most states that have a legal cannabis industry, state regulators realize that pesticides are being used and are caught in a quandary on how these uses should be regulated. The Nevada Legislature in 2015 required that the Nevada Department of Agriculture (NDA) work with the primary cannabis regulatory authority, the Nevada Division of Health (NDH), to establish a list of agrochemicals that could be used. The NDH initially required analytical laboratories to determine the concentration of each pesticide at the lowest tolerance of any food for which the agrochemical might be found. Approximately 80 chemicals were initially on the list. Since milk was often the food with the lowest tolerance, it required laboratories to determine the concentrations in the single digits, ug/kg, in a 5-10 gm sample, a difficult analytical task. Further discussions resulted in this list being pared to about 20 agrochemicals. However, because of the analytical issues, labs are required to determine the bulk of the pesticides on the list by LC-MS, which does not allow effective identification of other substances that may be used. Improvements in the regulatory oversight of laboratories are being implemented, but, in the absence of pesticide residue dissipation information on cannabis, substantial uncertainty exists as to which agrochemicals can safely be used.

AGRO 189

Regulation of agrochemical use on medical/recreational marijuana in Oregon

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In fall 2014, Oregon voters approved Measure 91. Measure 91 provided for “Control, Regulation and Taxation of Marijuana”. As is the case in each state in the US, there are no registered pesticides for marijuana and no tolerances established for any pesticides that are in current use. State regulators have been caught in a “cart before horse” predicament regarding rules for control and regulation of pesticide use. Measure 91 required Oregon Liquor Control Commission (OLCC), in cooperation with Oregon Health Authority (OHA) and Oregon Department of Agriculture (ODA), to establish regulations by January 1, 2016. Studies in spring 2015 uncovered dramatic use of pesticides that made the existing rules promulgated under the Oregon Medical Marijuana Act inadequate for existing cannabis production methods. In the absence of reliable data from multiple sources, the agencies were required to make decisions for their narrow mandates. The practical economics of moving an existing industry into compliance has been difficult. Oregon’s hindsight can be a valuable tool for decision makers in other states as they confront the same issues. Understanding the need to move from post WWII class designations of pesticides (carbamate, organochlorine, organophoshorous) to broad based screens for plant growth regulators and “natural” products such as neonicotinoids and pyrethroids is vital. Establishing lab protocols for additional consumer health protection has been a relatively speedy process in Oregon (months rather than years). How different agencies cooperate or don’t will determine the ability of states to administer to public health concerns as they begin to deal with this process. Looking at key scientific policy decision points in the Oregon process can be a valuable tool for non-scientists making future policy decisions.

AGRO 190

Navigating the pesticide related regulatory landscape with respect to individual state legal *cannabis* cultivation in the US

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The current regulatory environment around pesticide use for individual state legal *Cannabis* production in the US is complicated by the multitude of State and Federal Laws that are in play. This presentation will hopefully shed light on safety assessment concerns and on the ways analytical chemistry can help mitigate these concerns. Currently, no pesticide labels for agrochemical use on *cannabis* have been approved under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the federal laws governing pesticide usage. Thus legal growers have very few, if any, approved options for pesticide use during production. Pesticides generally used in commercial food and ornamental production agriculture are clearly prohibited as growers must adhere to strict label requirements. Therefore growers have resorted to over the counter or organic pesticide treatments. Many analytical laboratories routinely analyze for these pesticides products in food and feed applications but are just beginning to evaluate the analytical needs of the *cannabis* industry. Logistical, legal, and ethical concerns surrounding pesticide analytical support for this industry must be considered if the safety of these products from a pesticide exposure perspective is to be addressed.

AGRO 191

Current and potential future environmental liabilities considerations for the cannabis industry

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Although a number of states have created the appropriate laws legalizing cannabis production and use for medical and/or recreational use, under federal law, cannabis is still illegal and therefore regulated under the Controlled Substances Act. By virtue of the federal rules, federal regulatory agencies are unable to provide guidance and/or act to implement regulatory strategies in a manner analogous to similar industries (e.g., agricultural crops, tobacco cultivation and processing). As a result of conflicting state and federal laws as well as regulations, this new and rapidly developing industry faces significant challenges and unique hurdles. Banking, transportation, analytical product evaluation, and similar topics have dominated the conversations around these challenges. The discussion from an environmental perspective has not been a central theme in the cannabis movement until recently; even though, there are very important considerations that may influence potential environmental liabilities for cultivation operations. The significant load that indoor operations place on energy grids is a well-recognized consequence of this burgeoning industry. In addition, and not unlike many agricultural crops, cannabis requires a significant amount of water for proper growth and production (approx. 4 gal per plant per day). The recent issues associated with pesticide use, also raises the question of whether appropriate waste water discharge and associated mitigation measures are sufficiently delineated to meet both state and federal guidelines. This presentation will focus on the state regulations that specifically address environmental issues of the cannabis industry as well as federal regulations that govern water resources, waste water discharges, ecological impacts, and other associated environmentally relevant scenarios. This discussion is pertinent as the topics discussed may be important for contentious future liabilities the industry may encounter with the exponential growth that is expected as more states provide a path for legal production.

AGRO 192

Agricultural considerations in cannabis husbandry: Food, fiber & farmacy

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Introduction:
Cannabis has been “camp-follower” throughout human peregrinations, and for good reason: no other single plant could provide sustenance, fiber, and pharmaceuticals in one package, and so the plant has endured even modern prohibition and vilification. This functional versatility is coupled with an intricate biochemistry that the plant utilizes for ecological advantage in varied climates and against myriad predators. Additionally, its clandestine culture for three generations has presented numerous challenges in adapting a free-ranging plant to indoor culture. The move toward cannabis husbandry on an industrial scale has provided new challenges in preventing hemp diseases and pests. Its use as a medicine makes avoidance of pesticide residues mandatory, and strategies to accomplish these ends will be discussed.
Methods:
Current relevant historical literature was reviewed, and supplemented with online database review.
Results:
Cannabinoids, cannabis terpenoids and flavonoids all contribute to the plant’s innate protective responses, but additional human intervention is often required. Pitfalls to such pursuit, and alternative “clean culture” techniques will be presented. Additional focus will be undertaken on Good Laboratory Practice.
Conclusions:
Cannabis culture, whether for food, fiber or “farmacy” poses many challenges that 21st century agriculture must face and solve. Combining innate phyto-mechanisms through selective breeding and additional organic agents and integrated pest management portend to provide a promising future for the plant and those who will be involved in its production.

AGRO 193

Pharmacophore modifications for the discovery and optimization of biologically active molecules

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The modification of the pharmacophores of biologically active molecules can serve as the starting point for the discovery of new classes of active molecules. A number of different pharmacophore modification strategies may also be used in optimization of hits and leads which are discovered by any of a number of discovery platforms. A variety of different pharmacophore modifications including pharmacophore inversion, imine-amide bioisosterism, retroamides, and carbonyl transposition with new and known examples will be highlighted. Both successful and unsuccessful uses of these tactics from the past 30 years will be presented.

AGRO 194

Aminopyrazole fungicides

Jeffrey K. Long[1]*, jeffrey.k.long@dupont.com, Andrew Taggi*[2]*, James F. Bereznak*[1]*, Michael J. Mahaffey*[1]*, Cheryl E. Liberato*[1]*, Lora L. Geist*[1]*, Wonpyo Hong*[1]*. (1) DuPont, Wilmington, Delaware, United States (2) DuPont, Newark, Delaware, United States*

A new class of aminopyrazoles controlling important plant diseases has been discovered. Synthesis and biology of this class and its relationship to compounds acting on tubulin dynamics will be discussed.

AGRO 195

Bicyclic diaryl-pyrazoles as MAP / HOG1 kinase inhibiting fungicides

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It is projected that the world will need to increase food production by 60% in order to feed a global population which will be in excess of 9 billion people by 2050. Because of the ability of plant pathogens to rapidly develop resistance, it is imperative that new classes of broad spectrum disease control agents, with novel modes of action, be discovered in

order to prevent catastrophic yield losses, which can approach 80% in some circumstances. In this presentation, we will describe the design and synthesis of the bicyclic diaryl-pyrazole class of MAP / HOG1 kinase inhibiting agrochemical fungicides.

AGRO 196

Cyclopropyl carboxamides: A breakthrough in SDHi fungicides

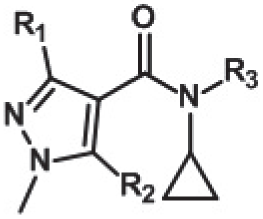
Christophe DUBOST, *christophe.dubost@bayer.com. Bayer, Lyon, France*

Cyclopropyl carboxamides is the new Bayer CropScience's proprietary SDHi chemical class that will deliver differentiated and new sustainable solutions to the growing world population and will meet our customer needs in key markets.

Recently, a number of SDHi compounds, such as Isopyrazam (Syngenta), Fluxapyroxad (BASF) and Bixafen (BCS), have been launched as fungicides. Cyclopropyl carboxamides will be a game changer thanks to their outstanding efficacy against major diseases at a very low application rate.

The unique feature of this family is the presence in its backbone of an unprecedented cyclopropyl tertiary amide which unsettles the SAR dogmas for SDH inhibitors.

This talk will give an overview on this chemical class starting with the genesis of the first weakly active hits that were modified using internal knowledge on SDHi chemistry and of the intensive chemical optimisation that ensued.

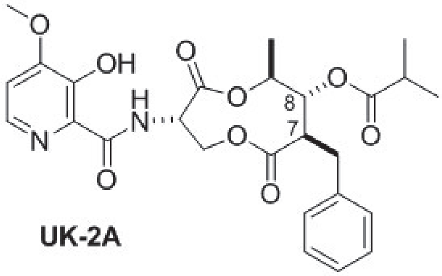


AGRO 197

Structure-activity relationship studies on the natural product UK-2A

Kevin G. Meyer, *kgmeyer@dow.com, W. J. Owen, Noormohamed Niyaz, Chenglin Yao, Richard B. Rogers, Gina M. Fitzpatrick, Fangzheng Li, Jamie Nugent, Michael J. Ricks, Thomas Slanec. Dow Agrosciences LLC, Indianapolis, Indiana, United States*

UK-2A is a natural metabolite isolated in 1995 from *Streptomyces sp*. 517-02. It has recently found utility as the precursor for Inatreq^{TM} active, a new active ingredient aimed at controlling major fungal pathogens in wheat and bananas. Substituent effects at positions 7 & 8 of the macrocyclic bis-lactone ring were studied through evaluation of physical attributes and against fungal pathogens of interest. The synthesis and biological efficacy of these molecules will be reviewed.



UK-2A

AGRO 198

Aqueous and soil fate of benzobicyclon and benzobicyclon hydrolysate under simulated California rice field conditions

Katryn Williams[1], *katwilliams@ucdavis.edu, Ronald S. Tjeerdema*[2]*. (1) Environmental Toxicology, UC Davis, Davis, California, United States (2) Environmental Toxicology, University of California, Davis, California, United States*

Herbicide resistance remains a prevalent issue impacting California rice production. Agents with alternative modes of action such as benzobicyclon are currently proposed for use on recalcitrant rice field weeds. Benzobicyclon, a pro-herbicide, forms the active compound, benzobicyclon hydrolysate, under aqueous conditions. Benzobicyclon hydrolysate inhibits the enzyme hydroxyphenylpyruvate dioxygenase (HPPD), subsequently halting the production of a carotenoid precursor within the plant. The loss of carotenoids results in the degradation of chlorophyll and ultimately plant death. Though benzobicyclon is anticipated to aid rice weed removal, the fate of benzobicyclon and its hydrolysate in the rice field is unknown. Hydrolysis is predicted to be a major contributor to the fate of benzobicyclon, while soil degradation may play a significant role in the loss of benzobicyclon hydrolysate in a flooded California rice field. As temperature is known to influence hydrolysis and soil degradation rates, experiments were conducted at varying temperatures to simulate typical California rice field conditions (15 – 35 °C). Rice management practices result in both anaerobic and aerobic soil conditions, depending on whether the field is flooded; therefore soil fate of the hydrolysate will be measured under oxic and anoxic conditions. This research will give insight into the fate of the parent and the active herbicide, which will aid policy makers and rice farmers in effectively managing the use of benzobicyclon and benzobicyclon hydrolysate on California rice fields.

AGRO 199

Exposure and risk assessment of pyrethroid insecticides in aquatic system

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Pyrethroids, a class of broad-spectrum insecticides, are the third most applied group of insecticides worldwide and are extensively used in agricultural and non-agricultural applications. Pyrethroids exhibit low toxicity to mammals, but have extremely high toxicity to fish and non-target invertebrates. Their high hydrophobicity, and pseudo-persistence due to continuous input, indicates that pyrethroids are tended to accumulate in sediments, pose long-term exposure to benthic invertebrates and ultimately cause significant risk to benthic communities. At the same time, short pulses of pyrethroids in aqueous phase are also evident to cause acute and chronic effects to stream macroinvertebrates. The exposure level and associated effects of pyrethroids in aquatic systems were mostly studied in California, US, followed by the Pearl River Delta, China. Pyrethroids were frequently detected in both agricultural and urban waterways, and bifenthrin and cypermethrin were identified as the main contributors to toxicity in invertebrates. Simulated hazard quotients (HQ) for sediment-associated pyrethroids to benthic organisms ranged from 3.91 ± 21.4 (esfenvalerate) to 23.5 ± 97.2 (cypermethrin), and this suggested significant risk. To date, very limited data is available to derive pyrethroid water/sediment quality criterion. The results provided strong evidence that pyrethroids are not only commonly detected in the aquatic environment, but also cause toxic effects to aquatic invertebrates; therefore, it is urgently needed to develop accurate criterions and effective ecological risk assessment methods for this important class of insecticides.

AGRO 200

Analysis of plant uptake and effects of pharmaceuticals using liquid chromatography tandem mass spectrometry

Rachel Mullen[2]*, rpetteys@buffalo.edu, Diana S. Aga*[1]*. (1) Univ at Buffalo, Buffalo, New York, United States (2) Chemistry, University at Buffalo, Williamsville, New York, United States*

There is great interest in agriculture to recover and reuse nutrients as fertilizer in a sustainable way. Recent research has focused on using wastewater or urine as a renewable fertilizer, yet it is known that they contain pharmaceuticals and the effects this could have on food crops and humans is not well understood. Previous studies involved the study of pharmaceuticals uptaken in lettuce and carrots, and it was found that all pharmaceuticals they were exposed to were uptaken, but this was not found to be the case for all crops. Specifically, it has been found that corn grown hydroponically and exposed to pharmaceuticals preferentially did not uptake the chlorinated pharmaceuticals chosen for this study in their native form. It is hypothesized that the glutathione in corn transferase catalyzes the conjugation of glutathione with the dechlorinated form of the pharmaceutical and essentially detoxifies it. The glutathione transferase has been extracted from the corn and used in-vitro studies with chlorinated pharmaceuticals. Additionally reactive oxygen species (ROS) has been measured using fluorimetry to determine if the concentration of ROS is greater in plants exposed to chlorinated pharmaceuticals. Preliminary results show that the plants exhibit more ROS when exposed to diclofenac and sulfachloropyridazine which could be indicative of upregulation of glutathione and glutathione transferase. This is to be confirmed or further explored using SDS-Page. All analysis of the corn is carried out using liquid chromatography tandem mass spectrometry to confirm the conjugation and structural information.

AGRO 201

Application of QuEChERS method for evaluating accumulation and metabolism of pharmaceuticals in vegetable

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Land application of animal manure and biosolids from wastewater treatment plants to agricultural fields could supply plant nutrients for crop growth, and improve soil quality as well. Agricultural irrigation with reclaimed water mitigates the stress of insufficient fresh water supplies. However, these practices can result in the potential uptake and accumulation of pharmaceuticals in plants. In this study, an efficient and more environmentally friendly QuEChERS method was validated and applied to quantify the accumulation of selected pharmaceuticals in lettuce. The extraction recovery of the QuEChERS method ranged from 91.7 to 119.2% for caffeine, carbamazepine, lamotrigine, trimethoprim, monensin and tylosin in lettuce roots, and from 86.3 to 105.3% in shoots. In the kinetic uptake study of pharmaceuticals by well-developed lettuce, the results revealed that the relatively large amounts of caffeine and carbamazepine were distributed in lettuce shoots than that in roots. Lamotrigine and trimethoprim manifested a greater accumulation in lettuce roots than in shoots. The relatively large-sized pharmaceuticals such as monensin sodium and tylosin demonstrated a weak affinity to lettuce root constituents, but were dominantly accumulated in lettuce roots. The analysis of pharmaceutical mass balance in the systems showed that the amount of caffeine and lamotrigine in lettuce roots and shoots kept on decreasing with time, suggesting the occurrence of formation of metabolites in the lettuce. To further elucidate the metabolic processes occurred in lettuce, we developed a reasonable work flow to identify and quantify metabolites of caffeine. This method could be also extended to analyze the metabolites of other types of pharmaceuticals and agrochemicals accumulated in agricultural crops.

AGRO 202

Non-extractable residues: Formation, extraction, and bioavailability

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Review mechanisms for formation of soil/sediment non-extractable residues (NER)
-Compare different test system types (soil, sediment, water)
-Discuss differences within test systems that can have effects on NER formation (microbial activity, soil organic matter, pH, texture)
-Discuss chemistries of pesticides that can affect level of NER
-Discuss immediate binding versus long-term binding
-Soil extraction efficiency experiment performed at dosing – where this approach falls short
Regulatory guidance for characterization of bound residues
-EPA guidance document issued September 2014
-Threshold for further investigation of NER
-Why has this become an area of emphasis?
Bioavailability of NER
-Which types of extracts should be considered bioavailable?
-What data should be included in degradation kinetics?
Summary of EAG/PTRL West's techniques/approaches for evaluation of NER
-Routine extraction (organic solvent, weak acid)
-Nonpolar extraction with 2 solvents
-Harsh extraction (Reflux, Soxhlet, or Microwave)
-Humic/Fulvic Partition
-Calculation of DT50, DT75 and DT90 using KinGUI/CAKE/other programs and data from routine and nonpolar extractions. Harsh extraction data is excluded from the calculations.

AGRO 203

Glyphosate extraction by different solvents and techniques from two agricultural soils

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Glyphosate is the most common herbicide for weed control in maize and soybean, and its usage in the US increased about 50 times in the last 20 years for both crops due to the availability of RoundUp Ready seeds. This upsurge of glyphosate usage has led to environmental concerns about this herbicide affecting water quality. Because glyphosate reaches the soil when applied, leaching of this herbicide from soils into water is of interest. In this study, we investigated the effect of different solvents (D.I. water, $KH2PO4$, $Na2B4O7$, methanol-$KH2PO4$ mix, and methanol-$Na2B4O7$ mix) and extraction techniques (shaking, sonication, microwave, and pressurized fluid extraction) to extract glyphosate and its metabolite aminomethylphosphonic acid (AMPA) from two glyphosate-treated soils under maize-soybean rotation. The soils consisted of an alfisol, a soil associated with hardwood forest, and a mollisol, a soil associated with grasslands. The results from this study demonstrated that glyphosate and AMPA extractions are dependent on the soil type, solvent, and technique. For both soils, the extracted glyphosate was similar when shaking with

K2PO4 and microwaving with Na2B4O7 solutions. In addition, the microwave technique with KH2PO4 solution extracted slightly more glyphosate than the aforementioned solvents/techniques from the mollisol. For the metabolite AMPA, the most effective solvent was KH2PO4 using either microwave or shaking from both soils.

AGRO 204

Nature of the residue study with Rinskor™ applied to rice

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Nature of Residue studies (NORs) are an important part of pesticide development, the studies provide information on metabolic pathways, residues at harvest in raw agriculture commodities (RACs), metabolites that require toxicology evaluation and analytes to include in analytical methods. A NOR was conducted on the novel rice herbicide, Rinskor™, (benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate). Three typical rice planting/application scenarios representing global uses of Rinskor™ were investigated. To separate plots of rice Rinskor™ was either foliar-applied at twice the maximum seasonal target rate of 60 g a.i./ha (two applications of 60 g a.i./ha for a total of 120 g a.i./ha) or water-injected at 400 g a.i./ha (two applications of 200 g a.i./ha). Target pre-harvest interval was 60 days; immature plants were collected 13 days after the second application. Mature white rice, hulls, and straw were harvested 59-70 days after the second application. A portion of the tissues containing >0.01 mg eq./kg was sequentially analyzed beginning with three different neutral extractions (each with increasing polarity) using accelerated solvent extraction (ASE), followed by an acidic water/acetonitrile extraction. Extracts were analyzed by HPLC and Mass Spectrometry to identify or characterize the composition of the residue. Mature samples were subjected to bound residue determinations, as necessary, such as starch, pectin, lignin, and acid/detergent fiber. In all cases, the residues in grain were significantly lower than those in straw; residues in hulls fell between the levels observed in grain and straw. In general the majority of the extractable radioactive residue in mature samples was present as Rinskor™, metabolites X11966341 and X11438848; minor metabolites X12131932 and X12431091 were present at levels less than 10% TRR. It was further shown that the residue of Rinskor™ becomes naturally incorporated as pectin, lignin and hemicellulose.

AGRO 205

Are muscarinic acetylcholine receptors the target of a new pyrazole oxime insecticide?

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The African malaria mosquito (*Anopheles gambiae*) is the most prolific vector of malaria in sub-Saharan Africa. Pyrethroid insecticides are the primary class of insecticides used to control *An. gambiae* populations, for which resistance is a rapidly growing problem. Therefore, there is a clear and urgent need to identify novel chemical insecticides with a unique or underutilized mechanism of action that has low toxicity to mammals. The neurotransmitter acetylcholine can activate nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors (mAChRs). Mammals have five subtypes of mAChRs (M1-M5), while insects have two subtypes of mAChR (Type-A and Type-B). Insecticide development of compounds towards the insect nAChR has been more successful than at the mAChR. A major drawback of experimental insecticides targeting the insect mAChR is cross-sensitivity with mammalian mAChRs. We have recently discovered a novel pyrazole oxime that is toxic to insecticide-susceptible and insecticide-resistant (pyrethroid and carbamate) *An. gambiae* (WHO paper assay range between LC_{50} = 0.122 mg/ml and 0.142 mg/ml), and the toxicity to mice is low (oral LD_{50} > 2,000 mg/kg). Injection of this pyrazole oxime and the non-selective mAChR agonist pilocarpine (50 ng/mosquito) resulted in the inhibition of flight followed by moribundity and death. Electrophysiological experiments in a *Drosophila melanogaster* CNS preparation, which follows changes in nerve firing when the CNS is exposed to neuroactive compounds, resulted in the non-selective mAChR pilocarpine (10 μM) having a biphasic effect on the CNS nerve firing. However, preincubation of this preparation with the novel pyrazole oxime (10 μM) extended the nerve firing of pilocarpine. Additional electrophysiological experiments will be discussed in addition to further studies examining which subtype of mAChR may be involved in oxime toxicity.

AGRO 206

RNAi for western corn rootworm management

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The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is the most important pest of corn in the United States Corn Belt. Economic estimates suggest that cost of control and yield loss associated with western corn rootworm damage exceed $1 billion annually. Historically, the management of western corn rootworm has been challenging because of its exceptional ability to evolve resistance to both insecticides and cultural control practices. The first transgenic Bt corn for rootworm management was introduced to the market in 2003, and novel events for rootworm control have been limited to Bt proteins. Currently, four Bt proteins are registered for western corn rootworm management. In 2011 field resistance was reported to Cry3Bb1 highlighting the importance of developing new modes of action for the management of this insect. Recently, the agricultural industry has recognized the potential to use RNA interference (RNAi) as a mechanism to control the expression of fundamental genes for insect survival that lead to insect death. The newest approach for western corn rootworm management is plant expression of long RNA hairpins that trigger an RNAi response. The first transgenic RNAi plant for rootworm control is projected to be released within the next decade and is likely to become an important management tool that will complement existing practices including synthetic pesticides and Bt traits. The success and durability of RNAi depends on our understanding of RNAi mode of action in the target species, improving dsRNA delivery and identifying potential mechanisms of resistance. This talk will focus on the current understanding of RNAi for western corn rootworm management including potential strategies such as lethal and parental RNAi. In addition, it will describe ongoing efforts to identify RNAi uptake and processing mechanisms in the western corn rootworm, and postulate the potential mechanisms of resistance.

AGRO 207

Through the looking glass: an opinion of pest management in an academic, government and industry setting

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Controlling arthropod pests in an important worldwide goal for the protection of human's structures, food sources and health. Each avenue of research, academic, government and private industry, has different objectives, methods and goals for controlling pests. As a researcher who has experience within all three different avenues, I will present the similarities and differences between each, and focus on areas where collaboration could occur.

AGRO 208

Peptide neurohormone receptors as insecticide targets

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Introduction:
Insecticide resistance is a recurring problem for most chemical forms of arthropod control, from agricultural pests to vectors of disease. Peptide neurohormone receptors represent a diverse but relatively unexplored array of targets for the development of new insecticide classes to circumvent current forms of insecticide resistance. In insects, peptide neurohormones control or modulate numerous biological processes, ranging from critical physiological functions to behavior. Several types of receptor classes are known to interact with peptide neurohormones, but most activate the diverse class of receptors known as the G-protein coupled receptors (GPCRs). Several peptide neurohormones have been matched to their cognate GPCRs in such model organisms as *Drosophila melanogaster*, but the presence and functionality of predicted homologous receptors in other insects, particularly in pest species in more distantly related taxonomic groups, requires confirmation in order to further develop strategies to target these receptors.
Methods:
Genetic sequences for candidate insecticidal target GPCRs were found through Basic Local Alignment Search Tool (BLAST) homology searches of available insect genome, transcriptome, and expressed sequence tag databases. These were confirmed by sequencing of cDNA amplified via PCR with specific primers. Receptors were expressed in reporter cell lines, and pharmacological profiles were characterized in response to predicted peptide ligands. In addition, potential non-peptide analogs were examined to determine the activity at these receptors and to explore their potential to disrupt normal signaling.
Results/Discussion:
This investigation provides a foundation for the exploration of peptide neurohormone receptors as viable insecticidal targets. Current progress on the characterization of these peptide GPCRs in pest insects will be discussed.

AGRO 209

Toxicological comparison of pyrethroids and sabadilla alkaloids on susceptible and resistant mosquitoes

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Insecticide resistance is an increasing problem in many insects that vector disease. Currently, the pyrethroid class of insecticides is the only approved compound class for insect treated bednets for the control of mosquitoes that vector disease. Target-site resistance has been attributed to the reduction in efficacy of standard use pyrethroid insecticides on the market. The incorporation of synergists, such as piperonyl butoxide, to insecticidal bednets has increased longevity and efficacy of the pyrethroid class of insecticides to both susceptible and resistant mosquitoes. The use of synergists to suppress metabolism-based resistance have been implemented as a pest management strategy to control resistant mosquitoes; yet, the insecticide resistance problem is ever present, and the need for novel target site resistance breaking mechanisms is even more imperative. To contest the increasing frequency of multiple resistance mechanisms in mosquitoes, we are proposing to examine the use of sabadilla alkaloid extracts as alternative synergist compounds for use in combination with pyrethroids as a novel resistance-breaking strategy. The goal of this research is to establish sabadilla alkaloids as alternative chemistries to mitigate pyrethroid resistance in mosquitoes that vector disease. This information will not only improve chemical control strategies for mosquitoes, but will guide the discovery and design of safe and selective mosquitocides in addition to the development of resistance-breaking chemistries for vector mosquito management.

AGRO 210

Gap junctions as potential new insecticide targets in the Yellow Fever Mosquito, *Aedes aegypti*

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Aedes aegypti is the primary vector of several medically important arboviruses, such as dengue, chikungunya, and Zika. Unfortunately, effective and affordable vaccines for most of these viruses are not available, which means we must instead rely on control of the mosquito vector. Conventional insecticides that target voltage-gated sodium channels and acetylcholinesterases are typically used to manage mosquito populations, but the evolution of resistance to these compounds is limiting their efficacy in the field. Thus, identifying new molecular and physiological targets to facilitate mosquitocide discovery and development is needed. Here we test the hypothesis that gap junctions (GJ) are molecular and physiological targets for insecticide development. GJ are intercellular channels that mediate direct communication between neighboring cells and are composed of evolutionarily distinct proteins in vertebrate (connexins) and invertebrate (innexins) animals. In this study, we show that topical application or hemolymph injection of pharmacological inhibitors of GJ in adult female mosquitoes elicits dose-dependent toxic effects. *In vivo* urine excretion assays demonstrate that the inhibitors impede the diuretic output of adult female mosquitoes, suggesting inhibition of excretory functions as part of their mechanism of action. When added to the rearing water of 1st instar larvae, inhibitors of GJ also elicit dose-dependent toxic effects. Finally, silencing the mRNA expression of multiple innexins in adult female mosquitoes via RNA interference decreases their survival. Taken together these data indicate that GJ may provide novel molecular and physiological targets for the development of new mosquitocides.

AGRO 211

Mosquitocidal activity and mode of action of the isoxazoline fluralaner

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Mosquitoes are important vectors of human diseases. Fluralaner, a recently introduced parasiticide, was toxic to *Aedes aegypti* mosquitoes in 24 hour exposures, with an LC_{50} in 4th instar larvae of 1.2 ppb and LD_{50} in adult topical assays of 1.3 ng/mg. This LD$_{50}$ value was 26-fold higher than fipronil. In time course studies, the insecticidal activity of fluralaner to adults increased by a factor of approximately two for each day of exposure, suggesting a slowly developing toxicity. The endpoint for fluralaner toxicity following topical application was about 5 days. In glass contact assay, fluralaner has an LC$_{50}$ value of 14 ng/cm^2, 47-fold higher than permethrin. These data show that fluralaner does not have exceptional toxicity to mosquitoes in typical exposure paradigms. In electrophysiological recordings on *Drosophila melanogaster* larval CNS, 1 mM GABA was used to block nerve activity and the EC$_{50}$ of fluralaner for restoring nerve firing (a measure of GABA antagonism) was similar in susceptible (0.34 μM) and resistant (0.29 μM) strains. The time to response of fluralaner was longer compared to dieldrin (6.3 ± 5.8 minutes at 10 μM), and sometimes required 30 minutes or more to appear, suggesting a slow action even when the blood-brain barrier is disrupted. Although dieldrin (logP = 5.4) and fluralaner (logP = 5.0) have similar lipophilicities, the large size (mw = 556) of fluralaner compared to dieldrin (mw = 381) might influence its penetration through the mosquito cuticle and into the CNS. Fluralaner has an IC$_{50}$ value >30 μM on α1β3γ2 GABA$_A$ receptor in HEK293 cells, providing additional evidence for selectivity and low mammalian toxicity. Efforts to improve contact activity of fluralaner used lipophilic amine complexation, which increased contact activity by 4-fold and 8-fold in topical and glass contact assays, respectively. The implications of these studies for vector control will be discussed.

AGRO 212

Targeted genome editing in *Aedes aegypti* using TALEN and CRISPR/Cas9

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Aedes aegypti (Ae. aegypti) is a primary vector for yellow fever, chikungunya, dengue, and Zika viruses. Given the severity of the diseases vectored by this insect it is crucial that novel control techniques are developed. TALE nucleases (TALENs) have been used with great success in a number of organisms to generate site-specific DNA lesions. We evaluated the ability of a TALEN pair to target the *Ae. aegypti* specific genes and confirmed that TALEN-based gene disruption can be a highly efficient process in *Ae. aegypti*, with editing rates between 20-40%. Type II clustered regularly interspaced short palindromic repeats or CRISPR/Cas9 has revolutionized gene editing with the ability to generate highly specific double stranded breaks within the targeted organism's genome. The CRISPR/Cas9 system has become an important tool for targeted gene disruption in many organisms, especially crucial disease vectors such as *Ae. aegypti*. We recently described that CRISPR/Cas9 is highly effective system in generating targeted mutations in *Ae. aegypti*. CRISPR/Cas9 or TALEN can be used to conduct screening to identify novel targets for insecticide use and also to confirm and validate current targets.

AGRO 213

Analysis of multiple neonicotinoids in small samples of honeybees combined with Quantigene® virology

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In a study done to monitor the exposure levels for neonicotinoids that occur under current conditions of use in agriculture, small numbers of adult honeybees were identified which showed symptoms reported to indicate pesticide poisoning, such as loss of hair and color (black bees), or loss of co-ordination, paralysis, trembling, stupefaction, disorientation and jerky, erratic behaviours (impaired bees). Since the same symptoms may indicate disease, mainly viruses, a proprietary assay based on the Quantigene® system with a validated battery of viral RNA targets was obtained from Affymetrix, Inc. This assay included targets for 8 major viruses, 2 replicating phase viruses and three reference genes from the bee genome. As the amount of sample was very small, ~1g, the sample preparation and extraction was adapted so that a representative subsample of only a few milligrams could be obtained for virology and the remainder of the sample could be used for pesticide analysis by LC-MS/MS. The compounds included and the limits of detection (ng/bee) were acetamiprid (0.008) clothianidin (0.032) imidacloprid (0.016), thiamethoxam (0.016) and metabolite thiazolylnitroguanidine (0.067). Samples as small as a single bee could be analysed with adequate sensitivity, but to reduce variability, these bees were collected when at least 10 individuals were found during hive inspections. The results from 14 samples of black bees collected inside the hives and 20 samples of impaired bees collected on the ground in front of the hives showed that the symptoms were associated with disease and not pesticides. The method will be presented and discussed.

AGRO 214

Collaborative epidemiological approach to investigate risk factors for diminished honey bee health in Ontario, Canada

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Diminished pollinator health has been increasingly reported by beekeepers, and others particularly as it relates to colony losses and weakened colonies. Symptoms of weakened colonies have been described to include elements such as: queen health issues, reduced production, increased susceptibility to pests and diseases, and reduced ability to overwinter. Risk factors for declining pollinator health are multifactorial and these risk factors and their interactions are poorly understood. Epidemiological analytical approaches to investigating diseases in other contexts (e.g., human health and health of other production animal species) have been powerful for investigating multiple risk factors and their interactions using a variety of data types, including field data. Epidemiological studies investigating honey bee health issues are limited and are warranted. The objective of this study was to investigate risk factors for colony losses and weakened colonies and their interrelationships using a collaborative, multi-sectoral epidemiological approach. Real world data from various sources including bee health incident reports, management information derived from beekeeper questionnaires, and geospatial bee-yard and crop (corn, soybean) locations as an indicator of neonicotinoid use in Ontario, Canada were integrated and investigated using multilevel, multivariable regression and spatial statistical analyses. Key results will be discussed and will demonstrate the value of a multi-stakeholder approach to field data integration and analysis. A stakeholder data platform approach to solving honeybee health issues will also be presented; this approach represents a novel framework for optimizing future honey bee health research.

AGRO 215

Managing risks of pesticides to bees

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Managing risk from pesticide exposure can include both mandatory and voluntary measures. EPA intends to use both of these tools to manage potential risk to pollinators. The Agency has been working to identify voluntary measures, in the form of precautionary language (*e.g.*, Bee Box), or Managed Pollinator Protection Plans (MP3s) - and BMPs that may be part of MP3s. As the Agency moves forward with compound-specific pollinator risk assessments and risk management decisions, science driven, label restrictions could also be part of the Agency's overall ecological risk management considerations. The Agency will provide an overview of its efforts to develop voluntary risk management measures (MP3s) and briefly discuss how the Agency may view, understand, and potentially manage pollinator risks identified through a quantitative risk analysis.

AGRO 216

Pollinator tier I risk assessment: A link between laboratory and field studies

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Pollinator risk assessment provides a mechanism for evaluating the potential risk of pesticides to bees, particularly honey bees (*Apis mellifera*). In June 2014, the United States Environmental Protection Agency (EPA), Health Canada Pest Management Regulatory Agency (PMRA) and the California Department of Pesticide Regulation (CDPR) published a guidance for assessing pesticide risk to bees. A Microsoft Excel-based model (Bee-REX version 1.0) was also developed as a screening level tool to use in tier 1 assessment. The European Food Safety Authority (EFSA) had published a similar guidance in 2013 for assessing the potential risk posed by plant protection products to bees, including honey bees, *Bombus spp*. and solitary bees. EFSA also developed an accompanying model called Bee Tool version 3 to aid in the tier 1 and tier 2 assessments. Both guidance documents propose a tiered testing scheme to provide data for risk assessment starting with simple and more conservative tier 1 studies to more complex higher-tier studies under semi-field, and field conditions. Laboratory-based tier 1 risk assessment provides a method to evaluate the potential effects of pesticides on terrestrial invertebrates as well as decision criteria regarding the need for performing higher tiered testing. A description of the use of tier 1 risk assessment will be presented as a tool for predicting the necessity of higher-tier semi-field and field studies following completion of laboratory oral/ contact and larval exposure testing.

AGRO 217

Pollinator risk assessment and risk management: impacts on product registration

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New methods and guidance for assessing risk of pesticide products to bees have recently been developed by the US EPA, Canada PMRA, and California DPR. Implementation of this guidance is a major new challenge for both regulatory agencies and product registrants, and has generated much interest among numerous stakeholder groups. In this presentation we review how the new pollinator risk assessment and risk management process is being implemented for products undergoing registration review as well as new products being introduced to the market. We focus on the how pollinator safety data and risk evaluations are influencing product labels and impacting product availability and use. We highlight recent evaluations of the risks to pollinators associated with use of imidacloprid in citrus and cotton as examples of where win-win solutions are possible for growers, beekeepers, regulators and registrants if science-driven actions are implemented, but where lose-lose outcomes may instead occur if politics, and not science, drives the process.

AGRO 218

Challenges of pesticide testing for privately owned cannabis testing facilities in Colorado

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The state of Colorado has recently enacted a legislative mandate to ensure the health and safety of Colorado residents from residual pesticides on recreational cannabis products. Currently in Colorado there are seven privately owned testing facilities that are certified to analyze cannabis products for potency, residual solvents and microbial contamination. There is no current certification process for the analysis of pesticides, however, which the state is currently working towards. One privately owned laboratory has gone out on a limb and started to provide this test; however, the results have been questionable and as of now, the Department of Agriculture is the only place to receive quality data. This test takes over a week to return data and is costly to the client. The challenge for a cannabis testing lab in Colorado at this time is the cost of bringing on such testing as the compounds needed to be identified require LC/MS/MS as well as the method development needed for analysis. The compounding issue is that the industry isn't willing to pay the cost of a typical pesticide test, requesting the test be less than $100 with a 2 day turn-around time. The real public health and safety in this issue is the concentration of pesticides in concentrate cannabis products.

AGRO 219

What's the catch? A comprehensive approach to testing cannabis for health and safety

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Cannabis testing laboratories face challenges that are unique to the legalized marijuana industry. Besides federal prohibition, we have to deal with varying regulations among states, unfair competition, unreasonable expectations, conflicting policies, and rapid change. While science cannot solve all of our problems, we gladly consider whatever it can offer to improve our services and profitability. For example, a technical challenge that can be overcome is the limitation imposed by less sample size to do more tests. Although we cannot disclose the details of our methods, we can provide the general approach we use to maximize the information that can be obtained for a nonhomogenous batch via multiple cross-sequential methods of analysis. Other technical problems in cannabis testing will be presented and discussed, so that interested research scientists can work on solutions that will have immediate economic impact, while at the same time advancing health and safety.

AGRO 220

Development of triazole fungicide resistance in powdery mildew disease of cannabis

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Many highly-valued cannabis cultivars are also highly susceptible to fungal diseases such as powdery mildew. High market demand for cannabis flower and the ubiquity of powdery mildew has promoted widespread use and heavy application of single mode-of-action demethylation inhibitors (DMI) such as myclobutanil. The predictable emergence of DMI-resistant fungal organisms has, in turn, intensified fungicide application during cannabis cultivation, leading to elevated fungicide residue in the final commodity. To assess the occurrence, regional prevalence and severity of DMI resistance, samples of powdery mildew were collected from cannabis cultivation sites across Colorado, including sites with no known history of DMI use. Isolates from each site were propagated on detached cannabis leaves, and tested for resistance against three common DMI fungicides: myclobutanil, propiconazole, and tebuconazole. Isolates from sites with no prior DMI use were sensitive to all three fungicides tested, whereas isolates from sites with prior exclusive use of DMIs exhibited variable levels of growth and cross-resistance to the DMIs tested. Identification of DMI resistance in powdery mildew of cannabis, and elucidation of the underlying genetic and biochemical basis, will inform integrated pest management planning and implementation by cultivators, and represents a necessary first step to address the current dearth of Good Agricultural Practices (GAP) in certain sectors of the cannabis industry.

AGRO 221

Challenges for multi-residue pesticide analysis in cannabis; extraction and cleanup strategies for LC-MS and GC-MS analysis

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With the recent widespread trend for acceptance of cannabis-based therapeutics, increasing attention is being focused on the quality of products available to consumers and patients. Due to the lack of EPA guidance for the use of pesticides for cannabis cultivation, unregulated, uncontrolled use of pesticides has resulted in contaminated products reaching the consumer market. Recent implementation of regulations, on a state by state basis, has targeted this issue, in an effort to ensure the quality of these cannabis-based therapeutics. Cannabis and cannabis infused foodstuffs present significant challenges for the pesticide residue chemist. Unlike many fruits and vegetables, cannabis contains a multitude of resinous constituents similar in physiochemical properties to many pesticides. Because these interfering resin constituents are so similar in solubility and polarity to the target analytes, standard methods of extraction and cleanup used for other dried commodities may not be effective for pesticide analysis in cannabis. The presence of this complex resin matrix also complicates the effective extraction of water soluble pesticides from cannabis. In this presentation options will be discussed for optimized sample preparation of cannabis for tandem LC-MS and GC-MS pesticide analysis. Topics will include modified QuEChERS methodologies and other alternative protocols for initial extraction of pesticides from cannabis products. Topics will also include strategies using dispersive solid-phase extraction (dSPE) and pass-through SPE for improved cleanup of the extracts.

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Quantitation of pesticide residues in cannabis by LC-MS-MS with modified QuEChERS extraction

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Legalization has resulted in a more than 10-fold increase in the production and consumption of cannabis (Cannabis sp.) in the last few years. This extremely high value crop is especially vulnerable to mold and insects, so growers may apply pesticides or antifungals to protect their plants. In order to protect consumers, sensitive and selective detection of pesticide residues in cannabis is required. We developed and validated an LC-MS-MS method using a modified QuEChERS extraction for detection and quantitation of more than 200 pesticide residues in dried cannabis flower.

Ground and homogenized cannabis was extracted with a modified QuEChERS and dispersive SPE cleanup. Detection was carried out by UHPLC-MS-MS using a Shimadzu Prominence HPLC with LCMS-8050 triple quadrupole mass spectrometer. Electrospray ionization was used with continuous polarity switching to measure analytes in both modes throughout the run. Pesticide recovery was determined using spiking experiments and matrix-matched calibration curves.

Matrix matched calibration curves were linear within the quantitation limits established for each compound, which was compound dependent, but ranged from as low as 1.5 ppb at the lower limit to 2000 ppb at the upper limit. Quantitation limits were required to have less than 20% RSD in triplicate, sufficient signal to noise, and freedom from interference in multi-lot matrix tests. Recovery was compound dependent; however, the majority were within the range of 70-120%.

In a subset of randomly tested cannabis flower samples offered for commercial sale, the three most commonly detected residues were piperonyl butoxide, myclobutanil, and imidacloprid. Concentrations for pesticides ranged from the detection limit up to the microgram/gram level. Measurements of a larger selection of commercially available cannabis samples offered for commercial sale are being carried out.

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Endemic pesticide use in Cannabis: Getting growers, labs, and regulators aligned through scalable and novel flash chromatographic remediation methodology

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The ubiquitous and unregulated agrochemical use by the cannabis industry represents a serious public health and product liability issue that varies State by State due to the complete lack of Federal regulation. In many cases, this is complicated by unclear or absent State laws. This has created serious problems including recalls and litigation in Colorado and Washington, as well as confusion for stakeholders. Sadly, the scale of the problem has become endemic. Extraction of active cannabinoids from plant material regardless of the approach, including supercritical carbon dioxide, hydrocarbons, or ethanol can exacerbate the possible risk of contaminants as agrochemical residues will inevitably be concentrated by the process. Consumers remain generally unaware of these issues while vulnerable populations are exposed. The attempts of the cannabis industry to evolve into a safe and regulated business model have been severely handicapped by a slowly evolving legal and political landscape. The application of unregulated agrochemicals by untrained personnel are only now being fully understood. This talk will discuss how the various stakeholders can be aligned in the short term by using economic incentives and remediation technology as opposed to waiting for enforcement or audit mechanisms. We report studies that address the use of large-scale preparative flash (normal and reverse phase) chromatographic methodology to remediate cannabis oil products containing agrochemical contamination. A panel of organochlorine and organophosphorus pesticides and fungicides were used to "spike" various extracts at high levels. Concentrations of these agrochemicals were reduced to a highly significant degree. Specifically, the reduction of residue levels of common, persistent, and problematic agrochemicals, as evaluated against MRL's for comparable industries (many pesticides were reported to be below detection limits), suggests these methods may represent a novel and scalable remediation and normalization process for the cannabis industry.

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Possible sources of discrepancy in interlaboratory reporting of THCA concentration in cannabis plant

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Despite the current prevalence of Cannabis *sativa* L. in both medical and recreational markets, analytical testing of potency is still in its infancy due to national and international bans on research for the past 40 years. Nearly all published methods (usually LC or GC methodology) focus on the identification of d9THC, the plant's main psychoactive ingredient, for the purpose of law enforcement, falling short of precise and accurate quantification (see UNODC 2009). As there are currently no federal organizations to standardize such analytical methods, vast differences exist among laboratories in their reported results. One major cause is sampling; the particular selection of which parts of the plant to include and which to discard is crucial, as some components do not have cannabinoids (stems, seeds, etc.) yet add to the overall weight (as in Mehmedic 2010). Different flowers on a single plant can also vary in potency up to 10%, hence random sampling is crucial if proper representation is required. Sample preparation can also lead to discrepancies-- beyond basic analyte recovery-- if not reported properly. As well standard calibrations must be adhered to avoid under-or-over-reporting analyte concentrations by extending the curve beyond its highest or lowest calibration points. A standardized reporting system will help immensely in avoiding discrepancies and confusion. Reporting wet weight vs dry weight on a sample can sway the final results by up to 15%. In addition some laboratories choose to combine the final results of certain cannabinoids and report numbers such as "Total THC" and "Total Cannabinoids" which have little scientific validity or use. As many markets for Cannabis across the US price their products solely on THC/THCA concentrations, standardized methods and reporting are necessary to avoid price manipulation and bring the plant up to the quality control standards required by western medicine.

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Chiral and isotope analyses for assessing the degradation and metabolism of fipronil in the sediment

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Fipronil is a chiral insecticide commonly applied as a racemate, and it has become an increasingly popular structural pest management insecticide for ant control. It can be easily transformed to fipronil sulfone and sulfide by oxidation and reduction, respectively. Fipronil and its metabolites are frequently detected in urban streams. Enantioselectivity may cause the different environmental fate and toxicity for the two enantiomers. Some investigators have found stereoselective degradation of fipronil in sediment, but currently there is no information about what metabolic reaction (reduction or oxidation reaction) caused this stereoselective degradation. In this study, the stereoselective metabolic reaction of chiral fipronil was investigated in two river sediments under oxic and anoxic conditions. The stable isotope analysis (SIA) was used to determine the pertinent metabolic pathways of fipronil enantiomers. The enantiomers of ^{13}C and^{12}C-fipronil were separated and collected on the Chiralcel OJ column under normal-phase conditions. The purity of the enantiomers was greater than 99.7%. The *R*, *S*, and racemic fipronil (mixed ^{13}C-(*S*) with ^{12}C-(*R*)) were spiked into two sediments. Degradation rate constants were determined by kinetic modeling. The isotope enrichment factor (ε) was obtained from fipronil and its metabolic product. The relationship between the enrichment factor and enantiomeric enrichment could explain which process caused stereoselective degradation in sediment. This study demonstrates that different environmental conditions may substantially alter the relative persistence of enantiomers of chiral pesticides. The stereoselective degradation of fipronil as well as the stereoselective formation of toxic metabolites may have some implications for better environmental and ecological risk assessment for chiral pollutants.

AGRO 226

Characterization of value-added biochemicals using mass spectrometry-based metabolomics in a non-model microalgae

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Microalgae is an effective source of lipids for biofuel production due to its high oil yield as compared to other plants such as corn and soy. When grown under limited nutrient conditions microalgae can accumulate certain lipids that are precursors for numerous value added products. Moreover, since microalgae can be grown in water, they do not use up arable land and therefore do not compete with other agricultural efforts. Using CO_2 from the atmosphere and nutrients from waste water, these organisms can produce many value added bioproducts such as lipids, vitamins, and phytosterols. The aim of this study is to analyze the changes in metabolites produced by microalgae under different growth conditions to better understand the metabolic processes that accompany these changes.

We chose a highly-adaptable, oleaginous, non-model microalgae *Ettlia oleaobundans*. We developed a mass spectrometry-based global metabolite profiling method to analyze the changes in metabolites under different growth conditions, specifically nitrogen replete (N+) and nitrogen deplete (N−). A biphasic solvent extraction was used to extract metabolites from *E. oleoabundans*. This extraction separates the hydrophobic and hydrophilic metabolites, which allows us to analyze these phases independently, thus broadening the metabolite coverage. We analyzed the hydrophobic metabolites using an integrated liquid chromatography – quadrupole – time of flight mass spectrometry (LC-Q-ToF MS) and gas chromatography tandem mass spectrometry (GC-MS/MS) platform. We showed that there were over 200 metabolites that changed > three-fold when grown under N− compared to N+. We annotated 81% of these species using MS/MS. Overall, triacylglycerols and sulfoquinovosyldiacylglycerols were accumulated, and chlorophylls and phosphatidylglycerols were depleted when grown under N− conditions. We expect that the method we developed will serve as both a resource and template for targeted and untargeted metabolomics to be used by the scientific community to better understand the bioproducts portfolio of model and non-model green microalgae.

AGRO 227

Mass Spectral identification of biomarkers of exposure to silver nanoparticles in corn roots

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Engineered nanomaterials have led to advances in technology and medicine due to their unique properties and characteristics. Their production and usage is growing rapidly, raising concerns over their impact on environmental and human health. Silver nanoparticles are used in many facets of the industry due to their antimicrobial properties. They can be found in clothing, food packaging, cosmetics and electronics. Due to their wide range of applications, silver nanomaterials containing products are expected to be disposed in landfills. Alternatively, they can leach out from textiles during washing, reach wastewater treatment plants, and accumulate in biosolids. The latter can be land-applied in agricultural fields and eventually be taken up by crops. Understanding the effects of silver nanoparticles on the growth and health of crops is paramount. The goal of our project is to determine the impact of silver nanoparticles on hydroponically grown corn. Root systems secrete chemicals to the environment, which dictates their interaction not only with soil, but with microorganisms present in the rhizosphere. When corn plants were exposed to silver nanoparticles, changes in the composition of polar metabolites such as carboxylic and amino acids were determined in the roots. Metabolite changes in corn roots brought about the exposure to silver nanoparticles and was performed using reverse phase liquid chromatography and high-resolution mass spectrometry. Evaluating the impact of metabolic changes will be of prime importance for the identification of a biomarker for exposure of affected plants to silver nanoparticles. The results will provide fundamental understanding of the interactions of nanomaterials exposed to an agricultural crop, which will provide further insight on the environmental impact of the new technology to agriculture.

AGRO 228

In Vitro Metabolism of [^{14}C]-Benalaxyl in Hepatocytes of Rats, Dogs and Humans

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The *in vitro* comparative metabolism study is now a data requirement under EU Directive 1107/2009. The comparative *in vitro* studies help determine the extent of metabolism of a chemical in each surrogate species and whether any unique human metabolite(s) is formed. In the present study, metabolism of [^{14}C]-benalaxyl (racemic) was investigated in cryopreserved rat, dog and human hepatocytes. The metabolites were identified/characterized by LC/MS/MS with radiometric detection and comparison with reference standards. [^{14}C]-glucuronide conjugates of benalaxyl metabolites in rat, dog and human hepatocytes were confirmed via additional experiments in which known reference standards were incubated with dog liver microsomes. After 4 hours of incubation, benalaxyl was extensively metabolized in all the species with the following trend: dog (100%) > human (86%) > rat (75%). In all species, the major metabolic pathway consisted of hydroxylation of the xylene (methyl) moiety to 2-hydroxymethyl- benalaxyl, further oxidation to carboxylic acid analogue (benalaxyl-2-benzoic acid), and hydrolysis of the methyl ester to yield benalaxyl acid. In addition, 2-hydroxymethyl-benalaxyl was found to be present as glucuronide conjugate, as major metabolite, especially in dog hepatocytes. No unique metabolite was observed in human hepatocytes. Also, benalaxyl did not undergo stereo-selective metabolism in rat or human hepatocytes.

AGRO 229

Probing the metabolomic impacts of chloroacetanilide herbicides on earthworm coelomic fluid

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Improvements in methods for monitoring environmental health and advancing our understanding of environmental effects depends on advances in technologies for chemical analysis and ecotoxicity assessment. Current limits of detection strain the true characterization of chemicals in the environment, some of which may hold significant toxicological implications at their low, unquantifiable levels. Thus, alternative methods to identify contaminants and evaluate ecotoxicity are needed. One such approach is environmental metabolomics, which seeks to understand how organisms respond to toxicants. Earthworms (*Eisenia fetida*) are vital members of the soil environment, serving as ecological engineers, decomposers, and a food source. Earthworms are also sensitive to contaminants and directly exposed to agents via soil contact, thus they are useful as bioindicators of soil health and ecotoxicity.

The chloroacetanilides are a class of broad spectrum, pre-emergent herbicides that are among the most widely used herbicides worldwide. These compounds are persistent and mobile in the environment, especially once transformed to their ethanesulfonic (ESA) and oxanallic (OA) acid analogs. In this study, the metabolomic impacts of five chloroacetanilide herbicides and one enantiomer (acetochlor, alachlor, butachlor, metolachlor, S-metolachlor, and propachlor) are examined in earthworm coelomic fluid using nuclear magnetic resonance (NMR) and mass spectrometry (MS).

AGRO 230

Radiovalidation of QuEChERS based on LC-MS/MS and LSC analysis

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The purpose of this research is to verify the analytical technique of extracting pesticides using QuEChERS. Analytical methods have already been developed and are fully established to determine residues of various matrices and are regulated under various governmental institutions. This research aids in the confirmation of QuEChERS extraction methods using radiolabeled [phenyl-^{14}C] Bensulfuron-methyl. QuEChERS primary ability to clean up samples can be further traced with radiolabeled ^{14}C which allows for identification and recovery on multiple analytical instruments. Liquid Scintillation Counting and HPLC-LC-MS-MS are the primary analytical instruments to determine residue concentrations. The LOQ is established to be 10 ppb. The primary matrices under investigation are soil, water, and tissue. Radioactive recovery values are within 110-90% on the LSC as well as mass spectrometer. 1) Specificity 2) linear dynamic range 3) limit of detection LOD 4) limit of quantification LOQ, 5) precision will be evaluated. With tighter recovery restrictions and multiple techniques, this research would prove useful to regulatory agencies as well as the agrochemical scientific community.

AGRO 231

Investigating the role of Trp86 residue of human acetylcholinesterase in interaction with organophosphate by docking, site directed mutagenic and molecular modeling approach

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Docking, site directed mutagenesis and molecular dynamic simulation (MDS) approach, was used to explore mode of binding and inhibition for human acetylcholinesterase (*h*AChE) and organophosphates (OPs). More than 200 OP molecules were investigated using Glide docking module of Schrodinger suitas co-crystal structure between two are not available in Protein Data Bank. In initial screening Trp86 was found to be involved in maximumPi-Cation interactionon anionic subsite of *h*AChEother than Ser203 (Catalytic site). With extra precision glide docking Phoxim Ethyl Phosphonate (PEP) lead among 200 OPs by interacting with Trp86, Gly121 and Ser203. Site directed mutagenesis at Trp86 (Trp86 to Ala86) shown the deterioration of the binding site in terms of size reduction, loss of electrostatic and geometric stabilization in binding cavity and significant reduction in binding of OPs in preferred orientation. Dock score of both wild and mutated *h*AChE shows a perfect qualitative agreement (R^2=64.1%) towards the study. Molecular dynamic simulation (GROMACS 4.5.5)of *h*AChE-PEP complex for 4 X 10^4 pico-second with SPC16 water system at 310K temperatureexplainedthe evident role of Trp-86 in stabilizing the ligand at P-site of the enzyme. Asp-74 and Tyr 124 were noticed in conveying H-bonds. Trp86 and Ser203 have shown consistent and better stability of bond based on distance factor (between residues and ligand). As residue Trp86plays significant role, it can be further explored for antidotes development in case of human poisoning.

AGRO 232

Accurate mass in agrochemical analysis. Understanding when to use ppm and when to use Da to express mass accuracy

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Accurate mass instruments are becoming more common in agricultural chemical analysis. The accuracy of the results is typically expressed in ppm. Understanding the true, practical meaning of ppm is critical to interpreting the data. Most accurate mass instrumentation actually produces data with accuracy that is more practically measured in mDa rather than ppm especially at low mass-to-charge ratios (smaller compounds). This poster will present examples of data showing that an error of 10 ppm, while sounding quite far off, can actually be quite good data at some mass values and on the other hand an error of 1 ppm on its own may not be good enough at other masses. It will also show how the use of isotope pattern information and the accurate mass MS/MS fragmentation pattern of a compound supplement the information found in the accurate mass value alone and can help to confirm or to deny the assignment of a chemical formula to an accurate mass value.

AGRO 233

Mass spectrometry based method for measuring vitellogenin in fish as biomarker of exposure to endocrine disrupting chemicals

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Important agricultural chemicals such as insecticides, herbicides, fumigants and fungicides have been suspected to cause endocrine disruption in fish and wildlife. In addition, synthetic and natural hormones that are also considered endocrine disrupting chemicals (EDCs) are present in animal manure and can enter the environment when manure is land-applied as fertilizer. It is not currently known which chemicals and what concentrations affect the endocrine system. One of the most effective ways to evaluate the presence of EDCs in the aquatic systems is to measure the induction of biomarkers in fish. A commonly used biomarker for EDC exposure is the elevated expression of vitellogenin (Vtg) in male fish, a liver-derived serum precursor protein of the egg yolk. In the present study, we are developing a liquid chromatography tandem mass spectrometry (LC-MS/MS) method to measure common peptides found in Vtg from different fish species, as an alternative to the commonly used enzyme-linked immunosorbent assay. Two experiments were designed. First, appropriate signature peptides resulting from the trypsin enzymatic hydrolysis of Vtg from three different fish species were identified using mass spectrometry. This process involved protein isolation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), in-gel enzyme digestion, and MS analysis using LC-quadrupole time-of-flight MS. Second, LC-MS/MS analysis of the identified signature peptides was performed under multiple reaction monitoring, as surrogate for Vtg levels in real fish samples that have been exposed to EDCs. Preliminary results from our study showed a possible signature peptide GILNILQLNIK that can be useful for quantifying Vtg by LC-MS/MS. Other signature peptides are being considered. After additional signature peptides are identified, a quantitative LC-MS/MS method can be developed. This work will demonstrate the

potential of LC-MS/MS as a generic method for measuring Vtg in various fish species that have been exposed to different types of EDCs.

AGRO 234

Advantage and limitation of combining met ID with quantitative analysis in the QTrap 6500 mass spectrometer

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Samples from metabolism rate assays are normally very dilute and rarely useful for metabolite identification (Met ID). The Qtrap 6500 triple quadrupole and ion trap functions coupled with fast scan speed provides the sensitivity to combine the quantitative MRM scan and data-dependent ms/ms fragmentation in a single sample injection. Met ID in discovery stage can provide insight for SAR to help candidate optimization. Combining the rate of degradation/metabolism with Met ID can also provide information on the dynamics of metabolite formation. This study was designed to evaluate the utility and limitations of combining MRM quantitation with one of the following data dependent Met ID scan modes in the routine 3-min LC/MS run: MRM plus full scan triggered data dependent ms/ms (MRM+EMS_EPI); MRM plus precursor triggered data dependent ms/ms (MRM+Precusor_EPI); and MRM plus predicted MRM (pMRM) triggered data dependent ms/ms (MRM+pMRM_EPI). Compounds used in this study were Oxathiapiprolin, Indoxacarb, Metaflumizone, flupyrsulfuron methyl and Hexazinone. Rat hepatocytes at 2 million cells/mL were incubated with 2.5 µM of the test compound. After the incubation was stopped with 10x volume of acetonitrile, a 2-µL aliquot sample was analyzed with Qtrap 6500 using one of these combined scan modes. These scan modes were evaluated by comparing their limit of detection, limit of quantitation, linearity, dynamic range, accuracy, precision and reproducibility, and the metabolite ID quality, such as number of metabolites detected and the quality of ms/ms spectra. Although the long duty cycle in pMRM scan makes it difficult to have enough data points for peak integration in the routine 3-min LC/MS run, our results demonstrate that the Qtrap 6500 is capable of generating good quality quantitation and metabolite ID from a single injection which was comparable to the quantitation by MRM only.

AGRO 235

Detection of ractopamine in sheep urine after exposure to trace levels of dietary ractopamine

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Ractopamine is a feed additive approved in the US for swine, cattle, and turkey usage to increase growth rates and improve feed efficiencies. Although ractopamine is approved for use with a zero day withdrawal period, trace levels detected by countries in which ractopamine is banned can resulted in import rejection. Our goal was to determine levels of ractopamine exposure that will result in its detection using qualitative and quantitative assays. Ractopamine was fed to sheep at 0 (ZERO), 0.001 (LOW), 0.01 (MED), and 0.1 (HIGH) mg/kg of diet (n = 4 per level, 0.5 kg of feed/day) for seven consecutive days and urine was collected daily about ~16 hrs post exposure. The HIGH dose represents 2% of the normal dose fed to hogs. On-site lateral flow assays (LOD ~1 ng/mL) of urine tested positive for ractopamine in each treatment group: ZERO (7.4%), LOW (0%), MED (82%), and HIGH (86%). With use of half maximum absorbance values (OD_{450nm} ~1.4, 4 trials), ELISA testing resulted 0%, 3.6%, 86%, and 93% positives for ZERO, LOW, MED, and HIGH, respectively. Parent ractopamine in urine was below the method LOQ (0.7 ng/mL) using LC-MS/MS in all samples. However, when total ractopamine (parent + hydrolyzed metabolites) was measured, LOW animals contained residues less than the LOQ for all samples with 7 out of 28 samples showed above LOD (0.22 ng/mL), MED animals had ractopamine levels of 1.08-9.13 ng/mL and HIGH animals excreted 4.85-32.82 ng/mL of total ractopamine. Ractopamine is rapidly eliminated; nevertheless urine from sheep exposed to as little as 5 µg/day (MED) of ractopamine had > 80% chance of having a positive ractopamine urine sample detected by the screening assays which was confirmed by LC-MS/MS.

AGRO 236

Adapting new techniques and instrumentation to improve the monitoring of > 150 veterinary drugs including aminoglycosides in food animal tissues

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Like its regulatory counterparts worldwide, the USDA Food Safety and Inspection Service (FSIS) monitors food animal tissues for veterinary drug residues. In support of this function, USDA Agriculture Research Service (ARS) conducts collaborative research to develop new analytical approaches that improve regulatory monitoring programs. Sample preparation to efficiently recover a wide range of drugs from diverse matrices is the first challenge, and it is inherently difficult to fully recover and isolate veterinary drugs without extensive matrix co-extractives that induce signal suppression and sensitivity losses during mass spectrometric detection. However, new cleanup materials on the market show promise to more selectively remove lipid components from extracts. Experiments have shown high recoveries of >150 veterinary drugs from bovine kidney using a streamlined method using EMR-Lipid for cleanup, which provides better performance than the current FSIS method using dispersive solid-phase extraction with C18. Unfortunately, aminoglycoside antibiotics are not able to be extracted from animal tissues at the same conditions as other drugs, but they can be analyzed in the same 10 min ultrahigh-performance liquid chromatography – tandem mass spectrometry (UHPLC-MS/MS) method by introducing an ion-pairing reagent into a combined final extract from each sample preparation method. This saves time and reduces the number of expensive UHPLC-MS/MS instruments needed in the monitoring laboratory. The final challenge in high-throughput monitoring of so many analytes to meet regulatory acceptable quantification and identification criteria involves reliable and accurate data processing and reporting without time-consuming human review of results. Implementation of the summation peak integration function followed by automatic post-run processing to make yes/no analyte identifications based on fixed, pre-defined criteria has been shown to yield more consistent quantification and fewer false negatives and positives than currently common data processing approaches, but without the need for human review of peak integrations.

AGRO 237

Residue of Fluquinconazole during Cultivation of Tomato

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In order to elucidate residual characteristics of fluquinconazole by application to tomato, and to generate the data for the maximum residue limit (MRL) establishment in an importing country of tomato for Fluquinconazole in or on tomato, fluquinconazole SC (10%a.i) was applied onto tomato according to its safe use guideline, and then tomatoes were harvested at 0, 1, 3, 5, 7, 10, and 14 days after final application from pesticide-applied plots at each of 6 sites. The tomato samples were extracted with acetonitrile. The extracted samples of fluquinconazole were analyzed by LC-MS/MS. The average recoveries of fluquinconazole at three different fortification levels (LOQ, 10LOQ, 70LOQ) ranged from 95.6 to 116.7% in tomato, and the coefficient of variations (CV) was less than 10%. The residue range of fluquinconazole was 0.13～0.78 mg/kg, and the residue was decreased to below MRL (0.7 mg/kg, KFDA) in 1 day harvest samples after final application. The biological half-lives of fluquinconazole were 14.3, 16.3, 9.0, 11.0, 6.4 and 10.1 days at each of the 6 test sites, respectively.

AGRO 238

Detecting pesticides in the cannabis plant: Complications and interferences

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Since the Cannabis plant has entered US markets as a legitimate crop, pesticide use has become a nontrivial issue. As with other crops, the increasing scale of cultivation has escalated the need for and use of pest control, compounded by the tendency of many cultivations to be indoors, isolated from the sunlight and climatic winds which normally aid in the degradation of chemical additives. Thus, the limits and recommendations for pesticide use set by the FDA -- intended mostly for outdoor crops -- lose pertinence. Cured cannabis flower has a very low water content and very high hydrophobic oil content, making the standard repertoire of validated analytical methods all but inapplicable. The author here aims to determine which specific pesticides can be quantified in Cannabis plant matrix easily using existing methods, and which will require development and/or refinement of analytical procedures. The pesticide monitoring list issued by the State of Nevada for cannabis crops was chosen as target analytes, covering a range of different chemical classes commonly employed in Cannabis cultivation. Of the 23 analytes studied, 16 were found to have acceptable recoveries in matrix spiked samples (LOQ 2ppb) using standard QueChers methods and LCMSMS with ESI. Five analytes responded more adequately to GCMSMS with API, one of which (Captan) required additional cleanup steps to prevent degradation in the inlet. For all analytes, matrix matched calibrations improved recovery by mitigating the effects of interferences from the complex chemical environment of the plant. It should be noted that, while acceptable methods have here been validated for 23 analytes, there exist hundreds of chemicals with potential for use in Cannabis pest control. Any legislation regarding chemical use and contamination will have to prove -- along with physiological safety -- the ability to be detected and quantified in the Cannabis matrix at the desired tolerance limits.

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Herbicide binding in plant acetyl-CoA carboxylase by homology modeling, MD simulation, and docking

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Abstract:

Cyclohexanediones (DIMs) and Aryloxypropionic acid analogues (FOPs) are the herbicides useful in controlling annual and perennial grass through inhibition of acetyl-CoA carboxylase (ACCase). They act by reversibly binding to ACCase and blocking a key step in fatty acid biosynthesis, i.e., conversion of acetyl CoA into malonyl CoA. Resistance of this enzyme to these inhibitors is a problem. Thus, understanding the binding mechanism of these molecules to ACCase helps in developing new efficient inhibitors. Since the crystal structures of plant ACCase are not available, we have used the crystal structure of yeast ACCase with bound herbicide ligands as the starting point for homology modeling of the corresponding plant ACCase. Alignment of published sequences of corn and other plant ACCases to the yeast sequence and structure were carried out with the Needleman-Wunsch algorithm and BLOSUM62. Molecular dynamics simulations were performed to refine the plant ACCase structures with the OPLS force field in Desmond from Schrödinger, Inc. and were compared with our earlier homology modeling with BioMedCache and the MM3 force field. We have docked analogues of aryloxypropionic acid (FOPs) and cyclohexanediones (DIMs) into the transcarboxylation domain of homology modeled corn and other plant ACCases using GLIDE with comparison to other docking software packages. Computed binding affinity scores are related to experimental IC50 and Ki values.

AGRO 240

Meta-analysis on parabens in sewage sludge

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Parabens are a group of compounds extensively used as preservatives in pharmaceutical and personal care products (PPCPs), food, beverages, and industrial products. Parabens also are considered to represent emerging endocrine disruptors and have raised concerns for human and animal health, as these compounds are toxic to aquatic organisms and also potentially may cause immune dysfunctions and affect human reproductive outcomes. Parabens continuously enter wastewater treatment plants (WWTPs), and despite their biodegradability, remain detectable at ng/L levels in treated plant effluent, and at ng/g levels in digested sewage sludge. Whereas parabens dissolved in WWTP process streams have been studied extensively, the number of studies on their occurrence and concentration in sewage sludge is still limited. However, monitoring of parabens in sludge is important for both ecological and human health risk assessments. Information on parabens in sludge is needed for risk assessments, since sewage sludge application on land as a common disposal practice can lead to contamination of soil, groundwater and surface waters. In this study, we reviewed literature data on parabens in sewage sludge, and estimated the estrogenicity contributed by parabens in sludge using as an indicator the Estrogenic Equivalent Quotient (EEQ). Parabens were found to be ubiquitous in sewage sludge, with methyl paraben and propyl paraben being the most frequently used and detected representatives of this chemical group. Overall, concentrations for parabens in sludge were moderate and varied between countries (*n*= 5) and sludge types. The

overall theoretical estrogenicity burden contributed by parabens in sludge was less than 1 pg/g 17β-estradiol equivalents or less than 0.1% of the burden posed by natural estrogens from food items present in sludge[RH1] . This study indicates that the risk associated with synthetic parabens in sewage sludge is minor and essentially insignificant due to the comparatively low concentrations and estrogenicity. Future studies should include metabolites and derivatives of parabens, which also may act as potential endocrine disruptors and can occur in sewage sludge at concentrations higher than those of the parent compounds.

[RH1]The units ng/g are no higher than those stated previously for synthetic parabens. Omit info or use correct units if applicable.

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Comparative analysis of organic contaminants in sewage sludge from the United States and China

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Municipal sewage sludge, the byproduct of wastewater treatment, is disposed of via several methods, including land application, incineration, and landfilling. Depending on the disposal methods, recalcitrant chemicals are reintroduced into the environment and can cause exposure to humans and wildlife through multiple pathways. The U.S. Environmental Protection Agency currently regulates toxic metals and pathogens in sewage sludge destined for land application, but no equivalent regulations exist for organic contaminants (OCs). The objectives of this study are to (i) summarize research efforts conducted toward OC characterization in U.S. sewage sludges to date, (ii) compare OC concentrations and trends found in the U.S. and China, (iii) identify and recommend priority OCs for monitoring and regulation, and (iv) inform on international research priorities. A comprehensive review of contaminants found in U.S. sewage sludge was performed via iterative literature searches using the Web of Science database. The search yielded four EPA sewage sludge surveys (1982, 1988, 2001, and 2006/07) and many other peer-reviewed publications. These results were then compared to an existing database of OCs found in Chinese sludges, and analyses were performed to quantify bi-national differences in chemicals assayed, chemicals detected, concentrations, and spatial and temporal trends. To date, some 500 OCs have been detected in US sludges reported in over 80 relevant studies. The most abundant group of chemicals were alkylphenols and their ethoxylates in US sludges, and phthalate esters in Chinese sludges. A meta-analysis of the literature showed a contrast in publication rate between the two countries. The United States had steady publication rates in the late 1990s and early-mid 2000s, but information has tapered off in the past decade. China has had steadily increasing publication rates from the mid-2000s, until now, each year publishing an increasingly higher number of studies. Also, land filling of sludges was more prevalent in China relative to the US. Based on the results from this study, a few target priority groups of OCs have been recommended for future US and China sewage sludge studies, and the database can be used for risk assessments and for the development of international monitoring policies.

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Detection of imidacloprid, fipronil and its degradates in wastewater and biosolids of eight wastewater treatment plants in Northern California

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Over the last decade, use of two relatively new insecticides - imidacloprid and fipronil has increased for pest control applications. In the present study, we explored the presence of fipronil, its degradates (total fiprole), and imidacloprid in urban wastewater before and after treatment, and in biosolids of eight wastewater treatment plants (WWTPs) of Northern California under low-flow drought conditions. Imidacloprid was detected at 100% detection frequency (DF) in influent and effluent samples at concentrations of 58-306 ng/L (influent per capita load of 120-310 nmol/L), whereas sludge analysis yielded no detections (<1 ng/g dw). Ninety-three ±17% of the mass of imidacloprid entering the plants remained in the effluent. Fipronil and its sulfone and sulfide derivatives were detected with 100% DF in all influent and effluent samples of all WWTPs monitored, with concentration ranging between 13-88 ng/L, 1-28 ng/L, and 1-5 ng/L, respectively (influent per capita total fiprole load of 45-65 nmol/L). Fipronil amide, a product of fipronil hydrolysis, was absent in all influent samples, but detectable in effluent of seven of the plants at concentrations ranging between 1-4 ng/L, suggesting hydrolysis during biological treatment. Total fiprole loading was partially removed from wastewater, apparently due to partitioning into sludge. Sixty-five ±11% of the mass of fipronil related compounds entering the plants remained in the effluent, whereas 35 ±11% was found in sludge. Concentrations of fipronil compounds detected in sludge ranged between 0.1-91 μg/kg dry weight. The ratio of sulfone and sulfide degradates to fipronil was higher in biosolids when compared to raw wastewater. This is the first regional study of imidacloprid and fipronil occurrence in California WWTPs. Although sampling didn't account for hydraulic retention time, results suggest that none of the treatment processes sampled provide significant removal, resulting in unwanted insecticide presence in wastewater effluent and (for fipronil) sludge.

AGRO 243

Molar distribution and correlation between fipronil and its degradates in wastewater and biosolids of eight California wastewater treatment plants

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Fipronil is a phenylpyrazole insecticide that forms toxic degradates under environmental conditions. However, the occurrence and generation of fipronil degradates in wastewater treatment unit operations have not yet been studied in great detail. The objective of the present study was to assess the distribution of fipronil and its degradates in wastewater and sludge or biosolids samples in eight wastewater treatment plants (WWTPs) located in California. Raw wastewater was separated into liquid and particulate fractions and analyzed separately to determine the total mass loading of fipronil and its degradates in sewage. Whereas fipronil amide was not detected in influent, fipronil desulfinyl

was found with 12.5% detection frequency (n=8) at concentrations of about 1 ng/L only in the liquid phase. In influent, the majority of the mass of fipronil was present in the aqueous phase, whereas its sulfone and sulfide degradates were found to be particulate bound. Overall, 62 ± 9% of total fiproles (fipronil and its degradates) were present in the dissolved state in influent, with the balance being bound to particulates. The molar distribution of fiproles was different in each WWTP process stream and phase, but this difference was relatively consistent across all sampled WWTPs despite the diversity of unit treatment processes among the WWTPs. Similarly, the molar distribution of fipronil and its degradates in sludge was consistent among WWTPs, but differed between anaerobically digested and untreated sludge, with significantly higher amounts of sulfide degradates being present in anaerobically digested sludge (biosolids). In influent and biosolids, linear correlations between the distributions of fipronil and its degradates were observed. The resultant molar distribution coefficients for process flows are helpful for estimating the occurrence and level of degradates when monitoring data is available only for the parent compound, fipronil.

AGRO 244

Novel study design for the performance of an aerobic flooded soil study utilizing natural sunlight and controlled temperature

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ABC Laboratories designed an outdoor study patterned after a laboratory OECD 307 aerobic flooded soil set-up where the temperature was maintained at 20-25°C, but the systems were subjected to natural sunlight during summer/fall months. This study was needed to investigate metabolites observed in field studies that were not replicated in a laboratory setting. The test systems were prepared by adding soil and water to specially designed jacketed vessels (500 mL borosilicate) that were sealed with a thin optical grade quartz lid, thus allowing the samples to be subjected to the natural sunlight. The systems were incubated in an air flow-through system, with the air leaving the systems drawn through a trapping series meant to capture any volatile ^{14}C-degradates. Radiolabeled (^{14}C) test substance was applied to the water phases at a rate of 0.3 mg/mL total water column, and samples were taken over a 60 day period. Dark controls were also prepared in the same manner, but were covered from the natural sunlight. The water and soil extracts were analyzed for total radioactivity, and then chromatographically profiled to determine the amounts of test substance and degradation products. The dissolved oxygen in the water confirmed aerobicity; pH remained slightly basic in the water and acidic in the soils; and redox potential was positive in the water and negative in the soil over time. Overall, temperature was maintained at 20-25°C even while subjected to natural sunlight, and mass balance was at desired levels (between 100 and 109% applied radioactivity (%AR)). Half-lives for the parent were roughly 20 days for parent subjected to sunlight and twice that for the dark controls. Seven metabolites were tracked and identified in the study, including the metabolites that could not be replicated in the lab. These results showed that the set-up was sufficient for the testing required.

AGRO 245

Outdoor water sediment study – Adding effects of sunlight to aquatic system exposure assessment

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Laboratory aerobic water sediment studies conducted in accordance with regulatory guidelines must be maintained in the dark during the in-life phase. For those agrichemicals demonstrating photolysis in solution, the standard study may not represent the real-world fate of the chemical in the environment. Integrating full-spectrum sunlight into the study design to assess its contribution may be important for determining route and rate of degradation as inputs to estimating environmental concentrations. An outdoor water sediment study was conducted on a new product using a design that followed the current study guidance except for substituting quartz glass laboratory vessels for borosilicate glass and exposing the system to sunlight out-of-doors. The results of the study showed that photolysis significantly altered both the route and rate of degradation of the test compound in the sediment systems tested when compared to results obtained in the dark incubated study.

AGRO 246

Experimental design of high tier aged sorption studies for pesticides

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In the “first tier” approach, conventional groundwater assessment with the FOCUS guideline assumes that a characteristic ratio between sorbed and dissolved pesticide is rapidly established after contact with the soil, implying that the strength of sorption is constant with time. However, in the real environment, the equilibrium for many pesticides is not reached after months, or even longer after application. Thus, achieving an acceptable regulatory risk assessment can be problematic for some pesticides using a ‘first tier’ approach.

An aged sorption study is one of the higher tier approaches being developed within the regulatory pesticide leaching assessment to refine the description of the pesticide behavior in soil. Currently, two documents “Guidance on how aged sorption studies for pesticides should be conducted, analyzed and used in regulatory assessments, CRD/FERA, 2012” and “Statement on the FERA guidance proposal, EFSA, 2015” are available as a general guidance on conducting an aged sorption study. The key components of the experimental design to derive aged sorption parameters will be reviewed and summarized. Key parameters such as soil selection, $CaCl_2$ extraction method, LOD/LOQ, sampling points needed for the modeling, etc. will be discussed. Other study design and data handling methods not covered in the guidance documents will also be proposed.

AGRO 247

Determination of the plant uptake factor for Oxathiapiprolin (DuPont™ Zorvec™) soil metabolites in tomato, potato and lettuce

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Oxathiapiprolin (DuPont™ Zorvec™) is a fungicide which provides exceptional control of oomycete diseases at very low use rates. Oxathiapiprolin degrades microbially to multiple soil metabolites some of which have low sorption coefficients that indicate potential mobility in soil. To improve estimates of the environmental behavior of these metabolites in cropped fields following normal application, the plant uptake of three oxathiapiprolin soil metabolites was extensively studied in tomato, potato and lettuce in buffered calcium chloride solutions. Plants were incubated hydroponically in test solutions treated with radiolabeled test material. The uptake of the test solution by the plants and the concentration of the test substance in the test solution were measured over a period of 8 days after which the mass of test item in the plant was determined by separately measuring the amount of radioactivity in the roots and stem/leaves. From these data, the plant uptake factor (PUF) was calculated. The most polar metabolite demonstrated only passive uptake with PUV values <1. The two less polar metabolites demonstrated uptake into the stem/leaves as well as concentrating in the roots with PUF values in some cases >1. These results indicate significant plant uptake which provides more realistic input parameters for the models used in calculating the predicted concentration in groundwater.

AGRO 248

Accurate determination of adsorption values for low adsorbing compounds

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The adsorption value is a primary input in leaching models for both parent compounds and metabolites. In the case of low sorbing compounds, any experimental error can lead to high uncertainty in the adsorption value and a questionable modeling endpoint. According to OECD 106, "Accurate determination is possible if the value which results by multiplying the K_d with the soil/solution ratio is > 0.3, when measurements are based on concentration decrease in the aqueous phase (indirect method), or > 0.1, when both phases are analysed (direct method)". The current regulatory approach in Europe is to test the reliability of the adsorption value according to this "p-criterion" calculation, and to consider an adsorption value of zero if the "p-criterion" is failed. Such conservative adsorption values create hurdles in the hazard-based European leaching assessment, and underpin the importance of experimental methodologies which can increase the accuracy of the adsorption value for low adsorbing compounds.

To this end, a high speed centrifugation plus filtration technique is proposed to enable the accurate determination of adsorption values for low adsorbing compounds. This technique, consistent with current OECD 106 guidance, can increase the soil/solution ratio from 1 kg/L for traditional sorption experiments to *ca.* 10 kg/L, increasing accuracy of the sorption measurement down to *ca.* 0.03 L/kg.

The high speed centrifugation plus filtration technique will be described in detail. Results will also be presented and discussed, and compared with the results of traditional (indirectly measured) sorption experiments.

AGRO 249

Comparison of photodegradation of selected agrichemicals on moist and dry soils

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Traditionally, soil photolysis studies have been conducted on dry soils only. Since the release of the Draft Document "OECD Guidelines for the Testing of Chemicals" in 2002, Agrochemical companies have revised their study protocols and incorporated the moist soil treatments in their soil photolysis study designs. In this presentation, results from the moist and dry soil photolysis studies of selected DuPont agricultural products, methomyl, nicosulfuron, Oxathiapiprolin, and triflusulfuron Methyl, were compared to illustrate the effects of moisture on their degradation rates and metabolite profiles. For the four compounds investigated, the degradation in moist soils was faster than in dry soils presumably due to the enhanced biodegradation and abiotic degradation, such as hydrolysis. Similar metabolites were formed in the moist and dry soils for nicosulfuron, Oxathiapiprolin, and triflusulfuron Methyl but the amount (% of applied radioactivity) of a certain metabolite in the moist soil was generally more than in the dry soil. For methomyl, the major component in the organic volatile traps from the moist soils was carbon dioxide while acetonitrile was the only component trapped from the dry soils.

AGRO 250

Survey of glyphosate in domestic and imported beer and wine

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One hundred and seven domestic and imported beers and thirty wine bottles purchased around the Philadelphia area were analyzed for glyphosate residues using ELISA. The beer samples consisted of ales, lagers, pilsners, mai bocks, IPAs, stouts, and porters, and wheat beers such as weissbiers, witbiers, weizenbiers. The wine samples consisted of white wines such as chardonnays, pinot grigios; red wines such as cabernets, merlots, tempranillos, and others. The limit of quantification (LOQ) of the method was 0.375 ppb. Glyphosate residues above the LOQ was found in >97% of the beers tested with a range of <0.375 ppb to 196 ppb, wheat beers exhibited the highest glyphosate concentration. All the wine samples tested contained glyphosate with a range of 2.6 ppb to 29 ppb.

The list of beer and wine samples and individual glyphosate concentrations obtained during testing will be presented.

AGRO 251

Practical implementation techniques for reliable and selective determination of glyphosate and AMPA in milk and urine using LC-MS/MS

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As analytical laboratories expand the types of matrices examined for determination of pesticide residues, such as glyphosate, there can be unique challenges encountered for each new matrix. Often sample handling, homogenization, extraction, and clean-up needs to be optimized to achieve the best performance of analytical methods. This presentation will focus on the practical laboratory techniques that were developed to prepare milk and urine matrices for reliable trace level quantitative determination of glyphosate residues. The outcome of this method development and validation process was simple, selective, sensitive, and robust procedures for the direct analysis of glyphosate [N-(phosphonomethyl)glycine] and aminomethylphosphonic acid (AMPA) in human and bovine milk and human urine matrices.

AGRO 252

Phosphate fertilizer impacts on glyphosate sorption by soil under different pH conditions

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Olsen-P can be used to estimate the amount of plant available phosphorus in soil. Phosphate fertilizers are annually applied on most North America agricultural soils, and the use of glyphosate is common in these soils. Phosphate and glyphosate have shown to compete for the same sorption sites in soils. The objective of this study was to examine the effect of Olsen-P levels in sandy clay-loam soil on the sorption of glyphosate applied to this soil. Soil samples were collected in 2013 from a research site that had received annual applications of mono ammonium phosphate (MAP) at 20, 40 and 80 kg P ha^{-1} and from products containing 0.4, 70 or 210 mg Cd kg^{-1} as an impurity. A series of batch equilibrium experiments were carried out to quantify glyphosate sorption distribution coefficient, Kd. Results indicated that extractable Cd concentrations in soil had no significant effect on glyphosate sorption. Olsen-P concentrations ranged from 13 to 99 mg kg^{-1} across plots, and glyphosate Kd values were significantly smaller for soils with higher Olsen-P concentrations regardless of the pH conditions studied. Glyphosate Kd values were also significantly reduced when MAP was added to batch slurries in the laboratory but less so for field soils already containing large amounts of phosphate. Glyphosate Kd values in soils ranged from 47 to 939 L kg^{-1} suggesting glyphosate is substantially retained in this sandy clay-loam soil and sorption was particularly strong under acidic conditions. Experiments repeated with a commercially available glyphosate formulation showed statistically similar results as the experiments performed with analytical-grade glyphosate suggesting that other ingredients in the commercial formulation had no impact on the sorption behavior of the active ingredient glyphosate in soil.

AGRO 253

Analysis of monitoring data for synthetic pyrethroids in U.S. surface water and sediment

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The Pyrethroid Working Group has compiled data from all available pyrethroid monitoring programs in surface water and sediment across the US. Many of the monitoring programs targeted agricultural and urban sampling locations where pyrethroid concentrations were expected to be high (e.g., irrigation channels and residential storm drain outlets), and included event-driven sampling intended to capture peak pyrethroid concentrations associated with storm events. The database currently contains more than 65,000 analytical results for nine key pyrethroids from approximately 5,000 whole water samples, 2,500 filtered water samples, and 2,400 sediment samples collected from 1992 to 2014. Nearly 90% of these records, including most of the highest measured concentrations, are from California. The goal of this analysis was to identify the factors that contributed to the highest measured concentrations at agricultural and urban monitoring sites. Monitoring reports were reviewed for information on site characteristics, co-occurrence of storm events, and other factors associated with sample collections. Site locations, drainage areas and surrounding features were examined using GIS software. Pyrethroid use in agricultural drainage areas was estimated using data from the CA Pesticide Use Reporting (PUR) database. Pyrethroid use in urban drainage areas was estimated based on population and housing density data from the US Census. Many of the highest measured pyrethroid concentrations were reported from agricultural drainage channels and urban stormwater outfalls, and most were associated with wet season sampling. The highest concentrations for most pyrethroids were 10-fold greater, and in some cases more than 100-fold greater, than 99% of all measured concentrations. Even the 99th percentiles of the measured concentrations were below nearly all of the 90th percentile concentrations predicted by standard pesticide exposure modeling, indicating the extreme conservatism of modeling assumptions.

AGRO 254

Kriging models for predicting atrazine peak concentrations for non-daily surface water monitoring

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An assessment of the performance of linear interpolation, ordinary kriging, and universal kriging methods in predicting maximum m-day (m = 1, 7, 14, 30, 60) rolling averages and the 95th centile of atrazine concentration in surface water where sample data were collected by every K-day sampling (K = 7, 14) is presented. Daily/near daily atrazine monitoring data obtained from 32 sites and 89 site-years by the Atrazine Ecological Monitoring Program in the Corn Belt region (2009-2014) and from 4 sites and 62 site-years by the National Center for Water Quality Research (1993-2008) were used in the evaluation. Covariates investigated included nonlinear functions of the deterministic Pesticide Root Zone Model (PRZM), and steam flow data.

Comparison of direct linear interpolation versus ordinary kriging or linear interpolation after log-transformation shows improved predictions on the log-scale for m>1. Predictions tended to improve and become less variable in comparison to linear interpolation on the original scale as the duration of the rolling average increased. For kriging methods, empirical semivariograms of daily atrazine time series suggest a nugget close to zero, and this is supported by replicate analysis of selected field samples which indicated negligible measurement error. Piecewise linear semivariogram models with a range equal to the sampling duration (i.e., 7 or 14 day) were found to perform best for sampled data in terms of prediction. Universal kriging with covariates chosen from nonlinear transformations of PRZM and flow data found a normalized Box-Cox transformation of flow to provide best predictions among all methods examined, although predictions were negatively affected by sampling variability in regression coefficient estimates. Revised models incorporating seasonal covariates and fully constrained regression parameters provided improved prediction in comparison to loglinear interpolation for all rolling averages, as is demonstrated using test and validation samples.

AGRO 255

Cyclic ketoenol insecticides: Retrospective consideration and prospects

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Cyclic ketoenol insecticides (KTEs) comprising tetronic and tetramic acid derivatives have been identified nearly 30 years ago based on a shift in the weed spectrum within an herbicidal program of PPO-inhibitors and finally by an indication shift leading to acaricidal activity. Up to now three KTEs have been commercialized, two compounds with a tetronic acid fragment in their molecules such as spirodiclofen and spiromesifen, and one compound containing a tetramic acid fragment, spirotetramat.

Retrospective reflections/considerations regarding molecular features of commercialized KTEs gave insight into general structural requirements for divers ring systems and their physicochemical properties, which have a remarkable influence on bioavailability and distribution in plants. The addressed insect pest profile is different between individual, non-systemic members of this chemical class, *i.e.*, spirodiclofen is active against spider mites, whereas spiromesifen demonstrates potency on spider mites and whiteflies. Spirotetramat has a much broader spectrum and can be used for the control of various sucking pests.
As non-neurotoxic, selective inhibitors the KTEs interfere with *de novo* lipid biosynthesis by inhibition of acetyl CoA carboxylase (ACCase) of insects and spider mites (own IRAC MoA Group 23).

All KTEs are proinsecticides which can be activated to their enol metabolites by hydrolysis of the O-acyl residue either *in planta* or in targeted pests. In this context, spirotetramat is readily taken up and can be transformed within the plant vascular system into its ambi-mobile enol metabolite, which demonstrates excellent insecticidal activity. Meanwhile, their promising acaricidal and insecticidal efficacies triggered extensive research programs within other research-based agrochemical companies.

Prospects of KTE chemistry will now be focused on the differentiation of compounds with access to new market segments, improved regulatory profile, on detection of novel chemistry with different physicochemical properties and on the structure elucidation of the ACCase as target for molecular modelling studies.

AGRO 256

Organism dependent binding of pesticides to Acetyl-CoA carboxylase

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Acetyl-CoA carboxylase (ACCase) catalyzes the first committed and rate-limiting step in fatty acid biosynthesis and thus is an attractive target for drug discovery against diabetes and cancer, but also for pesticide discovery against various pests including insects, fungi and weeds. ACCase is a biotin-dependent enzyme that catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA through its two catalytic domains, biotin carboxylase (BC) and carboxyltransferase (CT). ACCase is a multi-subunit enzyme in most prokaryotes and in the chloroplasts of many plants and algae, whereas ACCase is a single-chain multi-functional enzyme in the cytosol of most eukaryotes.

Several different pesticide classes acting on ACCase are on the market. These include the insecticidal cyclic ketoenols (KTEs) and the herbicidal aryloxyphenoxy propionates (FOBs), cyclohexanediones (DIM) and cyclic ketoenols (KTEs). All compound classes seem to have in common that they bind to the CT-domain of ACCase. We used crystal structures of the CT domain from yeast and our scoring function HYDE in order to investigate and compare the binding of different compound classes to the CT-domain of ACCase. In particular, an analysis will be presented which compares and explains the differences in binding of selected compounds to target organisms such as insects or a variety of different plants.

AGRO 257

Structure-based design of a novel class of herbicidal HPPD inhibitors

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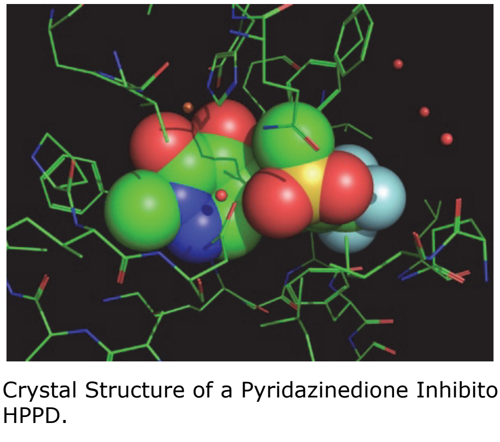
HPPD is the enzyme targeted by a range of commercial herbicides, including Mesotrione, Isoxaflutole, Tembotrione and Bicyclopyrone. The three-dimensional structures of a variety of herbicides complexed with HPPD had previously been determined by protein crystallography within Syngenta, giving us a detailed understanding of their binding. The talk will describe how this understanding has enabled the discovery of a new class of highly potent HPPD herbicides – the Pyridazinediones – using a structure-based design approach.



Crystal Structure of a Pyridazinedione Inhibitor bound to HPPD.

AGRO 258

Pyrimidinones and related carbonyl containing heterocycles as 4-hydroxyphenylpyruvate dioxygenase (HPPD) herbicides

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Inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD) were first developed in the 1980s as bleaching herbicides. The initially discovered molecules consisted of a substituted aromatic ring bridged by a carbonyl to a moiety containing an acidic hydroxyl group. In the search for alternative HPPD chemotypes carbonyl containing heterocycles were found to serve as suitable isosteres for the aromatic rings present in the original inhibitors. In addition to being isoelectronic with traditional aromatic rings, the carbonyls present in these heterocycles can serve in place of substituents on the aromatic rings. The pyrimidinone and triazinedione heterocycles we will describe in this presentation provided chemical novelty within this herbicidal mode of action and presented potential advantages over traditional aromatic rings including differing biological activity, improvements in physical properties and environmental fate. The synthesis as well as general structure activity relationships of these molecules will be discussed.

AGRO 259

Deuterated 6-aryl picolinate herbicides: Effect on potency and weed spectrum

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Arylex^{TM} active, a new broadleaf weed herbicide active ingredient, is representative of a potent class of 6-aryl picolinate chemistry for weed control in cereals and several other crops. Understanding uptake, translocation, and metabolism within this chemical class allows for the design of improved herbicidal compounds. Deuterium is often incorporated into herbicides to allow for monitoring of the parent molecule and metabolites thereby removing the need for radioactive samples when investigating uptake, translocation, and metabolism. Arylex^{TM} active as well as a set of 6-aryl picolinates were deuterated to better understand the metabolism of 6-aryl picolinate chemistry in both soils and plants. Appropriately incorporated deuterium atoms on 6-aryl picolinates resulted in an increase in both potency and weed spectrum. The observable phenotypic response represents a qualitative example of an *in vivo* kinetic isotope effect.
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AGRO 260

Synthesis and Herbicidal Activity of 3-Pyrazole Carboxamides

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We have found that 1-benzyl-3-pyrazole carboxamides are potent herbicides. Increases in herbicidal activity were found on replacing the benzylic group with a variety of heteroaryl isosteres. Surprisingly, we found two different sites of action for this area that depended on the nature of the amide and the position of a substituent on the pyrazole. We will detail the synthetic methods, biology, and SAR of these simple and interesting herbicides.

AGRO 261

High frequency monitoring of pesticides and water quality in PEI, Canada

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Fish mortality incidents have been a recurring problem in PEI. Some are believed to be related to pesticides in agricultural runoff, but while pesticides have been detected in fish and water samples from these incidents, the concentrations found have been too low to harm fish. A study was done to provide a more complete measure of water quality and pesticide concentrations continuously during the growing seasons of 2014 and 2015, at sites on the Barclay Brook the Little Pierre Jacques River and the Dunk River in PEI. Weather data including rainfall and barometric pressure were recorded. Water level, water temperature, turbidity, dissolved oxygen, pH, conductivity, total chlorophyll (Algae), Blue Green Algae, and Ammonia were recorded every 15 min. using EXO 2 Sondes. Water samples were collected for analysis using ISCO 6500R refrigerated autosamplers. Auxiliary pumps were set up to collect water continuously from the stream into a reservoir from which the autosampler collected samples at regular intervals. The pesticides included in the analysis were selected based on a risk quotient for fish above 0.5 and potential use in the area: ametoctradin, azoxystrobin, boscalid, chlorothalonil, cypermethrin, difenoconazole, fluazinam, fluxapyroxad, malathion, mancozeb, phosmet, pyraclostrobin, and zoxamide. Mancozeb was determined as ethylenethiourea (ETU). The analysis was done in two phases. In phase aliquots from samples were pooled and analysed, and in phase II the individual samples from the pooled sample with the highest residues were analysed. The analysis was sufficiently sensitive to compensate for dilution, ensuring that no significant events would be missed. Significant runoff events were profiled in each year. The design and results will be discussed.

AGRO 262

Placing EPA Tier II scenarios into national context in terms of runoff-erosion vulnerability after pyrethroid applications to agriculture

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US EPA regulatory evaluations of pesticides utilize crop-specific scenarios in a tiered exposure assessment framework. EPA defines the standard scenarios as "… best professional judgement sites expected to produce runoff greater than would be expected at 90% of the sites for a given crop/use". In order to evaluate standard scenario relevance for pyrethroid runoff and erosion, PWG has examined 15 crop scenarios using two different approaches. Firstly, the single field scale where 10-ha of a single soil is 100% cropped to the crop of interest was considered. The 90th percentile annual maximum runoff/erosion flux of a representative pyrethroid was estimated for each SSURGO soil shown by NASS CDL to have been cropped to each of the 15 crops over a 5-year period. This estimation used standard PRZM modeling with the nearest approved SAMSON 30-yr weather data and regional cropping dates. The second approach considered the real world catchment scale where surface waters receive runoff from multiple fields. The 90th percentile pyrethroid runoff/erosion fluxes were summed for all soil areas in each NHD+ catchment on which a given crop was grown. In both cases, the predicted runoff/erosion pyrethroid loads were

ranked to produce distributions against which the runoff/erosion loads from the corresponding standard EPA Tier II scenarios were compared to assess their relative vulnerability. The first approach indicated that across the 15 EPA standard scenarios, predicted runoff/erosion loading vulnerabilities varied with approximately half above and half below the 85th percentile. However, when compared to the summed pyrethroid runoff/erosion fluxes from soils growing each crop in real-world small catchments, 12 of the 15 EPA standard scenarios fell above the 99th percentile for runoff/erosion vulnerability for pyrethroids, and in two cases were more severe than any NHD+ catchment in the US. The approaches, results and implications of these analyses will be presented.

AGRO 263

Implementation of a portable small plot simulated rainfall and runoff collection system: GIS site selection, study methodology, and hydrologic results

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Surface water runoff can be an important transport mechanism for moving pesticides and nutrients from agricultural fields to adjacent surface water bodies. Measurement of hydrologic runoff response under relevant field conditions provides data that may be used to develop or refine runoff models. Such runoff measurements are often restricted to a single field site with (semi)permanent rainfall simulation and support equipment. Alternatively, attempts can be made to collect runoff data from multiple sites, but dependent on uncertain natural rainfall, which may lead to insufficient data collection within the time frame of the experiment. An existing portable rainfall simulator methodology was evaluated as a bridge between these designs.

A runoff study was conducted in three major corn and soybean producing Midwest states, Iowa, Illinois, and Ohio. A GIS analysis was used to identify candidate fields that met the runoff vulnerability criteria, including recent corn/soybean cultivation, moderate slope, and hydrologic soil group C or D. At each test site, nine test plots were established and characterized. Three test plots were located in each of three replicate blocks. The test plots in each block were subjected to simulated rainfall concurrently using three rainfall simulators. Two simulated rainfall events were conducted on each of the nine test plots, separated by 24 hours. During the runoff events, discrete samples and the entire runoff volume were sampled. Runoff volume was recorded for each discrete sample to calculate interval and cumulative runoff flow.

The experimental results indicate limited differences were observed in runoff volume and sediment transport between replicate blocks of test plots within each site. Therefore, the portable design appears suitable for hydrologic characterization and/or generation of samples that could be analyzed for pesticide or nutrient content.

AGRO 264

Multiresidue analysis for leaching of pesticides in groundwater of cotton cropping area

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Cotton in India is an important cash crop. About 50% of all pesticides used in India go for cotton cultivation. As cotton is a water intensive crop, it's the quality of the water that is most affected by its cultivation, due to leaching of chemicals and run-off in groundwater. Therefore a study was undertaken to analyze pesticide residues in cotton growing areas (Panniwala village, Sirsa, Haryana) and subsequent contamination of water resources. Groundwater samples were collected from eight tubewells of cotton (varieties: RD-8 and HD-123) growing farm fields.

According to the survey, Chlorpyrifos, Imidacloprid, Endosulfan, Methyl Parathion, Quinalaphos and Monocrotophos pesticides were used at different time intervals. Among the samples analyzed, 59% organochlorine, 45% organophosphorous and 29% synthetic pyrethroid samples were found to be contaminated. Analysis revealed that the concentration of various pesticides for organochlorine pesticides were from 0.032-11.6µg/l, organophosphorous pesticide from 0.033-7.829µg/l while synthetic pyrethroids analyzed from 0.02-0.602µg/l in groundwater samples. The results are alarming and show that either the use of banned pesticides (DDT and Endosulfan) is still continued or they are present in groundwater samples as they are very persistent. There is need of proper extension services to educate farmers about the judicious use of new molecules of pesticides along with Integrated Pest Management approach to avoid contamination of water resources.

AGRO 265

Flow-through experiments and algae population modelling as supporting tools within the pesticide risk assessment - results of case studies

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Algae flow-through experiments are not yet commonly known in risk assessment of pesticides, but are a useful higher-tier option to assess effects of pulsed pesticide exposure to algal populations.

The possibility of testing different exposure patterns in a flow-through system allows evaluation of more realistic time-variable exposure effects, compared to standard tests. In contrast to static experiments, it is possible to observe the inhibition of algal growth directly as visible loss of population biomass, as well as recovery of the population by re-attaining a steady-state level of cell density.

Combination with an algae population model extends the information obtainable with this experimental setup. Firstly, it allows simulating the impact of pulsed exposure on algae prior to performance of a flow-through test. If the simulated outcome is promising and a test is considered reasonable, the model can be used to design the flow-through test according to the specific needs in risk assessment. Application of a generic worst-case exposure profile in the flow-through test and successful simulations of the experimental results then enables validation of the model for predictions of population-level effects of the test chemical, and subsequently to use the validated model for extrapolations to other exposure regimes.

This approach has been tested with different algistatic toxicants and two standard algae test species, and by considering different types of pulsed exposure events, including a range of repeated peaks as well as more constant exposure periods. The experimental boundary conditions, the physiological properties of the algae and toxicity data obtained from standard growth inhibition tests, were used as the only model inputs for simulation of the experiments. No parameter fitting was necessary as standardized parameter sets for modelling were used.

Example experimental and modelling results will be presented and application of this approach within regulatory higher-tier risk assessments will be discussed.

AGRO 266

Water solubility and *n*-octanol/water partition coefficient measurements of pesticides, in freshwater and seawater

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Salinity has been reported to impact the water solubility of organic chemicals entering marine ecosystems; however, there is scarce data available on salinity impacts for pesticides potentially entering seawater. Impacts on water solubility would correspondingly impact chemical sorption as well as overall bioavailability and corresponding exposure estimates used in the regulatory assessment of pesticides. The insecticides fipronil, cypermethrins, bifenthrin and the herbicide atrazine are used heavily worldwide and can potentially be transported to water bodies through runoff or leaching. The water solubility and *n*-octanol/water partition coefficient (log K_{ow}) of these pesticides were determined in distilled water and artificial seawater using the column elution method. The water solubility of atrazine, fipronil, cypermethrins (a mixture of eight isomers), and bifenthrin were 56 ± 1, 4.8 ± 0.96, 0.78 ± 0.1, and 0.1 ± 0 in distilled water, versus 45 ± 0.24, 1.9 ± 0.25, 0.08 ± 0.02, and 0.04 ± 0.01, in artificial seawater, respectively. The log K_{ow} of atrazine, fipronil, cypermethrins, and bifenthrin were 2.55 ± 0.07, 3.48 ± 0.22, 5.99 ± 0.25, and 6.6 in distilled water, versus 2.74 ± 0.09, 4.24 ± 0.35, 7.9 ± 0.80, and 7.05 in artificial seawater, respectively. The seawater log K_{ow} values for the pyrethroids were estimated from their water solubility. Overall, the water solubility of pesticides were decreased in artificial seawater versus distilled water while the log K_{ow} of pesticides were increased in artificial seawater versus distilled water. Salinity appears to generally decrease the water solubility and increase partitioning potential, as indicated by the log K_{ow}. Dramatic differences in chemical partitioning behavior in seawater versus freshwater suggest salinity should be taken into account when conducting exposure estimates for marine organisms.

AGRO 267

Environmental behavior and metabolism of two chiral cis-nitromethylene neonicotinoid pesticides in aerobic soils by ^{14}C-labelings and Q-TOF MS

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The neonicotinoids are the most widely used class of insecticides in the world, and the growing problem has caused great concern because of their link to the bee colony collapses. Although neonicotinoid insecticides such as imidacloprid, have been widely used for decades, the understanding of their environmental behavior is far from comprehensive. Chiral insecticide paichongding (IPP) and cycloxaprid (CYC) are suggested as prospective substitutes to imidacloprid used in China due to their higher activity against imidacloprid-resistant insects. IPP contains two chiral centers with two couples of enantiomers and CYC has only one chiral center with two enantiomers. However, little is known about their behavior and metabolism in soils, including especially the different behaviors among their stereoisomers.
In this study, lab tests were conducted to elucidate the environmental fate and degradation of IPP and CYC in aerobic soils by ^{14}C-Labeling coupled with LC-MS/MS and high resolution MS tracking their residues and identifying major transformation metabolites. Entirely different behavior and metabolic patterns were discovered for neonicotinoid IPP and CYC. (1) For CYC, the enantiomers and racemate-CYC showed no significant stereoselective dissipation and metabolism in all three soils. CYC was transformed into three different degradation intermediates (IP1-IP3) in aerobic soils, which indicated soil preferential dynamic changes. Moreover, soil properties were found to have a noticeable effect on the metabolism pattern of CYC, with the fastest degradation in loamy soil and the slowest in red clay soil. Cleavage of the oxabridged seven-member ring, cleavage of C-N between the chloropyridinylmethyl and imidazalidine ring, carboxylation of alkene and hydroxylation of the imidazolidine ring, all contribute to the main transformation pathways of CYC. (2) While for IPP, the stereoisomers of IPP showed diastereoselective persistence in tested soils except an acidic clay soil. Significant specificity was observed for diastereoisomers of IPP in the formation of BR or mineralization in loamy and saline soils. Comparatively, no significant difference was found between IPP enantiomers. Moreover, IPP was transformed to a range of degradation intermediates (M1-M6), which also showed significantly epimer-selective and soil preferential formation. All these findings could provide useful information which can be incorporated into environmental assessments of chiral pesticides.

AGRO 268

Addressing analytical challenges associated with pyrethroid hydrophobicity

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Pyrethroids are extremely hydrophobic, and adsorption studies run in earlier decades indicated K_{OC} coefficients greater than 10E5. More recently, Solid Phase Micro-Extraction (SPME) technologies that only quantify concentrations of hydrophobic compounds freely dissolved in the aqueous phase have shown that true pyrethroid adsorption is characterized by even higher coefficients (1.1 to 7.2 10E6). We set out to measure comparative GLP quality K_{OC} coefficients for nine major pyrethroids under two sets of equilibration procedures using two sediments and both SPME and Liquid-Liquid Extraction (LLE) analyses. Before this could be completed successfully, we had to overcome two significant challenges.

Firstly, it has long been known that pyrethroids rapidly migrate from aqueous solutions to accumulate on vessel walls. Traditionally multiple wall washes with non-polar solvents in mixtures that ensure fully wetted vessel wall surfaces have obviated this problem. However, because SPME only “senses” the dissolved fraction rather than quantitatively extracting the compound, maintaining standard

concentrations during an auto-sampler run becomes problematic. This challenge was solved for pyrethroids using "staggered injections" of standards immediately before they were to be analyzed; however, this remains a potential issue with other chemical classes that SPME practitioners should consider.

Secondly, in contrast to batch adsorption studies where a sediment/pyrethroid/water mixture was shaken for 24 hours before phase separation and analysis, our second experimental design required aging pyrethroid sediment mixtures for ~14 days prior to static equilibration (analogous to a sediment ecotoxicity test). After sediment equilibration using these conditions, it was found that, among several factors, an extraction regime which worked with freshly treated sediments failed to provide quantitative removal of pyrethroid from the sediment phase and more stringent extractions were required. Once resolved, this effectively illustrated the phenomenon of bio-inaccessibility for pyrethroids in sediments. Results and implications will be discussed in addition to some additional analysis-related developments.

AGRO 269

Harmonized approaches to crop protection for minor uses: Past, present, and future

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Through the efforts of many, great advances have been made in crop protection and crop production to meet the food needs of a growing population. Minor uses of pest control products (uses on specialty crops) have also made great strides, which is important as they make the greatest contribution to a healthy diet. These great strides have also brought with it the ability to provide highly effective integrated pest management (IPM) solutions to growers which also mean a higher level of safety to applicators, consumers and the environment.

The United States has been fortunate to have the IR-4 Program that is recognized by the international community as a model program, to help specialty crop growers obtain legal access to safe and effective pest management technology. Over the years, IR-4 has developed numerous partnerships with other countries to share the burden of developing regulatory data to register products for minor uses. The global capacity building projects in the ASEAN region, the sub-Sahara Africa, and in Latin/South America will also lead to future collaborative research projects to address minor use needs and hasten IPM implementation. Many other “tools of harmonization” have been developed and are being implemented to provide tools for growers while enforcing the highest level of safety for consumers. Some of these tools include: crop grouping, extrapolation proposals or models that allow data on certain representative crops to be used to support registration on multiple crops within a group; and the OECD MRL calculator. The progress continues, but the needs remain high. The first Global Minor Use Workshop (September of 2015) identified nearly 3,000 grower pest control needs. Addressing these needs can only be accomplished through continued cooperation and expansion in the area of research and in the area regulatory policies that encourage robust decisions made as a global community.

AGRO 270

Evidence-based Initiatives for MRL alignment

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In the past few years, Health Canada’s Pest Management Regulatory Agency (PMRA) has significantly contributed to and collaborated on the advancement of various regulatory strategies to align pesticide Maximum Residue Limits (MRLs). More specifically, PMRA together with the US Environmental Protection Agency (EPA) have actively participated in NAFTA Joint Reviews while also being key participants in OECD Global Joint Reviews. These efforts have resulted in an increase in aligned MRLs since the first joint review in 2007. The Agency’s participation in the development of science policies on the alignment of crop groupings and on the interpretation of the OECD Guidance Document on Residue Definition has further minimized MRL differences. In regards to the Codex MRL setting process, Canada has enhanced its influence on the Codex deliberations and outcomes, promoted more effective work-planning by the Codex Committee on Pesticide Residues (CCPR) by ensuring priorities include Canadian stakeholders’ interests and promoted the timely development of standards by continuing to support participation on the Joint Meeting of Pesticide Residues (JMPR). Domestically, PMRA holds on-going discussions with stakeholders such as growers, registrants and regulatory bodies (i.e., AAFC, GAC) to collaborate on various initiatives to resolve trade irritants. Moving forward, PMRA will continue participating in Global Joint Reviews and science policy development, to avoid instituting new regulatory burdens, and continue contributing to Codex (CCPR/JMPR) activities to further align MRLs. The initiatives described above will be further discussed during the presentation.

AGRO 271

Pesticides registration in Ghana

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The regulatory framework for the registration of pesticides in Ghana came into force through the Pesticides Control and Management Act of 1996 (Act 528). The pesticides control and management provisions were combined with the Environmental Protection Agency (EPA) Act of 1994 (Act 490), and made Part Two of this Act. Act 528 has therefore been consequentially repealed.

It was however not until 2004 that the EPA-Ghana fully operationalized pesticides registrations in Ghana by putting in place the necessary structures and procedures.

There is increasing concern among the general public in Ghana about excessive pesticide residues in locally consumed food especially fresh vegetables such as tomatoes, cabbage and lettuce. Studies on residue of pesticides in tomatoes in Akumadan, one of the major tomato growing areas in Ghana, reveal unacceptable levels.

There is even greater concern about pesticide residues in agricultural produce destined for export. Ghana is a major exporter of dried cocoa beans, and to a lesser extent, pineapples, mangoes, papaya and chili peppers. In the absence of national MRLs, for various crop/pesticides combinations, Ghana relies on MRLs set by the importing countries. Agriculture in Ghana can therefore be said to depend on the setting of MRLs in key markets to facilitate the export of Ghanaian commodities. Japan, for example rejected a consignment of cocoa beans from Ghana due to unacceptable levels of residues of some pesticides.

This paper describes the regulatory process for the registration of pesticides in Ghana and presents data on residues of pesticides in some produce consumed locally. The paper also describes measures taken by EPA-Ghana and the Ghana Standards Authority and other relevant Agencies to address the issue of pesticide residues in food

AGRO 272

Establishing toxicological end-points for human risk assessment: challanges and opportunities

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The identification and characterisation of adverse effects of chemicals are the basis for defining the levels of exposure that are considered safe for humans. Toxicological risk assessment has relied heavily on animal testing; however, this approach is becoming unsustainable. In fact, it requires significant resources that are no longer available to test thousands of chemicals, and, in addition, there is societal and ethical demand for the reduction of the use of animals for toxicity testing. This has been recognised, for instance, by the US National Academy of Sciences (NAS) in its seminal reports in 2009-2012. Moreover, there in an increasing and urgent call for greater clarity and transparency in communicating matters related to human safety.

In addition, current approaches to risk assessment are mainly influenced by hazard assessment (i.e., identification of any adverse effect) without adequate consideration of potential human exposure. This has led to high-dose studies that, in many cases, bear little relevance to human exposures. This is particularly relevant for pesticides that require a relevant number of studies most of which are not used/useful for the final risk assessment. It has also been noted that long reviews, that are reiterated by different bodies in different countries, of highly studied, common chemicals monopolize valuable resources and often create confusion. Efforts are put into developing alternative methods that take advantage of previous knowledege, and of new computational and high throughput *in vitro* methods to conduct an efficient risk assessment that also poses greater value on exposura data or estimation.

Furthermore, the clarity of information and process that lead to any decision on chemical safety should be improved since there is a need to convey in transparent ways the process and the outcome: this will facilitate understanding the conclusions and the decisions in risk assessment.

AGRO 273

Regulation of pesticides in Mexico

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Coordinated Health Registration is a health authorization issued by the Federal Commission for the Protection against Sanitary Risk (COFEPRIS) of the Ministry of Health, in coordination with the Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA) and the Secretariat of Environment and Natural Resources (SEMARNAT), competent authorities on this matter in Mexico. This registration ensures the safety and efficacy of the registered product, and is an indispensable requirement for sale and use within the country. Based on Article 3 of DECREE amending, supplementing or repealing certain provisions of Regulations Pertaining Records, released the Import and Export Certificates Export of Pesticides, Fertilizers and Substances and Materials Household Hazardous, corresponds to COFEPRIS to authorize the registration and issue certificates of free sale and export of pesticides and plant nutrients; to SEMARNAT to issue technical opinion on environmental protection in cases introduced by that Regulation; and to SAGARPA to issue technical opinion on the biological effectiveness of pesticides and plant nutrients, as well as the phytosanitary aspects of maximum residue levels of pesticides in cases established by that Regulation. The Federal Plant Protection Act empowers the SAGARPA to monitor compliance with Mexican Standards related to marketing, formulation, assembly, import and aerial application of pesticides. Furthermore, Article 42a of the Act states the Secretariat shall establish and develop the National Monitoring Program Pesticide Residues in plant to determine that phytosanitary inputs are used in the expert advice of biological effectiveness granted. The National Health Service, Food Safety and Quality (SENASICA), decentralized agency of the SAGARPA body, the National Program Contaminant Monitoring in Primary Production Plant 2014, through sampling of fresh produce distributed in 22 states of the Mexican Republic, for analysis in laboratories SENASICA. Information derived from this program, assesses chemical contamination (pesticides) on crops in Mexico, make decisions to mitigate risks and improve the regulation of agricultural pesticides in Mexico.

AGRO 274

Farm to table: Pesticide residues in food and risk assessment

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The Health Effects Division (HED) of the US Environmental Protection Agency's Office of Pesticide Programs is responsible for evaluating human health risks associated with pesticides and determining appropriate maximum legal limits, or tolerances, for pesticide residues in or on foods. Prior to establishing tolerances for residues of a pesticide, the use of that pesticide must be shown to be safe as defined by the Food Quality Protection Act (FQPA) of 1996. This presentation will discuss residue chemistry from tolerance and risk assessment perspectives, including how HED (1) determines the residue definitions suitable for tolerance and risk assessment purposes, (2) derives tolerance levels for residues in foods and feeds, and (3) may use various sources of residue information to conduct dietary risk assessment.

AGRO 275

Consumers’ expectations of pesticide residues in our food

Philip A. Brindle, *philip.brindle@basf.com. Regulatory, BASF Corporation, Research Triangle Park, North Carolina, United States*

In the US, consumers’ expectations are high when it comes to fresh fruit and veggies. Namely: lots of variety, high quality produce, blemish-free, flavorsome, reasonable price, no pesticide residues, no microbial contamination, high in health-promoting “phytonutrients”, maintains freshness, dirt-free, prepared and ready to serve, locally and sustainably produced, preferably organic, recyclable packaging, low carbon footprint…. And, they expect all this 365 days a year. But, consider the expectations of all other people in the world. Many just need food! Any conscionable person today surely has an expectation that no man, woman or child should be without food.

Developing safe, efficacious products to help farmers grow sufficient food to feed the world’s growing population is the overarching mission of agrichemical companies, as is ensuring that food can be readily traded internationally so that no child does have to go hungry. Enter Maximum Residue Limits (MRLs)! To meet the US consumers’ lofty fruit/veggie expectations, as well as the world’s basic food needs, MRLs must be in place to support international trade. Furthermore, the MRLs need to be harmonized to prevent rejection of produce when it is monitored by importing countries. Without MRLs in their export markets, exporters face uncertainty and farmers are reluctant to use a particular crop protection product, regardless of how safe or efficacious it is.

Consumers are fearful of the "P' word and understandably want food without pesticide residues. They want to know, and have a right to know, that the registration process for pesticides is strictly regulated and controlled at the governmental level. And, they want reassurance that any remaining trace residues in their food are safe. These concerns are very valid concerns, but immensely complex to address. True answers only come through rigorous, science-based approaches; the emotive aspects of pesticide residues are extremely difficult to address.

AGRO 276

QSARs and computational chemistry in environmental risk assessment: Overview and historical perspective

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Quantitative structure activity relationships (QSARs) and computational chemistry have at least one common benefit -- they enable the prediction of properties, reactions, and effects of chemicals based on limited data. The foundation for QSAR was arguably the Hammett equation (1940), which inspired the development of many linear free energy relationships (LFERs) that correlated reaction rates and equilibria of one reaction series with related reaction series. The use of LFERs in physical organic chemistry expanded to the prediction of biological effects in medicinal chemistry and, eventually, toxicology, i.e., Biological Response = f(x), where x = some combination of electronic, steric, lipophilic, and structural fragment parameters and related molecular descriptors. In addition, the exponential increase in the power of computers has led to the application of quantum chemistry calculations to predict reaction rates, physical/chemical properties, and biological reactions. QSARs are also used to assess bioavailability, e.g., Lipinski's 'rule-of-fives' (1997). QSAR and computational chemistry techniques are important in assessing the human and environmental risks (fate and effects) of industrial and consumer chemicals in commerce, as well as pesticide metabolites. For example, QSAR application is critical for assessing potential risks of data-poor new chemicals pursuant to the US Toxic Substances Control Act (TSCA). QSAR analyses provide key input to decisions by the TSCA Interagency Testing Committee. The creation of the EPI-Suites QSAR modeling package by EPA contractor Syracuse Research Corp. provided a set of powerful QSAR tools in such areas of mobility, persistence, and toxicity as water solubility, vapor pressure, hydrolysis, atmospheric degradation, biodegradation, bioconcentration and fish toxicity. Pesticide manufacturers usually identify stable intermediate metabolites, but QSAR must often be used to characterize the environmental fate risks of these compounds. The 2007 EU legislation for the Registration, Evaluation, Authorization & restriction of CHemicals (REACH) now requires industry to evaluate the toxicity not just of new chemicals, but of around 30,000 existing chemical substances that are in use but which have never been subject to regulatory-approved testing. Where reliable experimental data on the toxicity of chemicals already exists, QSAR models can be used to predict the toxicity of other chemicals with related molecular structures.

AGRO 277

Predicting interactions of compounds and metabolites with toxicity-associated targets

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We will describe the development of quantitative structure-activity relationship (QSAR) models based on activity data from the ChEMBL database, to predict the interaction of compounds with protein targets associated with adverse outcome pathways and toxicities. However, systemic exposure to a compound will also result in the formation of metabolites, which themselves may be the cause of a toxic response. Therefore, we have developed an integrated system linking models that predict the enzymes responsible for metabolism of a parent compound and the resulting metabolites with QSAR models of target interactions. The combination of these models can predict potential toxicities resulting directly or indirectly from exposure to the parent compound. The initial implementation is focused on metabolism by Cytochrome P450 enzymes, but forms a framework that may be extended to other metabolic pathways and additional QSAR models of toxicity.

AGRO 278

New insights on the structural and molecular recognition properties of insecticides through computational chemistry: The challenging case of sulfoxaflor

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In the chemist arsenal, computational chemistry is an efficient and powerful tool to complement the experimental observations and predict the behavior of new compounds. For example, the conformational landscape of insecticides can be investigated with Density Functional Theory (DFT) calculations whereas hybrid QM/MM or QM/QM' methodologies can provide a comprehensive understanding of the binding of insecticides to their biological target.

In this contribution, we will present the application of these tools for the exploration of the properties of the various sulfoxaflor (**Figure 1a**, SFX) stereoisomers using a multiscale molecular modeling methodology. Indeed, since no experimental information is available with respect to the structure and binding of this new representative of insect nAChR agonists, such information are needed. Our strategy is based on the use of a wide range of methodologies from DFT, flexible docking, molecular dynamic simulations (40 ns period), to QM/QM' calculations for further refinement of the geometric and energetic parameters.

Our results first suggest that the *SR* stereoisomer is the one having the best affinity for the devised model of insect nAChR. Secondly, they point out the important role played by water molecules in the vicinity of the SFX nitrile group for binding with aminoacid residues of *Ac*-AChBP (**Figure 1b**). Lastly, they throw light on the possible role of the ligand sulfoximine group in the binding to the receptor through interactions involving aromatic residues (Trp147, Tyr188). On the whole, the present work provides relevant information for the understanding of the binding of SFX to *Ac*-AChBP. As such, it illustrates the potential of computational chemistry for the understanding of the action mechanism of insecticides at the atomic level and the design of new compounds.

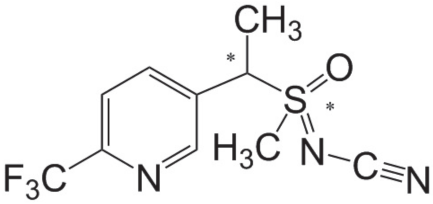
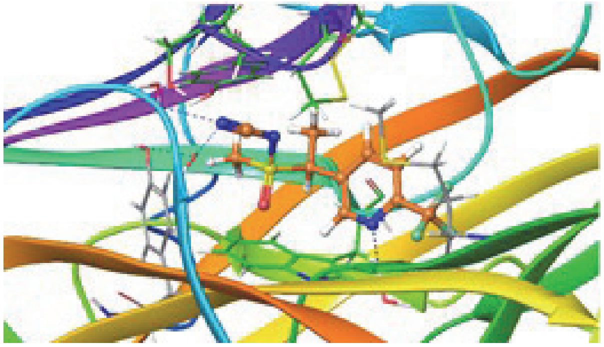


Figure 1. a)



b)

AGRO 279

Use of computational chemistry & toxicology tools and models for assessing chemicals under the Toxics Substances Control Act

***Tala R. Henry**, henry.tala@epa.gov, Kelly Mayo, Wen Lee, Yvette Selby-Mohamadu. USEPA, Washington, District of Columbia, United States*

The EPA's Office of Pollution Prevention and Toxics (OPPT) implements the Toxic Substance Control Act (TSCA). TSCA requires the assessment and, if necessary, regulation of all phases of the life cycle of industrial chemicals: manufacturing, processing, use, and disposal. TSCA regulates two broad categories of industrial chemicals: (1) existing chemicals in commerce on the TSCA Chemical Substances Inventory, and (2) new chemicals that are not on this inventory. Hence, OPPT conducts prospective evaluations (and risk assessment) of new industrial chemicals and retrospective assessments of existing chemicals. OPPT evaluates approximately 1000 new substances per year and has recently identified a "work plan" of over 80 existing chemicals to assess within the next three years, with the intention of assessing additional existing chemicals at some pace in years thereafter.

A great challenge to OPPT for all TSCA chemicals is that assessments are generally based entirely on existing and available data, which for new substances in particular may be next to nil. OPPT has for many decades relied on analog and read-across analysis, category approaches and predictive modeling (e.g., QSAR and expert systems) to assess the risks of both new and existing chemicals.

A number of these computational tools and models will be described in several presentations in this session, including the underlying basis/assumptions/computational approach, known limitations on domains of applicability, challenges/knowledge required for applying each tool/model. In addition, data needs for improving each tool and model in the future will be discussed.

AGRO 280

Use of computational chemistry & toxicology tools and models for estimating exposures under the Toxics Substances Control Act

***Mari Titcombe Lee**, Lee.Mari@epa.gov. Office of Pollution Prevention and Toxics, Environmental Protection Agency, Washington DC, District of Columbia, United States*

The Risk Assessment Division (RAD), in the EPA's Office of Pollution Prevention and Toxics (OPPT), performs risk assessments for over one thousand new chemicals every year, prior to allowing these chemicals to enter US commerce. To aid in this task, particularly when empirical data is unavailable, computational tools and models are used to provide quantitative occupational, general population, and environmental exposure estimates. Chemical releases, arising from industrial manufacturing, processing, and use are estimated using the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER). ChemSTEER also generates inhalation and dermal dose estimates, resulting from workplace exposures. The Exposure and Fate Assessment Screening Tool (E-FAST) uses environmental releases from ChemSTEER to estimate potential human exposures from chemicals in air, surface water, ground water, and via fish ingestion. E-FAST also contains a set of consumer exposure scenarios, to estimate inhalation and dermal exposure to chemicals in common household products. E-FAST and ChemSTEER input parameters are generated, when empirical data is unavailable, from the Estimation Programs Interface (EPI) Suite™. ChemSTEER and E-FAST computational tools use EPA methods and models to provide reasonable exposure estimates, using conservative assumptions. EFAST and ChemSTEER model outputs are used in conjunction with ECOSAR estimates to calculate risk. This talk will cover the key features, limitations, and data needs of ChemSTEER and E-FAST, and discuss the ongoing role of estimation tools in conducting new chemicals risk assessments.

AGRO 281

Development of screening assays and novel methods of mosquito control

***Subba R. Palli**, rpalli@uky.edu, Gopal Venu, Michael Sheetz, Das Sumistha. Entomology, University of Kentucky, Lexington, Kentucky, United States*

Juvenile hormone (JH) mimics are being used for mosquito larval control. We identified receptors for JH mimics, and DNA response elements these receptors bind to regulate gene expression. The JH response elements and JH-responsive *Aedes aegypti* cells were used to develop screening assays that could be used tp identify novel and more potent JH analogs. Homology models were developed for JH receptor, and the models were employed to perform virtual screening of chemicals structures available in Zinc database. RNA interference (RNAi) works well to silence genes and kill insects belonging to order Coleoptera. However, RNAi does not work well in *Ae. aegypti* larvae. To improve RNAi in this insect, we tested three nanoparticles, chitosan, carbon quantum dot (CQD), and silica complexed with double-stranded RNA to target two mosquito genes (SNF7 and SRC) for controlling *Ae. aegypti* larvae. Among the three nanoparticles tested, CQD was the most efficient carrier for dsRNA retention, delivery, and thereby causing gene silencing and mortality in *Ae. aegypti*.

AGRO 282

Pyrethrum and pyrethroids activate specific olfactory receptors and elicit spatial repellency in *Drosophila melanogaster* and mosquitoes

Peng Xu[2], Elizabeth Bandason[2], Xiangrui Tian[2], Yuzhe Du[2], Kamlesh R. Chauhan[1], ***Ke Dong**[2], dongk@msu.edu. (1) Invasive Insect Biocontrol Lab, ARS USDA, Beltsville, Maryland, United States (2) Michigan State University, East Lansing, Michigan, United States*

Pyrethroid insecticides are synthetic derivatives of pyrethrum, a botanical insecticide from *Chrysanthemum* species. Their insecticidal activity results from the disruption of the function of voltage-gated sodium channels in the nervous system. Currently, pyrethroids are the only class of insecticides that are approved for use in insecticide-treated bednets for malaria control due to their low mammalian toxicity. Besides their insecticidal activity, pyrethrum and some pyrethroids are widely used as mosquito repellents. However, the nature of pyrethroid repellency is controversial, and the underlying molecular mechanism remains elusive. In this study, we took electrophysiological, toxicological and behavioral approaches to evaluate the effects of pyrethrum and pyrethroids, including metofluthrin, transfluthrin and acetransfluthrin (ACTF; a derivative of transfluthrin) on the insect olfactory system. We discovered, for the first time, that these compounds elicited electroantennogram (EAG) responses and exhibited spatial repellency in fruit flies (*Drosophila melanogaster*) and yellow fever mosquitoes (*Aedes aegyti*). Pyrethroid-elicited EAG response was abolished in two *Drosophila* mutants of olfactory receptor cofactor, Orco, demonstrating that the EAG response is olfactory receptor (OR)-mediated. Further functional analysis using single sensillum recording (SSR) in *D. melanogaster* revealed these compounds activate specific OR neurons. Furthermore, pyrethroid-resistant mosquitoes carrying sodium channel mutations were less sensitive to spatial repellency, suggesting the involvement of sodium channels in pyrethroid repellency. Our study sets a new paradigm for the understanding of the modes of action of volatile pyrethroids in mosquito control and pyrethroid-treated bednets where pyrethroid repellency is predicted to be a critical component.

AGRO 283

Breaking pyrethroid resistance in *Aedes* mosquitoes

***Jennifer Williams**, jennifer.williams@mgk.com. R&D, MGK, Minneapolis, Minnesota, United States*

Resistance to traditional chemicals, greater risk of mosquito-borne diseases and increased public awareness are emerging simultaneously and driving the need for new and effective mosquito control strategies and solutions. Unique attributes of the biology and behavior of *Aedes* spp. that affect control and the biochemical basis of pyrethroid resistance will be presented. Research involving the development of novel active ingredients including synergists, plant-based adulticides and pupacides for *Aedes* spp. control will be discussed.

AGRO 284

Aedes aegypti adult control using aerially applied Dibrom Concentrate (naled)

***Charles A. Silcox**, charless@amvac-chemical.com, Peter Connelly. AMVAC Chemical Corporation, Lincoln University, Pennsylvania, United States*

Three field trials were conducted to evaluate recent modifications to application parameters of aerially applied insecticide with the objective of increasing *Ae. aegypti* control inside dwellings. Two of the trials were conducted at the Camp Blanding Joint Training Center in Starke, FL. The third was an operational field trial conducted in cooperation with the New Orleans Mosquito & Termite Control Board. The Camp Blanding trials used two test sites per trial. One site was the Military Operations Urban Training facility and the other was a mock village setting. In October 2013, applications were made on successive days using a C-130 aircraft operated by the USAF Aerial Spray Unit. In August 2014, applications were made on successive days with a Hughes 500D rotary aircraft operated by Manatee County (FL) Mosquito Control District. The NOMTCB trial was conducted during July 2015 and used a Briton Norman Islander aircraft. The Camp Blanding trials used caged, laboratory reared (USDA-Gainesville), susceptible *Ae. aegypti* adults. Results from the 2013 trial confirmed excellent activity by naled (94 and 112 g ai/ha) against *Ae. aegypti* adults with 100 percent control in outdoor, exposed settings (n=36). Control decreased with increasing challenges (indoors in open or closed buildings and/or inside a one cubic foot box). However, on the second day, using different nozzles, an average of 91.5 percent control was achieved inside open buildings. Similar results were observed using rotary aircraft in 2014 with an average of 86.7 percent control inside open buildings. The NOMTCB trial used caged, field collected (F-1) adults of both *Ae. aegypti* and *Ae. albopictus*. Cages were placed both in open settings and in sequestered locations including under buildings, tree canopies, etc. Results from this trial show naled (86 g ai/ha) averaged 99.7 and 89.9 percent control of *Ae. aegypti* in open and sequestered locations, respectively.

AGRO 285

Chemosterilization for SIT mosquito control: the case for thiotepa against *Aedes aegypti*

***Graham White**, gbwhite@ufl.edu. Entomology & Nematology, University of Florida, Gainesville, Florida, United States*

Among the aziridinyl alkylating agents, thiotepa (CAS#52-24-4) is an orphan drug employed for cancer chemotherapy since the 1960s. Also the USDA-ARS demonstrated its effectiveness for chemosterilization of mosquitoes and other insects for use in the sterile insect technique (SIT) for area-wide suppression of pest populations. Like irradiation, chemosterilization induces dominant lethal mutations in the gametes of insects, at dosages causing no loss of male vigor, but reducing fecundity of females. Adult mosquitoes can be chemosterilized by feeding or by contact with thiotepa, or by immersion of larvae or pupae in chemosterilant solutions of 100ppm or more, without deleterious somatic side-effects up to 0.2% for 24 hours. Cage population studies with *Aedes aegypti*, the mosquito vector of Zika, chikungunya, dengue and yellow fever viruses, demonstrate competitiveness of chemosterilized males throughout their normal life-span of several weeks. For vector control, chemosterilization is more economical and robust than irradiation and using genetically modified strains for mass production and release. Regulatory approval of thiotepa is recommended for field campaigns to suppress and eliminate pest and vector populations, since this aziridinyl compound has been approved by FDA and utilized for chemotherapy worldwide for decades.

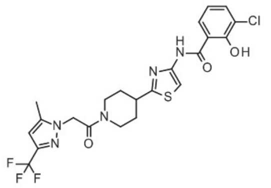
AGRO 286

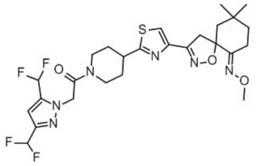
Synthesis and oomycete fungicidal activity of a new family of inhibitors targeting an oxysterol binding protein

***Martin Pouliot**, martin.pouliot@syngenta.com. Chemistry, Syngenta Crop Protection, Stein, Aargau, Switzerland*

Inhibitors targeting oxysterol binding protein have shown excellent fungicidal activity against late blight and downy mildew, plant diseases caused by oomycete pathogens. Oxathiapiprolin, discovered by DuPont researchers, have been the first compound of this class to reach the market and is commercialized by both DuPont and Syngenta under the trade names Zorvec^{TM} and Orondis^{TM} respectively. In this talk, we

would like to present research done in Syngenta on oomycete fungicide inhibiting oxysterol binding protein. The synthesis and antifungal activity of new classes of bicyclic and spirocyclic isoxazolines will be presented, along with that of the *N*-thiazol-4-yl-salicylamide class which distinguished itself by its unique capacity at controlling damping-off disease caused by *Pythium ultimum*.



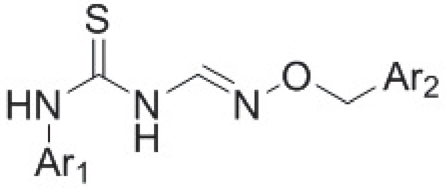


AGRO 287

SAR investigation of insecticidal thiourea amidines

Daniel Knueppel, *danielisaiahknueppel@gmail.com, Scott Castetter, David Demeter, Joseph D. Eckelbarger, Michael Sullenberger, Scott Thornburgh, Frank Wessels, Jeremy Wilmot. Dow AgroSciences LLC, Indianapolis, Indiana, United States*

A novel class of insecticidal (*E*)-*N*'-(benzyloxy)-*N*-(pyridin-2-ylcarbamothioyl) formimidamides, possessing an interesting thiourea amidine structural motif, was discovered through a targeted compound acquisition effort at Dow AgroSciences, LLC, using computer models for virtual screening. Structure-activity relationship (SAR) work led to the discovery of compounds that exhibited strong insecticidal activity against lepidopteran pests. Efforts to understand and optimize the biological activity and ultraviolet (UV) light stability of this class of chemistry will be discussed.



AGRO 288

SAR investigations into *N*-azinyl-*N'*-thiophenyl ureas as insecticides

Tony K. Trullinger, *ttrullinger@dow.com, Timothy Johnson, Ricky Hunter. Dow AgroSciences, Westfield, Indiana, United States*

While investigating a series of *N*-azinyl-*N'*-aryl ureas, a new *N*-azinyl-*N'*-thiophenyl urea scaffold was discovered which showed control of lepidopteran pests. Structure activity relationships (SAR) have been investigated to optimize the thiophene subunit as well as understand the optimal substitution patterns on the urea moiety. Chemistry developed to access these new molecules and their biological activity will be discussed.

AGRO 289

Synthesis and evaluation of insecticidal spinosyn mimics

Annette Brown[1], Kristy Bryan[1], Gary Crouse[2], Denise P. Cudworth[1], David Demeter[1], William H. Dent[1], Ricky Hunter[1], ***William T. Lambert***[1], *willyT1392@gmail.com, Casandra McLeod[4], Jack G. Samaritoni[3], Thomas C. Sparks[1]. (1) Dow Agrosciences, Indianapolis, Indiana, United States (2) Retired, Indianapolis, Indiana, United States (3) IUPUI, Indianapolis, Indiana, United States (4) KIPP, Indianapolis, Indiana, United States*

The spinosyns are macrolactone natural products which exhibit excellent control of a wide range of lepidopteran insect pests through interaction at an allosteric site in insect nicotinic acetylcholine receptors. Efforts directed toward the identification of insecticidally active spinosyn mimics led to replacement of the stereochemically complex spinosyn core with simpler triaryl ring systems. The nature of the linker between the conserved rhamnosyl moiety and the triaryl system was found to have a siginificant effect on the physical properties and insecticidal activity of these compounds. This talk with highlight the synthesis and SAR of these novel spinosyn mimics.

AGRO 290

Discovery and initial optimization of mesoionic pyrido[1,2a]pyrimidinones as a novel class of insecticides

Wenming Zhang, *wenming.zhang@dupont.com, Caleb W. Holyoke, Kenneth A. Hughes, Yvonne Bethel. Discovery Chemistry, DuPont Crop Protection, Newark, Delaware, United States*

A novel class of mesoionic pyrido[1,2-a]pyrimidinones has been discovered with exceptionally good insecticidal activity controlling a number of insect species. Here we report the initial discovery, preparation, and SAR study of this class of chemistry.

AGRO 291

Triflumezopyrim: A new class of nicotinic acetylcholine receptor inhibiting insecticides

Thomas Pahutski[2], *thomas.f.pahutski@usa.dupont.com, George P. Lahm[4], Caleb W. Holyoke[1], Wenming Zhang[3], My-Hahn-Thi Tong[2], Kenneth A. Hughes[5], Dan Cordova[2], Eric A. Benner[2], Daniel R. Vincent[2], Robert M. Leighty[2]. (1) Crop Protection, DuPont, Newark, Delaware, United States (2) DuPont Crop Protection, Elkton, Maryland, United States (3) Discovery Chemistry, DuPont Crop Protection, Newark, Delaware, United States (4) Stine-Haskell Research Center, DuPont Crop Protection, Newark, Delaware, United States (5) DuPont Crop Protection, Newark, Delaware, United States*

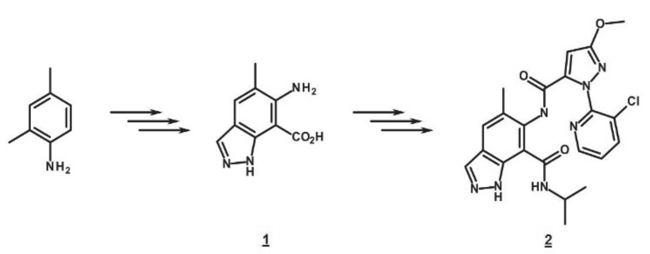
The discovery and development of new insecticides for the control of targeted pests will lead to increased yield and productivity of crops to meet an ever growing agricultural need. This presentation will cover the discovery and optimization of a new class of mesoionic insecticides leading to the identification of triflumezopyrim, a novel insecticide with exceptional activity for the control of hopper pests in rice. The chemistry, biology, and mode of action studies for this new class will be presented.

AGRO 292

Process chemistry aspects of indazole anthranilic diamide insecticides

***Regis Mondiere**, regis.mondiere@syngenta.com, Andre Jeanguenat, Olivier Loiseleur, Roger G. Hall, André Stoller, Andrew Edmunds. Syngenta Crop Protection Münchwilen AG, Stein, Switzerland*

The diamide insecticides belong to a recently discovered and successful class of broad spectrum insecticides. In particular, research into anthranilic diamides culminated with the introduction of chlorantraniliprole onto the market. The synthesis and structure-activity relationship of various bicyclic anthranilic diamides was recently reported. In this presentation, we will discuss process chemistry investigations towards anthranilic diamides such as **2**, and the key indazole intermediate **1**. The research synthesis of **1**, highlighting advantages and limitations will be presented; this will be followed by discussing approaches used in the process optimization towards **1**. Finally the selection of a robust route to prepare the anthranilic diamide **2** will be presented.



AGRO 293

Three estuarine mixing scenarios for pesticide risk assessmentt

***Stuart Z. Cohen**[1], ets@ets-md.com, Louis J. Thibodeaux[2], Craig Jones[3], Martin Williams[4], Sandra M. Haefner[5]. (1) Environmental & Turf Services, Inc., Silver Spring, Maryland, United States (2) Louisiana State University, Baton Rouge, Louisiana, United States (3) Integral Consulting, Santa Cruz, California, United States (4) Waterborne Environmental, Leesburg, Virginia, United States (5) Environmental & Turf Services, Inc., Wheaton, Maryland, United States*

The US EPA, the National Marine Fisheries Service, the US Fish & Wildlife Service, and pesticide registrants are focused on accurately assessing the potential risks of pesticides to threatened and endangered species. Currently, the EPA's Office of Pesticide Programs assesses risk with a modeling system, the Pesticide in Water Calculator (PWC), which couples the daily-time-step, field-scale numerical-solution model Pesticide Root Zone Model (PRZM) with the Variable Volume Water Model (VVWM). The VVWM has various receiving water mixing scenarios, including estuarine and coastal zones, but none of the scenarios consider tidally-driven sea water stratification and sediment transport processes. This creates a significant gap in understanding the exposure component of pesticide risk assessment, since the toxicology data component -- pesticide toxicity to marine and estuarine organisms -- is usually complete and available. We propose to create three distinct marine/estuarine mixing scenarios for incorporation into the VVWM: salmonids in the Pacific Northwest, coral in the Hawaiian Islands or the Florida Keys, and crustaceans in the Chesapeake Bay. The diverse scenarios include a coastal embayment or a marine shoreline, a salt wedge estuary, and a drowned eroded river valley. The biology of target species will drive the development of each scenario; e.g., the coral reef embayments will not have high TSS concentrations (coral doesn't grow in high TSS environments). A key consideration will be the exchange coefficient, i.e., the exchange of conservative substances due to tidal circulation. The full semi-diurnal tide will be considered so that times such as the slack tide period, when many of the organisms are feeding and there is low mixing, will be fully considered. The study will characterize the circulation, geometry, and aquatic chemistry of each habitat. Water, salt, and pesticide mass balances based on box model volume elements will be constructed that represent multiple vertical and horizontal segments. A key phase will be testing the models against field data for each scenario.

AGRO 294

Screening level and refined flowing water pesticide exposure modeling for use in endangered species assessments

***Michael Winchell**[5], mwinchell@stone-env.com, Lauren Padilla[4], Naresh Pai[4], Paul Whatling[3], Patrick L. Havens[2], Nick Poletika[1]. (1) Dow AgroSciences (formerly), Indianapolis, Indiana, United States (2) Regulatory Sciences and Government Affairs, Dow AgroSciences LLC, Indianapolis, Indiana, United States (3) FMC Corp., Arlington, Virginia, United States (4) Stone Environmental, Montpelier, Vermont, United States (5) Stone Environmental Inc, Montpelier, Vermont, United States*

The US EPA (EPA), Fish and Wildlife Service, and National Marine Fisheries Service (FWS/NMFS) have proposed generic aquatic habitats for which potential pesticide exposure must be determined as part of national endangered species risk assessments. In their recently released Biological Evaluations for three organophosphate insecticides, the US EPA concluded that new pesticide exposure modeling tools and parameterization approaches are needed for simulating pesticide exposure in the flowing water scenarios. The need for scientifically defensible models and tools for flowing water exposure modeling exists at both the screening level and the refined level of assessment. At the screening level, the model and parameterization requirements must be efficient and straightforward to implement, similar to how static water body simulation are currently conducted with EPA's standard tools. At the refined level, the modeling approach should incorporate spatially explicit data and additional realism to generate exposure predictions representative of specific locations throughout a river and stream network. This presentation will evaluate the use of the Soil and Water Assessment Tool (SWAT) and AGRO-2014 as the receiving water model for screening level exposure modeling of flowing water systems, and compare results with the Variable Volume Water Model. In addition, a watershed scale flowing water modeling approach that uses the SWAT model stream network and hydrologic and pesticide routing module was applied to demonstrate an approach for simulating spatially explicit pesticide concentrations which may be necessary in refined risk assessments for aquatic species. In both the screening level and refined modeling approaches, EPA's PRZM5 model provided the daily runoff, erosion, soluble, and sorbed pesticide fluxes from the landscape. The screening level modeling approach was found to generate realistically conservative predictions from high vulnerability exposure scenarios, while the refined approach led to more realistic predictions of pesticide concentrations in a wide range of flowing water bodies.

AGRO 295

Comparison of TOXSWA and AGRO-2016 as receiving water models for European pesticide exposure assessment

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Exposure modeling conducted following guidelines of the Forum for the Coordination of Pesticide Fate Models and their Use (FOCUS) uses the Toxic Substances in Surface Waters (TOXSWA) model to simulate pesticide behavior and fate in small surface water bodies. For runoff scenarios, TOXSWA receives pesticide fluxes in runoff/erosion from an adjacent field generated by the FOCUS Pesticide Root Zone Model (PRZM, version 4.63). For hydrophobic chemicals, with a strong tendency to adsorb to eroded soil and sediments, the suspended sediment concentration in receiving waters is a major factor in determining the proportion of chemical in sediment-sorbed and dissolved (bioavailable) states. In TOXSWA, the suspended sediment concentration is constant and does not depend on the flux of eroded soil predicted by PRZM during runoff events. In AGRO-2016, the suspended sediment concentration fluctuates with respect to eroded soil inputs and subsequent deposition and resuspension of sediment. The dynamic sediment processes included in AGRO-2016 lead to more realistic predictions of chemical partitioning for chemicals with high hydrophobicity. AGRO-2016 is an update of AGRO-2014 with an improved numerical integration method and parameterization options for FOCUS static (ponds) and flowing water bodies. Concentration predictions from AGRO-2016 and TOXSWA are compared for both high and low Koc compounds. PRZM simulations for various crop scenarios and the four FOCUS runoff scenarios provide runoff/erosion loading inputs to both models. Spray drift deposition inputs are derived by the FOCUS Drift Calculator. Simulations are conducted for both models with parameterizations for the FOCUS pond and stream receiving waters. In addition to differences in sediment processes, differences in other physical processes and assumptions are highlighted.

AGRO 296

Examination of PRZM5.0 storm rainfall depth and distribution algorithms compared to current U.S. storm trends

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The EPA model Pesticide Root Zone Model (version 5.0) is used to estimate pesticide concentrations in runoff, erosion and leachate for regulatory purposes. PRZM 5.0 uses the 2003 NRCS NEH-4 runoff curve number of antecedent curve condition II to estimate off-field runoff and the 1986 NRCS rainfall distribution region method to estimate off-field erosion. In 2015, NRCS released a draft revised "Chapter 4: Storm Rainfall Depth and Distribution" in the *National Engineering Handbook*. This chapter discusses NRCS' revisions to estimated US rainfall distributions and depth averages using recent meteorological data. Current research indicate that current storm rainfall distributions are more front-loaded or back-loaded in intensity than represented in the current PRZM 5.0 runoff and erosion methods. This paper will compare PRZM 5.0 daily predicted off-field runoff and erosion using both the current runoff and erosion algorithms and a revised PRZM 5.0 version with modified runoff and erosion algorithms per the recent NRCS findings.

AGRO 297

Direct and indirect air modeling based on dicamba field studies

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To maximize on-target applications, Industry, pesticide applicators, and government researchers have developed and employed a variety of application strategies and technologies to reduce spray drift. While most of applied product deposits in the targeted area, some can be lost from the intended area. When this applied product moves off target, it can travel various distances depending on environmental factors. These off target residues may then redeposit on the ground or other surfaces such as non-target plants. Some outside of industry have argued that large quantities of applied product leave the target area as vapor or as a volatilized substance. In order to evaluate the possibility of product transport as gas, we used both direct and indirect modeling approaches to evaluate field losses process. Loadings for the model came from a replicated field trial which used several sampler types. Results from measured field results, and resulting model predictions strongly indicate there was no gaseous loss mechanism from applications of dicamba that could be injurious to non target plants. These finding are consistent with guideline regulatory lab studies as well.

AGRO 298

AERMOD modeling for treatment period of sulfuryl fluoride residential structural fumigation

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Sulfuryl fluoride (SF) is a broad spectrum insecticide and has been used as a fumigant since the late 1950s. It is primarily used to fumigate sealed structures to control infestations of structural pests including termites, powder post beetles, and bedbugs. It can be also used to control insect pests in stored grains, dried fruits, tree nuts, and for use in food handling and processing facilities. A total 2,793,063 pounds of SF was used in California during 2014, making it the13th highest used pesticide. In 2007, the California Department of Pesticide Regulation listed SF as a toxic air contaminant and started developing mitigation measures to reduce its potential acute exposure to workers, bystanders, and residents. For this purpose, the air dispersion model AERMOD is used to estimate the distribution of SF air concentrations at the height of 1.5m around a fumigated structure. Firstly, the treatment periods of two air monitoring studies of 2004 are simulated to evaluate the performance of different modeling set-ups. The best modeling set-up uses 2-layer area source and assumes that (1) 50% SF mass loss escapes from the ground seal (area source height = 0 m), and (2) 50% mass loss escapes from the tarp at the height of 1.5 m. The mass loss estimated by this set-up is 1.06 -1.93 times of the mass loss measured in the monitoring studies. Secondly, SF indoor concentrations are collected in 23 structural fumigations of California. The monitoring results showed considerable variability of SF half loss time (11 – 60 hr) and mass loss (22 – 81%) during the fumigation treatment period. Indoor concentrations are then used to calculate the hourly flux of 24-hr treatment periods for various mass loss scenarios. Using all of these inputs, treatment periods of structural fumigations are modeled with 5-years of weather data in four California counties.

AGRO 299

Modeling agricultural spray drift using a coupled CALPUFF-AGDISP model

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Agricultural spraying applications of agrochemicals come with the risk of offsite drift and deposition. In particular, pesticide drift onto adjoining properties can negatively impact other agricultural interests, have health impacts on agricultural workers, and have impacts on surrounding residential populations.

AGDISP is a near-field spray model which predicts the motion of spray material released from aircraft, including evaporation and turbulent dispersion within the aircraft wake. CALPUFF is a non-steady state Lagrangian model which is capable of modeling transport over distances ranging from tens of meters to hundreds of kilometers. In order to utilize the capabilities of both modeling systems a direct coupling of the two models has been developed through the use of a hand-off emissions file and a new SPRAY source type in CALPUFF. The hand-off file is produced by AGDISP at a time after all turbulent vortices have died down and evaporation of the droplet volatile fraction is complete. AGDISP exports the position, elevation, final droplet diameter and emissions associated with the range of droplet sizes released from each aircraft nozzle. These values are used by CALPUFF version 7.3.0 to define a new SPRAY source type for the purpose of predicting further down-wind dispersion and deposition.
The implementation of this coupling between the two models through use of a hand-off file allows CALPUFF to have access to more precise information on spray characteristics and droplet size distribution as predicted by AGDISP. This presentation will examine the new coupled model system and give demonstrations of its capabilities and use.

AGRO 300

Pesticide residue and degradation formulations in vegetative filter strips for environmental exposure assessments

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Understanding and being able to simulate the fate and transport of pesticides from the application on a field, through a vegetative filter strip (VFS), and finally to adjacent receiving water bodies is critical for conducting environmental risk assessments. Previous research has proposed a modeling approach that links the US Environmental Protection Agency's (EPA's) PRZM/EXAMS with a well-tested process-based model for VFS (VFSMOD). More recent research updated this modeling approach to consider stored pesticide residue in the VFS and degradation prior to subsequent rainfall/runoff events. However, only one formulation for the pesticide degradation process was considered. The objective of this work is to update the modeling approach to consider four formulations of degradation rates to accommodate different regulatory environments. The formulations variably consider temperature and moisture content adjustments. This research also evaluated the importance of considering the type of degradation framework using three EPA scenarios (Illinois corn, Oregon wheat, and California tomato) and for two pesticides (mobile and rapidly degrading versus less mobile and persistent) with VFS lengths of 0, 1, and 9 m. Differences in acute estimated environmental concentration (EECs) were readily apparent between cases with and without a VFS except for cases where the VFS length was small (1 m) and the pesticides were mobile and rapidly degraded. Acute EECs for cases where pesticide residues were not considered in the VFS were lower than the corresponding values for cases where residues were considered in the VFS and available for transport in subsequent storm events. The form of the degradation equation used in environmental risk assessments was in general not as important as (i) considering the presence of a VFS especially for longer lengths of VFS, and (ii) accounting for pesticide residue and degradation processes in the VFS if considering less than 90% exceedance probabilities.

AGRO 301

Regulatory harmonization: Is it possible?

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The use of pesticide products in agriculture is regulated by national authorities throughout the world. The amount of residue legally allowed on agricultural crops (Maximum Residue Limits (MRLs)) is also established by many national authorities as well as the Codex Alimentarius Commission (CAC). Throughout the last decade numerous initiatives have been launched to address the harmonization of pesticide regulatory decisions and MRLs, the goal being to have a safe, affordable, available food supply without trade barriers as agricultural commodities are increasingly globally traded. The efforts to date, have led to progress in the harmonization of pesticide regulations. However, many issues still have yet to be resolved, leading to a question of whether harmonization is indeed achievable. Each year more authorities decide to establish country-specific MRL setting regulations with the potential to create trade irritants, different risk assessment methodologies, and pesticide use restrictions. Given the multiplicity of regulatory systems around the world, the question of whether alignment of regulatory decisions is even possible will be explored.

AGRO 302

Need for pesticides for pulse growers

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The Canadian pulse industry has recently increased its focus on the growing potential for technical barriers to trade associated with pesticide use. While local food production is critical to achieving the broader set of United Nations (UN) goals around hunger, trade plays a critical role. When local production fails to meet the demand for food 365 days a year, regional, national and international trade fills the gap. Under the UN-designated International Year of Pulse 2016, Pulse Canada is participating in the Global Pulse Confederation's advocacy efforts concerning the extent to which misaligned maximum residue limits (MRLs) may disrupt trade and constrain growers' productive use of pesticides in pulse growing regions around the world. This is an issue of growing concern for growers in developed and developing countries alike. While trade continues to work today, the risk is that more countries (a) develop national MRL lists without continuing to defer to Codex in cases where a national MRL has not yet been established, (b) apply unpredictable or unworkable defaults in the absence of an established MRL – even in cases where the MRL of neighbouring countries or Codex is orders of magnitude higher, and (c) apply testing with LODs much lower than were possible a decade ago. There is an emerging opportunity to develop a global dialogue on Recognition of Scientific Standards (RSS) – mutual recognition by regulators of other regulators' risk assessment work and MRLs. If an importing country accepted or referenced another country's tolerance in cases where it would otherwise apply a zero- or near-zero default because an MRL has not yet been approved, a large majority of MRL-

related trade risk would be eliminated. The opportunity includes the development of politically, technically and commercially workable RSS policies along with appropriate international outreach involving growers, governments and industry.

AGRO 303

Challenges and opportunities for California citrus exports

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TThe California citrus industry exports a third of its annual production and receives 40 percent of its total revenue from exports. Citrus trade has been beneficial to global consumers who don't have access to fresh citrus either because it can't be grown in that country or there is no domestic supply year round. Fresh citrus, such as lemons, oranges, grapefruit and mandarins must meet consumer expectations for quality and value, or consumers will substitute other available fresh fruits. However, shipping citrus over long distances can be a challenge because an array of decay organisms can degrade fruit and cause significant losses. The citrus industry uses postharvest fungicides to minimize the impact of losses from decay and rotted fruit. While use of this technology helps citrus growers and consumers it needs to be facilitated with international MRL standards that will enable trade and a strong effort to thwart efforts to establish barriers to trade. This talk will focus on the nexus of biology, economics and policy as it relates to international citrus trade.

AGRO 304

Market place considerations: The importance of harmonized MRLs

***Daniel A. Botts**, daniel.botts@ffva.com. Florida Fruit Vegetable Association, Maitland, Florida, United States*

Have you gone to your local grocery store and not been able to get the fruits and vegetables you wanted for yourself or to feed your family? Most likely the answer is no, as we have all come to enjoy a wide selection of fruits and vegetables through the year, regardless on local crop growing seasons. This trend of having a constant supply of produce to selection from in the grocery store is likely to continue and even expand to an increasing middle class around the world. Most consumers demand pest and disease free produce, and have no idea what a MRL is and why it has evolved into a hurdle for US agricultural trade. Growing healthy fruits and vegetables today is possible, but the barriers growers face to make the produce available to global consumers seems to be increasing. The US EPA has been establishing MRLs for years, way before many other nations even considered developing food standards programs. Since the US system of establishing MRLs has been determined effective in providing growers the pesticides needed to feed the US and ensure the pesticides are used appropriately in the field, why can't developing nations, new to creating a scientific body to review pesticide use, defer to the US system as a role model? There are no easy answers or solutions to the trade challenge MRLs have become; instead, US growers rely on organizations such as Florida Fruit & Vegetable Association, the USDA Foreign Ag Service, and EPA to advocate expanding import opportunities for US agriculture. I will share my experiences opening up markets for US crops and provide considerations for harmonizing MRL s.

AGRO 305

Plant protection products regulations in the EU - an overview

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In the EU, there are two major regulations on plant protection products in place. Regulation 1107/2009 deals about the placing of plant protection products on the market, whereas regulation 396/2005 is about maximum residue levels of pesticides in or on food and feed of plant and animal origin. Since applicability of regulation 1107/2009, EU moved from a risk-based assessment approach to a more hazard-based assessment by introducing cut-off criteria for active ingredient approval like, POP (persistent organic pollutants), PBT (persistent, bioaccumulative, toxic) or ED (endocrine disruptors). This hazard-based approach may affect setting of EU MRLs or Import Tolerances. Furthermore, evaluation timelines of active ingredient or products as laid out in 1107/2009 do not completely match with timelines foreseen in the MRL setting process according to 396/2005. This can lead to delays in either process. The presentation will give an overview about current activities and future aspects in the EU and the effects for the rest of the world.

AGRO 306

Purpose and aim of the new maximum residue limit (MRL) regulation in Mexico

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The Ministry of Agriculture (SAGARPA) and the Ministry of Health (COFEPRIS) elaborated the draft -PROY-NOM-000-SAG-FITO/SSA1-2013- where the aim of this regulation is to establish and review of Maximum Residue Limits (MRL's) of chemical pesticides for agricultural usage for purposes of registration and use in Mexico.

Since pesticides are monitored by several federal government agencies in order to ensure consumers its quality, effectiveness, and given its toxic nature, to prevent risks to public health, it is necessary to establish a level at which pesticide residues in imported and domestically-produced foods derived from an application of these to control pests do not pose a risk to human health.

This regulation will be mandatory for all Mexican territory and will apply for all entities applying for registration of chemical pesticides for agricultural usage with the purpose of use in Mexico and that require a MRL according to legal provisions. This project contains the technical guidelines and procedures for authorization of an MRL, and laboratory methods for risk analysis. Criteria to establish an MRL will be according to domestic regulations and considering international parameters like the ones published by the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO), through the CODEX Alimentarius, the Environmental Protection Agency of the United States (EPA), and others. This regulation will enforce the agreements done in the Technical Working Group for the harmonization of MRLs and minor uses.

AGRO 307

Opportunities to mitigate trade uncertainties related to MRLs

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Despite theoretical arguments that support the conclusion that regulatory convergence internationally is welfare enhancing and empirical evidence that there are large economic opportunities forgone arising from continued regulatory divergence, differences in maximum residual limits (MRLs) for pesticides persist among countries. The reasons why, despite economic evidence to the contrary, initiatives to promote regulatory convergence often fail to be successful are explored. These include misaligned bureaucratic incentives, scarcity of negotiating resources, and political precaution. The uncertainties surrounding MRLs are framed. Some suggestions on how to move MRLs toward international regulatory convergence are presented.

AGRO 308

Weighing benefits versus risk of pesticides in addressing food needs

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Why do pesticides have such a bad reputation? Whose responsibility is it to communicate the necessity pesticide? Are uses of pesticides really necessary to grow fruits and vegetables? Common misunderstandings are abundant and plentiful, such as: If an MRL is lowered does that mean the commodity is safer? Can't we feed the world without chemicals? How much land is required to grow the average amount of fruits & vegetables to feed a family of four in the USA? Why are the benefits of pesticides not recognized? Is communicating pesticide science the barrier to understanding the benefits? What is the registrant's role in promoting the benefits of pesticide use in agriculture?

AGRO 309

Use of computational chemistry & toxicology tools and models for assessing human health hazards under the Toxics Substances Control Act

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The Risk Assessment Division (RAD), in the EPA's Office of Pollution Prevention and Toxics (OPPT), is tasked with performing risk assessments for over one thousand new chemicals every year, prior to allowing these chemicals to enter US commerce. To aid in this task, particularly when data is unavailable, computational tools, assessment methods and databases are used to assess hazards and make risk determinations. Structure-activity approaches and interpolation between chemical category members, including read across from related chemicals (i.e., those with similar structural, physicochemical, and/or toxicological profiles), are used to fill data gaps. OPPT has developed a computerized method called the Analog Identification Methodology (AIM) to help risk assessors identify potential analogs to predict health effects that may be associated with exposure to untested chemicals. AIM uses a fragment-based structural similarity approach based on the premise that similar molecular features may lead to similar biological activity, and provides links to information sources where experimental data on related chemicals can be found. OncoLogic™ is another predictive tool that estimates cancer potential using decision logic and structure activity relationships. OncoLogic™ mimics the judgment of cancer experts by following 'knowledge rules' based on information gleaned from decades of cancer research. Efficient use of this tool requires experience with data interpretation and knowledge of chemical structures, especially reactive functional groups. This presentation provides an overview of structure-activity principles and predictive approaches used to inform human health hazard characterizations in EPA's New Chemicals Program. Key features, limitations, and future needs of AIM and OncoLogic™ will be discussed.

AGRO 310

QSAR in the evaluation of toxicity and environmental fate of novel explosives, propellants, and pyrotechnics

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The US Army and the Department of Defense are actively engaged in development of new energetic materials that will have reduced impact on human health and the environment when compared to legacy materials such as TNT, RDX, and ammonium perchlorate. The Toxicology Directorate of the Army Public Health Center (APHC) plays a key role in assessing the impact of new, developmental materials before they enter full production and are deployed in new munitions. Toxicological investigations are conducted in a progressive manner according to the stage of development of the new material. When no toxicological information is found following a systematic review of the literature, the first step in the assessment involves QSAR analysis. The goal of this analysis is to predict the fate, transport and potential for human and ecological toxicity using a combination of off-the shelf QSAR programs. Unlike many evaluations involving QSAR analysis that attempt to predict a single physical property, several are used in an integrative approach. Predictions are made for toxicological endpoints such as median acute oral and inhalation toxicity, dermal and ocular toxicity, developmental and reproductive toxicity, mutagenicity and carcinogenicity. Ecotoxicity endpoints are evaluated by combining estimates for physical properties with ecotoxicity endpoint models. Together, these data are used in making resource decisions to continue further research and for assisting in evaluating risk associated with emergency response operations. This presentation will show how data collected from *in silico* approaches can be used in a risk assessment framework to assist in decision making and assist in focusing future experimental data collection efforts.

AGRO 311

Use of computational chemistry & toxicology tools and models for assessing PChem properties, fate and aquatic toxicity under the Toxics Substances Control Act

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The Risk Assessment Division (RAD), in the EPA's Office of Pollution Prevention and Toxics (OPPT), performs risk assessments for over one thousand new chemicals every year, prior to allowing these chemicals to enter US commerce. To aid in this task OPPT has developed assessment methods, databases, and predictive tools to help evaluate new chemicals submitted with little to no physical chemistry, degradation or environmental toxicity data. Estimation methods and tools are useful for supplementing laboratory studies or monitoring data or filling data gaps when data are not available. EPISUITE (Estimation Program Interface Suite™) and ECOSAR (Ecological Structure Activity Relationships) are two tools used to provide estimates of physical/chemical and environmental fate properties and aquatic toxicity. EPISUITE is a Windows®-based program that estimates physical/chemical properties (melting point, water solubility, etc.) and environmental fate properties (breakdown in water or air, etc.) which can indicate what media a chemical will partition to and whether the chemical will degrade. ECOSAR uses quantitative structure-activity relationships (QSAR) to predict the toxicity of chemicals based on their structural similarity to chemicals with toxicity data. The program is designed to estimate a chemical's acute (short-term) toxicity and chronic (long-term) toxicity (LC_{50} and EC_{50}) to aquatic organisms, such as fish, aquatic invertebrates, and aquatic plants.

The purpose of this presentation is to provide a basic overview of how to use EPISUITE and ECOSAR as a screening-level application that allows the user to quickly screen for chemical properties, environmental fate, and aquatic toxicity of chemicals. The presentation will also address current model limitations with respect to certain chemical classes and future data needs required to improve model performance.

AGRO 312

Coupling metabolite predictions to pesticide toxicity in silico

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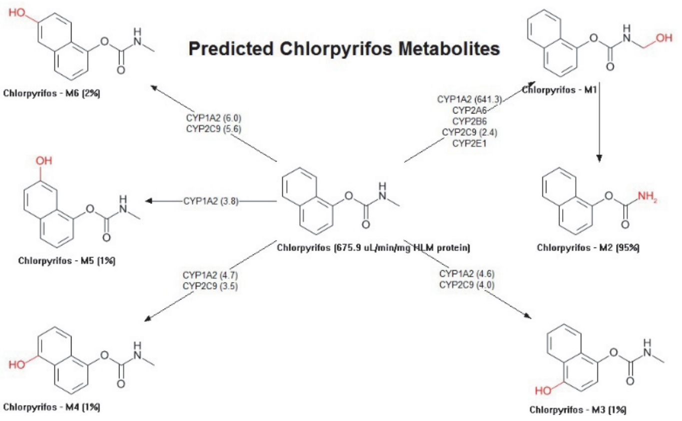
Analysis of quantitative structure/activity relationships (QSARs) has deep roots in agrochemical research and development. Historically the focus was on pesticide potency, whereas today applications that focus on elucidating generation of potential metabolites, human exposure to those metabolites and any toxicological risk associated with such exposure are at least as important. The many relevant endpoints involved must generally be modeled separately, so it is critical that predictions from the multiple models involved be integrated effectively into a single prediction. This talk will focus on how this is accomplished in general and, in particular, how the metabolic predictions can be tied in to predictions of toxicological properties.



AGRO 313

Case studies on identification of residues of concern in ecological risk assessment for conventional pesticides

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When pesticides degrade, transformation products of toxicological concern may be produced. The Office of Pesticide Programs (OPP) receives information on the toxicity of pesticide active ingredients to multiple taxa and sometimes receives toxicological information on selected degradates. For the risk assessment, OPP must decide which degradates to include as residues of concern and whether any may be excluded for particular taxa. Usually this decision is made with minimal data. An overview of the process used to identify which degradates are considered residues of concern and how those residues will be handled in the risk assessment will be presented using case studies. Tools used in the process such as read across using closely related active ingredients, quantitative structure activity relationship tools, and literature searches will be described. Sensitivity analysis is performed to show the impact of including degradates as residues of concern.

AGRO 314

Concluding discussion: quantifying uncertainty and identifying research needs

***Stuart Z. Cohen**, ets@ets-md.com. Environmental & Turf Services, Inc., Silver Spring, Maryland, United States*

This will be a structured, facilitated discussion. Each presenter is asked to submit thoughts regarding uncetainties and research needs in the areas of QSAR and computational chemistry and toxicology. These will be presented to the audience, who will be solicited for comments and suggestions. Notes will be taken, written, and distributed to the participants after the meeting.

AGRO 315

Toxicity of the natural insecticide matrine to *Aedes aegypti, Drosophila melanogaster* and *Periplaneta americana*

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Matrine is a Chinese traditional medicine extracted from *Sophora flavescens* and is used as the main ingredient of a number of natural pesticides in China. This study used traditional toxicity bioassays to assess insecticidal potency and electrophysiological techniques to investigate the mode of action of matrine. The glass contact assay displayed 20% death rate at 50 μg/cm^2 and 90% death rate at 10 μg/cm^2 against sensitive *Drosophila melanogaster* and *Aedes aegypti*, respectively. LC50 in this assay against *Aedes aegypti* was 4 μg/cm^2 with 95% fiducial limits of 2.6-6.6 μg/cm^2 at the end of 24h. Meanwhile, topical toxicity assay against *Aedes aegypti* adult females showed that the LD50 was 258 ng/mg with 95% fiducial limits of 218-336 ng/mg and KT50 was 27 min with fiducial limits of 25.16-28.97 min at the end of 1h. The paralytic action in headless mosquito larvae treated with matrine showed flaccid paralysis without evidence of neuroexcitation. The PC50 (paralytic 50% concentration) for larvae exposed to matrine was 7.62 ppm with 95% fiducial limits of 4.52-10.17 ppm at the 5h time point. For studing the effect of matrine on the heart contraction, American cockroaches were micro-injected with saline, 100 ppm, and 1000 ppm matrine. Average heart rate of the cockroach was 62 ± 3 beats/min. The heart rate was increased to 65 ± 5, 74 ± 7 and 80 ± 8 beats/min respectively after the cockroach micro-injected with 10 μl saline, 100 ppm and 1000 ppm of matrine. The experiment results demonstrated that matrine had weak effect on the heart rate of cockroach. Based on the above experiment results, matrine possess moderate toxicity on several observed insects. To better elucidate the target of matrine, different electrophysiological techniques will be used to study the mechanism of action.

AGRO 316

Mosquito repellents and larvicidal constituents based on natural products and their synthetic analogs

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Aedes mosquitoes are the primary vector for transmission of Zika virus. As part of joint efforts between USDA and DWFP (Deployed War Fighter Protection) program of DoD, natural products and synthetic analogs of natural products were investigated for mosquito control. Analogs of a chromene amide isolated from the leaves *Amyris texana* were synthesized and evaluated for mosquito repellent activity. Several of these analogs have shown better activity than the commercial repellent DEET. Among the chromene analogs that were synthesized and evaluated, the analog 2,2 dimethyl-2H-1-benzopyran-5-methanol showed the highest activity with three times longer duration of protection against female *A. aegypti* mosquitoes. Chroman analogs and some chromene analogs showed larvicidal activity against a permethrin resistant Puerto Rican strain of *A. aegypti* as well as the permethrin susceptible Orlando strain *A. aegypti*. Some of these analogs showed topical mosquito adulticide activity as well. From the ethyl acetate extract of seeds of *Ammi visnaga*, a member of Apiaceae family, two furanochromones were isolated via bioassay guided isolation and identified as khellin and visnagin by spectroscopic techniques. Phomalactone isolated from *Nigrospora spherica,* a plant pathogenic fungus showed adulticide and lavicide activites. Isolation, synthesis and mosquitocidal and larvicidal activities will be discussed.

AGRO 317

Chemical control of mosquitoes by re-purposed and modified agricultural insecticides

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Emerging and re-emerging infectious diseases are a growing human health concern, as epitomized most recently by the Zika virus outbreak. A major method of intervention in disease transmission is vector control of mosquitoes using insecticides. To date, significant reductions in malaria transmission have occurred from use of re-purposed pyrethroid insecticides for use on chemically impregnated bednets, with a lesser contribution from carbamate residual sprays. However, pyrethroid and carbamate resistance is common in mosquito populations, world-wide. In the search for alternatives, we have investigated a number of carbamate analogs with potent insecticidal and resistance-breaking properties. Moreover, a new formulation of an existing organophosphate insecticide, pyrimiphos-methyl has been introduced. This molecule has modest mammalian toxicity and circumvents the common G119S mutation in acetylcholinesterase that causes high levels of carbamate resistance. Recent results have been observed in our laboratories with the aphid feeding inhibitor, flonicamid. This compound impairs mosquito behavior and may act at a novel site in the insect nervous system. The implications of these findings for mosquito control will be discussed.

AGRO 318

Ultra-low rate application of deltamethrin for mosquito control

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Deltamethrin has been shown to be effective in the control of mosquitoes, including strains that are resistant to Type I pyrethroids and express the Kdr mutation. Bayer has developed a unique formulation of deltamethrin (DeltaGard Insecticide) that allows for uniform ultra-low volume (ULV) application at rates as low as 0.50 g ai/ha (0.00045 lb ai/A). The design and performance of a study to determine the potential residues in food and feed items following multiple applications of DeltaGard Insecticide will be discussed, along with the analytical methodology developed for the food and feed sample analyses. The potential impact of DeltaGard Insecticide use on human dietary, operator, and reentry exposure will also be discussed.

AGRO 319

Proven vector control methods to reduce the risk of dengue: Lessons for Zika

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Mosquito vectors of Zika virus and related pathogens are known as container breeding mosquitoes for their ability to rapidly complete larval development in small, well-defined water habitats. The principle vectors Aedes aegypti (Linn.) and Aedes albopictus (Skuse) successfully colonize both artificial man-made containers such as roof gutters, refuse, and outdoor pots, and natural plant-based pytotelma such as leaf axils, tree holes, and fallen leafs throughout their range. We hypothesize the proliferation of new habitats in urban areas may be contributing to the recent outbreaks of Dengue, Chikungunya and Zika disease.

Containers susceptible to mosquito colonization can be effectively treated with environmentally sustainable larvicides to prevent adult emergence and reduce the incidence of disease. The method of larvicide application either directly by hand or indirectly using a motorized sprayer into the container depends on the operational capabilities and goals of the government health district. A mix of larvicide formulations and application methods based on habitat heterogeneity may be necessary to achieve program goals.

Recent advances in larvicide formulations, application technology and GIS-based management provide for the economical and effective treatment of infested containers necessary to break the disease cycle. Current operational Public Health programs that successfully control Dengue vector populations serve as models for Zika programs because the vector species and habitats are identical. Examples of effective container breeding vector control and areas for further research will be discussed.

AGRO 320

USEPA regulatory framework for the ecological risk assessment of down-the-drain uses of pesticides

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The USEPA regulates the use of pesticides under the authority of two federal statutes: the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Since early 2005, when the Preliminary Ecological Risk Assessment for the Registration Eligibility Decision for permethrin was published, the USEPA has received numerous comments from stakeholders regarding the indoor down-the-drain uses of permethrin and other pesticides, and their potential to reach surface waters from POTW effluents and cause harmful ecological effects. During the second phase of the reevaluation of permethrin, the Agency has received additional and similar comments. In previous assessments, the Office of Pesticide Programs (OPP) has relied on the Office of Pollution Prevention and Toxics' (OPPT) Down-the-Drain module (DtD) in the Exposure and Fate Assessment Screening Tool (E-FAST) to estimate exposure. The DtD module relies on the total production volume of a down-the-drain chemical and the POTW treatment removal to provide Tier 1 estimated environmental exposure concentrations (EECs), which are then compared against aquatic ecological effects endpoints to obtain risk quotient (RQ) values. Since 2005, registrants have submitted

POTW monitoring data for a number of pesticides, although these data have been limited to only a few states. Currently, EPA is conducting a comparative environmental fate and ecological effects risk assessment for the Registration Review of eight synthetic pyrethroids plus the pyrethrins, most of which have down-the-drain uses. This presentation will cover conclusions from down-the-drain ecological risk assessments for pyrethroids and will include comparisons of monitoring and modeling results as well as inherent uncertainties of Tier 1 DtD assessments and how they can be addressed.

AGRO 321

Environmental risk assessment of down-the-drain chemicals in the European Union: Current approaches, strengths and weaknesses

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Down-the-drain is a key environmental emission pathway considered as part of the regulation of many chemicals in the European Union (EU). Relevant regulations include REACH and the Biocidal Products Regulation (BPR). This presentation will describe the standard exposure framework that is used in risk assessment of these chemicals, as implemented in EUSES, CHESAR and other related tools. Key assumptions, including those relating to per-capita water usage, wastewater treatment and dilution will be highlighted. Approaches to estimating emissions to wastewater will also be described in the context of biocides under BPR, including detailed description and evaluation of key assumptions described in emission scenario documents for biocidal Product Types 18 (insecticides), 19 (repellents) and 2 (disinfectants). This will include evaluation of the 'consumption data' and 'tonnage data' approaches and their suitability for emission estimation for different product types.

AGRO 322

Pesticides and POTWs: Opportunities and challenges

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Publicly Owned Treatment Works (POTWs) are not designed to remove pesticides. However, pesticides may be discharged to POTWs in conjunction with both indoor and outdoor pesticide applications. Pesticides can potentially interfere with treatment plant operation, ability to recycle water and biosolids, and compliance with National Pollution Discharge Elimination System (NPDES) permits. The potential for these impacts should be addressed in pesticide risk assessments.

When a pesticide is used, it can be discharged to a sewer, either because the use produces wastewater, or because an indirect pathway for sewer discharge exists (e.g., the treated surface is eventually cleaned with water or a pesticide-impregnated garment is laundered). Since POTWs are not designed to treat pesticides, treatment plant effluent and biosolids may contain the pesticide. Such pesticide releases may cause aquatic toxicity and exceedances of permit effluent limits.

To properly assess risks to POTWs, data on the environmental fate of all pesticides that may be discharged into the sewer should be required to sufficiently predict pesticide fate in POTWs and to support an evaluation of how the presence of pesticides in recycled water and biosolids may affect receiving waters and reuse. Pesticide data requirements for uses that discharge to POTWs should require both acute and chronic toxicity test results for at least one freshwater and one marine/estuarine invertebrate, vertebrate, and plant species utilizing US EPA standard water quality test species and procedures consistent with Clean Water Act implementation. If the results of the evaluation estimate concentrations that may impact receiving waters, cause aquatic toxicity, exceed NPDES permit effluent limits, or the ability to reuse recycled water or biosolids, mitigation measures should be included during the pesticide registration process.

This presentation will discuss conventional wastewater and biosolids treatment, variations in POTWs, how POTWs are regulated, intentional and unintentional pesticide sources to POTWs, pesticide compliance problems, and opportunities for improvement in the pesticide regulatory process.

AGRO 323

Wastewater discharge risk assessments: Importance and improvement opportunities

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Frequent detection of pesticides—and sometimes pesticide related toxicity—in municipal wastewater treatment plant effluents has revealed previously unknown and unexamined linkages between pesticide use and municipal wastewater. Municipal wastewater treatment plants are designed to treat human waste and, therefore, have reason to be concerned about the presence of toxicants like pesticides in their systems, due to past experiences linking a few notable consumer product ingredients with violations of water and air permits, increased operational costs, biosolids management restrictions, and, in rare cases, citizen lawsuits.

Long performed by cleaning product manufacturers, wastewater risk assessments are relatively new for pesticides and are revealing problematic shortcomings in current risk assessment methods, particularly for products with indirect discharge pathways. Common source identification approaches and models sometimes underestimate effluent chemical concentrations, omit important discharge sources, omit assessment endpoints (e.g., biosolids, air emissions, process interference, recycled water use), exclude other sources of the same chemical, or are not accurate enough to inform risk management actions.

Existing information from municipal wastewater science could improve risk assessment methods and ultimately provide better information for product design and regulatory decisions. For example, wastewater models should address zero-dilution discharges to effluent-dominated water bodies, update per-capita water use rates to reflect the effects of modern water-conserving fixtures, address both salt and fresh water discharges, employ more scientifically robust approaches for estimating removal efficiencies, and bring together conceptual models, transfer factors, and product use characteristics to create discharge estimates inclusive of direct and indirect discharges that address regional, seasonal, and other variability in consumer product use.

For regulatory consistency and to avoid product liability litigation, effluent effects assessments should address consistency with Clean Water Act compliance assessment requirements (e.g., aquatic toxicity test species, endpoints and time frames). More challenging topics, such as biosolids management risk assessment and recycled and downstream water use (e.g., crop irrigation, residential irrigation, human consumption) are priorities for future wastewater risk assessment improvements.

AGRO 324

Recurring U.S. national wastewater treatment plant survey and the Human Health Observatory at Arizona State University

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Over 2,000 new chemicals are introduced into US commerce each year, rendering monitoring of their annual loading, occurrence, persistence, and effects on wildlife and humans a very challenging and mostly unanswered task today. Already, some 40,000 chemicals have been identified as representing contaminants of emerging concern (CECs) with wastewater treatment plants (WWTPs) representing both the locus of treatment and discharge of these substances into the environment. To judge the sustainability of this chemical consumption and disposal, it is helpful to conduct national surveys of wastewater, to characterize chemical loadings and the efficiency with which existing treatment infrastructure attenuates potential threats. The US Environmental Protection Agency (EPA) has performed four sewage sludge surveys (1982, 1988, 2001, 2006-7) thus far, to identify inorganic and organic contaminants considered for risk assessment and regulation action. These surveys are important since about 50% of the biosolids produced annually in the US is reused as a soil amendment. However, past EPA surveys did not include screening of raw influent and treated effluent samples from WWTPs, thus preventing collection of information on chemical loading and the treatment efficiency of wastewater-borne chemicals used in commerce. The last EPA survey was conducted nine years back (2006/7), and surveying currently has been suspended. Determining current chemical consumption and discharges of US society requires continuation of wastewater process stream monitoring. The present study addresses this need by constituting a nationwide survey of WWTPs to determine (i) the current composition of US wastewater; (ii) the effectiveness of US wastewater treatment infrastructure in removing chemical constituents; and (iii) the quantity of chemicals discharged to surface waters and soils by representative US WWTPs via recycling of wastewater and biosolids, respectively. In addition to collecting nationally representative data, the study also serves to expand the National Sewage Sludge Repository, which is part of the Human Health Observatory (HHO) at Arizona State University. As illustrative data demonstrate, this shared resource for the scientific community is a valuable tool to extend current understanding of the occurrence of hazardous agents in wastewater and biosolids, their rates of removal during treatment, and their loading and fate in the environment.

AGRO 325

Occurrence and mass balances of neonicotinoid and phenylpyrazole insecticides during conventional wastewater treatment

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Neonicotinoids and phenylpyrazoles are two groups of systemic insecticides used for industrial agriculture, lawn treatment, indoor pest control, and pet flea treatment. These insecticides have been shown to adversely affect non-target sensitive aquatic invertebrate species, and also have been identified as chemicals of concern in the global collapse of honeybee colonies. The objective of this study was to evaluate the fate of six neonicotinoids and fipronil and its four major degradates through a wastewater treatment plant (WWTP) and an engineered wetland immediately downstream. Flow-weighted samples were collected for five-day monitoring period, subjected to solid phase extraction and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Among the six neonicotinoids targeted, thiamethoxam (<2 ng/L), thiacloprid (<2 ng/L), and dinotefuran (<100 ng/L) were not detected at all, whereas clothianidin was detected only inconsistently in the WWTP and wetland (80% detection frequency at >2 ng/L). Concentrations of imidacloprid in both WWTP influent and treated effluent were in the range of 45-55 ng/L; whereas concentrations of acetamiprid (1 to 10 ng/L) and total fipronil related compounds (20-35 ng/L) were comparatively lower. A mass balance over the WWTP showed insignificant aqueous removal of imidacloprid, acetamiprid, clothianidin, and total fipronil-related compounds. The constructed wetland located downstream from the WWTP and receiving treated effluent showed a lack of removal for both imidacloprid and acetamiprid; however, total fipronil was attenuated with an efficiency of 47 ± 13%. Additionally, composite samples of influent and effluent collected at 12 additional WWTPs corroborated the occurrence and persistence of neonicotinoids, suggesting annual discharges on the order of 1000-3400 kg/y of imidacloprid from US WWTPs to effluent receiving surface waters nationwide. These results indicate that discharged treated wastewater is an inadvertent source of recalcitrant pesticides to the environment.

AGRO 326

Consideration of subsurface pesticide degradation in groundwater assessments

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Available data regarding the fate and metabolism of pesticides in topsoil are much more robust than for subsoil layers or for aquifers. As a result, groundwater exposure models used by pesticide regulators rely on limited data inputs. This leads to standardized, default algorithms for a decline in pesticide degradation rate with depth in the soil profile and reliance on only a hydrolysis rate for degradation below 1 meter from the land surface. Yet the available research fails to confirm that use of such default assumptions consistently provides a reasonably accurate estimate of the amount of pesticide that remains available for leaching to ground water over time, particularly over multiple year scenarios. Field and laboratory study design options will be discussed for improving characterization of pesticide behavior throughout the vadose zone. Accounting for variations in the nature of abiotic and biological degradation rates and processes in the subsurface soil profile will help enable the Environmental Protection Agency's Office of Pesticide Programs to refine drinking water assessments to account for degradation processes more explicitly for a given chemical and also better account for spatial variation in these processes.

AGRO 327

Variations on a theme: Groundwater sensitivity

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Downward water movement and subsurface degradation are key factors in estimates of pesticide concentrations in groundwater. Since PRZM-GW estimated drinking water

concentrations (EDWC) are averaged over 30 years as opposed to estimating 90th percentile concentrations, timing of heavy (>2 inches) storms have a huge impact on EDWC for compounds with extremely short half-lives as compared to persistent compounds due to moving the pesticide out of the degradation zone. This presentation shows the sensitivity of various factors including timing of rain storms, type of application, and soil degradation was evaluated with PRZM-GW (USEPA's groundwater assessment tool) and PRZM-VADOFT. In addition, various soil degradation schemes were simulated and analyzed for the degradation in soil to the 1-m depth and degradation in soil below 1 m. The EDWC results were reduced 5 fold or to negligible concentrations as compared to results predicted following US EPA PRZM-GW guidance. The impact of these modeling assumptions will be tested with these two models.

AGRO 328

Impact of biphasic degradation on pesticide subsurface transport and groundwater exposure estimates

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Biphasic degradation patterns are increasingly observed in soil metabolism and other environmental fate studies. After application to soil, many pesticides with low use rates tend to show fast initial decline followed by a slower phase. These decline patterns deviate from the classical first-order kinetics and can be caused by many factors such as reduced bioavailability due to increased sorption and/or changes in microbial viability over time. Although widely recognized and confirmed in many regulatory guideline studies and scientific literature, the biphasic degradation process has never been incorporated into any of the current regulatory environmental exposure models. Instead, as a policy requirement, regulatory models must use the first-order kinetics fitted preferentially to the slow portion of the decline data while largely ignoring the fast initial phase. As a result, predicted exposure levels in groundwater are often artificially significantly elevated. This paper examines the impact of biphasic degradation on pesticide leaching behavior using a PRZM-based model synPRZM developed by Syngenta and Waterborne. The synPRZM model directly incorporates the kinetics of Double First-Order in Parallel (DFOP) to accurately take into account the complete biphasic decline profile. The inherent nature of DFOP reflecting the effect of time-dependent sorption on degradation is analyzed by equating the macro parameters in DFOP to the micro parameters that define the coupled sorption and degradation kinetics in the soil-pore water system.

AGRO 329

Predicting pesticide biphasic soil concentration decline under field conditions: Model-data comparison

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As part of the NAFTA degradation kinetics calculation process, the Double First-Order in Parallel (DFOP) model is considered along with the Single First Order (SFO) and the Intermediate Order Rate Equation (IORE). However, only SFO kinetics are represented in pesticide fate regulatory modeling tools like the Pesticide Root Zone Model (PRZM), thus hindering the model's capability of simulating chemicals with biphasic degradation characteristics. We have recently incorporated DFOP into synPRZM, a PRZM-based environmental fate model as an additional option to the sorption kinetics developed by Syngenta and Waterborne for predicting field soil residue declines. The model codes in synPRZM treat the chemical under simulation as two separate fractions which are determined by the DFOP degradation kinetics, with each fraction having a distinct degradation rate constant. Both fractions are simulated simultaneously each day, and the sum of their results is output as a whole for the chemical. Using DFOP parameters independently measured in laboratory soil metabolism studies, synPRZM was able to predict a number of field soil residue data sets reasonably well without elaborated calibration. Predicted soil pore water concentrations from synPRZM are compared with measured data from field lysimeters. The robust performance of synPRZM demonstrates the model's predictive capability as a useful and pragmatic option to handle biphasic degradation behavior frequently observed in pesticide field studies.

AGRO 330

Subsurface modeling of a pesticide using the leaching estimation and chemistry model for pesticides: A comparison of field results and modeled estimates

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Understanding the subsurface fate of pesticides can be significantly enhanced by coupling terrestrial field dissipation (TFD) studies and subsurface leaching models. In the United States, two models are typically used for registration purposes, including the USEPA Pesticide Root Zone Model for Groundwater (PRZM-GW) and the Leaching Estimation and Chemistry Model for Pesticides (LEACHP), the latter of which is used by the New York Department of Environmental Conservation (NYDEC) and the California Department of Pesticide Regulation (CDPR). The objective of this modeling study was to predict potential groundwater concentrations following pesticide application and characterize the uncertainties in Upstate New York. Two modeling studies were conducted. The first involved site-specific modeling using lysimeter data collected from TFD trial plots at a site located in California. Bromide tracer data were used in the model to confirm the model was parameterized appropriately to predict vertical water flow through the surficial soil. Measured concentrations of the primary degradation product were also compared to LEACHP predicted concentrations to determine if the DT50 calculated in the California TFD study resulted in accurate estimation of measured lysimeter concentrations. Finally, concentrations of the parent and metabolite were estimated using the NYDEC LEACHP model and DT50 values calculated from representative trial sites of similar climate, soil, and rainfall. Estimated bromide concentrations at the California site agreed closely with measured lysimeter results. Similarly, when the DT50 values for parent and metabolite, derived from the California TFD study were used in LEACHP, predicted and measured lysimeter concentrations were also in close agreement. Results from the modeling emphasize the importance of using a degradation rate that is representative of the soil, climate, and meteorological conditions where the product may be used.

AGRO 331

Comparison of modeling approaches in estimating total toxic residues (TTR) of pesticide in ground water

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Total toxic residues (TTR) of a pesticide in ground water are defined as the summation of toxicologically relevant soil metabolites in ground water and are required in support of drinking water risk assessment in the United States. The purpose of this study is to estimate the ground water

concentrations of TTR using three modeling approaches and then compare them with ground water monitoring data. The first approach is to treat soil metabolites as a single chemical, with the lowest sorption parameter of metabolites and TTR DT50. The 2nd approach is to simulate metabolites individually with metabolite-specific environmental properties, and then sum the results as TTR concentration. The third approach is to simulate pesticide degradation and metabolite formation dynamically by implementing degradation pathway in ground water modeling. A pesticide with ground water monitoring data at multiple locations was selected for this study. All the simulations were conducted with PELMO and PRZM-GW models parameterized with site-specific soil and weather conditions. The results of this study are intended to clarify which ground water modeling approach is adequate in support of drinking water risk assessment of pesticides.

AGRO 332

HYDRUS 2/3D applied to modeling transport of agrochemicals in drip irrigation scenarios

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HYDRUS 2/3D is an alternative to models that are commonly accepted for estimating fate and transport of agrochemicals in groundwater. One of the advantages of HYDRUS is the flexibility in creating scenarios to model agrochemicals fate in specific agronomic scenarios. User defined scenarios can be created in 1, 2 or 3D for a particular type of irrigation/chemigation method (e.g., drip, in furrow), product application technique (chemigation/spray/granular) with appropriate site-specific weather files. To investigate transport of agrochemicals applied via drip irrigation, scenarios in HYDRUS were designed for 1m width x 3m depth domains with a high groundwater table. Theoretical 'observation wells' (sampling points) were placed in the domain to track the concentration of hypothetical pesticides in soil and groundwater. Sensitivity analysis of input parameters (degradation rates, linear vs non-linear sorption coefficients, irrigation amount/frequency) was tested to illustrate capabilities of HYDRUS for single and multi-year simulations. Storm events and increased irrigation frequency (compared to spread out daily irrigation) also increased pesticide transport in groundwater. Overall analysis shows that HYDRUS can predict concentrations in a similar range to that of regulatory models and be a useful tool to predict pesticides fate in real agronomic conditions, such as drip irrigation, where current regulatory models fail to accurately define and model such situations.

AGRO 333

Why investing in international regulations and standards matters

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The USDA Foreign Agricultural Service (FAS) does not regulate, monitor, or enforce food safety standards. But, global agricultural trade absolutely depends on a reliable regulatory network among trading partner countries. Many emerging market countries are in the process of modernizing their pesticide laws, regulations, residue monitoring programs, and decision processes for adopting or establishing maximum residue levels (MRLs). Many of these counties request assistance from the United States to strengthen their systems by providing regulatory guidance or technical training. Is investment in these foreign countries' capacity beneficial to the US farmer and consumer? Absolutely. Creating a more stable, science-based trading environment provides confidence to exporters that their products will not be detained or rejected at ports, that regulations are understandable and fair, that standards are in place, and that analytical tests are accurate. Pesticide residues in trade is one of the most complex of these problems. It's global in scope; it is often regulated across multiple national agencies; and the problem is shaped by private sector investment interests, consumer health concerns, risk philosophies, economic and trade policies, government sovereignty, and food security. FAS will present on current efforts to help strengthen this complex regulatory environment for pesticide residues in trade, and pose questions about the future role of FAS in capacity building efforts related to standards development.

AGRO 334

Business of MRLs: A food and beverage industry perspective

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The ability of international food and beverage companies to efficiently navigate a range of Maximum Residue Level (MRL) regulations is a key business success factor. These regulations can have a significant influence on the procurement and distribution of agricultural commodities that are used in the production of food and drink products. This presentation will describe some of the steps food and beverage companies, and related associations, have taken to work with suppliers, regulatory agencies and crop protection technology manufacturers to raise awareness of these needs and address specific MRL-related barriers. For consumer-facing companies, regulatory compliance plays a key role in maintaining consumer confidence in the quality and safety of packaged foods and beverages.

AGRO 335

Addressing Food Waste in the World with Pesticides

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It can take months to grow the perfect fruit that ends up on our kitchen table. Commodities are at their most delectable, highest quality, and have the potential to receive the greatest monetary reward for growers at harvest. Getting the freshly harvested crop to the consumer is a challenge in this globally traded market place. It is possible to have bountiful access to fresh, top quality fruits and vegetables without disease or rotting via cold storage, speedy transportation, and local consumption. However, these simplistic methods of ensuring quality have limitations in a world that expects an abundant supply of healthy food year-round. The greatest opportunity for meeting the desires for a wide variety of foods without food decay and disease occurrence during storage and shipping is through the use of crop protection chemicals at harvest. The pesticide industry has met the challenge of preserving food quality by developing what are called post-harvest pesticides. These chemicals are applied at or just after harvest and are expected to remain on the fruit or vegetables until sold to the consumer where it is often washed off or removed by peeling. The difference between post-harvest chemicals and other pesticides is often a matter of the timing of the application. Significant advances in reducing food waste are realized with postharvest pesticides.

AGRO 336

Retailers' secondary standards: What they are and why they exist

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Abstract: Growers, packers, shippers and processors regularly face country standards related to Maximum Residue Levels (MRLs) when handling and processing food. Increasingly, retailers are becoming more active in this area, and creating additional secondary standards that are more restrictive than government standards. These secondary standards are done by an individual retailer, and they can vary based upon their geographic location and the company. They have little advantage for consumer safety.

This presentation will give an overview of some of the major secondary standards that exist with retailers, and how that impacts growers' and processors' ability to effectively use crop protection products to deal with major pests in their area. It will also cover how these secondary standards impact their decision to ship to specific retailers, and determine how to market their crop.

AGRO 337

Strategies to meet export maximum residue limits for Michigan apples and cherries

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Profitability in global food markets requires meeting high food quality standards, often through the judicious use of crop protection materials, including pesticides. At the same time, many export targets for US fruit crops set maximum residue limits (MRLs) that are lower than the domestic tolerances held by the USEPA. Meeting this challenge is especially difficult with the recent prevalence of late season invasive pests, like the Brown Marmorated Stink Bug and Spotted Wing Drosophila. Michigan State University scientists are conducting residue decline studies on apples and cherries to help fruit growers determine which compounds hold the highest risks for export-bound crops. These data will also support establishment of "Export PHIs" that growers can use to avoid load rejections from export-target countries. Treatment regimens with minimum and maximum seasonal applications, addition of adjuvants, and the use of post-harvest water rinsing were tested for their effects on residue levels at harvest.

AGRO 338

Contemporary MRL issues for California specialty crops: things that make you go hmm?

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Several economically important maximum residue level (MRL) case studies are reviewed. Challenges, inconsistencies, and deficiencies associated with instrumental analyses are presented in the context of MRL establishment as well as enforcement. One case will be highlighted which involves fosetyl and phosphorous acid/phosphonate residues on specialty crops exported from the United States to the European Union. Novel analytical approaches, involving extraction with methanolic solvent followed by capillary electrophoresis-tandem mass spectrometry (CZ-MS/MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS), were developed to accurately quantify such residues, and the results were evaluated relative to methods used by the European Union (i.e., QuPPe-Method Version 7.1 and Version 8.0) for enforcement. We discuss the juxtaposition of political, regulatory, and economic MRL factors versus (presumptively) sound instrumental analytics that confound field, environmental, and methodological variability.

AGRO 339

Monitoring pesticide residues at the federal level

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The Pesticide Data Program (PDP) was established as a result of the 1996 Food Quality Protection Act (FQPA) to test agricultural commodities in the US food supply for pesticide residues. The PDP emphasizes on commodities highly consumed by infants and children. A variety of domestic and imported foods are tested using rigorous statistical sampling protocol and current laboratory methods. The data show that overall pesticide residues found on foods tested are at levels well below the tolerances established by the Environmental Protection Agency (EPA). The PDP data enables the EPA in assessing dietary pesticide exposure and tolerance assessments as well as providing guidance to the US Food and Drug Administration (FDA) to make informed decisions when enforcing EPA tolerances. The USDA's Foreign Agricultural service (FAS) also uses PDP data to demonstrate MRL compliance in order to facilitate global marketing of US grown agricultural products.

AGRO 340

Use of PBPK models in risk assessment of agrochemicals

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Risk assessment requires the connection between hazard identification and knowledge regarding exposure through an understanding of the dose-response relationship for a health effect. Dose-response relationships are often inferred from laboratory animal toxicity or less often, *in vitro* studies. For human health risk assessment, these dose-response relationships need to be extrapolated to the human-relevant exposure region. The number and quality of epidemiology studies evaluating agrochemicals studies is increasing and thus playing a larger role in human health risk assessment. Laboratory animal studies often do not cover the low exposure region that humans encounter and can show different findings, either qualitatively or quantitatively, compared to laboratory animal studies. To reduce the uncertainty in extrapolations, physiologically based pharmacokinetic (PBPK) models can be a valuable tool to evaluate the low dose effects in humans and in interpreting biomonitoring data from epidemiology studies. In recent years, several PBPK models have become available for agrochemicals. The development of a PBPK model, however, can be resource-intensive and time-consuming since chemical-specific PBPK models require significant amounts of in vivo and in vitro data for calibration and evaluation. Given the number of agrochemicals that require risk assessment to support decision-making, there is a critical need for a strategy to efficiently develop PBPK models using contemporary approaches such as read across and *in vitro* to *in vivo* extrapolations, allowing PBPK models with different levels of confidence to be used in risk assessment. This presentation will provide case studies using agrochemicals on the value of PBPK models in improving low dose extrapolation, species extrapolation, and interpretation of biomonitoring data.

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Utilising *in vitro* to *in vivo* extrapolation and PBPK modeling demonstrates how a better understanding of human systemic exposure can establish margins of systemic exposure and be used to refine agrochemical risk assessments

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The application of default composite uncertainty factors in human risk assessments is widespread, but with such an approach, efficacious compounds in the field that in reality pose no threat to human safety can fail these generic risk assessments and be prevented from progressing further. However, by characterizing intra and interspecies variability in systemic exposure in both the toxicology species and in humans margins of exposure that can be quantified, we can move towards the application of Chemical Specific Adjustment Factors and Data Derived Extrapolation Factors to provide refined and more accurate risk assessments.

Obtaining toxicokinetics to describe systemic exposure within the toxicology species is becoming more routine within the agrochemical industry. However, the difficulty remains in obtaining human systemic exposure data. Not only that, but for this approach to be successful, variability within a human population must be explored, and safety within specific sub populations such as children must also be addressed. One way to approach this lack of human data is through the use of *in vitro* to *in vivo* extrapolation and PBPK modeling. Simcyp is a Population-Based Simulator containing extensive demographic, physiologic and genomic databases which include algorithms which account for human variability. This enables the user to predict compound behavior in virtual populations instead of a single virtual reference human, allowing individuals at extreme risk to be identified.

By using *in vitro* and *in vivo* toxicokinetic data from rat to build a PBPK model in simCYP to demonstrate understanding of a test compound *in vivo* and then translate these learnings to a human model, we will demonstrate an approach that supports the goals of data-derived extrapolation factors to maximize the use of available data and improve the scientific support for a risk assessment.

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Integration of toxicokinetic parameters for molecular design and safety assessment

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Plant protection active substances and formulations are tested rigorously at higher doses to assess potential toxicity towards mammalian and environmental species prior to registration and marketing. The toxicity potential, if any, identified in these studies along with the no-observed-adverse-effect levels and uncertainty factors are used to drive the overall human health risk assessment. In recent years, a greater emphasis has been placed to assess systemic exposure in relation to exposed dose levels to obtain meaningful information for extrapolation across species and routes and further to reduce uncertainty for human health risk assessment. In the current presentation, specific repeat-dose toxicity case studies will be presented to demonstrate the significance of integrating toxicokinetic parameters for dose level selection of plant protection active ingredients in relation to administered dose. These studies demonstrate the utility of kinetically derived maximum dose and avoid dosing inappropriately outside of the kinetic linear range in the subsequent repeated dose toxicity studies. In addition, the current presentation includes an overview of the performance of a mechanistically-based *in silico* PBPK model to evaluate systemic bioavailability in relation to prior generated experimental data from the oral, dermal and inhalation routes of exposure. Overall, this presentation demonstrates the significance of integrating toxicokinetic parameters for appropriate safety characterization and reducing uncertainty for risk assessment.

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Features and application of the ILSI/HESI RISK21 exposure framework

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The ILSI Health and Environmental Sciences Institute (HESI) Risk Assessment in the 21st Century (RISK21) project was initiated to address and catalyze improvements in human health risk assessment. RISK21 is a problem formulation-based conceptual roadmap and risk matrix visualization tool, facilitating transparent evaluation of both hazard and exposure components. The RISK21 roadmap is exposure-driven, i.e. exposure is used as the second step (after problem formulation) to define and focus the assessment. The exposure component is a tier based framework that enables mapping of existing exposure information to the risk assessment problem being analyzed. Exposure data, organized by physical chemical characteristics, exposure screening tools, exposure models, and biomonitoring data are considered as needed with toxicity data to assess risk of the health problem being considered. This enables the user to employ readily available exposure and toxicity data in a coordinated fashion for decision making and avoid the trap of requiring additional toxicity data to improve the assessment without consideration for the context of exposure. The framework can be visualized with the risk matrix visualization tool to assess confidence in the assessment and to explore aspects of uncertainty in the data. The net result of this approach is an efficient, economical, and transparent analysis of risk.

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Determining the adequacy of drinking water monitoring data for exposure modeling in risk assessments using the Risk21 framework

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Risk assessment for pesticides is the intersection of toxicological data and estimated exposures. For human risk assessments, exposures are estimated from a combination of scenarios by which a population could be exposed (dietary intake, drinking water intake, and/or residential handling). Dietary exposure via residues on food is often the most prevalent exposure scenario; however, for certain substances, drinking water can be the major route of exposure. Sampling frequency of water systems is a balance between water system characteristics, potential chemical exposure and cost. For certain widely used pesticides, extensive water monitoring including daily/near daily samples may be available; however, for most pesticides, monitoring data are very limited. Various methods have been used to fill non-sampled days (filling the gaps) to allow for better temporal resolution for risk assessments. Using atrazine as a case study and its daily/near daily monitoring data, we show the performance of various methods (linear interpolation, statistically based bias factor and kriging) by evaluating the uncertainties (deviation from the true) of various estimates of assessment endpoints with specified likelihood (i.e., to minimize false negatives). Risk21 provides a framework for comparing the adequacy of the toxicological and exposure data in a more quantitative manner. For the existing toxicological data for atrazine, dose metrics of human exposure via drinking water were calculated using the physiologically based pharmacokinetic model. Risk21 analysis was used to examine the interaction of the dose metrics and the water sampling frequency. The kriging-predicted daily exposures were shown to be robust and provide sufficient resolution for calculating exposure dose metrics, negating the need for daily monitoring. This case study using Risk21 analysis demonstrated that the existing exposure and toxicological data are sufficiently robust for risk assessment and regulatory decision making.

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Dosimetry modeling approach to refining inhalation risk assessment

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Inhalation dosimetry models are used to extrapolate dose across species, for example to predict human health effects from animal inhalation toxicology studies. Standard methodology for estimating inhalation reference concentrations (RfCs) for assessment of chronic non-cancer toxicity includes the dosimetric adjustment factor (DAF), applied to the observed exposure concentration in a particular laboratory species to estimate the Human Equivalent Concentration (HEC). Determination of delivered dose equivalence between animal and human requires the comparison of the dose at different parts of the respiratory tract, as determined by the particular target site for the chemical of interest. Traditionally, this is handled by calculating a regional deposited dose ratio (RDDR), using the calculator of the same name, to derive interspecies Point of Departure (POD) adjustments. The EPA Office of Pesticide Programs (OPP) has been using the RDDR model to derive the HEC from animal inhalation toxicity studies. With the development of more sophisticated dosimetry tools like Multiple Path Particle Dosimetry (MPPD) and Computational Fluid Dynamics (CFD) models, the use of RDDR is becoming obsolete. MPPD is a widely accepted model that allows the input of a particle size distribution that is relevant for the activity being assessed, while the RDDR model assumes the same particle size distribution as that of the inhalation toxicity study. Consideration of human activity relevant inhalation particle size distribution results in significant refinement in deriving the RfC for inhalation risk assessment. This presentation describes a case study in which MPPD is used to refine systemic inhalation risk assessment from exposure to non-gaseous particles.

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Open-source workflow for predicting *in vivo* outcomes

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Access to a comprehensive database of high quality reference data is essential for the development, validation, integration, and implementation of *in vitro* and *in silico* models that reduce and replace the use of animals in toxicity testing. Available information must often be pooled from a variety of disparate sources to efficiently link an outcome and/or hazard classification to a set of assay responses. To address the need for a centralized data access point, NICEATM has developed a set of web resources to retrieve curated data, spur hypothesis generation through data exploration via interactive graphics, and download open source workflows and models to use with local data. In this presentation, we will describe the processes of obtaining data from the data portal, combining downloaded and local data sets, and executing downloaded NICEATM workflows to make predictions on potential hazards based on available data. In this example, we combined *in vitro* assay data and pharmacokinetic (PK) modeling to estimate *in vivo* exposure at which an adverse outcome might occur (i.e., *in vitro* to *in vivo* extrapolation). Using data from EPA's ToxCast estrogen receptor pathway assays, we show how our PK workflow applies population PK models to estimate the daily equivalent administered dose (EAD) that would result in a steady state blood concentration equivalent to the lowest effective *in vitro* concentration of a given assay. The estimated EAD is compared to the lowest effective levels from curated *in vivo* uterotrophic assay data obtained from the NICEATM database. Together, these web resources provide an open source solution to create integrated approaches that combine available data and information to computationally predict potential hazards and/or estimate risk. Technical support was provided by ILS and Sciome under NIEHS contract HHSN273201500010C

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EPA's Exposure Forecasting (ExpoCast) Project

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High-throughput screening (HTS) programs such as ToxCast and Tox21 have profiled the potential hazard for thousands of chemicals. These chemicals are tested in part because most have limited or no data on hazard, exposure, or toxicokinetics (TK). The US EPA's Exposure Forecasting (ExpoCast) project aims to provide rapid, provisional exposure predictions and TK for these thousands of chemicals. ExpoCast models reduce uncertainties associated with HTS prioritization based on putative hazard to HTS by inclusion of exposure and TK. To provide rapid exposure information, the EPA is developing mathematical models, organizing and analyzing extant data, and using new tools such as screening-mode mass spectrometry (MS) to collect new data on chemical properties, use, and occurrence. The pilot phase of the ExpoCast data collection has focused on four activities: 1) high throughput physicochemical property measurements, 2) new biomonitoring data, 3) chemical emissivity data for articles of commerce, and 4) chemical deformulation of consumer products and articles of commerce. To address TK, we have been using steady-state and physiologically-based TK (PBTK) models that can be parameterized with chemical-specific *in vitro* measurements and physico-chemical properties predicted from chemical structure. These PBTK models can further be tailored with physiological parameters to emulate specific human populations. We simulate population physiological parameters based on data from the most recent NHANES, which describe distributions of demographic and anthropometric quantities in the modern U.S. population. This approach estimates margins between predicted bioactive doses and predicted exposures for the most sensitive portion of the population. Ultimately, we work to identify the chemicals for which these new tools may be used with confidence, and to identify those chemicals where alternative approaches are needed. We have statistically evaluated our tools to identify chemical classes for which our methods and models perform reasonably and those where predictive ability is poor. The health risks posed by the chemicals in our environment depends on both chemical hazard and exposure – we are working to provide defensible high throughput forecasts for exposure and TK. *This abstract does not necessarily reflect US EPA policy.*

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Application of the carcinogenic mode of action/human relevance framework to agrochemical compounds

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The mode of action involves the functional or pathological changes that result in the adverse effects of exposure of a living organism. As a result of the revised USEPA cancer guidelines in 1996 a more transparent and scientifically based approach to linking rodent cancer results from chemical treatment to human risk. This mode of action framework provides a weight-of-evidence approach based on considerations for causality in experimental studies. The criteria to judge a mode of action hypothesis involves consideration of dose response and temporal concordance, consistency, specificity, biological plausibility, and coherence. Since most rodent carcinogens involve the induction of liver neoplasms. An approach has been constructed for rodent hepatic carcinogen mode of action. These include genotoxic and nongenotoxic mode of action. The genotoxic mode of action involves the interaction of the chemical or its metabolite with nuclear DNA resulting mutation and eventual neoplasm formation. Nongenotoxic liver mode of action are further subdivided in to nuclear receptor medicated or non-receptor mediated processes. However common key events of nongenotoxic modes of action involve the induction of hepatocyte proliferation with a subsequent expansion of preneoplastic hepatocytes to neoplasia. Most agrochemicals that have been demonstrated to induce rodent hepatic tumors function through nongenotoxic modes of action. Most rodent carcinogenic chlorinated insecticides have been demonstrated to function through a CAR receptor mediated mode of action. Similarly herbicides that induce rodent liver tumors function through PPARalpha and cytotoxic mode of action. While the genotoxic mode of action is linked to potential human risk since the damage of DNA and resulting mutations are applicable to human cancer induction. In contrast most of the hepatic nongenotoxic modes of action seen in rodents are not likely to be applicable to human risk.

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Mouse liver tumor mode of action via CAR activation in mice, and ability to achieve a weight of evidence assessment with *in vitro* methods: A case study with the triazole fungicide cyproconazole

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Cyproconazole, a triazole fungicide used globally as a crop protection chemical, caused a higher incidence of liver tumors in CD-1 male mice at dietary dose levels of 100 and 200 ppm, when compared to the control group. The mode of action (MOA) for cyproconazole-induced liver tumors has been studied extensively via regulatory submitted MOA studies (US EPA, 2007) and in the literature (Peffer et al., 2007; Tamura et al., 2013; 2015). These mechanistic studies demonstrate that the short-term key events leading to liver tumors are mediated by activation of the constitutive androstane receptor (CAR), leading to altered gene expression, Cyp2b and Cyp3a induction and increased cell proliferation. After long-term exposures, the mitogenic stimulation leads to altered foci and an increase in hepatocellular adenomas and carcinomas at the tumorigenic dose levels (≥100 ppm), whereas lower dose levels up to 15 ppm showed none of these changes. In CAR knockout mice, the short-term effects and the eventual development of altered foci and/or tumors in an initiation-promotion model were essentially blocked at dose levels up to 200 ppm, demonstrating that CAR activation is a critical key event in mice. To help advance the progress in reducing animal usage and demonstrating the utility of 21st century toxicology methods, cyproconazole data from *in vitro* methodologies are compared to the outcomes of *in vivo* studies for this data-rich molecule. In summary, comparisons in this presentation show that the early key events in this MOA as well as the ability to exclude many alternative MOAs can be demonstrated based on *in vitro* technologies. While the late key events (e.g. altered foci and eventually tumors) cannot be replicated *in vitro*, assessment of those endpoints plus other supporting data for a particular MOA can be part of standard guideline carcinogenicity studies, either retrospectively or prospectively, if samples are obtained that facilitate these mechanistic analyses. This case study illustrates how high throughput assays, *in vitro* data and further analyses of existing samples obtained *in vivo* can help to prove a well-documented MOA in rodents without the need for extensive additional animal testing.

AGRO 350

Application of toxicokinetics in regulatory-mandated toxicity testing of plant protection products (PPPs): From concept to application

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Guideline toxicology testing programs are designed to characterize toxicity of PPPs under various conditions (e.g., dose, duration, genders, routes) in multiple animal species to understand potential harmful effects to humans, if any, prior to their public use. Correlation of toxicity to systemic rather than nominal (administered) doses is the most meaningful assessment of toxicity. During the course of toxicological studies, rate, extent and duration of systemic exposure are best interpreted by the use the absorbed (*e.g.,* AUC, C_{max}) rather than administered dose. Generating systemic-dose data facilitates understanding toxic mode-of-action; avoiding additional studies defining human relevance of toxicity observed in animal studies. In recent years toxicologists have pushed for more relevant toxicity testing strategies for PPPs, increasing relevance to human exposure and risk assessment. For example, by selecting high doses based on non-proportionality of the systemic dose, or kinetically-derived maximum dose (KMD), rather than traditional maximum-tolerated dose (MTD). In 2001, ILSI/HESI-ACSA proposed a tiered approach to toxicity testing of PPPs with emphasis on determining systemic dose of parent and/or metabolite(s) in study animals. Saghir et al. published results of their proof-of-concept study in 2006, proposing collection of ≤3 blood samples from core study animals to determine diurnal systemic dose (AUC_{24h}) at steady-state. Their approach has successfully been used in the toxicity testing program of a number of PPPs. This presentation will touch on the improvements made to the published approach in the last few years, including the impact of age-related changes on the systemic dose and late-occurring toxicities. Additionally, differences between dose-linearity and dose-proportionality will also be discussed.

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Cheminformatics approaches to inhalation toxicity

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This seminar will review the state of the science for *in silico* approaches to predict mammalian toxicity via the inhalation

route. An overview of our work will be provided with an emphasis on current approaches for read-across as applied to acute lethality. This will include (1) development of an acute toxicity database that includes data from multiple routes and species, (2) models that simulate absorption to predict systemic bioavailability, (3) approaches to distinguish and bin point-of-contact driven effects versus those due to specific enzyme/receptor-type mechanisms, and (4) potential use *in silico* tools including the OECD Toolbox to profile compounds for their reactivity.

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Use of toxicokinetics to improve the current extended one-generation reproductive toxicity (EOGRT) study design

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An important aspect of safety assessment of chemicals (industrial chemicals, agro-chemicals and pharmaceuticals) is determining their potential developmental and reproductive toxicity (DART). A number of guidelines have outlined a series of separate DART studies, from fertilization through adulthood and in some cases to second generation. The EOGRT study is the most recent and comprehensive guideline in this series. EOGRT study design makes toxicity testing progressive, comprehensive, and efficient by assessing key endpoints across multiple life-stages at relevant doses using minimum number of animals, combining studies/evaluations and proposing tiered testing approaches based on outcomes. EOGRT study determines toxicity during preconception, development of embryo/fetus and newborn, adolescence, and adults with specific emphasis on the nervous, immunological, and endocrine systems. EOGRT study also assesses maternal and paternal toxicity. However, EOGRT study guideline is complex, criteria for selecting doses is unclear, and guidance for monitoring systemic dose during the course of the study for better interpretation and human relevance is vague. This presentation will discuss potential simplification of EOGRT study and suggest procedures for relevant dose selection and monitoring systemic dose at multiple life-stages for better interpretation of the generated data and human relevance.

AGRO 353

Sedaxane: Use of nuclear receptor transactivation assays, high content imaging and toxicokinetics as part of a mode of action framework for rodent liver tumors

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Sedaxane, a succinate dehydrogenase inhibitor (SDHI) fungicide, produced increased incidences of liver tumors in male CD-1 mice (7000 ppm) and male Wistar rats (3600 ppm) following chronic exposure. Experimental data demonstrate a mode of action (MOA) for sedaxane-induced liver tumors that is initiated by CAR and/or PXR activation, which triggers key events of altered expression of pro-proliferative and anti-apoptotic genes and an early transient increase in hepatocellular proliferation. After long-term exposure, the mitogenic stimulus results in an increase in hepatocellular foci and eventually an increase in hepatocellular adenomas and carcinomas. Associative events in this MOA include increased expression of CAR/PXR responsive genes coding for Cyp2b and Cyp3a isoforms, increased hepatocellular hypertrophy and increased liver weights. Measurement of plasma concentrations of sedaxane at steady state showed that very fast clearance of sedaxane in mice caused very low levels of parent compound, whereas in rats sedaxane and its demethylated metabolite were the major circulating moieties. This kinetic difference produced much lower sensitivity of mice to the liver effects and general toxicity of dietary sedaxane. Other novel approaches in the mechanistic work with sedaxane included: 1) testing in CAR and PXR transactivation assays for mouse, rat and human; 2) pathway analysis of whole mouse genomic microarrays vs. model CAR activators; and 3) comparative results of liver proliferation index by BrdU labeling vs. zonal Ki67 labeling plus high content imaging. Based on comparative techniques in this case study, a move toward use of *in vitro* methods to demonstrate a well-understood liver MOA (e.g., CAR) is envisioned for future compounds.

AGRO 354

Mode of action framework: Bridging the gap between animal and human data

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Over the past decade, the human health risk assessment paradigm has been rapidly changing. Risk assessments have shifted from a default assumption-based approach to one that is evidence-based and data-derived. During the course of this evolutionary change, regulatory risk assessors are not only asked to identify the toxic endpoints, but also its mode of action, how the chemical substance elicits its overall toxicity at various biological levels of organization.

The IPCS *Mode of Action Framework* contextualizes how disease incidence may occur from chemical exposure by integrating biological mechanistic data and empirical information, along a cascade of key events. Collectively, these data inform the selection of a point of departure, a precursor key event that would be protective of adverse effects. The MOA framework and the Tox21 advancements have revolutionized animal toxicity data. Available tools to measure early key events include new *in silico*, *in vitro* and high throughput assays, but methods to measure later key events remain largely unchanged. Traditional whole animal apical endpoints and observational human epidemiology data are used to inform the adverse outcome at the individual organism and population levels. While animal experimental data are supplemented with the advancements of Tox21 tools in an integrated weight of evidence consideration, human epidemiology data have continued to provide a single “stand alone” observational dataset. This disparity gap between the advancements of animal toxicology and traditional human epidemiology data must be bridged before advancements in risk assessments can become fully realized.

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Pesticides in California’s wastewater - Science needs

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California Department of Pesticide Regulation (DPR) has initiated efforts to better understand pesticide sources to wastewater catchments to allow for a science-driven approach to develop potential mitigation strategies. The California state regulatory framework to address the occurrence of pesticides in surface water is driven by distinct mandates and authorities of two state entities: DPR and the State Water Resources Control Board (SWRCB). The SWRCB has begun to develop, and in some cases implement, discharge limits for pyrethroids in wastewater effluent. Concentrations of pyrethroids and fipronil in wastewater effluent have been reported at levels that exceed USEPA Aquatic Benchmarks, posing a potential risk to the aquatic organisms in surface waters receiving wastewater discharge. Although there are limited data on treatment efficacy, available data suggest pyrethroids and fipronil persist at toxicologically relevant concentrations despite wastewater treatment technology used. Published conceptual models have suggested pesticide use patterns that could result in down-the-drain transport; however, there have not been studies conducted to quantify the relative contribution of potential sources. A study has been completed that quantifies the portion of fipronil-containing spot-on treatments that wash off dogs during routine bathing. DPR has also initiated a wastewater source identification study to directly measure the relative contribution of pesticides (pyrethroids, fipronil, imidacloprid, and others) from residential, commercial, institutional, and industrial sources within a sewershed. Study results may be used to establish a larger monitoring framework to evaluate trends of pesticide use patterns with down-the-drain potential, and to evaluate efficacy of possible voluntary mitigation efforts, pesticide product registration changes, or other regulatory requirements. As a part of product registration, DPR is in the process of developing a down-the-drain model that will allow DPR scientists to better evaluate the transport potential of pesticides into a wastewater catchment, and take decisive registration actions (e.g., denial of registration) on products that that could ultimately pose a threat to surface waters receiving wastewater discharge.

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Modeling the fate of down-the-drain chemicals at large geographic scales

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Many potentially-harmful chemicals arising from human activities are continuously introduced into waterways and can pose significant risks to human health and/or ecosystems. To best manage such compounds and to mitigate any risks associated with their introduction into waterways, we need to better understand their origin, how they are propagated, and the nature and magnitude of the risks they pose. Unfortunately, at present, data is lacking on many emerging down-the-drain chemicals with respect to their rates of release and subsequent environmental presence. In the absence of such data, regulators and other stakeholders in jurisdictions around the world require realistic and reliable exposure models to readily predict environmental concentrations. To address this need, a first-of-its-kind contaminant fate model was developed to support both local-scale decision making and impact assessments at regional or continental scales. The model is particularly suited for assessing substances for which measurements in the environment are unavailable, sparse or unreliable and for substances for which robust monitoring techniques have not yet been developed. The model accounts for the flows and concentrations of contaminants introduced into surface waters from point sources, such as urban areas served by wastewater treatment plants, as well as from non-point sources based on population densities. The transport of pollutants is modelled along the river network, accounting for depletion processes as well as dilution experienced in rivers and lakes, to provide estimates of predicted environmental concentrations of contaminants in the river network and lakes. The model is designed to work with existing hydrographic and hydrologic data at very high spatial resolution (500 m pixel size) and is implemented within a standard Geographic Information System (GIS) to facilitate easy application to regions with limited resources and data availability. The feasibility of the model was demonstrated by assessing the impact of selected pharmaceuticals to all river reaches and lakes located in province-wide regions of Canada and the release of anthropogenic steroidal estrogens to waterways of mainland China. Efforts are currently underway to expand the approach in order to develop a truly global-scale contaminant fate model for down-the-drain chemicals.

AGRO 357

Modeling the sustainability of using treated water containing active pharmaceutical ingredients for reuse in irrigation applications

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The use of treated water for irrigation is gaining increased recognition as a sustainable option to conserve water supplies that are stressed or diminishing. Interest has also increased in using treated irrigation water to achieve zero discharge. Active pharmaceutical ingredient (API) interactions in irrigated soil–plant systems are complex and literature indicates that APIs in irrigation water may persist in soil for several months after the irrigation is stopped for the season. In this paper, we demonstrate a tool to simulate the fate and transport of treated irrigation water containing APIs in order to optimize sustainable water reuse. The tool estimates API residues in soil, runoff, and leachate on a daily time step over multiple years following single or repeated applications of treated water when applied to turf. An optimization routine solves for optimal irrigation volumes that are unlikely to result in exceedances of user defined API thresholds in soil, groundwater, and runoff. Modeled estimates are compared to measured API concentrations in soil and groundwater obtained from the open literature, including field studies of reclaimed municipal waste-water irrigated turf fields. Close agreement was observed between modeled and measured results, validating the utility of the tool as a valid approach to optimize sustainable reuse of treated water for irrigation purposes.

AGRO 358

Ecological exposure assessment approaches for indoor use pyrethroids in POTW effluent

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Indoor use pesticides are regulated for potential environmental impacts after movement down the drain and eventual release from publicly owned treatment works (POTWs). Pyrethroids are commonly used within the home for applications such as general household insect control (including ants, cockroaches, fleas and bedbugs) by both professional and homeowner application, for houseplant protection, as an insect repellent on clothing, and for the maintenance of pet health. Depending on the application type, some portion of the pyrethroid may be disposed of down the drain via cleaning of hard surfaces after application, washing of clothing or bedding after treatment, and bathing pets indoors. As pyrethroids traverse the sewer system and eventually are released from wastewater treatment plants, a large fraction is removed from the effluent water due to the extremely high hydrophobicity of pyrethroids and the prevalence of organic material to which it can adsorb. However, some pyrethroid residues do pass through to the POTW effluent along with dissolved organic matter and these are mixed into river flow. It is at this point (as well as for downstream river reaches), that an assessment of the potential ecological effects of the bioavailable fraction of pyrethroids must be conducted. Using results for several pyrethroids, this presentation will discuss the relevant aspects that should be considered in an ecological risk assessment. In addition, a tiered approach for conducting an exposure assessment using models at various spatial scales will be presented along with an evaluation of the potential impact of various sources of uncertainty in these assessments. Comprehensive pyrethroid POTW monitoring data are available to help provide context for refined model output.

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Degradation of pyrithiobac sodium in soil and sediments

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Degradation of pyrithiobac sodium [PE350] was investigated in a number of soils and sediments using ^{14}C-PE350. It degrades primarily via microbial degradations which lead to the separation of the two rings of the molecule. This investigation has led to identification of several metabolites which were previously not understood. In addition, non-extractable residues and ^{14}CO2 accounted for a significant portion of the degraded residues. We have attempted to characterize these non-extractable residues via application of kinetics modelling tools. Although such modelling tools are routinely used to clarify degradation pathways, we describe use of such tools in shedding light on the origin of non-extractable residues. Our work demonstrated that overall degradation in soils and sediments proceeded via similar pathways. Kinetics modelling was used to clarify all degradation pathways including the formation of bound residues and CO_2

AGRO 360

Integrating advances in environmental fate and exposure into regulatory frameworks: Learning from RISK21

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Environmental risk assessments are often based on laboratory studies conducted according to regulatory guidelines. These studies determine the significance of each environmental fate process by studying it in isolation. Mathematical models are then used to reintegrate the contribution of each process to predict environmental concentrations. This can result in highly conservative outcomes as the contribution of different degradation processes acting together is not considered. Furthermore it is questionable whether the degradation products identified in such studies are an accurate representation of those formed under use conditions where multiple degradation and processes take place simultaneously. Terrestrial field dissipation (TFD) studies can provide useful data to refine risk assessments; however, these data are rarely used for this purpose or to do so require designs that isolate a single degradation process in the same way as laboratory studies.

This presentation will outline a number of approaches that can be used to provide realistic data on the rate and route of degradation of compounds under close to use conditions. By integrating the key environmental fate processes under a range of agro-climatic conditions these studies provide data that can reduce the uncertainty in risk assessments. To support and facilitate the use of such studies for risk assessment a mechanism is required by which stakeholders can agree in advance the key questions to be addressed and the study designs required to do so. A collaborative framework that combines elements of RISK21 and the EPA conceptual model approach will be outlined to achieve this.

AGRO 361

Are additional solvent extractions in soil/sediment laboratory studies really necessary?

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In September 2014, the Environmental Fate and Effects Division (EFED) of the United States Environmental Protection Agency published guidance for addressing unextracted residues in laboratory studies involving soils or sediments. The Agency promulgated an approach aimed at removing weakly sorbed residues using solvents of different polarities since laboratory biodegradation or photolysis studies often use one set of extraction solvents, generally polar solvents such as acetonitrile, methanol, water, and an acid such as hydrochloric acid. EFED indicated in their guidance document that if a substantial component of the applied test substance remained in the soil or sediment following extraction (e.g., 10% or more), additional extractions with non-polar and less polar solvents should be used in addition to the highly polar solvents. For instance, these solvents should belong to three different dielectric constant groups, namely, non-polar solvents (e.g., hexane, chloroform and others with dielectric constants from 1.9 to 4.8), less polar solvents (e.g., dichloromethane, ethyl acetate and others with dielectric constants from 6.0 to 9.1) and more polar solvents (e.g., methanol, acetone, acetonitrile, water, formic acid and others with dielectric constants from 18 to 80). As a result of this guidance, we conducted a number of laboratory studies (aerobic soil and aerobic and anaerobic aquatic metabolism) with pesticides and pharmaceuticals incorporating these additional solvent extractions. We found that if the initial extraction procedure involved polar solvent components such as acetonitrile, water, and hydrochloric acid, the additional non-polar and less polar solvents did not remove appreciable soil/sediment residues (i.e., < 5% of the dose and often much less). We will discuss these results in detail and offer an explanation as to why the additional extractions did not remove much more residue than the initial extractions using more polar solvents.

AGRO 362

Aerobic mineralization in surface water: Study design, challenges and regulatory issues

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An aerobic mineralization study in surface water according to OECD 309 is a new guideline requirement for registration of agrochemicals in European Union under the new regulation 1107/2009/EC, Annex Part A, Point 7.2.2.2 (March 01, 2013). The guideline permits several options within study design for conducting this study, especially the option to conduct the experiments either in darkness or under diffuse light, and the option to use surface water only (pelagic test) or surface water amended with small amounts of suspended solids/sediments (suspended sediment test). The study is conducted at two very low concentrations in order to obtain biodegradation kinetics that reflect those expected under realistic environmental conditions. However, it is not clear how the results of this study will be interpreted and whether DT_{50} values of the test substance and its metabolites obtained from the test will be used as input parameters for running surface water models in order to facilitate exposure/risk assessments. Also, the issues concerning using of kinetic

results obtained from two studies-- aerobic mineralization in surface water and transformation in aquatic sediment systems for surface water modelling will be explored from modelling and regulatory perspectives. This session aims to present and analyze the overall study design and study requirements with regulatory acceptance criteria. The presentation will also include the results based on recently conducted studies as examples and experience with regulatory agencies. In addition, the challenges concerning analysis of aqueous test samples at low concentrations, reliable quantification and identification of polar metabolites will be examined.

AGRO 363

Determination of substance specific Plant Uptake Factor (PUF) for use in regulatory fate modeling

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Environmental fate leaching models, such as PEARL and PELMO, incorporate a plant uptake factor (PUF) as a measure of the ability of the plant to absorb chemicals from the soil. When taken up into the plant, the absorbed material is removed from the soil and no longer available for leaching into ground. A default plant uptake factor of 0 is used in the models due to the absence of an experimentally determined value. To refine these models, a study design was created in order to experimentally determine the plant uptake factor. To validate the new study design, ten laboratories conducted round robin testing of the plant uptake factor using 1,2,4-triazole in wheat plants by means of a hydroponic system as a test case. The data generated will be shared in order to aid in the creation of a guidance document for generating experimental plant uptake factor values as input for leaching models as uses in the EU regulatory process. The oral presentation will detail the proposed study design and the results obtained from the round robin assessment.

AGRO 364

Transformation of organic chemicals in environmental fate metabolism studies: A comparison between aquatic sediment (OECD 308) and surface water test systems (OECD 309: simulation biodegradation test)

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Among a broad range of laboratory environmental fate test methods, higher-tier assessments include metabolism studies in aquatic sediment systems under aerobic and anaerobic incubation conditions (OECD 308) and aerobic degradation/metabolism studies in natural surface water both with and without suspended sediment (OECD 309). In the natural environment, during and after application of crop protection agents, drift to water bodies with (suspended) sediment present is most prevalent. Hence, a possible influence of sediment on the transformation of organic chemicals in environmental fate metabolism OECD 308 and OECD 309 studies has been investigated, which involves evaluating the degradation results of a significant amount of crop protection compounds being subjected to both study types. One objective has been the screening of test compound patterns for the potential formation of degradation products in significant amounts in OECD 309 test systems, while not being formed in OECD 308 test systems, in which sediment is present. The second purpose has been to examine the influence of sediment on the test compounds degradation velocity. The results provide an insight into the effect of sediment on the degradation pattern and velocity of plant protection products in water/sediment when compared to water only test systems.

AGRO 365

FDA pesticide monitoring program

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The goal of FDA’s pesticide monitoring program is to carry out selective monitoring to protect public health. FDA uses a three-fold approach to monitor pesticides in foods: Regulatory Monitoring, Focused Sampling, and Total Diet Study (TDS). Under Regulatory Monitoring, FDA samples individual lots of domestically produced and imported foods and analyzes them for pesticide residues to enforce the tolerances established by EPA. As for Focused Sampling, it is generally used to follow-up on suspected problem areas or to acquire residue data on select commodities that are not usually covered during regulatory monitoring. Focused sampling is carried out by short-term field assignments. TDS is distinct from Regulatory Monitoring and Focused Sampling because it determines pesticide residues not in the raw commodity, but in foods that are prepared table-ready for consumption. Data from TDS are used to calculate exposures to the pesticides analyzed in the Regulatory Monitoring program. In addition to monitoring foods for human consumption, FDA also samples and analyzes domestic and imported animal foods for pesticide residues. The monitoring focuses on foods for livestock and poultry animals that ultimately become or produce foods for human consumption. Key findings from the fiscal year 2013 pesticide monitoring program will be discussed.

AGRO 366

Digest of dietary exposure methodologies in support of global MRLs

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Discussion of the causes of globally disharmonized Maximum Residue Limits (MRLs) often focus on crop residue trials, crop groupings or statistical calculation procedures. Less attention is typically paid to the variations of approach by regulatory bodies across the globe in regard to the dietary exposure assessments which support an MRL.

This talk will compare and contrast assumptions, input and regulatory complexity of several key dietary models in use around the world, including the publically-available models of EU PRIMo, JMPR IESTI/WHO GEMS diets and US DEEM-FCID. An understanding of purposes and limitations behind the common dietary models used can inform stakeholders regarding opportunities for better data collection or input. Use of dietary monitoring data can be used to assess real world exposures.

AGRO 367

Acute risk assessment trends in EU: a case of compounded conservatism

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Dietary risk assessment models used by authorities and international bodies such as JMPR to protect consumers from the potential presence of trace pesticide residues in their food have the difficult challenge of safeguarding diverse populations while still representing realistic exposure scenarios. Many risk assessment models support a tiered approach which starts with a very conservative initial estimate of exposure, followed by more realistic refinement, if necessary. The EU, based on recommendations made at an EFSA scientific workshop, is considering changes to their acute risk dietary model which will significantly increase the conservatism of the exposure estimates to unrealistic levels while precluding meaningful refinement. This application of the precautionary principle will ultimately result in the loss of MRLs and have a negative societal impact on the food supply. This presentation will examine the compounded conservatism of the current and EFSA-proposed changes to the international estimated short-term intake (IESTI) equations from a mathematical perspective. Alternative modifications to the equations will be explored which could provide a more reasonable level of conservatism that is protective for consumers, but represents a more realistic exposure scenario.

AGRO 368

Comparing Pesticide Data Program (PDP) and registrant-generated residue data

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An important component of human health risk assessments for pesticides is an estimation of residue concentrations in raw agricultural commodities (RACs). The US Environmental Protection Agency (EPA) requires registrants to generate residue data from field trials for food commodities for registration of pesticides. The required regulatory field trials are designed to reflect the use profile from the product label, i.e., maximum application rates, maximum number of applications and minimum pre-harvest intervals (PHIs). Therefore, the information generated under these conditions represent the high-end of residue concentrations expected in RACs and are used by the EPA to set legal residue limits (“tolerances”) on the amount of pesticides in or on foods. The residue data generated from regulatory field trials are used in human safety assessments, which are mandated by the Food Quality Protection Act (FQPA) of 1996. To obtain a realistic view on exposure to pesticides, the USDA Pesticide Data Program (PDP) monitors pesticide residue levels found in food grown in regions across the United States. This national program collects residue data on agricultural commodities in the food supply based on a rigorous statistical design to ensure that sample collections provide reliable estimates of consumer level pesticide residues in the US food supply.
The objective of this work is to test the hypothesis that residue levels in RACs from regulatory field study will be significantly higher than the PDP for a set of selected pesticides/RAC combinations. We used parametric and non-parametric statistical approaches conduct the comparison analysis. For these selected pesticide/food combinations, summary statistics of the left-censored data set (mean, median, quartiles) were calculated and compared with summary statistics of corresponding field trial data sets. The results show that the mean and median PDP residue levels of the examined pesticide/food combinations are 7X-70X and 4X-400X lower, respectively, than the corresponding statistic of field trial residues. This work supports the hypothesis and furthermore supports the notion that the likelihood of exceeding legal residue limits at the consumer level is very small and regulatory assessments based on field trial residues are conservative in nature.

AGRO 369

Harmonizing pesticide assessments to allow for free and open trade

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Why are nations not using risk assessments from other countries? What are the barriers to using other national reviews and assessments? How can using other national assessments become an acceptable practice in the global world today? What should registrants, growers, and regulators do to encourage mutual acceptance of risk assessments?

AGRO 370

Investigating the impact of exposure to pesticide mixtures on the metabolomic profile of amphibians

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Understanding the consequences of agricultural use pesticides on non-target species is necessary to assess any ecological risk these compounds pose. Pesticides are often applied as mixtures to better control or eradicate agricultural nuisances. In amphibians, a non-target species to pesticide applications, absorption of these compounds occur readily through the dermis. Previous work in our laboratory has begun to elucidate the uptake of pesticides as well as their hepatic metabolism in both terrestrial and aquatic amphibian species. The purpose of the current study was to investigate the impact of pesticides, individually or in combination, on the biochemical profiles of amphibians. Amphibians were exposed to pesticide contaminated soil or water for 8 and/or 24 hrs. Livers were excised for biomarker analysis and body burdens were determined using mass spectrometry. Body burdens were often higher following exposure to pesticide mixtures when compared to those observed in single compound exposures suggesting modulation of the metabolic capacity in amphibians. Metabolomic profiling of livers support this, and pesticide mixtures appear to synergistically influence biochemical perturbations in the current study. Loadings plots coupled with t-test filtered chromatograms were used to identify metabolites of interest. Correlations of the significant changes in these metabolites will aid in identifying potential biomarkers associated with exposure to agricultural use pesticides in these and other non-target species.

AGRO 371

Integration of metabolomics and other OMICS approaches to elucidate cytotoxicity of agrochemicals: 2,4-D case study

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The pro-oxidant 2,4-dichlorophenoxyacetic acid (2,4-D) is the most widely used herbicide in the world. Mechanistically, 2,4-D is a synthetic auxin mimic that kills dicots without affecting monocots. While, the function of 2,4-D in agriculture is relatively well defined; its impact on human and other living species as well as on overall ecosystem is still questionable due to differing interpretations of epidemiological data and the inability to irrefutably link exposure to specific disease or cellular toxicity. Therefore, it is essential to gain a better understanding of the physiological and molecular alterations induced by exposure to 2,4-D. The present study using *S. cerevisiae* (grown to stationary phase on OXPHOS-dependent carbon sources to closely represent post-mitotic aerobically-dependent cellular processes) and a cohort of “OMICS” technologies correlated to the physiological data revealed unusual, autophagy-mediated cell toxicity and death. An LC_{50} dose at six hours post exposure was determined by assessing membrane integrity and housekeeping protein activity. Both global and targeted metabolomics approaches were used to assess overall changes in metabolism and activity of specific metabolic pathways. Results showed primary impact on the energy and lipid metabolism as well as on the redox homeostasis and ROS formation indicating mitochondrial dysfunction. To further determine the nature of these changes, damage to the proteins through irreversible oxidative modifications known to alternate the protein activities were investigated using 2D DIGE and hydrazide derivatized fluorophores. Results demonstrated 2,4-D-induced oxidation to the proteins involved in the electron transfer, redox homeostasis and ubiquinone biosynthesis. Cell death mechanism was assessed using Annexin V-FITC for PS externalization, and null mutants. Interestingly, mutants lacking Yca1, Nuc1, or AIF apoptotic genes showed no significant difference in 2,4-D-induced cell death compared to the wild type, suggesting limited role of the apoptosis. On the other hand, evaluation of mutants lacking autophagic proteins, Atg8 and Atg12, displayed increased sensitivity and decreased sensitivity to 2,4-D exposure, respectively, suggesting a dual role for autophagy as cytoprotective and cytotoxic upon 2,4-D exposure. Overall, these data indicate that 2,4-D causes majority of the cell death through mitochondrial dysfunction, ROS generation and autophagy mediated pathways.

AGRO 372

Metabolism of the xenobiotic compound benzotriazole in *Arabidopsis* plants

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Benzotriazoles (BTs) are xenobiotic contaminants of emerging concern. Indeed, BT has been measured in recycled water sources that could be used for crop irrigation, even following reverse osmosis treatment. In this work, we investigated the uptake and metabolism of BT in the model plant *Arabidopsis*. Assimilation and metabolism of BT was rapid, repeatable, and nearly complete in a hydroponic system. Using LC-QTOF-MS untargeted metabolomics, we discovered two main types of novel transformation products: glycosylated BT conjugates and conjugation with amino acids via incorporation into the tryptophan biosynthesis pathway. The former are presumed detoxification products, whereas the latter form structural analogues to tryptophan and auxins (important plant growth hormones). Because of the structural similarity of BT plant transformation products to some phytohormones, we conducted exposure bioassays with synthetic BT-metabolites. BT-metabolites exhibit a weak but significant auxin mimic effect on plants, consistent with many synthetic auxins. Glycosylated BT generated *in planta* was subsequently excreted from the plant tissue, a phenomenon not previously observed. We synthesized four major metabolites and were able to close >60% of the mass balance on a BT model system. We also conducted a proteomics investigation to target BT incorporation into plant proteins; examination of >10,000 peptides revealed no significant incorporation. In a field investigation, we were able to measure BT and novel BT metabolites in crop tissues. Overall, this work helps to reveal insights into the plant metabolism of an emerging water contaminant with implications to phytohormone functioning in floral agro-ecosystems.

AGRO 373

GC-TOF-MS based root exudates metabolomics revealed defense mechanism of cucumber plant to nano-Cu

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Copper-based nano pesticides are increasingly used in modern agriculture. It is important to understand their environmental implication, especially the impact on edible plants. In addition, plants have various tolerance and defense mechanisms under biotic and abiotic stress. Root exudates, as an important but hidden part of plant defense, has been underestimated and less studied. In this study, three-week-old cucumber plants were expose to 10 and 20 mg/L nano-Cu for one week. The leaf tissue and root exudates were collected for non-target metabolomics study by GC-TOF-MS at harvest. Metabolomics and partial least-squares discriminant analysis (PLS-DA) revealed that the metabolite profile in cucumber leaves and root exudates was distinctively altered due to exposure to nano-Cu. This indicates that nano-Cu triggered significant metabolic changes in cucumber leaves and root exudates. In root exudates, eleven amino acids, including Alanine (Ala), beta alanine (β-Ala), Glycine (Gly), Isoline (Ile), Leucine (Leu), Lysine (Lys), Phenylalanine (Phe), Proline (Pro), Serine (Ser), Threonine (Thr), and Valine (Val), were apparently up-regulated in response to nano-Cu in a dose-dependent way. It is also shown that citric acid in root exudates was significantly down-regulated. The up-regulated amino acids and down-regulated citric acid are possibly an active defense mechanism against excess nano-Cu. Here, we demonstrate that non-targeted GC-TOF-MS based metabolomics is a powerful tool to detect plant response to nanoparticles at the molecular level. In addition, the root exudates metabolites study could supply important information of plant defense mechanisms.

AGRO 374

Dual- and single-retention behaviors of solutes in linear programmed temperature gas chromatography

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The current theory of programmed temperature gas chromatography (PTGC) considers that solutes are cold trapped at the column head completely and does not explicitly recognize the different effects of initial temperature (T_o) and heating rate (r_T) on the retention time or temperature of a homologue series. In the present study, *n*-alkanes, 1-alkenes, 1-alkyl alcohols, alkyl benzenes and fatty acid methyl esters standards were used as model chemicals and were separated on two non-polar columns, one middle polar column and one polar column. Retention behaviors of the un-cold-trapped and cold-trapped solutes can be explicitly described with exponential quadratic equation and cubic equation models, respectively. When the solutes were in the un-cold-trapped status, the dual retention behaviors of solutes were observed. Dual retention behaviors are complicated, sensitive to temperature changes from both T_o and r_T values and cannot be described by any explicit expression. When the solutes were in the cold-trapped status, the single-retention behavior of solutes was observed. It is simple, dependent upon r_T only and can be well described by the cubic equation model which was visualized through four sequential slope analyses. These observed dual- and single-retention behaviors of solutes were validated by various experimental data, physical properties and computational simulation. This study presented a broad framework of retention behaviors of solutes under PTGC conditions and supplemented the current PTGC theory and practices. Reproducibility of retention data such as retention indices in PTGC can be significantly improved, when it is operated at a single-retention behavior region.

AGFD DIVISION

AGFD 158

Pterostilbene in blueberries and PPARα activation

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The discovery of pterostilbene in blueberries and that it acts a ligand for the peroxisome proliferator-activated receptor alpha-isoform (PPARα) caused resurgence in interest on this compound. In our initial study, we demonstrated that pterostilbene produced greater induction of PPARα than ciprofibrate, a known hypolipidemic drug, at 100 μM in H4IIEC3 cells. We have further demonstrated that pterostilbene significantly and dose-dependently (at 10, 20 and 50 μmol) increased PPARα gene expression in H4IIEC3 cells, and the effect at 10 μmol was greater than 100 and 200 μmol of fenofibrate. In a later study using age-accelerated mouse model, pterostilbene incorporated in the diet (120mg/kg of diet) was shown to positively modulate markers of cellular stress, inflammation, and Alzheimer's disease pathology, and were associated with up-regulation of PPARα alpha expression in the hippocampus. In yet another feeding study, Syrian Golden hamsters fed a diet fortified with blueberry peel ethanol extract (2% of diet), exhibited up-regulation of hepatic PPARα mRNA expression along with deceases in plasma LDL and total cholesterol. Whether the observed PPARα up-regulation is due to pterostilbene or other constituents (such as known anthocyanins in blueberry) will be discussed.

AGFD 159

Physiological effects of pterostilbene and blueberries in animal models of obesity

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Pterostilbene and resveratrol are two highly bioactive naturally occurring stilbenes. Their chemical structures are less complex yet share most of the important physical and physiological characteristics of complex flavanols found in many fruits. They have low solubility in water and are not highly bioavailable yet have considerable physiological effects. In hamsters and mice on high fat diets, supplemention with low levels of pterostilbene or resveratrol prevented increases in plasma cholesterol and body weight and other characteristics of obesity related metabolic dysfunction. Blueberry pomace also showed similar physiological response. The amount of pomace fed was much higher because the pomace contains large amounts of dietary fiber and flavanols as well as resveratrol.

AGFD 160

Berry bioactives: the health benefits of color

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Berry fruits provide a rich source of dietary anthocyanins to the human diet. Epidemiological evidence has identified dietary anthocyanins as specific flavonoids associated with lower cardiovascular risk and metabolic health. These data are consistent with in vitro and animal model findings. Human clinical trials are emerging to show how and in what amounts berry-rich anthocyanins need to be consumed to provide their benefits. This presentation will provide a brief overview of the berry and anthocyanin research to date focused on metabolic health and potential links to their benefits centrally.

AGFD 161

Effects of blueberries on cognition and neuroplasticity

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It has been demonstrated that consuming blueberries can prevent and even reverse the occurrence of neurochemical and behavioral changes associated with aging. Recent research suggests that consuming a high-fat diet (HFD) may result in behavioral deficits similar to those observed in aging animals. To determine if supplementation of a HFD with blueberries offers protection against behavioral declines, nine month old C57Bl/6 mice were fed low-fat diet (LFD) or HFD (60% calories from fat) with and without 4% freeze-dried blueberry (U.S. Highbush Blueberry Council) for 5 months. Novel object recognition memory was tested after 2, 3, and 4 months on the diets and the Morris water maze was used to assess spatial learning and memory after 5 months. Recognition memory was impaired in the HFD-fed mice at all time points, but mice fed HFD + blueberry showed a reversal of memory disruption after 4 months. Probe trial performance in the water maze was impaired in animals consuming the HFD, while animals on HFD + blueberry were not different from those on LFD. Brains of the mice were assessed for microglia activation, neuroplasticity by measuring brain-derived neurotrophic factor (BDNF), and neurogenesis by measuring doublecortin (DCX). There was significantly less microglia staining with Iba1 in mice fed HFD + blueberry. Moreover, BV-2 microglial cells treated with blood serum from mice fed HFD + blueberry produced less nitric oxide in response to stimulation with LPS compared to cells treated with serum from mice fed HFD. BDNF levels and the number of DCX positive cells were enhanced in the hippocampus of mice fed HFD + blueberry. Overall, this study demonstrated that supplementation of a HFD with blueberry was associated with reduced indices of microglia activation and increased neuroplasticity and neurogenesis; these changes may underlie the reduction of memory deficits observed in these animals.

AGFD 162

Phytochemicals against oxidative stress and inflammatory responses in microglial cells

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Microglia are the major immune active cells in the brain and are known to play multiple functional roles, not only for scavenging cellular debris, but also for the ability to release pro-inflammatory and other factors that may affect neighboring cells. Activation of microglia has been implicated in a number of neurological diseases including traumatic brain injury and stroke. It is immensely important to keep microglia healthy in both young and old brains. Many dietary fruits and vegetables are known to contain phytochemicals with antioxidant properties. Our recent studies have identified compounds such as quercetin from berries that can effectively mitigate oxidative and inflammatory responses in activated microglial cells. Bacteria endotoxins (lipopolysaccharides, LPS) activate the Toll-like receptors in microglial cells leading to induction of the NF-κB transcriptional pathway and resulting in production of pro-inflammatory cytokines and nitric oxide. However, some of these phytochemicals not only are capable of inhibiting LPS-induced NF-κB pathway, but also are effective in stimulating the antioxidant pathway involving Nrf2 which mediates transcription activation of the Antioxidant Response Element (ARE) and synthesis of antioxidant enzymes such as heme oxygenase-1. There is evidence that phytochemicals that effectively inhibit the NF-kB pathway also are effective in stimulating the Nrf2 pathway. Our goals are focused on understanding mechanism mediating the cross-talk between these two signaling pathways and to identify novel phytochemicals effective in enabling healthy microglia to fight against neurological diseases.

AGFD 163

Quest for indirect modulators of the endocannabinoid system from natural products

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The endocannabinoid system has emerged as a popular pharmacologic target due to its diverse roles in physiological function. Anecdotal reports of nutmeg being used as a substitute for marijuana suggest its components may interact with the endocannabinoid system. The objective of this study was to utilize bioassay-guided fractionation to isolate pure nutmeg compounds and evaluate them for *in vitro* fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) inhibition; key endocannabinoid degradation enzymes. Ten nutmeg fractions were screened for fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) inhibition All ten nutmeg fractions showed greater than 50% fatty acid amide hydrolase (FAAH) enzyme inhibition, with the enzyme recovering by 48 hours. From these, three active compounds, MF 30-7, MF 109-5 and MF 117-3, were isolated; showing an IC_{50} value of 7.02 μM ± 2.02, 4.57 μM ± 0.66 and 33.10 μM ± 5.35, respectively. Additionally, MF 30-7, MF 109-5 and MF 117-3 showed acetylcholinesterase (AChE) inhibitory activity, with percent inhibitions of 64.08 ± 7.88, 35.13 ± 1.89 and 71.80 ± 0.96. Of the three purified compounds, MF 109-5 possesses unique characteristics of highest potency and selectivity making it a viable candidate for *in vivo* testing in behavioral animal paradigms.

AGFD 164

Traditional plant breeding vs molecular plant breeding

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The human food supply is contingent on modified plants. Modern crop varieties are partly the result of plants that have been modified by incorporating numerous desirable traits into one variety. At the advent of agriculture, plants were selected for various traits, including better taste, storage ability, disease resistance, and yield. These initial efforts centered on selection of naturally occurring mutations that provided desired traits. Plants containing desirable mutations were crossed together, and the progeny were sorted through to identify the plants having the desired combination of traits; the great majority of the progeny would show undesired traits, and get discarded along the way. A limitation has been that since genes for traits are transferred as chromosomal segments rather than single genes, undesirable linked genes always get crossed in with desirable ones. Alternatively, when the needed traits are not present, attempts are made to create them via mutagenesis, or to resort to extraordinary measures to obtain them by crossing the crops with their distant relatives.

Understanding the genetic changes that accompanied the selection of desired traits makes it possible to rationally design future changes. For such molecular breeding, the necessary genes for desired traits can be obtained from any other organism, and then get engineered into a crop variety. As opposed to conventional breeding that works with traits, molecular breeding works with the genes themselves, thus requiring an extensive up-front characterization of the gene and its function. With such knowledge in hand, hundreds of engineered plants are created and evaluated. Those that do not show the desired trait or that show undesired traits get discarded, and only one (the lead event) is subjected to extensive safety tests prior to commercialization.

AGFD 165

Biotechnology innovations and solutions for sustainable agriculture

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Biotechnology has proven to be versatile tool to combat many of the challenges farmers face. It enables growers to sustainably improve the opportunity for yields while protecting biodiversity. Insect control traits provide an improved way for controlling pests above and below ground, and have led to a reduction in the amount of insecticide used. Below-ground insect control traits have also indirectly helped plants better withstand water stress. Integrating biotechnology with other key advancements such as those in molecular breeding, crop protection technologies, digital agriculture, microbials and other technologies will be key as we look to feed our growing world population. Monsanto's SmartStax® PRO, currently in phase 4 of the R&D Pipeline, demonstrates the ability to help improve crops with Bt combined with RNAi. Companies across the industry are leveraging RNAi to develop products, like the genetically modified Arctic Apple® and J.R Simplot Company's Potato, which bring additional benefits like reduced food waste, to farmers and society. Through an integrated solutions approach, the agriculture industry can bring farmers more options for sustainably managing on-farm challenges.

AGFD 166

Herbicide-resistant crops: Past, present and future

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Glufosinate- and bromoxynil-resistant crops were introduced in 1995, but had little impact. In 1996, the first glyphosate-resistant (GR) crops (soybean and canola) were introduced, and these and later GR crops (cotton, maize, sugar beet, and alfalfa) had a huge impact on weed management, with more than 90% adoption where they were available. GR crops have been the most commonly used transgenic crops, and, as a result, glyphosate became the most used herbicide globally. The strong selection pressure from the almost exclusive use of glyphosate in GR crops led to the evolution of many GR weed species. This is leading to greater adoption of glufosinate-resistant crops and crops made resistant to auxinic herbicides (2,4-D and dicamba), with other herbicide-resistant crops on the way. In most cases, the GR trait is included with the genes for these additional herbicide resistance traits. So, farmers are still using large amounts of glyphosate to kill non-GR weeds in GR crops. Some scientists have indicated that glyphosate alters the mineral composition of GR crops, but most studies have not found this to be true. Additionally, there have been claims that glyphosate makes GR crops more susceptible to plant pathogens, but this has not been substantiated, and there is better evidence that glyphosate reduces infection by some plant pathogens in GR crops. Glyphosate and its degradation product, aminomethylphosphonic acid, are found in the harvested seed of GR soybeans treated with glyphosate, but in the few published studies, the levels found have been below what is allowed by the US FDA. Other weed management technologies are under develop that will probably reduce glyphosate use in the future.

AGFD 167

Challenges for the production and acceptance on transgenic wheat

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Transgenic crops have been grown in many countries for many years, including maize for food, feed and industrial use. However, although trasgenic wheat has been procuced as a research tool for over 20 years, it has not led to any commercial varieties. This is surprising as wheat is the most widely grown staple crop and the dominant crop in temperate zones, accounting for up to 70% of the calories in some developing countries. I will therefore discuss progress in developing trandgenic wheat as a commercial crop in the context of wheat production and utilisation. This will incude attitudes to the acceptability and safety of transgenic wheat, and the importance of focusing on improving traits which are of value to consumers due to benefits for health and quality of life.

AGFD 168

How basic research can lead to development of improved cereal crops: But where are they?

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Thioredoxin (Trx), a small molecular weight protein, catalyzes reactions via a dithiol-disulfide exchange mechanism involving redox-active cysteine residues. The Trx redox center, with its disulfide (S-S) bridges, interacts with specific target proteins, facilitating changes in their structure and activity. Trxs are present in all living organisms, including large, complex families in plants. Work, primarily in cereals, established the role of one family member, Trx *h*, as a central regulatory protein in seeds, due its reductive effects on seed storage proteins, enzymes and enzyme inhibitors. Our applied efforts involved up- or down-regulating Trx *h* expression in protein bodies or the cytosol of cereal grain endosperm. In barley, embryo germination was accelerated and starch-degrading enzymes were enhanced when Trx *h5* was overexpressed, important traits for the malting and brewing industry. Trx *h5* was also overexpressed in wheat endosperm, leading to increased solubility of disulfide proteins and decreased allergenicity. Using an antisense Trx *h9* construct, underexpression of Trx *h9* was achieved in wheat, resulting in the engineered grain germinating slower with a dramatic delay in preharvest sprouting, which causes costly losses in grain yield. But where are these improved cereals? Regulatory costs for introducing genetically engineered foods into the market is outside the financial resources of academic scientists and small companies. And the regulatory pathway to commercialization is not always clear. And once in the market concern and possible backlash by consumers raises concerns with food marketers. Will use of genome editing to improve crops and foods lead to a different scenario?

AGFD 177

How to eat a plant--phytochemical detoxification in bees vs butterflies

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In 1961, H.T. Gordon postulated that the propensity of herbivorous insects to develop resistance to pesticides is "the result of selection for endurance of prolonged biochemical stresses" associated with dietary phytochemicals. Thirty years later, at the 202nd national meeting of the American Chemical Society, Rene Feyereisen and his colleagues presented their findings on patterns of expression of CYP6A1 in the house fly *Musca domestica* -- the first cytochrome P450 associated with insecticide resistance. A year later, the first P450 involved in phytochemical detoxification, CYP6B1, which detoxifies furanocoumarins in *Papilio polyxenes*, was characterized. In 2016, the 25th anniversary of the characterization of the first xenobiotic-metabolizing cytochrome P450, entire CYPomes are available from insects with a broad diversity of diets and a substantial literature suggests that plant-feeding insects vary enormously in how they detoxify the phytochemicals in their food. The structural, functional, and regulatory diversification of cytochrome P450s in herbivorous insects is reviewed in the context of the Gordon's remarkable insight.

AGFD 178

Dietary pterostilbene is a novel chemopreventive and therapeutic agent in prostate cancer: Pre-clinical studies

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A potential strategy for chemoprevention and cancer therapy is to discover/develop new plant-derived bioactive molecules with anti-inflammatory and anticancer activities. Studies in our laboratory have shown that dietary stilbenes such as resveratrol and its analogs inhibit oncogene and epigenetic modifier metastasis-associate protein 1 (MTA1) expression in prostate cancer cell lines and xenografts. Pterostilbene, a dimethoxy analog of resveratrol that is found in grapes and blueberries, showed the highest MTA1 and MTA1-signaling inhibitory activity in prostate cancer cells compared to resveratrol and other analogs. The purpose of this study was to determine the MTA1-targeted chemopreventive and therapeutic efficacy of pterostilbene in *Pten*-loss pre-clinical model of prostate cancer that have elevated MTA1 levels compared to wild type animals. Prostate-specific *Pten* heterozygous ($Pten^{+/f}$) and *Pten* knockout ($Pten^{f/f}$) mice, which represent chemoprevention and intervention scenarios, respectively, were utilized. We found that pterostilbene both as a dietary supplementation and interventional daily injections, through targeting MTA1 and MTA1-dependent oncogenic network, inhibited inflammation, proliferation and angiogenesis and induced apoptosis in prostate tissues. These effects resulted in reduction of prostatic intraepithelial neoplasia (PIN) lesions and adenocarcinomas in precancerous *Pten*$^{+/f}$ and cancer-prone *Pten*$^{f/f}$ models, respectively. We believe that pterostilbene is a novel MTA1-targeted chemopreventive and therapeutic natural agent that may have potential clinical applications in prostate cancer management.

AGFD 179

Topical pterostilbene prevents UV-B-mediated skin damage

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Skin is the human body's first and best defense against environmental exposures including solar ultraviolet (UV) radiation. Exposure to UV light is a key factor in the development of many skin disorders. Skin cancer is the most prevalent type of cancer in the United States, affecting an estimated one out of every seven Americans. UV radiation is one of the most powerful (and common) environmental factors that can cause a wide range of cellular disorders by inducing mutagenic and cytotoxic DNA lesions.

Resveratrol, a natural polyphenol found in grapes, berries and other plants, has been proposed as an ideal chemopreventative agent due to its putative plethora of health promoting activities. However, despite its promise as a cancer prevention agent its success in human clinical trials has been limited, in part due to its poor bioavailability. Thus, interest in other natural polyphenols is intensifying including the naturally occurring dimethylated analog of resveratrol, pterostilbene, which has been proposed to have better bioavailability in humans.

In this study, a SKH-1 (hairless) mouse model was used to examine if pterostilbene, like resveratrol, was effective in preventing UV-mediated skin damage. Mice were exposed to 180 mJ/cm^2 UV-B radiation every other day for two weeks or left unexposed as a control. Thirty minutes prior to each exposure, topical pterostilbene, resveratrol, or vehicle was applied to the back of each mouse. We clearly show that pterostilbene prevented UV-B-induced damage in mouse skin. Visually, pterostilbene prevented redness on back skin and associated nicely with the prevention (or reduction) of hyperplasia, skin thickening and transepidermal water loss as compared to vehicle + UV-B treated mice. Furthermore, pterostilbene prevented UV-B mediated DNA damage (both cyclobutane pyrimidine dimer formation and 64-pp) in the skin of mice as compared to vehicle + UV-B. Interestingly, pterostilbene was more effective than resveratrol in all of our experiments.

AGFD 180

Health benefits of natural tocopherol mixtures

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Tocopherols, members of the vitamin E family, are lipophilic antioxidants present in vegetable oils, such as cottonseed, corn and soybean. The tocopherols consist of four forms, designated as α, β, γ, and δ variants. Recently, γ-tocopherol, the most common form of vitamin E in the diet in the United States, has shown anti-inflammatory and anti-cancer activities in experimental animal models. We have found that dietary administration of natural tocopherol mixtures (high in γ and δ forms) suppresses mammary tumor growth and tumor multiplicity in Sprague Dawley rats treated with N-methyl-N-nitrosourea, inhibits MCF-7 estrogen dependent xenograft tumor growth and reduces estrogen-induced mammary carcinogenesis in ACI rats. In our studies, treatment with natural tocopherol mixtures increases the expression of p21, p27, caspase-3 and peroxisome proliferator activated receptor-γ (PPAR-γ), while inhibits estrogen signaling in mammary tumors. In ACI rats implanted with slow release estrogen, natural tocopherol mixtures have also shown anti-estrogenic action and anti-tumorigenic activities. Our mechanistic study suggests that tocopherols activate PPAR-γ and Nrf2, while antagonize estrogen action in animal models of mammary tumorigenesis. Overall, natural tocopherol mixtures high in γ- and δ-tocopherols may be potentially beneficial agents in the prevention of breast cancer.

AGFD 181

Chemistry, safety and caloric value of partially hydrolyzed guar gum

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Partially hydrolyzed guar gum (PHGG) is obtainded by partial hydrolysis of guar gum which comes form the Indian cluster bean (Cyanopsis tetragonolopus). PHGG is composed of galactomannan polymers with a molecular weight ranging from 200 to 300 kDa. The intact and partially hydrolyzed forms have multiple food applications. Guar gum can form gels or be used as a thicking agent in foods. The intact material can be used to control the viscosity, stability, and texture of foods. PHGG is highly soluble and has little physical impact on foods. Both forms are indigestible but are excellent sources of fermentable dietary fiber. The caloric value of intact guar gum is accepted as 2.0 kcal/g. This paper will review the chemistry, safety, in vivo effects, and caloric value of PHGG.

AGFD 182

Fifty years of smelling sulfur: From the chemistry of garlic to the molecular basis for olfaction

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Smell is one of five senses through which we perceive the world. By one estimate, humans can sense more than one trillion olfactory stimuli. My talk will focus on strong-smelling sulfur-containing odorants, whose chemistry I have been studying for more than 50 years, with a major focus being on sulfur compounds from garlic, onions and related species ("*Allium* chemistry"), particularly newly discoverd thiolane derivatives. Humans and animals have an exquisitely sensitive sense of smell toward low-valent, volatile sulfur compounds. In 1887, Emil Fischer wrote that concentrations of ethanethiol as low as 0.05 ppb are "clearly perceptible to the sense of smell". In 2005 we identified (methylthio)methanethiol ($MeSCH_2SH$; MTMT) as a garlic-smelling social-signaling compound found in mouse urine. We subsequently identified the mouse odorant receptor (OR), MOR244-3, responding robustly to MTMT, finding that it employs Cu as a cofactor. Extending this research, I will describe the identification of a Cu-requiring human OR, responding robustly to 2-methyl-2-propanethiol, the odorant in natural gas, and related thiols. Taking a broader look at the sense of smell, I will discuss the vibrational theory of olfaction, focusing on musk odorants and their human ORs. Our collaborative OR studies use QM/MM modeling and site-directed mutagenesis to understand the molecular basis for olfactory detection of strong-smelling compounds.

AGFD 183

Rancidity development in roasted almonds (*Prunus dulcis*): Relationships between chemical changes and sensory descriptive analysis

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Almond quality is dependent upon many factors including the development of rancidity. To date, little is known regarding correlations between chemical markers of rancidity and consumer acceptance/perception of roasted almonds. Raw almonds (cv. Nonpareil) were roasted for 60 min at 115 C to give a light roast, and 20 min at 157 C to give a dark roast. Markers of oxidative stress including: peroxide value (PV), free fatty acids (FFA), vitamin E, and volatile profiles were measured in roasted almonds stored at 39 ± 2 C and relative humidity of 15 ± 3% over 12 months. Marked changes in volatiles related to lipid oxidation (e.g. hexanal, octanal, nonanal, (E)-2-octenal, 1-heptanol, 1-ocatanol, etc.,) were noted by 3 months of storage. PV values did not exceed 5 millieq until 4.5 months. Levels of FFA did not change significantly during the 12 months of storage. Sensory descriptive analysis indicate that degree of difference vs. control, total oxidized flavor, cardboard flavor, painty/solvent flavor, soapy flavor, bitter taste, pungent/irritation/burning mouthfeel and astringent mouthfeel increase with storage whereas clean nutty aroma and flavor, and clean roasted flavor decrease. Significant sensorial changes were notable by 6 months of storage and correlated with markers of oxidation.

AGFD 184

Chemical mechanisms for 3-MCPD ester formation

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3-MCPD fatty acid esters are a group of processing-induced food toxicants. The free radical mediated chemical mechansims for 3-MCPD ester fromation from triglyceride, diglyceride, and monoglyceride were investigated. ESR determination demonstrated a free radical mediated mechanism for 3-MCPD ester formation, whereas FT-IR examination suggested the involvement of a carbonyl group during the reaction(s). Possible mechanisms and radical intermediates were investigated and will be discussed. In addition, the possible catalytic role of Fe in 3-MCPD ester formation and thermal decomposition were studied and will be discussed.

AGFD 185

GMO crops may contribute to decline of monarch butterfly populations

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Recent trends indicate a significant decline in the monarch butterfly populations that migrate from summer breeding stocks to overwintering sites in Mexico and the California coast. Contributing to the ‘disappearing monarchs’ are habitat alteration, including deforestation, loss of milkweed flora which serves as a larval host for the monarchs, as well as drought and altered agricultural practices.

The use/overuse of herbicides that are active against perennials, such as milkweeds, can be a factor for the decline as monarchs depend on this plant for vital components of their life cycle including egg laying and larval food. Planting of crops that have been genetically modified for resistance to Roundup (glyphosate) herbicide has facilitated the decline of weeds. This presentation will focus on factors that might come into play to reduce Monarch populations as a result of this GMO-inspired technology and changes in farming practice. The populations of milkweeds in the US had declined significantly during the same period when Monarch populations declined, and the use of GMO crops expanded (roughly from the 1990s to the present).

Milkweeds harbor cardiac glycoside poisons that can poison livestock, causing the plants to be undesirable for ranchers, leading to use of Roundup and other herbicides. Glyphosate is also effective against invasive weeds—whose populations have also increased during the same time period and Senecio spp. Loss of milkweeds and Senecio to herbicide treated acreage, as well as overlaying drought, results in fewer plants to serve as hosts for monarch larvae, thus reducing the availability of cardiac glycoside poison for sequestration, which defend monarchs from predators during the reproduction, feeding, metamorphosis, and migration cycles.

A compelling scenario can be postulated that links herbicide use, drought effects, and changing agricultural/forestry practice to monarch decline. Various solutions to counter these threats to Monarch populations are under discussion.

AGFD 186

Impressive progress, opportunities, and obstacles in the use of genetically engineered trees

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Genetic engineering (GE) provides options for circumventing some of the considerable difficulties of breeding trees, including their delayed onset of reproduction, limited capacity for hybridization/introgression, and intolerance of inbreeding. The capabilities provided by GE are likely to become increasingly important in a world characterized by rapid environmental change, climatic stress, and associated shifts in tree pests. I will review the scientific progress in creating and studying GE trees, including new opportunities provided by RNA interference and gene editing advances. I will also review the case for market and regulatory reforms, which are badly needed if the potential benefits of GE to society are to be realized.

AGFD 187

Progress on transgenic approaches to solving citrus greening disease

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Huanglongbing (HLB) has become the dominant disease threatening the Florida citrus industry. HLB affects all cultivated citrus varieties and causes a rapid decline of trees and produces unmarketable fruit. This disease is caused by a phloem limited nonindigenous bacterial pathogen and causes substantial economic losses to every commercial citrus cultivar. Incorporation of gene(s) via genetic engineering can potentially confer resistance in susceptible cultivars, while maintaining the varietal fidelity. Several antimicrobial peptide gene constructs (AMPs) and the SAR inducing genes NPR1 and SABP2 have been tested in commercial sweet orange cultivars with varying degrees of success. Field trials in an HLB endemic environment as well as greenhouse based insect vector transmission trials have identified a few genes that can potentially confer tolerance to HLB. Initially, the AMP genes looked promising, but the tolerance response faded over time. The most successful genes to date are the SAR induction genes NPR1 and SABP2, driven either by a constitutive d35S promoter or a phloem specific Arabidopsis SUC2 promoter.

Independent transgenic lines with these genes continue to show tolerance to HLB after four years in the field at a challenging field site. We will also discuss our current citrus improvement strategies to develop more consumer-friendly transgenic plants by the utilization of plant based genetic constructs. We have developed a transformation system to generate reporter gene expression free citrus by coupling a visual citrus derived anthocyanin producing transcriptional factor gene with an embryo specific promoter to regenerate genetically modified plants that have the marker gene switched off. In addition, we are also evaluating a large number of citrus derived genes, promoters, genes and terminators to create an all citrus transformation vector. These constructs are also being incorporated using our protoplast transformation system, which allows the incorporation of linear DNA pieces into the citrus genome. HLB-resistant transgenic citrus containing all plant DNA should have quicker regulatory approval as well as improved public acceptance.

AGFD 188

American chestnut research and restoration project

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The American chestnut (Castanea dentata) and chestnut blight is the classic example of what happens when our forests succumb to exotic pests and pathogens. Because of its environmental, economic, and social importance, many tools have been brought to bear on the chestnut blight problem. We have focused on enhancing blight resistance by adding only a couple genes to the approximately 40,000 gene pairs in the chestnut genome using the tools of genetic engineering. Although we have eight candidate genes that putatively enhance blight resistance slightly, one stands out among the rest. The most promising gene to date encodes an oxalate-detoxifying enzyme, called oxalate oxidase (OxO). This gene comes from bread wheat (Triticum aestivum), but is also a common defense gene found in many plants including all grain crops as well as bananas, strawberries, peanut, and other familiar produce. The OxO is not a pesticide, not an allergen, and is not a gluten protein. According to chestnut leaf and small stem assays that predict the level of blight resistance, this OxO has raised resistance levels in American chestnut at least as high as those found in the blight-resistant Chinese chestnut (C. mollissima). This will be the first time an ecosystem restoration program will use the tools of genetic engineering. The next step is to have the trees reviewed by three federal regulatory agencies, the USDA, EPA, and FDA. Once approved, these blight resistant American chestnut trees can be used to rescue the genetic diversity and local adaptation in the remnant, surviving population of American chestnut and be an additional tool for the restoration of this important keystone tree.

AGFD 189

Transgenic and gene edited animals for use in agriculture: Where are we now?

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There have been many developments in technology to create transgenic since the early 1980s, including somatic cell nuclear transfer-based cloning, effective use of PGCs in chickens, lentiviral vectors, and among others. The development of gene editing methodologies has improved our ability to precisely alter the genome of animals and, with the large number of sequenced genomes, we have unprecedented access to sequence information, including, control regions, coding regions, and known allelic variants. We now can create new transgenes that will express when and where we want and can target precisely in the genome where we want to make a change or insert a transgene. However, to date we have only one GE animal approved for use as food anywhere in the world, the AquAdvantage Salmon. World-wide there has been a failure of the regulatory processes to effectively move forward. Estimates are that we will need to increase our current food production by 70 to 100% by 2050; that is we will have to produce the total amount of food each year that has been consumed by mankind over the past 500 years. The combined use of transgenic animal technology and gene editing will become increasingly more important tools to help feed the world. However, the practical benefits of these technologies have not yet reached consumers in any country and in the absence of predictable, science-based regulatory programs it is unlikely that the benefits will be realized in the short to medium term.

AGFD 190

Microalgae derived ingredients, oils and the future of foods

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At TerraVia, we are redefining the future of food. By harnessing the power of algae, our technology brings better, next generation, plant-based nutrition to people along with economic and environmental stability for the planet. Microalgae, the mother of all higher plants and the earth’s first triglyceride oil producers, have evolved over hundreds of millions of years to very efficiently convert light and/or simple sugars into biomass and oils; often in very inhospitable parts of our planet, making algae the earth’s original superfood Algae are poised to play a growing role in the world’s food supply. Microalgae are extremophiles and can be found thriving in environments such as polar and high alpine regions, thermal vents and hot springs, soda lakes as well as natural salt lakes. Provided adequate energy and nitrogen sources are available microalgae direct their efforts to reproduce; thereby, driving biomass production. In the presence of an energy source but the absence of nitrogen, microalgae respond by producing triglyceride oils that are stored inside the cells for future use. Carbohydrates, vitamins and pigments critical for human and animal nutrition are also routinely produced by microalgae.

In this presentation, we will explore how TerraVia has harnessed the robustness, efficiency and productivity of heterotrophic microalgae to develop technology platforms that vastly expand the functionality of microalgae ingredients to create healthy, nutritious and appealing foods, improving the lives of people and the planet. In particular, we will discuss utilizing native microalgae to produce protein rich and lipid rich whole microalgae ingredients as well as oils and the composition, properties and usefulness in foods of oils and butters from tailored microalgae. We’ll also take a closer look at how algae-based platforms offer a superior sustainability profile as well

AGFD 214

Chemical synthesis of optically pure rhizopines: Steps towards engineering a synthetic symbiosis between bacteria and crops

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In agriculture addressing crop productivity while ensuring sustainability has become crucial. Despite this, with nitrogen as one of the main limiting nutrients for efficient crop growth, unsustainable nitrogen fertilisers are still being extensively used. Leguminous plants form a symbiotic association with diazotrophs called Rhizobia. Rhizobia resides in legume root nodules, and converts atmospheric nitrogen into its biologically accessible form. Therefore, engineering a synthetic nitrogen-fixing symbiosis between Rhizobia and crops, such as corn and wheat, would lead to more sustainable agriculture. One major challenge is ensuring that symbiotic bacteria are able to efficiently colonise crop roots. In addition, Rhizobia should acquire sufficient carbon to energise nitrogen fixation. Rhizopines (+)-5, (−)-5 and 6, are uniquely able to mediate the growth of Rhizobia, facilitating the formation of a symbiotic relationship. Engineering rhizopine synthesis in crops therefore represents an exciting target for enhancing productivity and sustainability. We aim to characterise the biosynthetic and catabolic pathways of rhizopines. This can only be achieved using synthetic rhizopines (+)-5, (−)-5 and 6. We have thus completed the first enantioselective synthesis of (+)-5 and (−)-5, starting from myo-inositol 1. Both enantiomers were synthesised, in 13 steps via racemic triol (±)-2, using a novel diastereoisomeric protection-resolution strategy. This route was also applied to the synthesis of 6. Chemically synthesised rhizopines (±)-5 and 6 were used as standards in GC-MS studies to gain further insight into the biosynthetic pathway towards 5. A new biosynthetic pathway was therefore proprosed. In addition, synthetic SI 6 was shown to serve as a sole carbon and nitrogen source for Rhizobia. Finally, using the enantiomerically pure Rhizopines synthesised, the natural enantiomer of 5 will be identified.

AGFD 215

Engineering a bypass of 1-deoxyxylulose-5-phosphate synthase in *Esherichia coli* for the conversion of pentose sugars to isoprenoid chemicals and biofuels

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Despite significant advances in the microbial conversion of plant biomass to simple alcohol biofuels via microbial engineering, several challenges remain in the industrial conversion of lignocellulose to bio-based chemicals and drop-in replacements of gasoline for transportation energy. With regards to lignocellulosic feedstocks, the co-utilization of pentose and hexose sugars in plant hydrosylate to sustain growth and chemical production in genetically tractable microbes is a major challenge to the field. Likewise, the efficient capture of this energy into valuable chemicals and so-called advanced biofuels at high productivity and titer remains elusive. Here we engineered the metabolism of *Escherichia coli* K12 strain MG1655 to produce 1-deoxyxylulose-5-phosphate (DXP) from D-arabinose while bypassing the native enzyme DXP synthase (or DXS). A *de novo* bypass of DXS for DXP synthesis has, to our knowledge, not been reported and promises to allow increased carbon flux into the isoprenoid pathways of the cell, which in turn allows for production of mid-sized alcohols (C5-C10) or hydrocarbon biofuels and high-value isprenoids common in fragrances and medicines.

AGFD 216

Genetically programmed functional bacterial biofilms

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Biofilm engineering is an emerging field of synthetic biology for the design of functional bionano-interfaces. Escherichia coli, Bacillus subtilis, and Pseudomonas aeroginosa are among the bacteria that attach to surfaces and form biofilms through the expression of curli-related proteins. E. coli produces curli proteins with two structural components, the major subunit csgA and minor subunit csgB proteins. The csgB subunit helps the csgA subunit to stack on top of each other and form fibrous superstructures. We aimed to control the morphology, mechanical properties and electrical properties of the amyloid nanofibers to be utilized as new generation of protein based materials. We used native and functionalized curli subunits to produce electrically conductive curli fibers in bacteria. Anhydrotetracycline-inducible riboswitches were used to control the expression of curli subunits, allowing the on-demand synthesis of protein-based bio-nanomaterials at the biotic/abiotic interface with desired functionalities such as controlled electrical conductivity and controlled mechanical strength. We also built AND, A NIMPLY B, B NIMPLY A and NOR logic gates to control the expression of curli subunits and the morphology of curli nanofibers. Initial studies with aromatic acid functionalized scaffold peptides guided us for the modified versions with aromatic amino-acid insertions of curli nanofibers for bio-conductive structures. Following the selection of the most conductive aromatic aminoacid residues we achieved peptide insertions into the curli nanofiber monomeric protein CsgA inspired by conductive Geobacter metallidurans PilA protein. We kindly thank to TUBITAK 115Z217 and 114M163for its support and funding.

AGFD 217

Novel combination of megaTAL nuclease-driven genome engineering with a drug selection cassette increases efficiency of HIV gene therapy

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Human Immunodeficiency Virus (HIV) infection remains a substantial health problem worldwide. The human C-C chemokine receptor 5 (CCR5) gene, which encodes a co-receptor required for HIV entry into CD4+ T cells, is a promising alternative therapeutic target. Early clinical trials using CCR5-disrupting nucleases in patients are promising, with the exception of two limitations: (a) a need for higher levels of CCR5-disruption and (b) preferential selection of gene modified cells. The CCR5-targeting megaTAL is a nuclease architecture that combines a LAGLIDADG homing endonuclease scaffold with an eleven repeat transcription activator-like (TAL) effector array to achieve efficient site-specific cleavage. We are coupling megaTAL nuclease treatment with drug selection in order to disrupt the CCR5 locus and select modified CD4+ T-cells. The mutant human dihydrofolate reductase (DHFR) construct renders cells resistant to lymphotoxic concentrations of the drug methotrexate (MTX) at 0.02 μM. For optimal cell viability we deliver nucleases via mRNA and selection-constructs via adeno-associated virus (AAV). Electroporation with megaTAL mRNA demonstrated robust CCR5 disruption: 95% in GHOST-Hi5 cell lines and 70-90% in human CD4+ T-cells. Gene-modified human T-cells were transplanted into NOD/SCID/γc-null 'humanized' mice and subsequently challenged with HIV-1 infection. CCR5-null modified cells preferentially survived during active HIV infection in vivo (100-fold increase). Initially, the HIV plasma viremia was significantly lower in the nuclease-treated mice. However, the virus levels rebounded over time. We hope to address this by selectively transplanting cells that have been CCR5 disrupted to achieve therapeutically relevant levels of HIV-protected cells. In our preliminary experiments, primary T-cells lentivirally transduced with a Tyr-22-DHFR cassette at 15% efficiency showed an enrichment of >90% over 7 days in 0.02 μM methotrexate. More recently, we have demonstrated AAV-mediated targeted gene insertion followed by chemoselection and reached >50% enriched populations of human CD4 T cells. The CCR5-megaTAL nuclease platform produces very high levels of gene-modified CD4+ T-cells and protects these cells from subsequent HIV infection *in vivo.* We are able to knock-in a selection cassette at the CCR5 disruption-site and specifically enrich for gene-modified cells.

AGFD 218

Analysis of the everninomicin gene cluster and dichloroisoeverninic acid biosynthesis in *Micromonaspora carbonacea* var. *aurantiaca* in pursuit of novel everninomicin analogs

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The rapid emergence of antibiotic resistant illnesses is a growing threat to human health that could eventually lead to a post-antibiotic era. Currently, there is a renewed interest in not only discovering new antibiotics but also revitalizing previously described scaffolds. One such class of molecules is the everninomicins, complex polysaccharides produced by Micromonospora carbonacea var. aurantiaca. They contain a number of unique structural features, such as orthoester linkages, a methylenedioxy bridge, a dichloroisoeverninic acid moiety, and a nitro sugar. During previous development, the everninomicins showed strong activity against antibiotic resistant strains of bacteria via inhibition of protein translation. However, the everninomicins' development was hindered by an inability to access analogs via total synthesis or semi-synthetic methods. We propose to derivatize the everninomicins using the natural biosynthetic machinery via genome editing to develop novel analogs with increased antibacterial potency. All natural everninomicin analogs contain at least one iterative type I polyketide synthase (iPKS)-derived dichloroisoeverninic acid (DCE) moiety. This conserved functionality's biosynthesis and role in activity are poorly understood. In order to elucidate everninomicin biosynthesis, the four genes associated with DCE biosynthesis will be deleted using methods specifically designed to avoid disruption of the overall everninomicin gene cluster. The enzymes will also be biochemically characterized and their substrate specificity explored to evaluate the incorporation of non-natural substrates. Genomic editing and incorporation of non-natural substrates will allow for investigations into the ability of M. carbonacea var. aurantiaca to produce everninomicin analogs with diversified everninic acid moieties containing bulkier alkyl groups or non-chlorine halogens. Analogs properly designed with useful synthetic handles could eventually be utilized for coupling reactions to increase everninomicin potency and study ribosomal inhibition. The proposed genetic manipulations and enzymatic studies will elucidate the biosynthesis of everninomicins and provide novel analogs in an attempt to revitalize this potent class of antibiotics.

AGFD 219

It is about safety

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In general, the first set of questions relating the chemistry and safety of a GMO-derived crop are whether the GMO food or feed source has the equivalent chemical composition of the original crop source, other than the intended effect of the genetic change. The second set of questions focuses on any unintended consequences directly or indirectly resulting from the novel and intended change made in a GMO crop plant. A third aspect meant to relate the chemistry with the safety of a GMO food can rely on a precautionary principle that one cannot predict all of the long-term consequences that might happen with a GMO crop source of food or feed. Application of the precautionary principle effectively has reduced the number of GMO crops commercialized by adding significant cost and time requirements that are only met by a small number of applicants. Whether these approaches have increased food safety; whether these methods have ever failed to protect the consumer; and whether these approaches have unfortunately resulted in stifling a safe and sufficient global food supply are subjects for discussion.

AGFD 242

Unintended effects associated with GM crops are both expected and low risk

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One concern often raised for GM crops is the occurrence of unintended effects. Such unintended effects may result from random DNA modification or might be a direct or indirect consequence of the intended trait. In reality, unintended effects are most often attributable to plant breeding itself, and not specific to GM crops. Conventional breeding has always been accompanied by unintended effects, and plant breeders must always select plant lines without adverse agronomic or anti-nutritional characteristics. GM crops are not immune to these biological realities, although a better knowledge of the mechanisms that underlie GM traits reduces unexpected effects compared with less comprehensively characterized traits selected based on phenotype from non-GM variants or mutants. Extensive experience with unintended effects from both non-GM and GM traits, including pest resistance and herbicide tolerance, suggest low food, feed, and environmental risk.

AGFD 243

Assessing the risks of resistance evolution for transgenic crops for insect control: Capitalizing on successes and learning from mistakes

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Transgenic plants for insect pest control have become an important component of a number of crop production systems. Resistance management has been and continues to be an important consideration in the registration of transgenic plants expressing protein toxins from Bacillus thuringiensis. In some instances, target pests have remained susceptible to these technologies and even resulted in area wide population suppression. However, instances of field-evolved resistance to Bt crops has increased substantially in recent years and has become a major obstacle to continued success of this technology. Examining the factors that have resulted in both successful and unsuccessful resistance management should provide insight into sustainable approaches to using the technology.

AGFD 244

FDA's safety evaluation of foods from genetically engineered plants

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Developers of new plant varieties have a rich history of introducing new traits into plant varieties using various techniques. By law, food producers are responsible for ensuring that they produce food that is safe and nutritious. The development of biotechnology posed new challenges for government based on the ease of introducing traits from different species into other organisms. In 1992, as part of its response to the Office of Science and Technology Policy's Coordinated Framework for the Regulation of Biotechnology,

FDA published a policy that describes the responsibilities of developers of new plant varieties and FDA's encouragement for them to consult with the agency to ensure the safety and regulatory compliance of their products. Since 1994, FDA has consulted with developers on over 150 varieties. The varieties encompass many vegetables and some fruits, primarily soybeans, corn, cotton, canola, and potatoes, with various traits, predominantly herbicide tolerance and insect resistance. The specific applications of food law to the challenges of genetic engineering are discussed, as well as the types of products, regulatory and scientific challenges, and specific examples from completed consultations.

AGFD 245

Intellectual property issues of GMO food crops

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Genetically-modified organisms have brought intellectual property (IP) rights into the focus of the agricultural industry. All commercialization hinges on IP rights, and this is no different for transgenic food crops. Research dollars must be recovered with profits in the marketplace. If you cannot assert legal ownership of your crops, then you cannot commercialize your product. IP rights are key to the success – or failure – of genetically-modified agriculture. However, GMO-related IP issues are not just about making a profit, there is also the question of farmers' rights to their own crops. This paper will provide a brief overview of legislation governing genetically-modified food crops, followed by a survey of legal controversies pertaining to GMOs and IP rights. The necessity (or possible lack thereof) of GMO labeling will also be included.

AGFD 246

Communication of GMO issues to non-technical audiences

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One of the major short comings of the scientific community has been effective communication of the technology and risks associated with GMO foods. Transgenic proteins for drug therapy are well accepted but there remains fear and resistance to GMO foods. Advocacy groups have effectively raised public concern about the dangers of GMOs. As a scientific community we have to find ways to communicate what GMOs are, why they are produced, the safety and environmental concerns, and risks to human health. We need to establish clear language that explains the technology in clear non-technical terms while providing assurance about the safety, environmental concerns and health risk issues. The long term benefits to the consumer need to be explained. Comparison to conventional plant breeding, weed control and insect resistance should be carefully described. The reduced use of chemicals and direct gene insertion or blocking can be compared to conventional techniques.

ENVR DIVISION

ENVR 9

Power of novel metal oxide-carbon nanotube heterostructures: enabling microwave to disinfect water for aquaculture

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Over the past half a century, global food consumption has increased significantly, rising by nearly 400 kcal/person/day. Such growth has put tremendous stress on the food chain, and access to a high calorie diet should be at the forefront in ensuring global food security. Fishes (global aquaculture production of 50M metric tons of $100B per annum value) are effective protein sources for humans with the highest edible yield (~70% of the body mass utilized) and protein retention (31%) compared to red meat (18%). Since water is the life-blood of aquaculture industry, ensuring suitable water quality is essential to the success of this industry. The Norwegian virus attack of 1984 as well as recent reports from FAO demonstrated the ability of pathogens to have severe consequences on aquaculture production. The current best practices in aquaculture involve ultraviolent (UV) disinfection to ensure biological quality of water. UV radiation is mutagenic to pathogens by breaking molecular bonds within micro-organismal DNA and producing thymine dimers. While this technique is largely successfully it suffers from three key limitations: (i) efficiency drops precipitously in turbid water; (ii) required irradiation time can be prolonged to inactivate certain pathogens, rendering this technology to be cost-prohibitive; and (iii) some pathogens are resistant to UV irradiation, e.g., viruses. Since chemical disinfection is not an option for aquaculture (due to the bioaccumulation risks of carcinogenic disinfection byproducts and other chemicals) development of a more effective irradiation-based technology is desired. This study presents a novel nano-enabled disinfection system, which utilizes low intensity energy waves, i.e., microwaves (MWs), to effectively disinfect water. Metal oxide-carbon nanotube heterostructures have shown to establish a synergy between adsorptive ability of microwave with the upconverting ability of the metal oxides to successfully generate reactive oxygen species and disinfect water. The synthesized heterostructures have inactivated pseudomonas aeruginosa, when irradiated with MW for 10 s. *F. columnare* inactivation is currently being studied under water quality composition, relevant to aquaculture.

ENVR 10

Engineered Water Nanostructures (EWNS): A chemical free, nanotechnology based antimicrobial platform for inactivation of foodborne microorganisms across the farm-to-fork continuum

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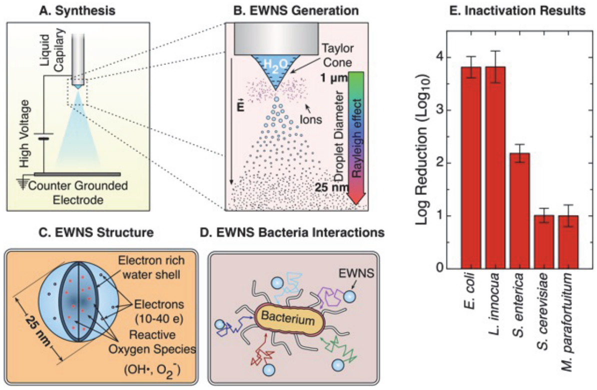
Foodborne diseases caused by the consumption of food contaminated with pathogenic microorganisms or their toxins have very serious economic and public health consequences. Here we present the effectiveness of a recently developed nanotechnology based intervention method for inactivation of

microorganisms on fresh produce, and food production surfaces using Engineered Water Nanostructures (EWNS). EWNS are synthesized using an integrated process which is based on electrospraying and ionization of water. EWNS possess unique properties; they are 25 nm in diameter, remain airborne in indoor conditions for hours, contain Reactive Oxygen Species (ROS) and have very strong surface charge (between 10 - 40e$^-$/structure). More importantly, it was demonstrated that EWNS properties can be "fined-tuned" during their synthesis to optimize their inactivation potential. Taking advantage of their high surface charge, we developed and characterized, a "draw through" Electrostatic Precipitator Exposure System (EPES) for the targeted delivery of the EWNS on surfaces. We explore their efficacy in inactivating representative foodborne bacteria such as Escherichia coli, Salmonella enterica, Listeria innocua, Mycobacterium parafortuitum, and Saccharomyces cerevisiae on the surface of organic cherry tomatoes. The inactivation was facilitated using the moderate dose of 40,000 #/cm^3. The preliminary results presented here showcased that EWNS can achieve microbial removal rates between 1 and 3.82 $\log_{10}$ after 45 mins of exposure. Electron Microscopy imaging revealed that the EWNS could destroy the bacteria membrane leading to their inactivation. The observed destruction of the membrane lipid due to the presence of ROS was assessed with a lipid peroxidation assays (LPO), identifying the ROS as the primary mechanism of inactivation. More importantly, this antimicrobial platform has the potential to be scaled up and used across the farm to the fork continuum to enhance food safety.



ENVR 11

FRET-based quantum dot sensor for detection of botulinum neurotoxin serotypes A and B

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Botulinum neurotoxin (BoNT) - a zinc-dependent endoprotease - is one of the most potent toxins known to humans. Foodborne botulism results from oral ingestion of biologically active toxin, and only a few nanograms of the toxin are sufficient to elicit symptoms. The gold standard method of BoNT detection is the mouse bioassay. It is highly sensitive and selective but is also time consuming and poses ethical concerns over the use of laboratory animals. This highlights the need for new rapid and sensitive screening methods for detection of BoNT, particularly ones that are sensitive to active forms of the toxin. We developed a detection method for biologically active BoNT based on Förster resonance energy transfer (FRET) between quantum dots (QDs) and photoluminescent quenchers (PLQs) that were linked by peptides. Each peptide contains a sequence of amino acid residues that act as enzymatic cleavage sites for a specific BoNT serotype, and possess terminal oligo-histidine regions that allow binding of the peptide to QDs. In the presence of biologically active BoNT, the specific peptide was cleaved and the quenching was removed, resulting in the recovery of QD photoluminescence. The detection capability of this sensor was demonstrated with BoNT/A light chain (LcA), which is the catalytic domains of BoNT/A. LcA in buffer was detected in 3 h with a detection limit of 4 nM (10 ng per sample). A linear relationship was found between the QD photoluminescence recovery and LcA concentration in the range of 4 nM to 200 nM. BoNT/B detection was demonstrated by observing the recovery of QD photoluminescence due to the cleavage of the BoNT/B-specific peptide by LcB. Active BoNT holotoxins were also detected using the sensor. The specificity of the sensor toward each BoNT serotype was also evaluated. The performance of the sensor indicates its potential application as a rapid screening method for biologically active BoNT.

ENVR 12

Nanoscale micronutrients suppress plant disease and increase crop yield

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In the United States, plant pathogens reduce agricultural productivity by 20%, resulting in billions of dollars of annual losses from reduced yield, wasted resources, and compromised food quality. The added stresses from a changing climate and increasing population will further challenge agricultural productivity. Novel approaches to suppress crop disease and increase productivity are needed; nanotechnology offers tremendous promise as a new tool for increasing food security. Plant micronutrients are critical to disease resistance through multiple pathways but unfortunately, element availability in soil is low and foliarly applications are often poorly absorbed and not effectively translocated. However, data have shown that nanoparticle (NP) forms of copper are not only absorbed more effectively through plant shoots, but movement of the nutrient throughout the plant is also increased. Investigations were initiated in which NP Cu, Mn, and Zn oxides were compared to bulk equivalents or salts for effects on the growth and yield of eggplants in soil infested with the pathogen *Verticillium dahliae*. Foliar applications (1000 µg/ml distilled water) were made to transplants before inoculation with *V. dahliae.* All Cu compounds reduced disease progression, but the NP CuO treatment delayed the onset of symptoms until week 3, resulting in disease severity that was significantly less than the control or the bulk Cu. Root Cu concentrations were higher in plants treated with NP CuO, suggesting significant basipetal NP transport. Separately, treatments were also applied to young plants that were then transplanted into field plots infested with *V. dahliae.* The largest canopy was that treated with NP CuO; although disease progression did not differ with treatment, the yield from plots treated with NP of CuO was increased by 34% over the untreated control. Although NP are currently being incorporated into numerous agricultural products, their possible role as amendments for disease management holds much promise.

ENVR 13

Applications of cerium oxide nanoparticles for plant salt stress enhancement in agriculture

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Limited high quality water resources and growing population are forcing growers to use water with relatively high salt concentrations for crop irrigation. It is well recognized that salinity affects almost every aspect of the plant physiology and biochemistry and crop yield, and represents one of the most serious threats to sustainable agriculture. Meanwhile, the rapid development of nanotechnology in recent decades, in particular, the integration of engineered nanoparticles (ENPs) in fertilizers provides a unique opportunity to enhance sustainable agriculture in adverse environmental conditions. The goal of this study was to investigate whether low concentrations of cerium oxide nanoparticles (CeO_2NPs) could induce physiological and biochemical changes in agricultural crops which would enhance plant salt stress resistance. Two salinity levels: 0 (control) and 100 mM NaCl, and three CeO_2NPs concentrations: 0 (control) 200 and 1000 mg/kg dry soil, were investigated. *Brassica napus* L. cv. 'Dwarf Essex', an important vegetable oil plant, was used as a model crop. Measurements of the plant growth rate and biomass indicated strong physiological and biochemical adjustments in salt stressed plants. Changes in proline, chlorophyll content and efficiency of the photosynthetic apparatus all demonstrated that CeO_2NPs led to changes in the growth and physiology of *Brassica napus* which alleviated the plant salt stress. This study provides important insights on the potential applications of nanotechnology for sustainable agriculture in the context of global climate change and explosive population growth in the world.

ENVR 14

Impact of metal and metal oxide nanoparticle speciation and solubility on their bioavailability to terrestrial and aquatic plants

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Nanotechnology has the potential to increase the efficacy of delivery of micronutrients, macronutrients, and pesticides to plants. This can greatly decrease the required agrochemical inputs for food production, thereby lowering energy and water inputs and environmental impacts. Currently, there is little mechanistic information available regarding the interactions of plant roots with nanomaterials. This makes it difficult to optimize agrochemical formulations for delivery of nanomaterials to plant roots. We investigated the effect of speciation and solubility of Ag and Cu nanoparticles on their bioavailability in soils, association with plant roots, and biouptake by the terrestrial plant *M. sativa* and the aquatic plant *L. punctata*. Synchrotron X-ray fluorescence and XANES analysis are used to determine the spatial distribution and speciation of metal in plant roots exposed to either ionic or nanoparticulate forms of the metals. For hydroponic exposures, we find that the nanoparticulate forms of the metals initially adhere to the mucilage of the plant roots while ionic forms of the metal are more readily taken up. Over time, the dissolution of the NPs leads to metal uptake, even for the relatively insoluble metal sulfides. For Ag, uptake occurs through both ionic metal uptake and uptake of the nanoparticles. Cu and CuO NPs slowly dissolve in soils over ~30 days, suggesting that bioavailability will increase with time. This is confirmed with extractions using DTPA and with XANES analysis of metal speciation over time. The speciation of metal inside the roots of aquatic plants exposed to CuO and CuS NPs appears different than either NP form, suggesting that uptake occurs only after dissolution. Together, these studies are increasing our understanding of fate and bioavailability of metals from metal and metal oxide NPs in terrestrial and aquatic systems.

ENVR 15

Advanced nanomaterials for catalytic dephosphorylation and phosphorus recovery

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Before the early 1900s, most of the world's phosphorus was derived from animal waste. Today the vast majority (nearly 80%) of phosphorus in modern fertilizers comes from phosphate rock. Although there are smaller deposits around the world, the majority of the Earth's phosphate rock is harvested in the remote Western Sahara. Despite changes in diet and improved agricultural efficiency, peak phosphate rock production is expected to be reached by 2040, and supplies will be completely depleted by the end of this century. This could have major impacts on fertilizer production and, as a direct consequence, food production in order to feed the population of nearly 10 billion by 2050. The world is now presented with the pivotal challenge of developing innovative ways to sustainably produce phosphorus from renewable sources. This issue is quickly gaining the attention of scientists and policymakers. Here we report on the development of heterogeneous catalytic technologies for dephosphorylation. Cerium oxide (ceria) nanocrystals was synthesized and applied as an "artificial phosphatase" to cleave the phosphate ester bond and generate inorganic-*P* from biomass feedstocks. This concept was initially demonstrated by studies on *para*-nitrophenylphosphate (*p*-NPP) as a model of biomolecules containing phosphorus, which was hydrolyzed to form *para*-nitrophenol (*p*-NP) and release one phosphate ion. Systematic kinetic studies were performed on ceria nanocrystals of different sizes, morphologies and surface defects, in order to reveal the structure-property relationship of the active sites and improve the activity and recyclability of the catalysts. The optimized catalysts were then used to treat phospholipids and nucleic acids. The released phosphates were ultimately collected by using zeolite adsorbents and regenerated as pure phosphate chemical streams for further applications.

ENVR 16

Starch stabilized silver nanoparticles, synthesis and their adsorption-desorption pattern for dichlorvos insecticide

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In this study, the ability of cassava starch nano-silver particles (CVNPs) to adsorb Dichlorvos (VOS) from aqueous solution was investigated through batch experiments. CVNPs were prepared by physical gelation and synthesis monitored with UV-Vis spectrophotometer for the Surface Plasmon Resonance (SPR) of silver nanoparticles. CVNPS were characterized by UV–Visible Spectrophotometry, Fourier Transform- Infrared (FT-IR), Field Emission Scanning Electron Microscopy (FESEM), High Resolution Transmission Electron

Microscopy (HRTEM), Energy Dispersive X-Ray (EDX), Selected Area Electron Diffraction (SAED), Powder X-Ray Diffractometry (PXRD), Differential Scanning Calorimetry (DSC) and Thermogravimetry Analysis (TGA). The SPR of CVNPs was 403nm. The morphology was spherical, monodisperse and average size range of 7- 11nm. EDX, SAED and PXRD confirmed the silver (Ag) presence with the revelation of reflections of silver nanoparticles indexed as (111), (200), (220) and (311) with the corresponding 2θ values of 38.4^{O}, 44.4^{O}, 64.1^{O} and 77.7^{O} respectively. The adsorption of dichlorvos onto CVNPs has been found to depend on initial concentration and contact time. The experiments were carried out at pH of 6.5. Equilibrium data fitted well with the Langmuir and Freundlich models with values of r^2 of Langmuir better with a maximum adsorption capacity of 33.33 mg/l. The experiments showed that the highest adsorption rate was 92% for VOS. Desorption pattern showed hysteresis, while sorbed dichlorvos depicted slow release, thus making CVNPs suitable matrix for slow release formulation of pesticides.

ENVR 180

Managing challenges of the food-energy-water nexus

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Food, energy, and water are essential for our existence; however, which of these is the most important can be a subject of debate. The interrelationship of food, energy, and water presents us with a multidimensional problem that links resources, national policies, politics, and quality of life for all of us. We need to better deploy these resources to address our needs now and for the 9 billion people in the next 40 years (1). Detailed investigations are needed to determine our food, energy, and water needs collectively, rather than individually. After air, water is the most essential requirement for human survival. Also, water from a variety of sources is used to produce food and energy. At times, food such as corn is used to produce ethanol, which can be used as fuel in automobiles. A great deal of water is required to produce hydroelectric energy; however, the water sent over a dam can still be used for other purposes. Also, the water used for thermal and nuclear energy requires cooling before it can be returned to the water source. The water used for fracking requires significant treatment before it can be used for other purposes. The necessity of water in the production of food and energy clearly indicates that human survival is not possible without water. It should be noted that energy is necessary to produce and distribute water for domestic use and for irrigation of food crops; i.e., there is a nexus among these three important resources. Together, water, food, and energy help us maintain and improve our lifestyle. Addressing these complex issues will require collaborative efforts among federal and state agencies, private enterprise, and nongovernment organizations. More investment is necessary to develop sound science and policies that address these complex issues that affect food security, sustainable energy, and adequate availability of water.

ENVR 181

Techno-economic assessment of desalination technology for application in agriculture

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Agricultural yields will need to increase drastically to meet the expected growth and development of the global population in the coming century. Clearing additional farmland must be avoided, however, as land use change is a major drive of global climactic change. One strategy currently considered to increase yields on agricultural land is the use of small-scale, distributed desalination technologies in arid regions. These technologies offer two primary benefits to the producer – (1) they decrease soil salinization and thus increase yields and (2) they increase water supply by accessing previously impaired sources such as drainage or saline groundwater. If drainage is used as the feed water in these systems they also confer environmental benefits to downstream ecosystems. These benefits must be weighed against other costs in the FEW nexus – if systems are powered by fossil fuels they will have climate and human health impacts. Weighing both public and private costs and benefits is crucial for understanding the net effect of the technology. By combining high resolution data from satellites, crop models, and empirical relationships from field experiments we develop and implement a methodology to quantify economic benefits of desalination technology at high resolution across wide areas. We take advantage of modern computing power to apply this method at a 30 meter scale over the Central Valley of California for crop year 2013. We find that the value of augmented water supply exceeds that of improved water quality in most of the state, and that during drought the private benefits exceed costs in some areas, indicating that the technology may be adopted in the private market during periods of extreme water stress. We also find, using the Air Pollution Emission Experiments and Policy analysis (APEEP) model, that the specific technology is important – solar driven processes avoid significant climate and health impacts over grid and natural gas powered facilities. By accounting for both public and private costs and benefits, we are able to predict how technology adoption may happen under certain conditions and inform how policy makers should guide technology adoption in order to ensure that both growers and environmental systems benefit.

ENVR 182

Integrated energy-water planning in the eastern interconnection

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Water is an essential resource to the thermoelectric power sector, which is the largest user of water in the United States. As water-related issues continue to grow due to drought, climate change, and tension between multiple sectors, it is becoming increasingly important that water resources are considered in long-term energy planning. This study seeks to evaluate projected implications on water resources as energy demand grows and new power plants are built. Capitalizing on recent energy-demand projections from the Eastern Interconnection Planning Collaborative (EIPC) we are identifying watersheds at highest risk for future water stress. Three future policy scenarios—business as usual, federal portfolio standard, and carbon reduction—are being compared. Energy expansion is calculated for each region defined by the North American Electricity and Environment Model (NEEM). This work will enable the energy sector to factor watershed stress into future energy expansion, which is not currently done.

ENVR 183

Food, energy water nexus, complicated by global climate and the need for new technology

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By the year 2050, the Earth's population is expected to exceed 9 billion people, posing significant challenges to meet human needs for food, water, and energy. We must utilize technology to meet these needs while minimally affecting the environment. To support the increasing population, we will need secure and safe sources of food, energy, and water. The nexus of food, energy, and water is one of the most complex, yet critical, issues that face society. There is little or no new land to exploit; the supply of fresh water in many areas of the world limits the use of land for agriculture. Any solutions must also deal with the overlay of global climate change. Meeting the current and future requirements for the increased population's needs will require secure and renewable sources of food, energy, and water supplies. A nexus approach is needed to improve food, energy, and water security, integrating the management of limited resources while transitioning to a more "green" economy, which provides adequate food, energy, and water for the expanding human population.

ENVR 184

Multi-objective optimization model for minimizing cost and environmental impact in shale gas water and wastewater management

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Unconventional resource extraction from shale involves complex operations for water and wastewater management. These water management operations are expensive for companies and emit significant quantities of criteria air pollutants and greenhouse gases that impact human health and the environment (HHE). We present a multi-objective mixed integer linear programming (MILP) framework for assessing the tradeoffs between financial cost and HHE costs for shale gas water acquisition, transport, storage, and treatment under realistic scheduling, operational, and regulatory constraints. We formulate objective functions to identify water management strategies that minimize financial cost, minimize HHE cost, and minimize combined financial and HHE costs. The model was applied to a 14 wellpad case study that is representative of shale gas extraction in the Marcellus Play. We observe significant variation in the financial and HHE costs under different objective functions and regulatory scenarios.

ENVR 185

Engineered natural treatment systems at the food-energy-water nexus: The influence of vegetation on micropollutant fate

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Vegetated systems are prominent at the food-energy-water nexus. Indeed, water used for growing crops is the largest use of water, and alternative low-energy approaches are required for irrigation needs. Engineered natural treatment systems (ENTS), which are often vegetated, hold the promise of reclaiming otherwise unused or underutilized sources of water that can then be used for irrigation or to offset other consumptive uses. Plants are important for determining micropollutant fate at the food-energy-water nexus because 1) crops receive the reclaimed water and 2) many ENTS feature vegetation as a means of pollutant removal. Therefore, understanding micropollutant fate and transformation is critical to improving ENTS and understanding exposure risk. In this work, we present the study of 2-mercaptobenzothiazole (MBT) fate in a plant system and elucidate pollutant transformation mechanisms. MBT is a tire rubber vulcanizer present in both stormwater and wastewater effluent, both of which may be used as reclaimed water sources. We present work on the uptake kinetics and elucidation of novel transformation products. We discovered that MBT is efficiently and rapidly assimilated by plants and transformed into glycosylated and amino acid conjugated metabolites. The results of MBT plant transformation are analogous to our previous work with benzotriazole, suggesting the pollutant pathways we discovered are likely more broadly applicable. Understanding plant transformation of micropollutants will be key for sustainability at the food-water energy nexus.

ENVR 186

Unexpected ion-exchange reactivity of nanometric scheelite: Applications in food, energy, and water sectors

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It was recently discovered that nanometric scheelite, $CaWO_4$, undergoes unexpected ion exchange with heavy metals and actinides, sorbing the dangerous metal ions and releasing benign calcium ions into solution. Selectivity is for larger metal ions over smaller ones and is especially high for oxocations such as uranyl, and hydroxocations such as those formed by zirconium. Sorption capacities are extremely high – exceeding 100% by weight in several cases. The novel reagent has been used to remove heavy metals such as lead and cadmium and radionuclides such as strontium and uranium from water. Applications in treatment of contaminated water, milk, and juice will be discussed. Notably, uranium isolated from drinking water or "ocean mining" can be isolated and used for nuclear power. The elaboration of a sustainable process whereby the sorbent is recycled and reused while the target ions are isolated as separated solids will be described.

ENVR 187

Impact of cerium oxide nanoparticles on plant water use efficiency at different environmental conditions

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The growing global demand for food, energy and clean water, accompanied by dwindling arable lands and global warming, are imposing unprecedented challenges on our society. Plant water use efficiency is a particularly important consideration because it directly relates to the global food security and is heavily affected by environmental conditions. Rapid development of nanotechnology significantly increased the possibility of accumulation of engineered nanomaterials in agricultural soils, which have been shown to significantly affect plant growth. However, whether and how the engineered nanomaterials may affect plant water interactions has not been explored. The primary goal of the research was to investigate the impact of cerium oxide nanoparticles on the plant water use efficiency and plant photosynthesis at both water sufficient and deficient conditions. Cerium oxide nanoparticles with two different surface charges (+ and -) and soybean as a model plant were used in the study. The results indicated that cerium oxide nanoparticles indeed had a significant impact on plant water use efficiency as indicated by the C^{13} isotope discrimination in the soybean tissues. The impact of cerium oxide nanoparticles on plant water use efficiency varied with the concentrations and surface properties of nanoparticles as well as the soil moisture content. Detailed analysis on the performance of photosynthetic apparatus and associated physiological processes provided mechanistic insights on the impact of engineered nanoparticles on plant water interactions. This study demonstrated that rapid nanotechnology development may have a great impact on the water exchange in different compartments of the food energy and water nexus and demands further investigation.

ENVR 193

Caterpillar cross tolerance/resistance to *Bacillus thuringiensis*: Don't forget our history

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There has been a history of the discovery of new insecticides representing different classes of synthetic chemistry and modes of action (organochlorines, organophosphates, carbamates and pyrethroids) and the evolution of caterpillar resistance to these chemistries. Today, the use of insect-resistant transgenic crops is now wide-spread and essential to pest management. To delay the evolution of resistance to insect-resistant transgenic crops, the industry currently relies on pyramiding events expressing two or more insecticidal proteins in the same cultivars. Looking at the lessons from the past, we should not be surprised if caterpillars are eventually able to also overcome different protein insecticides. Our research has been interested in this possibility and the impact of increase feeding rates as one method of potential cross tolerance/resistance to protein toxins. We have found in cotton bollworms and tobacco budworms field populations in the Southeast US that increased feeding rates were associated with decrease susceptibility to Bt, that decreased temperature and feeding rates increased *Bacillus thuringiensis* (Bt) susceptibility, that a laboratory derived tobacco budworm Bt resistant strain demonstrated increased feeding on non-Bt artificial diet, and that a field collected fall armyworm Bt-resistant strain also demonstrated increased feeding on non-Bt artificial diet. Also when we compared slow versus fast feeders of the cotton bollworm at a single temperature, the fast feeders have a reduced susceptibility to Bt toxin. These studies suggests that increase feeding could be one mechanism for cross-tolerance or resistance to proteins and that insect selection by Bt crops could results in caterpillar populations with enhanced feeding rates regardless of whether they are on Bt plants or not. More work is needed to better understand this mechanism and its relative potential for impacting the use of Bt cotton for caterpillar control but at least suggests caution is needed in the management of this technology.

ENVR 194

Pros and cons of the first 20 years of GMO cotton production

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Genetically engineered cotton varieties were introduced in 1995 with herbicide and insect resistance. The technology was adopted widely and rapidly by producers in the United States. Economics and convenience were primary reasons for rapid adoption of these technologies by growers. The compatibility of herbicide resistance with reduced tillage systems that save time, labor and fuel as well as reducing soil erosion and sand blasting was also attractive to many growers. Cotton producers have encountered some problems such as reduced pollination, poor fiber quality, inadequate bollworm control and rapid changeover of varieties with the technologies, all of which have been corrected to some degree in subsequent versions. The major concern with genetically engineered varieties for growers is pest resistance at this point. Resistance to insecticides and herbicides are not new to cotton producers, as they encountered pesticide resistance before biotechnology was introduced. Herbicide resistance in particular has been prominent in the news in recent years with a new term "superweeds" used to describe the phenomena known simply as weed resistance prior to biotechnology. Current weed resistance issues are not isolated to genetically engineered crops as resistance is also seen in crops like wheat that are not like genetically engineered. The number of new resistance species discovered per year has decreased since the introduction of biotechnology, but is still very concerning to agriculturalists. Many feel that what makes weed resistance such a serious problem at this point in time, is not the level of resistance occurring in "GMO" crops versus "nonGMO" crops, but the lack of investment in new pesticide chemistry. The lack of investment by companies in new pesticide chemistry is probably partially due to the initial success of "GMO" weed control systems, but just as much or more due to the cost associated with the regulations involved in registering new chemistry and bringing it to the market.

ENVR 195

Local vs. global population editing: A novel and responsible approach to gene drive

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RNA-guided gene drive elements are predicted to spread through all wild populations connected by gene flow, posing challenges for ethical development, popular consent, and international governance. Here we detail daisy-chain gene drive systems that bias inheritance in a manner limited by the number of elements in the chain. Our models suggest that releasing "daisy drive" organisms constituting a small fraction of the local wild population can drive a useful genetic element to regional fixation for a wide range of fitness parameters without resulting in global spread. We additionally report numerous highly active sgRNA sequences sharing minimal homology that may enable evolutionary stable daisy drive as well as global CRISPR gene drive. Daisy drives could simplify decision-making and promote ethical use by enabling local communities to decide whether, when, and how to alter shared regional ecosystems.

ENVR 196

Starting a dialog about GMOs with non-majors through three editions of Chemistry in Context

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Current popular opinion on the topic of genetically modified organisms (GMOs) demonstrates the woeful lack of public understanding and comfort with fundamental chemical concepts. Arguments in this arena are often based upon fear rather than informed discussions using the scientific principles underlying new technologies. While chemistry faculty can and should continue to include information on topics of public importance such as GMOs as well as communication skills into our major courses, we miss a large opportunity to educate a broader population immediately. Many college students have the option or requirement to take at least a single semester of general education chemistry. This course requirement presents a valuable opportunity to integrate preliminary understanding of genetics and genetically modified organisms into the knowledge base of a broader population. Chemistry in Context is a textbook that provides a platform for interaction with this broader audience. This presentation will discuss the process of adapting content on genetics and biochemistry from the context of health to the context of GMOs over three editions of the text. Three different tactics for presentation of information will be discussed along with sample student responses. These tactics are valuable to target information for both non-majors and majors. We require new tactics to reach an educated and questioning, yet often misinformed, public to shift the questions asked and the basis of public argument.

ENVR 197

Public and policy engagement on synthetic biology

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Synthetic biology is a convergence of technologies enabling faster, cheaper, more precise and more sustainable processes in medicine, energy, agriculture, and the environment. Yet even as 'synbio' reaches the marketplace, the research community has largely failed to engage public stakeholders about what synthetic biology is, the motivations of researchers, and the challenges and opportunities of engineering biology. To make matters worse, the media tends toward hyperbole and fear rather than careful exploration with stakeholders about possible futures. These factors may give rise to public misconceptions about issues like safety, security and ethics, which may in turn diminish support for engineering biology research and US scientific and economic leadership in the coming era of biology. In this talk, I will describe the experience of Synberc (the Synthetic Biology Engineering Research Center) and share our efforts to authentically engage public and policy audiences about this fast-growing field. Then I will preview the newly established EBRC (Engineering Biology Research Center), which aims to be the leading organization bringing together academics, industry, policymakers, funders, and public stakeholders to create shared visions and roadmaps for engineering biology and catalyze research to address national and global needs.

The Engineering Biology Research Consortium, or EBRC, aims to be the leading organization bringing together an inclusive community committed to advancing biological engineering to address national and global needs. We showcase cutting-edge research in engineering biology, identify pressing challenges and opportunities, and articulate compelling research roadmaps and programs to address them. We promote dialogue to create unified, shared visions to realize the very best potential of this fast-evolving field.

EBRC will sustain the traditions and work of Synberc by broadening our membership and strategic aims.

ENVR 198

Engineering biology for the U.S. bioeconomy

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US revenues related to genetically-modified organisms are over $300B annually. Current bioprocessing technologies, including those used to convert biomass resources to fuels and chemicals, require significant resources to reach market. Estimates suggest that it can cost up to $150M to bring one biologically-produced molecule to market, a figure of merit that applies to the pharmaceutical, energy, and chemistry sectors. A significant part of the cost is the time it takes to make a product: thousands of person hours are spent developing a molecule through trial and error and some of the most successful bio-based products took 10-15 years to reach commercial production scales. However, new integrated engineering-biology research platforms promise to greatly accelerate the discovery of solutions to national and global needs, and in the process, democratize engineering biology to enable researchers in academia, government and industry to drive advancement across fields and industrial applications. Critically important to the success of this new approach is the development of standards, guidelines and safeguards for new products, processes, and systems of engineering biology that will lay a solid foundation on which to build a robust and responsible biomanufacturing future.

ENVR 199

First things first: What is a GMO?

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Developing sound policy and defensible positions requires accurate information, disciplined use of language, dispassionate analysis, and clear thinking. Many of these requirements were missing when governments around the world developed various regulatory policies related to “GMOs.” As a result, the diverse and extraordinary benefits that genetic engineering could provide to society have been greatly curtailed, especially in food and agriculture. As ACS committee members consider developing an official policy on “GMOs,” their training as scientists will lead automatically to conversations characterized by dispassionate analysis and clear thinking. This presentation is intended to provide a supply of the two other necessary ingredients for good policymaking: accurate information and appropriate terminology. Thoughtful and productive discussions about the technical aspects of "GMOs" and the risks they might pose can occur only if they are grounded in history. For thousands of years, humans have genetically modified the plants, animals, and microbes that we rely on. As a result, the term "GMO" could encompass all organisms that humans use. This presentation will describe 1) some the many, but unfamiliar, ways that humans depend on products provided by microbes; and 2) the continuum of genetic modification techniques that have been used to improve crop plants. Biologists agree that the way the term "GMO" is used in contemporary culture does little to inform discussions and debates because people use the same term to mean very different things. This has led to a variety of regulatory definitions of GMOs that unintentionally capture many products, some of which have been in commerce for well over a century. This presentation will dissect the various, diverse concepts people have in mind when they use the term "GMO" and will describe the ways in which these concepts have led to legal definitions that fail to provide clarity to regulators and the regulated community.

ENVR 200

Legal and regulatory implications of genetic engineering for the chemical community

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The pathway to market for new products utilizing genetic engineering can be difficult to navigate, posing a challenge for companies, researchers, and other chemical community stakeholders. While it is understood that the novelty posed by some genetically engineered products can make it difficult for regulators to evaluate risks, the product review and approval process needs to be rationalized. This presentation will consider the regulatory oversight of genetically engineered products in the United States. This presentation will also review the Obama Administration's efforts to modernize the governance of biotechnology as outlined in the July 2, 2015, memorandum issued by the White House Office of Science and Technology Policy directing federal agencies to update the 1986 Coordinated Framework for the Regulation of Biotechnology. The effort has three components. The Administration will: update the Coordinated Framework to clarify the "roles and responsibilities" of the federal agencies that regulate the products of biotechnology; commission an external analysis of the future landscape of biotechnology products (the Administration has asked that The National Academies of Sciences, Engineering, and Medicine conduct such an analysis); and formulate a long-term strategy to ensure that the federal regulatory system is equipped to assess efficiently the risks, if any, associated with future products of biotechnology. The last component is essential and holds considerable promise in developing a game plan for achieving success. The chemical community's participation in the multi-year process envisioned by the White House directive is critical as the outcome of this process will have significant and long lasting implications for researchers, innovators, and other members of the chemical community.

ENVR 201

Genetically engineered governance: Why international governance systems need their DNA engineered to keep pace with genomic technologies

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In 2012, estimates of the US industrial biotech sector's revenues were $105 billion, $66 billion of which came from the biochemical sector (Carlson, 2016). More and more start-up companies are entering the market providing designer organisms which are changing the way traditional chemicals are being developed, produced and regulated. Meanwhile, genomic applications designed to combat human/animal disease and agricultural pests are challenging regulatory structures that were designed over 30 years ago. Society is rapidly moving from applications (and in some instances companies) that traditionally were treated as point source pollution towards living, moving targets. In addition, lower barriers to entry and access to funding (through crowdfunding platforms) has expanded who can participate in genetic engineering, further challenging the regulatory system. How the US and other international governing bodies coordinate and address new genomic technologies will impact how they are developed, who is able to maneuver the system and thus participate in the market, and whether applications are introduced at all. Over the last year the White House released a memo directing federal agencies to review their authorities under the Coordinated Framework for the Regulation of Biotechnology; the European Commission released three opinions on synthetic biology (definition, risk assessment methodologies and research priorities), and the United Nations Convention on Biological Diversity concluded its Ad-Hoc Technical Expert Group on Synthetic Biology. This talk will explore how the U.S. and international governance systems are trying to keep pace with genomic technologies and how it could impact the field moving forward.

ENVR 243

Rotavirus control for safe and sustainable production of leafy greens

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In the US, most food consumed by individuals is produced on an industrial scale. While this has decreased the cost of food, it has significantly increased energy and water use. We propose that a return to local growing of vegetables and fruits through community-based gardens will significantly ease energy consumption used for industrial scale farming and shipping of produce from farms to tables. Furthermore, the strategic use of otherwise ignored water sources (e.g., rainwater and storm water, or RSW) for irrigation will ease competition for water consumption and municipal water treatment. The safety of the fruits and vegetables irrigated by this water must be ensured to remove one barrier towards implementing community gardening. We conducted microbial risk assessment, solar disinfection of RV and sanitation of RV attached to leafy greens. We found that (1) SODIS inactivation of human RV correlates with the formation of hydroxyl radicals in solutions containing natural organic matter (NOM), such as those that would be found in storm water, (2) SODIS is targeting the VP4 and VP7 proteins (the major capsid proteins), greatly inhibiting capsid translocation and entry into cells, (3) genetic basis for SODIS susceptibility can be used to predict the effectiveness of SODIS of irrigation water, and (4) effectiveness for produce sanitation depends on both chemical properties of the leaf and the sanitizer. The results of this study confirmed that combination of SODIS and post-harvest produce sanitation can be used to ensure safety of produce irrigated by unconventional water treated by solar energy.

ENVR 244

Advances and challenges in recycling of high strength organic waste and wastewater for clean water and energy

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Agro-food processing industries such as distilleries, sugar refineries, dairies, fruit canning, meat processing, and pulp & paper mills are facing a series of daunting challenges for wastewater treatment due to high concentrations of COD (~ 190 g/L) and mineral salts (potassium ~ 12 g/L and sulphate ~ 9 g/L), and a large amount of dark brown pigments such as lignin, phenolic derivatives, and melanoidins. The high strength organic waste and wastewater (HSOWW) is an industrial pollution source, but it is an underutilized resource. Hence, the development of technologies and management approaches for energy recovery at high rates and recycling of the treated wastewaters from complex and refractory wastewater streams such as municipal wastes and landfill leachate is important. In this study, recent progress in recycling of the HSOWW for production of clean water and energy as well as challenges rated to the recycling of the HSOWW will be discussed.

ENVR 245

Evaluation of Microbial Fuel Cell implementation at the advanced wastewater treatment plant at Blue Plains, Washington DC

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Application of Microbial Fuel Cells (MFC) for energy recovery from wastewater (WW) is a promising sustainable technology. MFCs are made up of an anode biofilm that maximizes the catalytic activity of bacteria in converting a substrate to CO_2 at the cathode while producing electrical current. WW contains a large amount of chemical energy in the form of organic matter that can be recovered by bacteria. In the US, 44 trillion l of WW is produced annually with an energy content equaling 1-2% of the annual total electricity consumption in the US. The potential for MFC implementation can be quantified by availability of biodegradable material shown as Biological Oxygen Demand (BOD), Total Suspended Solids (TSS) and the presence of exoelectrogenic bacteria. During the biological treatment process, aeration is applied to oxidatively remove organic matter with simultaneous growth of microorganisms thus requiring high-energy usage. The objectives look to explore MFC applications for specific sources of wastewater that will reduce oxygen usage, enable energy recovery and identifying indigenous exoelectrogenic bacteria. This will be done by studying four specialized high BOD WW streams: primary effluent, centrate, digestate and belt press filtrate. Samples were collected monthly, BOD and TSS were determined and DNA was extracted and sequenced with Illumina. DNA sequencing results demonstrated the existence of Delta-and Gamma Proteobacteria that are related to known exoeletrogenic bacteria (such as Geobacter and Shewannella) shown in other MFC studies. Preliminary tests in batch MFCs show a correlation between BOD concentration and current generation. Substrates with higher BOD content such as digestate (BOD: 600 mg/L) seem more efficient for current generation than lower BOD concentration streams (primary effluent). Further research will focus on the relationship between current generation potential, organic matter content and the diversity of the exoelectrogenic bacterial community in pilot scale MFC reactors located at Blue Plains.

ENVR 246

Identifying data gaps in understanding feasibility of reuse of nanoparticles-containing wastewater in aquaculture

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Nanoparticles (NPs) have been reported to occur in wastewater effluent and also in the environment. As there is a recent trend towards using wastewater effluent for aquaculture, it is important to know the impacts of nanoparticles in wastewater effluent. The objective of this study was to identify data gaps in understanding the feasibility of reuse of nanoparticles-containing wastewater in aquaculture. To understand data gaps, the following scenario was considered: nanoparticle-containing wastewater effluent is being used for growing fish where fish tissue bio-accumulates nanoparticles and nanoparticles-related ions. Humans consume these fish inadvertently and thus are exposed to these nanoparticles. For assessing risks and understanding knowledge gaps, first a 6-step human health risk assessment framework consisting of hazard identification (parameters: nanoparticles and its associated toxic effects on health), exposure assessment (parameters: nanoparticles concentration in fish; fish consumption/bioaccumulation factor), dose-response assessment (parameters: reference dose or target toxicity dose), risk estimation (metric: hazard quotient and lifetime incremental risk of cancer) and uncertainty characterization (data uncertainty and variability), and risk communication and management to stakeholders was developed in a spreadsheet environment. Then parameters required at every step were compiled and literature review was conducted to obtain their values. An example calculation was conducted for exposures of silver nanoparticles through consumption of fish. Preliminary risk estimation indicated that risks due to exposures of silver NPs could vary depending on silver NPs concentration as well as silver NPs uptake by human organs from fish consumed. This example calculation illustrated the challenges in estimating health risks from exposures of NPs from fish grown during aquaculture. More efforts are required in reducing data gaps at every step of risk assessment in order to protect human health from this exposure pathway and utilize the benefit of water re-use for practicing aquaculture.

ENVR 247

Air emission implications of expanded wastewater treatment at coal-fired generators

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Over the past decade, the Environmental Protection Agency (EPA) has promulgated a series of rules targeting emissions of criteria air pollutants, greenhouse gasses, and wastewater discharges from thermal power generators. One rule the EPA has promulgated is the effluent limitation guidelines (ELGs) that mandate stricter treatment of power plant wastewater. The ELGs require that flue gas desulfurization (FGD) wastewater must be treated with chemical precipitation and biological treatment (CPBT) or with zero liquid discharge (ZLD) treatment. Both CPBT and ZLD systems require chemicals and auxiliary electricity, resulting in additional emissions and damages that reduce the benefits of the ELGs. The EPA incorporated additional damages from auxiliary emissions resulting from CPBT treatment in their cost-benefit analysis for the rule, but did not account for damages from

chemical manufacturing and from auxiliary electricity to achieve ZLD. Including these damages may lead to costs for the ELGs in excess of their benefits. We first use process models of CPBT and ZLD treatment to estimate emissions from additional auxiliary electricity demand and chemical manufacturing. We then use an integrated assessment model, AP2, to calculate damages from additional air emissions. We find that CPBT treatment imposes $210 million in damages from increased air emissions. Of these damages, $200 million are from chemical manufacturing and unaccounted for in the EPA's cost-benefit analysis. These damages exceed the health benefits from treating FGD wastewater by an order of magnitude. We estimate that meeting ZLD standards would impose $610 million in damages that are significantly higher than the benefits of ZLD treatment of FGD wastewater. While it makes sense to treat FGD wastewater given the health and environmental benefits, treatment systems should avoid relying on auxiliary electricity or chemicals in order to avoid the unintended consequences of air emission damages associated with water treatment processes.

ENVR 248

Trace element allocation across air pollution control devices in coal fired power plants

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The characterization of trace elements in coal fired power plant (CFPP) wastewater is of vital importance in the design of wastewater treatment systems and in understanding the health and environmental impacts of wastewater discharge. Unfortunately, the lack of water quality monitoring in US CFPPs makes it difficult to estimate the concentration and speciation of trace metals in effluent. This work combines mass balance and process modeling approaches to assess the partitioning and speciation of several trace elements regulated by the Environmental Protection Agency under the Effluent Limitation Guidelines for CFPP wastewater (e.g., mercury, selenium, arsenic, chloride). We use published studies of coal composition, coal combustion, and environmental control technologies (e.g., selective catalytic reduction systems, electrostatic precipitators, fabric filters, and flue gas desulfurization scrubbers) to build the model and account for uncertainty in fuel inputs and process performance. The final model allows users to select coal inputs and process configurations in order to estimate the speciation and concentrations of trace elements in exhaust, ash, and wastewater discharges. We use this model to perform a Monte-Carlo analysis on the plant-level emissions of trace elements at all U.S. CFPPs with a capacity of at least 10MW. These results provide first-order estimates of CFPP wastewater composition that may reduce the need for costly monitoring systems, guide testing of wastewater treatment schemes, and serve as an input into water quality transport models to calculate downstream trace element concentrations.

ENVR 249

Rice uptake of organic arsenic species: Competition with silicon

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Rice (*Oryza sativa* L.) is a staple food crop for billions of people, and its contamination by arsenic from natural sources, industrial pollution and contaminated groundwater constitutes a substantial health risk for many consumers. Under reducing conditions, arsenite is predominant in flooded paddies and enters rice roots through a silicic acid transporter, which has prompted silicon competition studies at field scales. However, less is known about how organic forms of arsenic enter rice. Dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) have been widely found in rice grains, particularly in rice grown in the southern United States. Researchers have suggested that DMA and MMA also enter the rice root via silicic acid transporters. To test the ability of silicic acid to compete with MMA and DMA at relevant concentrations, we performed hydroponic experiments with varying levels of silicic acid additions. MMA was found to exhibit severe toxicity at 5 μM, regardless of silicon treatment. Silicon treatment did not affect plant biomass for control or DMA, although DMA significantly affected grain yield and plants showed symptoms of straight-head disorder. The decrease in grain yield caused by DMA exposure was rescued by increasing silicon concentrations. The addition of silicon was also correlated to lower arsenic concentrations in both the husk and grains. Collectively, this research provides insights into how silicon amendments can decrease rice uptake of organic arsenic species.

ENVR 258

Dealing with dual use: Risk governance in synthetic biology

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Biotechnology is diffusing rapidly and globally. Efficient methods for reading, writing, and editing genetic information, producing genetic diversity, and selecting for traits are becoming widely available. These advances are causing a decoupling of information and material flows, and decreasing knowledge barriers. New communities of practice are gaining the power to act on timescales and geographies that fall outside current systems of oversight. Governments and scientific communities in turn are struggling to appropriately respond. Recent controversies have brought these issues to light: "gain-of-function" research may risk causing the very pandemics it aims to help mitigate; "CRISPR kits" are being sold on Kickstarter; and the development of "gene drives" may drastically alter ecosystems. The lack of suitable mechanisms to assess the benefits and risks of such work has prompted new federal regulations and triggered blunt solutions including scientific and government-lead moratoriums. This presentation examines key assumptions underlying new federal policies that concern the capacity for individuals and institutions to assess safety and security risks in research. It compares institutional processes for risk identification and assessment employed by different organizations, including the multi-university NSF Synthetic Biology Engineering Research Center, the international Genetically Engineered Machine Competition, and the DOE Joint Genomics Institute. Each assigns different roles and responsibilities to researchers and review committees and provides different training and incentives. I will discuss how the design of these processes impacts their responses to risks and ambiguities in existing policies. Lastly, I will discuss how these case studies expose long-standing governance challenges concerning the feasibility, ethics, and outcomes of controlling access to new information and knowledge, as well as how the scientific and policy communities might respond.

ENVR 259

Regulating the unregulatable: Policy considerations for the national security threats posed by advances in genetic engineering

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Recent advancements in genetic engineering hold great promise for areas, such as personalized medicine, environmental protection, and food security. However, those same advancements in biological synthesis are creating significant challenges to US national security. Existing normative frameworks for addressing these challenges are insufficient. A fresh look at policy and regulation development is needed to position the US and international community to remain alert, prepared, and ready to defend against unwanted products of a rapidly evolving technology. The field of synthetic biology is creating new classes of agents based upon nucleic acid sequences and synthetic peptide engineering that greatly increase the list of specific threats and create properties that are dynamic and often unknowable. It is becoming increasingly difficult to predict what an adversary may create, thus undermining our ability to detect--and therefore secure or regulate--products or activities of concern. This presentation will explore the impact of these threats on US national security, framed in the context of a growing, yet still inadequately understood, global problem. It will also discuss challenges associated with synthetic biology and make recommendations for policies and technology development that could help address them. The very nature of synthetic biology is shortening development times and enabling rapid evolution of the field, thus increasing the likelihood of technical surprise, undermining the effectiveness and relevance of existing policies and regulations. Further, the so-called democratization of biotechnology enabled by synthetic biology presents another policy and regulatory challenge. The simplification of complex bioengineering processes through the provision of design rules, instructions, and parts, combined with greater accessibility, lower cost devices, and automation are reducing the costs and necessary skills for genetic manipulation. As a result, aspects of this type of work can be done in more, new, under- or unregulated locations, such as community labs, homes, and the cloud. The transnational synthetic drug trade is also growing. Consequently, our struggle to detect nefarious activities and keep up with technology's advancements with effective regulations presents a serious national security concern, warranting new approaches to policy development and regulation enforcement.

ENVR 323

Mechanism and applications of black carbon-mediated microbial contaminant transformation

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Black carbon has been recognized as an important class of geosorbents and catalysts that control the transport and abiotic redox transformation of organic contaminants. In contrast, the microbiological role of black carbon has been less explored. Black carbon such as biochar is widely suggested as a soil amendment and as a sorbent for remediation, and its effects on microbial transformation of nutrients and contaminants are often speculated, but seldom demonstrated. In this study, we proposed and experimentally demonstrated a mechanism(s) through which black carbon can enable or promote microbial transformation reactions. We will show that the mechanisms explain both laboratory and field results and may allow better design of engineered systems for stormwater treatment and subsurface (bio)remediation. The microbiological roles of black carbon, and the environmental implications and applications of our findings, will be discussed.

ENVR 324

Heavy metal remediation via biologically driven calcium carbonate precipitation

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Contamination of water resources by heavy metals as a result of natural resource and energy extraction is a persistent and ongoing environmental and health challenge that will depend upon innovative methods for effective remediation. Waste streams of concern include leachate from mining and nuclear waste, mine tailings and selenium laden wastewater from flue gas desulfurization processes at coal-fired power plants. Microbially induced calcium carbonate ($CaCO_3$) precipitation (MICP) has been employed previously for applications such as immobilizing radionuclides, enhancing the reliability of subsurface carbon sequestration and manipulating flow and transport in porous media. MICP occurs by way of the microbially catalyzed hydrolysis of urea, which produces alkalinity and increases carbonate saturation. Carbonate minerals precipitated as a result of MICP can co-precipitate other metals, such as cadmium and strontium within the solid phase. This research is an ongoing effort investigating the potential of MICP for co-precipitation of radionuclides (strontium, cesium and barium) and heavy metals (i.e., selenium, zinc, cadmium) for potential remediation of wastes from mining and coal operations. In this research, the ureolytic bacterium *Sporosarcina pasteurii* was cultivated to evaluate ureolysis driven MICP for removal of mixed radionuclides and heavy metals. Both synthetic groundwater containing contaminants and wastewater from a coal power plant were used in laboratory tests. Laboratory systems included batch reactors and flat plate porous media reactors that allowed visualization of precipitate formation. Metals in solution were measured by inductively coupled plasma mass spectrometry (ICP-MS) and minerals were analyzed by scanning electron microscopy and x-ray diffraction. In batch studies of mixed contaminants, ureolysis occurred in the presence of all metals tested. Removal of Sr, Ba, Zn and Cd was observed, but Se was not removed via co-precipitation. These studies demonstrate the potential of MICP for remediation of radionuclides and heavy metals based on a microbially driven, chemical precipitation process. Ongoing investigations are underway to evaluate MICP for prevention of leaching and stabilization of mine tailings.

ENVR 325

Microbial response to antimony contamination in severelyantimony-contaminated environments and bioremediation thereof by an onsite field-scale bioreactor

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Antimony (Sb) is a naturally occurring toxic metalloid that has been classified as a suspected carcinogen as well as a priority

pollutant by the US Environmental Protection Agency (USEPA). *In situ* microbial transformations that promote precipitation and immobilization of Sb compounds are promising strategies for remediating of Sb contaminated sediments and waters. Although Sb and arsenic (As) share similar physical, toxicological and chemical properties, studies of Sb biotransformation have been limited, compared to the extensive studies on ecology, genetics, and biochemistry of microbial As cycling. We selected several Sb-contaminated environments including river sediments, mine tailings, and agricultural soils in Dushan, Guizhou Province, Southwest China to study the microbial response to elevated Sb contamination. All the Sb contamination in these environments originated from the same active Sb mine. High-throughput sequencing of 16S rRNA genes revealed a broad diversity of microorganisms in these environments. Different dominant taxonomic groups were dominant in different environments. For example, Fe-metabolizing bacteria (e.g., *Shewanella* spp., *Halomonas* spp., and *Geobacter* spp.) were dominant in river sediments just downstream of the Sb mine while sulfate-reducing bacteria such as *Desulfurivibrio* species thrived in the river sediments downstream of the tailings dump. Sulfur-oxidizing bacteria such as *Thiobacillus* spp. and *Limnobacter* spp. were elevated in abundance in the tailings but some As-metabolizing bacteria such as *Achromobacter*-related and *Methylobacterium*-related bacteria predominated in the contaminated agricultural soils. These observations suggest that the microbial communities are dependent on site-specific (historical) factors. Statistical analyses suggested that, in addition to Sb and its extractable fractions, geochemical factors also significantly structured the overall microbial community, particularly the dominated taxonomic groups. We also investigated passive treatment as a means of remediating Sb contamination. An onsite field-scale bioreactor operated for one year treating runoff from this active Sb mine removed more than 90% of total aqueous Sb and Sb(III). Further microbial community analysis indicated that a number of Fe-oxidizing and As-oxidizing bacteria may be responsible for the removal of Sb, suggesting that remediation is due to a combination of biological processes with chemical reactions.

ENVR 326

Effect of phospholipid coating on pyrite oxidation and bacterial communities under simulated acid mine drainage (AMD) conditions

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Acid mine drainage (AMD) is a severe environmental problem impacting streams and rivers in areas with present or past mining activities. AMD-impacted waters have low pH and high metal content. The root cause of AMD is the exposure of mining waste containing high percentage of pyrite (FeS_2) to air, water, and microorganisms. Current remediation schemes include the use of alkaline reagents, such as limestone or caustic soda, to neutralize the acidity, which is costly and personnel intensive. An alternate remediation strategy is based on chemical coating of the pyrite surface to inhibit oxidation and decomposition leading to AMD. Research in our laboratory has showed that specific phospholipids can bind to reactive regions of pyrite to form robust hydrophobic coatings that reduce iron and sulfur oxidation under both abiotic and biotic environments. The objective of this study was the test the effect of phospholipid coating on the biogeochemistry of pyrite oxidation. Experiments were conducted using laboratory columns packed with actual coal mining pyrite-containing overburden/waste rock (OWR), with and without phospholipid treatment. OWR material was initially treated with two types of phospholipids - 1,2-*bis*(10,12-tricosadiynoyl)-*sn*-glycerol-3-phosphocholine and commercial Phospholipon® 80H (phosphatidyl- and lysophosphatidylcholine) - and exposed to flowing pH-neutral water over a 3-year period. Untreated OWR resulted in acidic effluent (pH = 2 - 4.5) with total iron (Fe) and sulfate (SO_4^{2-}) ≥ 20 and ≥ 30 mg/L, respectively. In contrast, phospholipid-treated OWR produced near-neutral effluent (pH ~6.5) with Fe and SO_4^{2-} ≤ 2.0 and 1.6 mg/L, respectively. The prokaryotic communities developing in treated and untreated OWR samples over 3 years were characterized using 16S rDNA metagenomic pyrosequencing. Resulting prokaryotic profiles showed higher abundance of AMD-species - i.e., acidophilic organisms commonly associated with AMD - representing 40 - 45% of total species in untreated samples as compared with phospholipid-treated samples (2.5 - 20% of total species). Moreover, untreated samples contained 20 - 40% of acidophilic iron and/or sulfur oxidizing species - responsible for pyrite oxidation and AMD generation - while treated samples did not contain significant numbers of these species. These results indicate that phospholipid treatment can prevent the development of AMD-like bacteria and the associated generation of AMD-like effluents.

ENVR 327

Sustainable technologies for mine influenced water treatment in different water chemistry

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Mine influenced water (MIW) from abandoned mine sites is a prevailing environmental problem, deteriorating water quality and ecosystems in many areas of the world. Recently, the concept of sustainability has been applied to the mining industry processes, including MIW remediation. MIW has been traditionally been treated using active processes requiring chemicals, energy, and labor. A more practical alternative involves the use of passive technologies, which do not require those additions. But, in order to determine which treatments could be considered sustainable, we need to understand the principles behind each applied technology. In the present study, we evaluated several technologies that use the mechanisms of sorption, precipitation, complexation, chelation, and bioremediation for their sustainability. To highlight the feasibility of MIW bioremediation, we present a case study of mine water treatment using different substrates in sulfate reducing bioreactors with different influent water chemistry. We concluded that bioremediation using sulfate reducing bioreactors can be an important sustainable alternative for MIW treatment, particularly in mine sites that are periodically inaccessible, due to weather or other factors, because these reactors can be implemented as underground trenches in the mine sites operating only by gravity. Crushed crab shells, wood chips, hay, and manure, are among the tested substrates with successful Zn, Cd, Cu, and Mn removal.

ENVR 328

Biofilm covered activated carbon particles enhance bioremediation of polychlorinated biphenyl (PCBs) in sediment

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Removal of polychlorinated biphenyls (PCBs) from contaminated sediments is a priority because of their ability to enter the food chain and due to their toxicity. Commonly adopted remedies include dredging and capping which are associated with challenges including disruption of existing habitat and high cost. While *in situ* microbial degradation of PCBs represents an improvement, previous attempts have failed because of PCB stability, low bioavailability, low abundance and activity of indigenous PCB-degrading microorganisms. The high efficiency of activated carbon (AC) and other porous substrates to quickly adsorb PCBs from sediments has been demonstrated. Co-localizing PCB-degrading microbes onto surfaces of adsorptive particles as biofilm utilization as a delivery system provides a novel approach to address PCB contamination. In this study, biofilm covered adsorbent materials including AC were evaluated for enhancement of PCB dechlorination. Biofilms of anaerobic Dehalobium chlorocoercia, enrichments from wastewater and aerobic Burkholderia xenovorans were formed. Mature biofilms were inoculated into PCB contaminated sediment mesocosms. PCB concentrations and individual congener concentrations were determined by GC. Techniques included: DNA extraction, q- PCR with specific 16S rDNA primers, identification by DHPLC and Illumina sequencing and microscopic analyses with DAPI, PNA-FISH and CLSM. Biofilm formation of DF1, LB400 and enrichments was observed via microscopic techniques. Biofilms inoculated into sediment mesocosms showed a 2-fold increase and enhanced PCB degradation (1.5 chlorines/biphenyl for biofilms (31%) vs. 0.3 chlorines/biphenyl for planktonic inoculum (6%) over 200 days. The bacterial diversity, with up to eight indigenous species of organohalide respiring bacteria, was not affected thus not causing the difference in dechlorination. The application of biofilm covered AC particles enhanced the PCB degradation in the mesocosms. This was likely due to PCB adsorption onto AC ensuring direct contact between the PCB degrading biofilms and the adsorbed PCBs. The factors involved in this mechanism are currently being evaluated.

ENVR 329

Transformation of carbon tetrachloride and chloroform by tetrachloroethene and trichloroethene respiring anaerobic mixed cultures

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Carbon tetrachloride (CT) is a toxic and recalcitrant groundwater contaminant with the potential to form a broad range of transformation products through cometabolism and abiotic processes. These include carbon dioxide (CO_2), chloroform (CF), dichloromethane (DCM), and carbon disulfide (CS_2). CT and CF are also potent inhibitors of microbial processes, which complicates bioremediation strategies at complex contaminated sites. Since abiotic CT transformation readily occurs, it would be possible to spatially separate remediation activities that utilize different strategies. Results will be presented from batch experiments with the Evanite (EV), Victoria Strain (VS) and Point Mugu (PM) anaerobic mixed cultures, which are grown in chemostats and reductively dechlorinate tetrachloroethene (PCE) and trichloroethene (TCE) to ethene (ETH) via dehalorespiration. Cells and supernatant not previously acclimated to CT and CF were harvested from the chemostats and spiked with varying concentrations and an excess of formate (EV and VS) or lactate (PM). CT transformation was complete with 30-40% of the mass accounted for as CF, DCM, and CS_2 with the remainder unknown (likely CO_2). CF was subsequently transformed at slower rates and inhibited H_2 (from formate or lactate) utilization by the culture above approximately 0.3 μM $[CF]_{aq}$. When batch reactors were established with culture that was poisoned with 50 mM sodium azide (NaN_3), CT transformation was unaffected with no CS_2 or DCM formation. This suggests a limited importance to live cells in the reduced medium, suggesting that abiotic processes dominate CT transformation. However, the observed shift in the product distribution shows how the chemical environment can be manipulated to limit the formation of undesirable end products. The complete transformation of CT and CF by a reductively dechlorinating anaerobic mixed culture looks promising for possible remediation of sites contaminated with chlorinated methanes and ethenes.

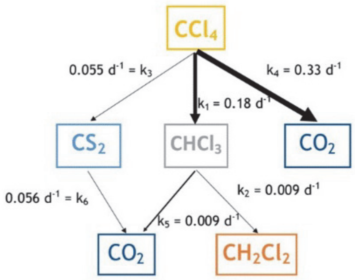

Figure 1: Biochemical pathway representing CT transformation and observed pseudo-first order rates for the Evanite culture (EV-SL) and 2.6 μM $[CT]_{aq}$.

ENVR 330

Enhanced microbial sulfate removal and recovery through a novel electrode-integrated bioreactor

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Excess sulfate in freshwater is a concern due its adverse impacts to wetland ecosystems and possible contributions on methylmercury production and eutrophication. Biological sulfate removal has potential to be very cost effective and versatile in terms of applications. To effectively apply biological process for sulfate remediation, the supply of defined growth substrates to treatment systems is a key requirement that can present technical challenges. In this study, a novel electrode-integrated bioreactor has been developed to provide electron donor and acceptor substrates to biological sulfate treatment in a controlled manner. We determined the efficacy of electrolysis of water and/or iron to enhance microbial sulfate reduction and sulfur recovery from high sulfate in water impacted by mining operations in Northern Minnesota. Particularly, sulfur and iron species produced and consumed was monitored during reactor operation to compare performance under different conditions. Changes in the anaerobic and microaerophilic microbial communities in the bioreactors were also examined by culture-independent methods.

ENVR 331

Electrically conductive particles supporting direct interspecies electron transfer in anaerobic microbial communities

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Electron flow within mixed microbial communities controls the types and rates of chemical reactions. Mediated electron transfer through the generation of soluble intermediates, such as hydrogen and formate, is associated with many environmentally relevant transformations, such as the anaerobic degradation of organic materials. During this process, complex organic substances are converted to methane and carbon dioxide through interspecies hydrogen transfer from syntrophic bacteria to methanogens. Recently direct interspecies electron transfer (DIET), a non-mediated form of syntrophy which enables direct cell-to-cell electron exchange, was discovered. Laboratory co-culture studies have shown that DIET is associated with methane generation via syntrophic fermenters and methanogens. Providing an electrical connection between microorganisms with electrically conductive particles can also promote DIET. However, little information is available on the ability to stimulate DIET in the mixed microbial consortia typical of remediation and treatment processes. Our objective, therefore, was to determine the impact of a variety of conductive materials on two distinct mixed microbial communities. Specifically we focused on the addition of different particle types and loadings on the anaerobic digestion of two high-strength wastewaters: swine wastewater and activated sludge solids. Our initial results showed that methane production rates increased by ~280% when the wastewater was supplemented with conductive particles. Some particles, especially highly porous carbon materials such as biochar, led to a ~97% reduction in volatile fatty acids, likely due to adsorption. For high solids containing wastewater, particles were found to have little to no impact on methane generation rates relative to no-particle controls. These results suggested that conductive materials could promote wastewater treatment efficiency and biogas production, and thus enhance energy recovery during the anaerobic treatment of some high-strength wastewaters. Further work is needed to identify the potential synergistic role that particle-based sorption processes may play in promoting or inhibiting DIET.

ENVR 332

Microbial reductive dechlorination of selected PCB tracker pair congeners in the Hudson and Grasse River sediment microcosms without nutrients amendment

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Polychlorinated biphenyls (PCBs) are classified as persistent organic pollutants. As an environmental friendly remediation approach, microbial reductive PCB dechlorination has been studied for 30 years. However, the knowledge of dechlorination pathways, patterns, rates, extents and the potential of PCB natural attenuation remains limited. The main reasons include: 1) limitations of instrumental analysis make it difficult to qualify and quantify every single possible PCB product, and 2) dechlorination is sediment specific. To overcome these obstacles, in the present study, two mixtures of typical PCB tracker pairs including PCB 5/PCB 12, PCB 64/PCB 71, PCB 105/PCB 114, PCB149/PCB 153/PCB 170 in Mixture 1 and PCB 5/PCB 12, PCB 64/PCB 71, PCB 82/PCB 97/PCB 99, PCB 144/PCB 170 in Mixture 2 were carefully selected. Microcosms were prepared with PCB spiked sediments and natural river water under anaerobic conditions. Duplicate sediment slurry samples were collected at different time points during an one-year incubation period. Congener specific PCB analysis was conducted using GC-ECD and the presence and shifts of putative PCB-related microorganisms were investigated using quantitative polymerase chain reaction (Q-PCR) targeting 16S rRNA genes of putative dechlorinating organisms in the phylum *Chloroflexi*, the *Dehalococcoides* genus, and two PCB degrading organism strains *o*-17 and DF-1. The results showed that 1) spiked PCB congeners were significantly dechlorinated in microcosms with river water in both sediments; 2) different dechlorination rates and preferred pathways were found; 3) shifts of tracker pair ratios were observed in all studied treatments; 4) after an one-year incubation, 21.1±2.4 % (mole percent) and 20.1±8.0 % of spiked PCBs left in the Hudson sediment with Mixture 1 and Mixture 2 respectively, while 10.2±2.4 % and 10.4±1.5% of spiked PCBs left in the Grasse sediment with Mixture 1 and Mixture 2 respectively; 5) PCBs dechlorinated faster and more extensively in the Grasse River sediment than in the Hudson River sediment. Regardless of the congener composition, total mass losses of around 30% and 20% were found in the Grasse and Hudson River sediments, respectively; and 6) putative dechlorinating microorganisms were detected in all samples and significant increases of the target microorganisms were observed when dechlorination started. The findings provided a direct evaluation of natural attenuation as an alternative *in situ* PCB remediation approach.

ENVR 442

Combining high throughput omics tools with targeted DNA, RNA and protein quantification techniques to model respiration rates of specific organohalide contaminants by *Dehalococcoides* strains

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Discovery of pathways for biodegradation relies heavily on first enriching cultures followed by intensive studies to confirm responsible enzymes. Once key “functional” enzymes are discovered, they can serve as highly specific “biomarkers” that can be used to document *in situ* bioremediation is occurring and improve fate and transport modeling. Strains of *Dehalococcoides* (Dhc) are notable bioremediation agents that respire diverse organohalides - chlorinated ethenes, dioxins, biphenyls, benzenes, furans and ethers. We used high-throughput omics surveys to discover general and specific Dhc respiratory biomarkers, then explored use of RNA and protein biomarkers in quantitative rate modeling. For RNA biomarkers we first established empirical correlations between biomarkers’ levels and respiration rates then used those correlations in biokinetic models. For protein biomarkers, we utilized a targeted proteomics method (Multiple Reaction Monitoring) to quantify actual respiratory enzyme levels in cultures and infer *in vivo* Michaelis-Menten-type rate parameters that could be used directly in models.

ENVR 443

Biomarkers for validating 1,4-dioxane biodegradation in contaminated groundwater

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Biodegradation of 1,4-dioxane, a carcinogen and emerging water contaminant, has been reported in several laboratory and field studies, but advanced molecular monitoring tools to support attenuation of 1,4-dioxane by the intrinsic or augmented microbial communities were recently developed. Our previous research identified genes associated with the dioxane monooxygenase (*dxmB*) and an aldehyde dehydrogenase (*aldH*) as suitable biomarkers for 1,4-dioxane in pure cultures as well as in an activated sludge system treating high concentrations of 1,4-dioxane. This study extends our research to evaluate and validate biomarkers as indicators of 1,4-dioxane biodegradation in microcosms established using impacted groundwater and soil as well as in the direct analyses of groundwater contaminated with 1,4-dioxane concentrations ranging from 0 – 300 µg/L. While increased expression of dxmB was related to the concentration of 1,4-dioxane in microcosms, upregulation of both *dxmB* and *aldH* was associated with active 1,4-dioxane biodegradation. Direct analyses of contaminated groundwater resulted in a high correlation between the presence of *dxmB* and *aldH* in monitoring wells with historically decreasing concentrations of 1,4-dioxane suggesting biodegradation was actively occurring at these locations. This study further supports *dxmB* and *aldH* as specific biomarkers to indicate biodegradation of 1,4-dioxane in microcosm treatments and in the direct analysis of contaminated environmental samples.

ENVR 444

Catabolic biomarkers for sensitive and fast quantification of 1,4-dioxane biodegradation activities at impacted aquifers

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1,4-Dioxane (dioxane) contamination has emerged as a compelling global groundwater issue due to its carcinogenic potentials and widespread occurrence at sites historically impacted by chlorinated solvents. *In situ* biological treatments, including Monitored Natural Attenuation (MNA) and Bioremediation, exist as environment-benign and cost-efficient options to manage the large and diluted plume that are commonly formed by dioxane, but their field application is restricted due to the lack of the capability to monitor onsite biodegradation activities. To overcome this technological hurdle, a primer/probe set was developed to target bacterial genes encoding the large hydroxylase subunit of tetrahydrofuran/dioxane monooxygenases that is in charge of initiating dioxane catabolism. The probe set targets conserved regions surrounding the active sites that govern the enzymatic substrate preference, thus enabling detection of multiple dioxane degraders. Quantitative PCR (qPCR) with genomic DNA from reference strains demonstrated the high selectivity and sensitivity of this probe with no detection of false positives using Taqman Chemistry. Microcosm tests prepared with groundwater samples from 16 monitoring wells at five different dioxane-impacted sites showed that enrichment of this catabolic gene was significantly correlated to the amount of dioxane degraded. Notably, a significant correlation was also found between biodegradation rates and the abundance of *thmA/dxmA* genes. In a further study to research the effectiveness of various auxiliary substrates (e.g., 1-butanol and tetrahydrofuran) on stimulating dioxane biodegradation, this set of genetic probes was employed to monitor the dynamics of dioxane metabolizers over the incubation time. Amendments with specific and non-specific substrates to the tetrahydrofuran/dioxane monooxygenases exhibited distinct effects on dioxane removal revealed by bench-scale microcosm tests and *in situ* Biotrap™ assays. All these results corroborated that this novel catabolic biomarkers have significant potential to rapidly assess the performance of natural attenuation and bioremediation of dioxane plumes and provide a scientific basis for regulatory agencies and decision makers to select or reject these cost-efficient biological remediation techniques to mitigate dioxane contamination at impacted fields.

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Understanding the metabolism of 4-OH-2',5'-dichlorobiphenyl by the model plant *Arabidopsis thaliana* using whole-genome expression microarrays

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Higher plants have been shown to contribute to PCB remediation, as they are capable to take up and partially metabolize lesser-chlorinated PCBs - a process referred to as *phytoremediation*. The metabolism of PCBs in higher plants frequently begins by their oxidation into hydroxylated PCBs (OH-PCBs), which may exhibit higher toxicity than the parent PCB and are increasingly considered as a new class of environmental contaminants. The objective of this study was to understand further the metabolism of 2,5-dichlorobiphenyl (2,5-DCB) and its primary OH-metabolite, 4-OH-2',5'-DCB, in the model plant, *Arabidopsis thaliana*. The transcriptomic response of exposed plants was investigated using whole-genome microarrays (Affymetrix). Exposure to sub-lethal concentration of 2,5-DCB (50 mg/L) and 4-OH-2',5'-DCB (5 mg/L) resulted in significant up-regulation of 210 and 22 genes, respectively, and down-regulation of 510 and 83 genes, respectively. A large proportion of differentially-expressed genes were found to be involved in response to stress, and biotic and abiotic stimuli, reflecting the deleterious effects of both compounds on plants. Many genes involved in response to oxidative stress, including catalases, peroxidases, and superoxide dismutases, were overexpressed, suggesting that these toxicants act on plants through generation of reactive oxygen species (ROS). Exposure to 2,5-DCB and 4-OH-2',5'-DCB also resulted in upregulation of genes potentially involved in metabolic transformation of PCBs, including cytochrome P-450 monooxygenases, glutathione *S*-transferases, and glycosyl transferases, suggesting a detoxification mechanism following the traditional sequence, activation (phase I), conjugation (phase II), and sequestration/excretion (phase III). Although distinct expression patterns developed upon plant exposure to 2,5-DCB and 4-OH-2',5'-DCB, a significant overlap of differentially-expressed genes was observed between the two treatments, suggesting either that the two compounds – due to their structural similarities – induced similar genes, or that 2,5-DCB was partially transformed *in vivo* into 4-OH-2',5'-DCB, resulting in a gene expression profile similar to the one developing upon direct exposure to 4-OH-2',5'-DCB. This study illustrates the potential of high-throughput gene expression analysis for understanding better the fate of environmental contaminants.

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Micropollutant biotransformation in activated sludge: Exploring linkages between observed reaction types and microbial community characteristics

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For the majority of wastewater-born, polar micropollutants, biodegradation by microorganisms in activated sludge is the predominant removal pathway. This process is difficult to predict since it depends as much on the composition and activity of the microbial community as on the molecular structure. In our work, we seek to gain a more mechanistic understanding by relating the micropollutant biotransformation capacity of different microbial communities to their functional features. In the present study, a series of six sequencing batch reactors was operated at solids retention times (SRTs) between 1 and 15 days. The six microbial communities were exposed to a mixture of 59 polar organic micropollutants, comprising different chemical classes and types of hypothesized main biotransformation reactions. Rate constants were determined from concentration-time series measured with liquid chromatography coupled to high-resolution mass spectrometry. Complementarily, the oxygen uptake rate (OUR) was monitored and measurements of ammonia, nitrate and nitrite were conducted. Whereas we can confirm previous findings that higher SRTs lead to overall higher micropollutant biotransformation, our results show that trends for individual compounds and for specific classes of compounds significantly differ from this average trend. The group of p-aminobenzenesulfonamides, for instance, showed a decrease of transformation rate constants with SRT and exhibits a strong correlation with OUR instead. Also, aliphatic thioethers (for which we suspect oxidation at the S atom) showed a distinct trend with fastest biotransformation at intermediate SRTs of 7 or 10 days. Additionally, 16S rRNA amplicon sequencing was recently performed, allowing us to include measures of biodiversity and taxonomic composition in our discussion. We will apply different multivariate statistical methods to this extensive dataset of biotransformation rate constants, observed biotransformation products and microbial community parameters to uncover the relative roles that different microbial community functional features play in the removal of specific classes of micropollutants in activated sludge.

ENVR 447

Novel oxygenase detoxifies benzalkonium chlorides in the environment

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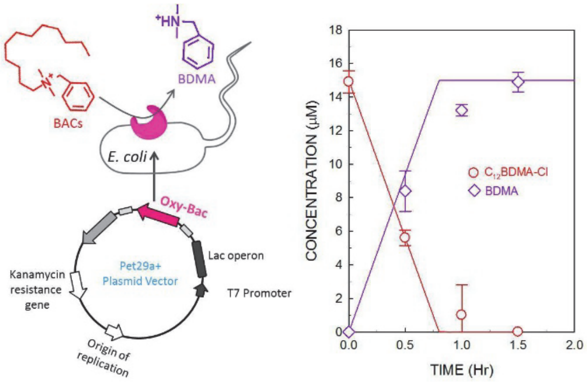
Benzalkonium chlorides (BACs) are emerging pollutants of concern due to their implications on both human and environmental health. Although biodegradation of BACs by various strains of *Pseudomonas* spp. demonstrated, genes and enzymes involved in the biotransformation is not extensively elucidated. In this study, in order to determine the specific genetic markers of BAC degradation, four phenotypes of *Pseudomonas sp.* BIOMIG1 isolated from sewage that can do BAC mineralization ($\text{BIOMIG1}^{\text{BAC}}$), BAC to benzyldimethyl amine (BDMA) transformation ($\text{BIOMIG1}^{\text{BDMA}}$) or only BDMA degradation ($\text{BIOMIG1}^{\text{BD}}$) were isolated along with a phenotype that cannot degrade BACs ($\text{BIOMIG1}^{\text{N}}$). DNA of the phenotypes was extracted and shotgun sequenced using the Illumina HiSeq2000 platform. A gene encoding a Rieske-type oxygenase (Oxy-Bac) was identified by comparing the genomes of these four phenotypes. A phylogenetic analysis demonstrated that *Oxy-Bac* clustered with homolog oxygenases involved in the degradation of naturally occurring QACs like carnitine and stachydrine, however had only 26% aminoacid similarity to its closest biochemically characterized homolog. A plasmid containing Oxy-Bac gene was constructed and inserted into an *E. coli*. *E. coli* having the *Oxy-Bac* containing plasmid could transform BAC to BDMA with up to 90% efficiency and a rate of 13 μM/hour whereas E. coli that did not have *Oxy-Bac* could not. Our results suggest that Oxy-Bac is a novel oxygenase catalyzing dealkylation of BACs, thus highly important for the fate of BACs in the environment.



Biotransformation of BACs by an E.coli with a plasmid containing Oxy-Bac

ENVR 448

Differential sensitivity of wetland-derived nitrogen cycling microorganisms to copper nanoparticles

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Metallic nanoparticles (NPs), the most abundant nanomaterials in consumer and industrial products, are the most probable class to enter the environment. In this study, wetland-derived microcosms were incubated with copper nanoparticles (Cu-NP) and ionic $CuCl_2$ to investigate acute (10 days) and chronic (100 days) exposure towards nitrogen cycling microorganisms. The microbial ecology of wetlands play a crucial role in balancing nitrogen in pristine environments as well as in areas impacted by high nutrient loads (e.g., at wastewater effluent discharges). Gene abundance and expression changes were monitored using the GeoChip 5.0 high throughput functional gene microarray and metatranscriptomic shotgun sequencing (RNA-seq), respectively. After 10 days, the Cu-NP impacted microbial communities experienced structural shifts within microorganisms associated with dissimilatory nitrogen reduction accompanied by lower nitrate removal as compared to the unexposed controls. By day 100, these differences were largely resolved and nitrate removal was similar to the unexposed control. Furthermore, the Cu-NP exposed microcosms tolerated copper and were more resilient and adaptive than the unexposed controls based on the abundance and expression of other functions, including electron transfer, metal homeostasis, and stress response. These findings suggest sudden influxes of Cu-NPs into wetland systems may impair nitrogen removal initially, but long-term microbial shifts and functional redundancy would promote the net flux of total nitrogen out of the wetlands.

ENVR 510

Biotransformation and biodegradation of insensitive munitions compounds in soil

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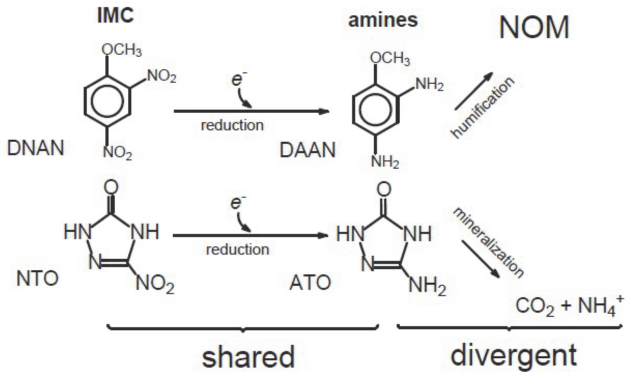
Two new insensitive munitions compounds (IMC), 2,4-dinitroanisole (DNAN) and 3-nitro-1,2,4-triazole-5-one (NTO), are replacing conventional explosive formulation ingredients. Yet little is known about the environmental fate of these IMC. Both compounds are readily reduced under anaerobic conditions to amine-containing daughter products such as 2-methoxy-5-nitroaniline (MENA) and 2,4-diaminoanisole (DAAN) from DNAN, and 3-amino-1,2,4-triazole-5-one (ATO) from NTO. Unfortunately, the toxicity of some daughter products is similar and, in some cases higher, compared with IMC. Thus, there is a great need to understand and predict processes in soil that lead to the conversion of IMC daughter products to environmentally safe end-points. The amine daughter products of DNAN can become irreversibly covalently bound with the soil natural organic matter (NOM), however, the geochemical conditions promoting this transformation are not well understood. ATO, on the other hand, can be completely mineralized by biodegradation provided that suitable microorganisms are present. DNAN and ATO share a common pathway in soil in that both are readily reduced to the amine-containing daughter products. However, their pathways diverge when considering the fate of the daughter products. Based on ^{14}C-labelled DNAN experiments, intermediates become incorporated into the insoluble fraction of humus (humin). Novel reactions occurring under strictly anaerobic conditions contribute to covalent coupling of intermediates forming azolinked dimers. The daughter product of NTO (ATO) on the other hand was observed to become mineralized by microorganisms under aerobic conditions yielding CO_2 and NH_4^+, the latter becomes nitrified to NO_3^-. Clone libraries of ATO-degrading enrichment cultures has provided some insight on the microorganisms involved. The results taken as a whole indicate that a sequence of reductive and oxidative (or nucleophilic substitution) events can convert IMC to environmentally benign end products either inert humus or mineralized C and N compounds.



Shared and divergent pathways of NTO and DNAN (bio)transformation in soil

ENVR 511

Biotransformation and inhibitory effect of furanic and phenolic compounds in the anode of a microbial electrolysis cell (MEC)

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Lignocellulosic biomass has been increasingly used as a feedstock for biofuel production. Pretreatment of lignocellulosics results in the formation of furanic and phenolic compounds, which are known inhibitors to H_2- and ethanol-producing bacteria, and represent a challenge in biorefinery wastewater treatment. Bioelectrochemical systems have recently shown to be an alternative method to convert problematic furanic and phenolic byproducts in the bioanode to electricity (MFC) or H_2 (MEC). This study assessed the biotransformation pathways and inhibitory effect of two representative furanic (furfural, FF; 5-hydroxymethylfurfural, HMF) and three phenolic compounds (syringic acid, SA; vanillic acid, VA; 4-hydroxybenzoic acid, HBA) in a MEC bioanode. Biotransformation of the five compounds occurred via fermentation, resulting in the production of acetate, which became the main electron donor for exoelectrogenesis. Based on the amount of electron equivalents of the five parent compounds and the measured current, the extent of transformation was estimated to be higher for the furanic compounds (67% of FF and 64% of HMF) than for the phenolic compounds (50% of SA, 14% of VA and 10% of HBA). The transformation of the phenolic compounds was via a sequence of demethylation and decarboxylation reactions, based on the structure of products, verified by LC/MS-MS. Catechol and phenol were persistent transformation products of VA and HBA, respectively. All five parent compounds were inhibitory to exoelectrogens (IC_{50} = 1.9 - 3.0 g/L). Individual, non-inhibitory concentrations of the five compounds, when in mixture, resulted in severe inhibition. Microbial bioanode community analysis using next generation sequencing of the 16S rRNA gene, showed the presence of known degraders of furanic and phenolic compounds, fermentative bacteria, and exoelectrogens, in potential syntrophic partnerships. This study advances our understanding of the biotransformation potential and inhibitory effect of furanic and phenolic compounds under redox conditions relevant to MEC bioanodes.

ENVR 512

Microbial transformation of tetracycline and sulfonamide antibiotics

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Certain microbes can transform antibiotics in the environment. However, little is known about the identity of these microbes and their antibiotic biotransformation processes. The objectives of this study were to (1) isolate bacterial strains capable of transforming antibiotics, (2) determine the biotransformation kinetics of antibiotics, (3) characterize the effects of background carbons on the biotransformation kinetics, and (4) identify and characterize the biotransformation products under various environmental conditions. We isolated one bacterial strain that is capable of transforming tetracycline (i.e., *Stenotrophomonas maltophilia* strain DT1) and enriched one mixed culture that is capable of degrading several sulfonamide compounds. Based on the 16S rRNA gene sequence, the mixed culture was primarily made of members of the genera *Brevibacterium* and *Castellaniella.* Biotransformation rates were measured under various environmental conditions, such as pH, temperature, and background carbon composition. The kinetics of tetracycline biotransformation can be described by the difference of two first-order reactions: overall degradation (hydrolysis plus biotransformation) and hydrolysis. The kinetics of sulfadiazine biotransformation can be described using a mirrored logistic function. The identity and antimicrobial potency of the biotransformation products were determined. For tetracycline, a potential biotransformation pathway was proposed that included sequential removal of N-methyl, carbonyl, and amine function groups. The tetracycline biotransformation product(s) had lower antimicrobial potency than the parent compounds. For sulfadiazine, 2-aminopyrimidine (2-AP) was identified as the primary biotransformation product and 4-aminobenzenesulfonate, the remaining structure after the cleavage of 2-AP from sulfadiazine, was confirmed to not be a biotransformation product. Results from this study can lead to better estimation of the fate and transport of antibiotics in the environment and has the potential to be utilized in designing engineering processes to remove tetracycline and sulfonamide antibiotics from water and soil.

ENVR 513

Aerobic and anaerobic biotransformation of N-ethyl perfluorooctane sulfonamide (N-EtFOSA) in soil from a constructed wetland

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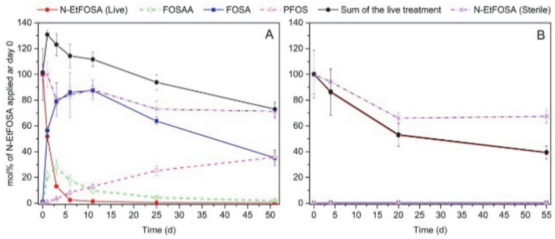
The aim of this work was to conduct a microcosm study to investigate the aerobic and anaerobic biotransformation of N-ethyl perfluorooctane sulfonamide (N-EtFOSA). Slurry was collected from rhizosphere of *Typha angustifolia* in a reed bed of a constructed wetland facility in Singapore. The incubation times for aerobic and anaerobic biotransformation studies were 90 and 180 days respectively. Microbial community in live treated slurry and live control bottles were analyzed at the end of the incubation periods to look into the effect of N-EtFOSA on microbes.

In the aerobic biotransformation study, N-EtFOSA was rapidly transformed ($T_{1/2} < 2$ d) and perfluorooctane sulfonic acid (PFOS) was formed steadily over time, accounting for 35 mol% of initially applied N-EtFOSA by day 51 (Figure 1A). The apparent decrease of N-EtFOSA in the sterile control of anaerobic study (Figure 1B) may be due to abiotic transformation or incomplete extraction due to irreversible sorption. Compared to the sterile control, N-EtFOSA in the live treated bottles of anaerobic study decreased more significantly, making it reasonable to speculate that part of N-EtFOSA was biologically transformed with relatively slow rate in comparison with aerobic transformation. The three aerobic biotransformation products including perfluorooctane sulfonamide acetic acid (FOSAA), perfluorooctane sulfonamide (FOSA) and PFOS, were not detected in the anaerobic system. There may exist unknown intermediates in anaerobic system and further study is required to identify them.



Molar yields of individual biotransformation products during N-EtFOSA aerobic biotransformation (A) and anaerobic biotransformation (B)

ENVR 514

Effects of residual antibiotics in groundwater on survival and pathogenicity of *Salmonella*

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A complex transposon library of *Salmonella* was generated and exposed to several groundwater conditions with residual antibiotics to identify changes in pathogen fitness and survival under these conditions. The transposon library was monitored using a novel screening strategy and analyzed by high-density microarrays. Representative concentrations of amoxicillin, tetracycline, and a cocktail of antibiotics were tested. Antibiotic susceptibility analysis and pathogenicity of *Salmonella* were determined by using human epithelial cells *in vitro* and nematodes *in vivo*. The results showed that the similar sets of housekeeping genes are required for survival among the environmental conditions tested. These genes were generally involved in universal stress response regulation, carbon starvation and source depletion, osmotic shock, sugar uptake, and cell division. Tested *Salmonella* cells remained viable for long periods of exposure to antibiotics containing groundwater, yet viable-but-nonculturable-state (VBNC) was also observed among some of the isolates.

ENVR 532

Interaction forces between microalgae cells and membrane surface based on XDLVO theory in algae harvesting using axial vibration membrane

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In recent years, fossil fuel depletion and food security have aroused people’s concern. Many studies have shown that microalgae can be destined to biofuel and food. However, there are still many challenges in large scale algae harvesting. Membrane filtration in microalgae harvesting not only can recover microalgae, but also yield clean effluent water. Now, more and more researchers have studied how to make better use of the membrane technology to harvest microalgae. But this technique has some problems to be solved, and the primal challenge is membrane fouling. The axial vibration membrane (AVM) can effectively control membrane fouling by increasing vibration frequency. In addition, AVM can also improve the membrane flux in algae harvesting.

In membrane filtration, whether microalgae cells deposit on membrane surface is largely determined by the Lewis acid-base force (F_{AB}), Lifshitz-van der Waals force (F_{LW}), and electrostatic double layer force (F_{EL}), i.e., “extended Derjaguin, Landau, Verwey, Overbeek” (XDLVO) forces (F_{XDLVO}), inertial lift force (F_{IL}) and permeate drag force (F_D). The interaction forces between algae cells and membrane surface are computed to reveal the mechanism how AVM controls microalgae depositing on membrane. Vibration frequency has a significant influence on microalgae cells deposition on membrane surface, consequently affecting the membrane fouling process. Increasing frequency can improve F_{IL}, which can enhance the repulsive force between microalgae and membrane, and thus prevent algae cells from approaching membrane surface. In fact, at a high frequency the total interaction force is a long-range repulsive force, which can prevent microalgae from depositing on membrane. With the decrease of vibration frequency the inertial lift force reduces, and the interaction force presents a long-range attractive that will draw more microalgae cells to approach membrane. Therefore, increasing frequency can reduce membrane fouling and improve membrane flux in microalgae harvesting.

ENVR 533

Effect of ozonization on biochar and its organic compounds

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Biochar is a carbon-rich solid product from biomass pyrolysis, which may be used as a soil amendment to help retain soil water and nutrients for enhanced crop productivity, while sequestering carbon to help control climate change. Since conventional biochars often have quite limited cation exchange capacity (CEC), and some of them may contain potentially toxic dissolvable organic compounds (DOC) that could lead to even undesirable effects in crop fields, they may not be entirely suitable for use as soil amendment. To achieve this mission, advanced clean hydrophilic biochar with higher CEC value needs to be developed to better retain soil water and nutrients, such as ammonium and potassium cations. This presentation reports our latest work on biochar ozonization and its effect on biochar properties and on the associated water-extractable organic compounds. In this study, pinewood-derived biochar materials (pine 400: pine wood pyrolyzed at 400 °C) were treated with various dose of ozone (no ozone treatment, 30-minute, 60-minute, and 90-minute ozone treatments were performed). Biochar properties including pH, CEC and water-holding field capacity were measured. Biochar columns were made for water filtration study. Biochar water-extractable organic compounds were collected from biochar filtration columns by running water over biochar treated with different levels of ozonization. The filtrate from each of the biochar samples was tested for DOC concentration. Then, algal bioassays with varying concentrations of filtrate DOC (0, 50 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm) from each biochar sample were performed using multi-well plates. The toxicity effect of biochar DOC will be assessed by measuring the rates of photosynthetic oxygen evolution and respiration with a Clark-type oxygen electrode system, and by measuring algal chlorophyll fluorescence induction curve in algal cells.

ENVR 534

Nutrient cycling in arid river corridors: Advancing the food-energy-water nexus by closing nutrient loops

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Closing nutrient loops in terrestrial and aquatic ecosystems is integral to achieve resource security in the food-energy-water (FEW) nexus. We performed multiyear (2005-08), monthly sampling of instream dissolved inorganic nutrient concentrations (NH_4-N, NO_3-N, soluble reactive phosphorus - SRP) along an ~ 300 km arid land river corridor (Rio Grande, NM, USA) and generated nutrient budgets to investigate how the net source/sink behavior of wastewater sources and irrigated agriculture can be holistically managed to improve water quality and close nutrient loops. Treated wastewater on average contributed over 90% of the instream dissolved inorganic nutrients (99 kg/day NH_4-N, 1224 kg/day NO_3-N, 656 kg/day SRP). During growing seasons, the irrigation network downstream of wastewater outfalls retained on average 35% of NO_3-N and 33% of SRP inputs, with maximum retention exceeding 60% and 80% of NO_3-N and SRP inputs, respectively. Nutrient retention in the irrigation network and instream processing together limited nutrient export from the watershed during growing seasons, with total retention of 33-99% of NO_3-N inputs and 45-99% of SRP inputs. From the results of our synoptic analysis of the Rio Grande, we identify tradeoffs associated with wastewater reuse for agriculture within the scope of the FEW nexus and propose strategies for closing nutrient loops in arid land river corridors globally.

ENVR 535

Water quality and public health: Role of wastewater

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The quest to sustain modern civilization and food security amid a world population explosion has resulted in unprecedented levels of water contamination from anthropogenic sources. Also, natural phenomena such as climate change are set to worsen the already grave situation. Public health is under threat from emerging and reemerging water-related illnesses. In developed countries, thriving economies and advances in technology had rendered water-borne illnesses "a-thing-of-the-past". The problem was left for developing countries to deal with - but, not anymore! Water contamination is, once again, a global issue thanks to an increase in pollution in the midst of an increase in demand for water, poor economies, and faltering infrastructure across the globe. Donald Hopkins of the Carter Center once described the situation almost perfectly - "Throughout parts of the developing world, some people work hard "just to get dirty water" for drinking, cooking, and for other personal needs.... Although the overall quality of water is far better, developed countries also face challenges in maintaining drinking water quality standards."[1] Streams of raw wastewater in streets and residential areas are now a common site in some countries, both developed and developing, and water is inevitably getting contaminated by a slew of environmental pollutants. A former EPA administrator once said of the United States drinking water: "For years, people said that America has the cleanest drinking water in the world……That was true 20 years ago. But people don't realize how many new chemicals have emerged and how much more pollution has occurred. If they did, we would see very different attitudes."[2] This manuscript discusses the source and health impacts of water contaminants. The biogeochemistry, speciation, toxicity of selected heavy metal(loid)s, as influenced by wastewater and microplastics, will also be discussed.

ENVR 536

Analysis of ground turmeric samples with a handheld X-ray fluorescence analyzer

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Turmeric powder is acquired from the rhizome of the turmeric plant which is found throughout tropical Asia. The powder is generally used as a spice for cooking or rituals. Twenty samples of turmeric with various orange or yellow hues were purchased from stores in the Detroit Metropolitan area. The locations of where the samples were grown were far more widespread. The samples were analyzed with a handheld X-ray fluorescence (XRF) analyzer using the soil fundamental parameters calibration method that reports results for 37 metals. The concentrations of the metals present in each of the samples were investigated. The most common metals found in some of the samples were potassium, calcium, titanium, iron, manganese, and zinc.

ENVR 539

Photocatalysis of triclosan and triclocarban by tetrapod zinc oxide and nitrogen-doped reduced graphene oxide

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Triclosan and triclocarban are antimicrobial agents that are widely used in personal care products and hospitals. They are environmental contaminants of concern and are currently listed among the top ten identified organic wastewater materials in the US. Removal of these two compounds is challenging due to the two benzene rings and Cl-C bonds of these compounds. Recent studies reported that graphene oxide-based materials, like nitrogen-doped reduced graphene oxide (N-RGO), can effectively sorb as well as promote photocatalytic degradation of contaminants in an environmentalyl friendly approach. Furthermore, some transition metals like zinc oxide (ZnO) have interesting chemical and physical characteristics that might promote efficient degradation for different persistent contaminants. Accordingly, we hypothesized that triclosan and triclocarbon can be degraded under UV irritation using N-RGO or tetrapod ZnO as photocatalysts. Our preliminary results showed that the tetrapod ZnO can effectively dechlorinate triclosan under UV (365 nm) light exposure. Ongoing experiments are to evaluate the efficacy of photocatalytic degradation of these compounds using ZnO and N-RGO under different catalytic conditions, and to determine degradation metabolite, as well as possible degradation mechanisms. Results of this study are expected to offer new treatment options for effective triclosan and triclocarban removal.

ENVR 540

Investigating the photochemical fate of triclosan as a function of water quality parameters

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The increasing environmental risks imposed by the presence of pharmaceutical and personal care products (PPCPs) in environmental waters is of great concern. Certain PPCPs intended for human use are being introduced into aquatic ecosystems from human disposal and wastes. It has been found that sewage treatment plants (STPs) do not remove all PPCP chemicals and, therefore, PPCPs are often found in environmental surface waters. Triclosan (5-chloro-2 (2,4,-dichlorophenoxy)phenol, or TCS) is a common antimicrobial agent that has been found in relatively high concentrations in aquatic ecosystems. The photo-degradation of triclosan has previously been shown to produce dioxin-like products, and the rate of degradation may be altered by various interactions within an aquatic system. The work presented here aims to determine the effect of common water quality parameters on triclosan photo-degradation, including variables such as pH, ionic strength, and the presence of dissolved organic matter. Our preliminary results indicate that increasing ionic strength leads to faster degradation rates of TCS and the presence of dissolved organic matter also accelerates the rate of photo-degradation of TCS in aqueous conditions, likely due to indirect photochemical processes. Here we will discuss the impact of water quality parameters on TCS photochemical degradation.

ENVR 541

Environmental influences and fate of triclosan in a Southeastern Pennsylvania watershed: Sources in the East Branch of the Brandywine Creek

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The presence of a wide range of pharmaceutical and personal care products (PPCPs) as contaminants in the environment is of concern. Triclosan, an antimicrobial agent, is reported to enter surface and stream waters most commonly through waste water treated effluent, agricultural runoff, leach fields, and leaking sewage systems. In this study, the measurement and source determination for triclosan delivery into environmental waters in a rural to suburban watershed is of interest. Samples from twelve locations in the East Branch of the Brandywine Creek (EBBC) for triclosan measurement were collected in June 2014 and extracted for quantification. Collected samples were determined to have triclosan of detectable concentrations in the range of 0.131 to 274 ng/L. A second sampling in the EBBC from the same locations was performed in drier, low discharge conditions in October 2015 with the purpose of narrowing down potential non-municipal sources for triclosan delivery into EBBC waterways. Therefore, the significance of seasonality on triclosan concentrations in the EBBC will be highlighted with the comparison of measurements from different sampling times. With previous triclosan studies having primarily sampled downstream of waste water treatment facilities, sampling sites such as the Beaver Creek tributary above a treatment facility in the EBBC with significantly high levels of triclosan are of interest for source determination. The results of this study focus on the identification of non-traditional sources such as septic systems and small privately-owned sewage treatment facilities as sources of triclosan delivery into EBBC waterways.

ENVR 542

Efficacy of multilevel antimicrobial coating in reducing vancomycin-resistant *Enterococci* in hospital ward

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Vancomycin Resistant Enterococci (VRE) is an important nosocomial pathogen that is becoming more prevalent in the hospital environment. VRE can survive months on inanimate surface to infect susceptible host. This study evaluate the use of a multilevel antimicrobial coating in reducing the viability of VRE in ward environment. The coating material consists of

encapsulated gaseous chlorine dioxide for the "release-killing" properties, while a reactive dyad provided "contact-killing" activity. The use of "anti-adhesion" polymers as encapsulate also prevented the adhesion of VRE on the surface. The study was carried out in isolation rooms for VRE patients at a Hong Kong public hospital. The control group was subjected to rigorous hospital cleaning routine twice daily using approved disinfectants by trained cleaning staffs wearing protective garments. The treatment group was coated once daily with the multilevel antimicrobial coating. A total of 668 samples were taken and analyzed during the 4 weeks study period with the results showing that the treatment group had 80% lower microbial contamination on its surface and 60% less likely to have viable VRE than the control group.

ENVR 543

Photolytic fate of poultry antibiotics in agricultural wastewater

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Antibiotics are regularly used as feed additives in the poultry industry to prevent disease and increase feed efficiency. A significant portion of the ingested antibiotics are excreted unmetabolized in poultry litter. Land application of poultry litter provides a route of entry for antibiotics into the environment. This study examined the photolytic fate of ten antibiotics (*i.e.*, azithromycin, chlortetracycline, ciprofloxacin, norfloxacin, ofloxacin, oxytetracycline, roxarsone, sulfadoxin, sulfamethoxazole, and tylosin) that have been identified in poultry litter. Antibiotic solutions were irradiated at 310 – 410 nm in batch reactors. Since dissolved organic matter (DOM) is known to promote formation of reactive species (*e.g.*, HO*, $^1O_2$, 3-DOM*) during irradiation, DOM from water extracts of poultry litter was used at environmentally relevant concentrations to represent agricultural wastewater matrices in photodegradation experiments. Suwanee River natural organic matter was used as a surrogate DOM standard. At pH 6.8, the fluence-based pseudo-first order rate constants in deionized water ranged from 1.8×10^{-7} to 1.0×10^{-3} cm^2mJ^{-1}, and the formation of reactive species in the presence of DOM enhanced the apparent degradation kinetics of select antibiotics. For example, the steady state concentration of $^1O_2$ increased with DOM (dissolved organic carbon ranging from 0 mg/L to 400 mg/L), thereby increasing the apparent rate constant for photodegradation of roxarsone from 1.8×10^{-7} cm^2mJ^{-1} to 6.0×10^{-7} cm^2mJ^{-1}. This result demonstrates that photolysis of antibiotics occurs in spite of high DOM levels, and can potentially form transformation products that retain antibiotic activity. This result is significant since at high DOM levels, light screening and scavenging mechanisms dominate, and are thought to inhibit photolysis. Ultimately, the study contributes towards understanding the role of photodegradation in removal of antibiotic residues in natural systems.

ENVR 544

Identification of flouroquinolone antibiotics and resistant bacteria in Indian sewage treatment plants

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Antibiotic resistant bacteria are a major concern in almost every country including India. Unused and excess use of antibiotics accumulated in Sewage Treatment Plants (STPs) is considered as a major hub for most of the antibiotic resistant bacteria. This study focused on flouroquinolone (FQ) antibiotics and resistant bacteria in Indian STPs. Analysis of STP samples showed that significant number of bacteria were resistant to three classes of FQ antibiotics (Norfloxacin, Ofloxacin and Ciprofloxacin). Further screening of identified FQ resistant bacteria with other antibiotics showed that they are also multidrug-resistant in nature. Ribosomal DNA sequencing revealed that the resistant bacteria belong to *Enterobacteriaceae* family. To identify the genetic determinant for FQ resistance, we have found that the signature mutations at the quinolone resistant determinant region (QRDR) in Gyrase A gene. Solid phase extraction followed by high performance liquid chromatography detected significant amount of FQ antibiotics in the sample.

ENVR 545

Bioavailability of soil-sorbed tetracycline to *Escherichia coli* bioreporter: Agar diffusion assay and direct microscopic observation

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Environmental residues of antibiotics could be responsible for the ever-increasing antibiotic resistance in microbial communities. A large proportion of antibiotics administered to animals are discharged to manure; land application of animal manure as auxiliary plant fertilizers renders a predominant portion of antibiotics bound to soils/sediments. The bioavailability of soil-sorbed antibiotics to bacteria for development of antibiotic resistance in soil bacterial communities is largely unknown. In this study, a whole-cell *E.coli* bioreporter was employed to detect and quantify the bioavailable fraction of tetracycline sorbed by three surface soils. The results of agar diffusion assay indicate that soil-sorbed tetracycline was desorbed and still bioavailable to activate the antibiotic resistance genes in the *E.coli* bioreporter. Desorption of soil-sorbed tetracycline to agar medium manifested a concentration gradient for tetracycline. The combination effects of uptake of tetracycline by *E.coli* bioreporter and the growth of bacterial numbers along the concentration gradient resulted in the formation of a bright fluorescent ring with the center of the tetracycline-sorbed soils. As for the bacteria residing at the soil surfaces, they could still access the soil-sorbed tetracycline, and the amount of tetracycline associated with the soil determined the extent of the antibiotic response of the bacteria. We will discuss the influence of soil types, incubation time and water contents on the bioavailability of soil-sorbed tetracycline to *E.coli* bioreporter.

ENVR 589

Enhanced dechlorinization of highly chlorinated solvents in groundwater through amendment with hydroxypropyl-beta-cyclodextrin

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In a field study, hydroxypropyl-β-cyclodextrin (HPβCD), a cyclic sugar capable of forming inclusion complexes with hydrophobic organic contaminants (HOCs), was previously investigated for its ability to extract trichloroethylene, 1,1,1-trichloroethane, and dichloroethene from a surficial sandy aquifer. After completion of that effort, a network of wells was used to monitor the fate of the cyclodextrin left within the aquifer along with the levels of the target pollutants and common terminal electron acceptors (TEAs). While dissolved oxygen levels were unaffected because background levels are nearly anaerobic, the distribution of residual HPβCD was found to correlate with an area of depressed TEAs: sulfate (HPβCD > 1mM, avg. = 0.059mM ± 0.015, n=6; HPβCD < 1mM, avg. = 0.0085mM ± 0.009, n=8), and to a lesser extent those of nitrate and iron.

In a series of monitoring rounds, the apparent consumption of this sugar aligned with loss of highly chlorinated solvents TCE and 1,1,1-TCA. During 425 days of observation, cyclodextrin decreased from 214 to 93kg, coinciding with a loss of 1,1,1-TCA (381 to 97g) and TCE (828 to 14g) without a net production of DCE (decrease 22 to 16g). From this work, it is apparent that the presence of the residual CD improved the bioavailability and acted as suitable substrate leading to temporary anaerobic conditions and co-metabolic degradation of highly chlorinated solvents. While these conditions are known to be favorable to degradation of less-chlorinated daughter products, the low-levels of these compounds inhibited confirmation of that process. These observations imply that cyclodextrin has promise as an agent to facilitate the anaerobic conditions and greater availability in the aqueous phase which has been shown to lead to the loss of highly chlorinated solvents in groundwater systems through acting as substrate to create anaerobic conditions and complexing with the solvents.

ENVR 590

Effect of surface treatment on GAC as an electron acceptor in microbial transformation reactions

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Granular activated carbon (GAC) is a common adsorbent in remediation and water/wastewater treatment processes that can facilitate microbial transformation reactions. The goal of this study is to understand how surface functional groups on carbon surfaces affect the ability of bacteria to utilize GAC. Experiments tested three different pure cultures (*Geobacter metallireducens* strain GS-15, *Geobacter sulferreducens* PCA, and *Shewanella oneidensis* MR-1) in solutions containing minimal media, carbon substrate (acetate /lactate) and treated GAC granules (oxidized, non-oxidized, acid-treated, and reduced) as the sole electron acceptor. We monitored the rate and extent of substrate degradation and compared differences in kinetics between different treatments. Afterwards, GAC granules were additionally analyzed for surface area (BET) and external functional groups. These experiments provide a range of governing factors that control the electron-transfer processes between GAC and microbial species and identify optimal surface treatments of GAC for enhanced microbial utilization.

ENVR 591

Extracellular iron reduction by the Gram-positive fermenter *Clostridium beijerincki*

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Gram-positive fermentative bacteria comprise a substantial portion of the microbial diversity in subsurface environments and play an important role in the reduction of uranium or other heavy metal contaminants and iron oxide minerals. Currently, the mechanism of metal reduction by fermentative bacteria is poorly understood. *Clostridium beijerinckii* is an obligate anaerobe that is known to reduce ferric iron during fermentative growth. In this study, we examined the mechanism of iron reduction by *C. beijerinckii* and investigated the extracellular metabolites involved in the electron transfer process. Experiments were conducted to elucidate the physiological and metabolic state of *C. beijerinckii* cells during iron reduction. We applied a metabolomics approach and monitored the extracellular metabolome of *C. beijerinckii* during glucose fermentation. The results indicate that direct contact is not required for solid-phase iron oxide reduction. Extracellular iron reduction occurs mainly when the organism has reached stationary phase. The growth rate and yield were similar in the presence or absence of iron oxides. Analysis of the extracellular metabolites revealed the presence of redox-active dinucleotide molecules in the spent medium. The role of redox-active dinucleotide molecules in extracellular iron reduction by *C. beijerinckii* will be discussed in this presentation.

ENVR 592

Analysis of polychlorinated biphenyls in effluent discharged from a wastewater treatment plant during dry and wet weather periods

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Polychlorinated biphenyls (PCBs) generated from industrial activities are toxic, persistent, and bioaccumulative in the environment, where they, due to their hydrophobicity, can cause reproductive impairment and thus population decline. PCBs can also be found in wastewater (WW) effluent. The amount of PCBS present in the large volume of effluent exceeds the Total Maximum Daily Load (TMDL) despite very low PCB concentrations. In this study, the presence of PCB congeners and associated toxicity were evaluated for dry weather and storm water-effluent for a large WW treatment plant by using three years data from 2013-2015. Effluent samples were collected as composite samples over 24 h during eight dry and wet weather periods (annually) from the main outfall and as grab samples from the bypass outfall. The main outfall discharges the fully treated WW and the bypass outfall discharges partially treated WW during rain events. All 209 PCB congeners were analyzed at a certified laboratory using EPA method 1668A. A heat map was developed to provide information of the PCB congeners at each sampling to improve the understanding of these complex data sets. The data showed differences in PCB congener concentrations during wet and dry weather periods. It was found the storm water-effluent had a higher average chlorination level with 5.01 Cl/biphenyl vs a dry weather-effluent level of 4.22 Cl/biphenyl. Moreover, the bypass outfall had a significantly higher level of the very toxic 12 dioxin-like PCB congeners resulting in a higher total toxicity equivalent concentration (75 pM) vs. 9 fM for dry weather-effluent. In addition, the storm-effluent from the bypass outfall indicated the presence of more of the 12 dioxin-like PCBs. Future research will focus on designing solutions that will bring the amounts of PCBs below the TMDL by combined chemical and microbiological approaches thus evaluating PCB transformation during the treatment processes.

ENVR 710

Kinetic studies of ceria nanocrystals for catalytic dephosphorylation

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An essential element in living systems, phosphorus is crucial for growth, energy storage, and other processes in plants.

Phosphorus is found naturally in soil, but concentrations can fall unacceptably low depending on geography and climate. Commercial phosphorus fertilizers containing orthophosphate are commonly employed to meet the phosphorus demand of many crops, enabling the spread of agriculture in adverse conditions. Today the vast majority (nearly 80%) of phosphorus in modern fertilizers comes from phosphate rock. Although there are smaller deposits around the world, the majority of the Earth's phosphate rock is harvested in the remote Western Sahara. Despite changes in diet and improved agricultural efficiency, peak phosphate rock production is expected to occur before 2040 and supplies will be completely depleted by the end of this century. This will have profound impacts on fertilizer production, and as a direct result, food production for the 10 billion people expected to be alive by 2050. Thus, there is significant interest in sustainably extracting phosphorus from phosphorylated molecules found in biomass via robust nanocatalysts that can function as efficient replacements for temperature- and pH-sensitive enzymatic systems. Here we report on the development of heterogeneous catalytic technologies for dephosphorylation. Cerium oxide (ceria) nanocrystals were synthesized and applied as an "artificial phosphatase" to cleave the phosphate ester bond and generate inorganic phosphorus from biomass feedstocks. This concept was demonstrated on para-nitrophenyl phosphate (p-NPP) as a model of biomolecules containing phosphorus. This model reactant was hydrolyzed to form para-nitrophenol (p-NP) and free phosphate ions. Systematic kinetic studies were performed on ceria nanocrystals of different sizes, morphologies, and surface defects in order to reveal the structure-property relationship of the active sites and to improve the activity and recyclability of the catalysts. The optimized catalysts were then used to dephosphorylated phospholipids and nucleic acids. The released phosphate was ultimately collected by using zeolite adsorbents and regenerated as pure phosphate chemical streams for further applications.

ENVR 713

Levels and distributions of organophosphorus pesticides in agricultural soils from the Yangtze River Delta of China

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Organophosphorus pesticides (OPPs) are used worldwide and pose a great threat to human health. With their persistence, OPPs can persist in the environment for long periods of time due to their frequent application in intensively cultivated areas. It has been reported that continuous and excessive use of OPPs has led to the pollution of several environmental mediums in different parts of the world. However, our understanding of the contamination status of OPPs in soils is generally limited. Thus, the distribution of OPPs in soil is needed to evaluate possible human and ecological risks. In order to characterize the regional-scale spatial distribution of OPPs in agricultural soils from rapidly developing regions, we conducted an extensive survey in the core Yangtze River Delta (YRD) region. We assessed the contamination status of OPPs in agricultural soils, as well as the possible ecological and human health risks. The results showed that the concentrations of Σ9OPPs ranged from <3.0 to 521 ng/g with a mean of 64.7 ng/g and a detection rate of 93% in all samples. Dimethoate was found to be the most abundant compound, followed by methyl parathion, parathion, disulfoton and thionazin. Jiangsu Province showed the highest loadings of OPPs due to the frequent agricultural activities and pesticide factory emissions. The contamination of OPPs is also highly correlated with land-use types. The lower concentrations of OPPs found in vegetable fields could be attributed to their easy photodegradation or hydrolysis in aerobic soils. There was no significant difference in microbial biomass and community structure among soils with different concentrations of OPPs. The hazard indexes (HIs) of OPPs in all soil samples were below 1, suggesting an absence of noncarcinogenic risks of OPPs in the YRD soils. This study provides valuable information for a better understanding of the pollution status of OPPs in agricultural soils and a scientific basis for soil quality assessments.

ENVR 714

Contamination and risk assessment of DDTs in agricultural soils from the Yangtze River Delta of China

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Organochlorine pesticides DDTs were widely used in China because of their high insecticidal efficiency and low cost. In 1983, DDTs were banned for agricultural production in China due to their carcinogenic and endocrine disrupting effects on human health. It has been reported that DDTs residues in both agricultural soils and products have significantly decreased in the past decade. However, these studies generally focused on limited number of samples and/or relatively small sampling area. Few studies have characterized the regional-scale spatial distribution and the associated exposure risks of DDTs in rapidly developing regions, such as the Yangtze River Delta (YRD) in south China. Thus, to evaluate the status and associated ecological and health risk of DDTs in soils, we conducted an extensive survey in the core YRD area. An evenly distributed sampling network composed of 241 sites was schemed to cover a terrestrial area of approximately 45,800 km^2. We assessed the contamination status of DDTs in agricultural soils, as well as their plant accumulation and potential risks.
The results showed that the residues of DDT, DDD and DDE were in the range of <0.2-3500 ng/g (average 63.0 ng/g). The concentrations of DDTs in most soils were low. More than 94% of the soil samples satisfy the less stringent Grade II Limits for DDTs according to the Environmental Quality Standard for Soil (GB-15618−2008). DDTs are mainly located in tillage layer of the soil (0-30 cm), decreased vertically in the soil profile, thus not likely to pose a threat to groundwater. The accumulation of DDTs in vegetables is very limited. DDTs were only detected in three of total twenty vegetable samples, with concentrations at 9%-18% of those in the corresponding soils. Low DDTs residues had no significant effect on soil microbial biomass, community structure and diversity, suggesting that DDTs may not cause significant soil ecological risks. The non-cancer and carcinogenic risks of DDTs to human were evaluated with the methods recommended by the US EPA. Both risks were very low for children and adults. This study provides the basic information to environmental risk management of DDTs pollution using this fast developing region of China as a representative example.

ENVR 715

Atrazine contamination in agricultural soils from the Yangtze River Delta of China and associated health risks

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Atrazine (2-chloro-4-ethylamino-6-ethylamino-1,3,5-triazine) is one of the most widely applied, persistent and toxic herbicides in the world for controlling pre- and post-emergent weeds in crops. Atrazine not only affects the growth of sensitive succeeding crops, but also poses a potential threat to soil ecosystem and environmental health. There is a growing concern about the risks of atrazine to human health including severe irritation of eyes and skin, birth defects, and cancers. The environmental behavior of atrazine and its ecological effects have become an important research topic. This study investigated the spatial distribution and environmental impacts of atrazine concentrations in agricultural soils collected from the Yangtze River Delta (YRD) as an illustrative analysis of rapidly developing regions. The results showed that the concentrations of atrazine in the YRD agricultural soils ranged from <1.0 to 113 ng/g dry weight, with a mean of 5.7 ng/g and a detection rate of 57.7% in all the soils. Relatively high concentrations of atrazine were found in Changxing County of Zhejiang Province. Pesticide factory might be a major source for the elevated levels of atrazine at nearby locations. The contamination of atrazine was closely associated with the land use. The concentrations and detection rates of atrazine were higher in corn fields and mulberry fields than in paddy rice fields. Overall, the concentrations of atrazine in the YRD region were lower than those in North and Northeast China regions due to low application rates. There was no significant difference in compositions of soil microbial phospholipids fatty acids (PLFA) among the areas with different atrazine levels. Soil type and land management practice can have significant impacts on soil microbial communities. Human health risks via exposure to atrazine in soils were estimated according to the methods recommended by the US EPA. Atrazine by itself in all the soil samples imposed very low carcinogenic risks ($<10^{-6}$) and minimal non-cancer risks (hazard index < 1) to adults and children.

ENVR 716

Catalyitc hydrodechlorination of diclofenac on Pd/CeO_2 catalysts

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Diclofenac is a commonly used analgesic and anti-inflammatory drug, which is proven to be harmful to the liver, kidney and gills of rainbow trout. In the present study, palladium catalysts supported on different supports (e.g., Al_2O_3, activated carbon (AC), SiO_2 and CeO_2) were prepared using the impregnation and deposition–precipitation methods. The liquid phase catalytic hydrodechlorination of diclofenac on the catalysts was investigated, and the toxicity of the original and treated diclofenac solutions was evaluated using *Daphnia magna*. Characterization results indicated that the Pd catalyst supported on CeO_2 had a higher Pd dispersion than those supported on Al_2O_3, AC and SiO_2. The binding energy of Pd 3d5/2 in Pd/CeO_2 was higher than Pd/Al_2O_3 with a similar Pd loading amount. Additionally, for Pd/CeO_2 prepared by the deposition–precipitation method the binding energy of Pd 3d5/2 slightly decreased with the Pd loading amount. As for catalytic diclofenac reduction, Pd/SiO_2 exhibited a nearly negligible catalytic activity, whereas diclofenac concentration decreased by 100, 86, and 29% within 50 min of reaction on Pd/CeO_2, Pd/Al_2O_3, and Pd/AC, respectively, indicative of a catalytic activity order of $Pd/CeO_2 > Pd/Al_2O_3 > Pd/AC > Pd/SiO_2$. The hydrodechlorination of diclofenac on Pd/CeO_2 could be well described using the Langmuir–Hinshelwood model. Diclofenac hydrodechlorination processed via a combined stepwise and concerted pathway, and increasing Pd loading amount in Pd/CeO_2 favoured the concerted pathway. In comparison with original diclofenac, catalytic hydrodechlorination of diclofenac led to markedly decreased toxicity to *Daphnia magna*.

ENVR 717

Occurrence and distribution of pharmaceutical compounds in the vadose zone of a wastewater irrigated field in Northern China

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Wastewater irrigation is a common agricultural activity in arid- and semi-arid regions to minimize water scarcity. However, this practice can lead to the accumulation of pharmaceutical compounds in soil and the contamination of the vadose zone. In the present work, we investigated the occurrence of 15 pharmaceuticals belonging to 8 categories in vadose zone in an agricultural field receiving wastewater irrigation. Targeted pharmaceuticals were discovered with concentrations ranging from 0.05 to 124 $\mu g\ kg^{-1}$. Chloramphenicol was found with the highest median concentration of 0.81 $\mu g\ kg^{-1}$, while caffeine with the highest mean concentration of 3.09 $\mu g\ kg^{-1}$. Carbamazepine, caffeine and chloramphenicol were the mostly frequently detected compounds in vadose zone with frequencies over 50%. Vertical distribution of these chemicals were studied to evaluate their penetration throughout the vadose zone. 8 pharmaceuticals were detected in 15 m depth samples and carbamazepine, caffeine, gemfibrozil and chloramphenicol continuously distributed in the soil profile. At the same time, physico-chemical and mineralogical properties were measured for soil core samples. Significant correlations were found between pharmaceutical compounds and soil organic content and the percentage of several clay minerals, respectively. Comparing to groundwater irrigated field, concentrations and detection frequencies of pharmaceuticals in deep layer samples were higher in wastewater irrigated field, suggesting that accumulation and migration of pharmaceutical compounds in vadose zone can be a result of wastewater irrigation. This work provides fundamental data and contributes to the research on migration and risk of pharmaceutical compounds in sub-surface environment.

ENVR 718

Photochemistry of dissolved black carbon released from biochar

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Dissolved black carbon (DBC), the water soluble fraction of black carbon, is an important constitute of the natural dissolved organic matter (DOM) pool. The photochemistry of DBC is expected to be important for understanding its own fate and that of priority pollutants. This study revealed that DBC released from biochar can be one of the more photoactive components of DOM. Under simulated sunlight irradiation, DBC can efficiently generated reactive oxygen species (ROS) including singlet oxygen ($^1O_2$) and superoxide (O_2^-). The apparent quantum yield of $^1O_2$ was 4.07 ± 0.19%, 2−3 fold higher than many well-studied DOM. Carbonyl-containing structures other than aromatic ketones were involved in the singlet oxygen sensitization. The generation of superoxide apparently depended on electron transfer reactions mediated by silica minerals in dissolved BC, in which phenolic structures served as electron donors. Our study also indicated that DBC underwent fast phototransformation and slow mineralization under solar irradiation. After a 169 h simulated sunlight exposure, DBC underwent 56% loss of absorbance at 254 nm, almost complete loss of fluorescence, and 30% mineralization. Solid-state nuclear magnetic resonance data suggested that the photoreactions preferentially degraded aromatic structures and methyl groups, generating $CH_2/CH/C$, and carboxyl/ester/quinone functional groups. The phototransformation of DBC was

mediated by both self-generated ROS (> 25%) and direct photolysis. Photobleaching of dissolved BC decreased its ability to further generate ROS due to lower light absorption.

ENVR 719

Selective sorption removal of phenanthrene by resins from anionic and nonionic surfactant solutions

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Surfactant enhanced remediation (SER) is a promising technology for removal of hydrophobic organic compounds (HOCs) from contaminated soils and groundwater. However, the operation costs of SER are expensive due to the large surfactant dose used in SER and the additional treatment costs of waste water, i.e., surfactant solutions contaminating HOCs. Selective sorption removal of HOCs from surfactant solutions could be a potential technology to recycle surfactant solutions and thus lower the operation costs of SER. Therefore, in this study, we examined sorption of phenanthrene, a nonionic surfactant (TX100) and an anionic surfactant (SDBS) by ten more resins from water, and selected several resins with large sorption capacity of phenanthrene but limited capacity of TX100 and SDBS for selective sorption removal of phenanthrene from surfactant solutions. These selected resins can sorption phenanthrene efficiently with percent removal of more than 95% at added resin dose of 1.0 g/L from surfactant solutions at TX100 and SDBS concentrations up to thousands mg/L, indicating these resins can be used as adsorbents for selective sorption removal of HOCs from surfactant solutions in SER to recycle surfactant solutions and lower the operation costs. Partitioning of phenanthrene into the selected resins is the key mechanism for sorptive removal.

ENVR 740

Metagenomic survey of antibiotic resistance genes in four paired reclaimed and potable water distribution systems

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Reclaimed water is widely used in the US for non-potable purposes. As water reuse infrastructure expands, there is growing interest in understanding the presence of antibiotic resistance genes (ARGs) in reclaimed water, and their potential for regrowth and gene exchange in distribution pipes represents a knowledge gap. The purpose of this study was to conduct a survey of paired reclaimed and potable water distribution systems and to compare the relative abundance of ARGs. Bulk water samples were collected seasonally from the reclaimed and potable water distribution systems of four participating utilities before treatment, at the point of entry to each distribution system, and at five points throughout each system. Biofilm samples were also collected for two of the utilities. Data was collected for physical (pH, temperature, turbidity, dissolved oxygen, and conductivity) and chemical (metals, nutrients, organic carbon, sulfides, and disinfectant residual) properties for bulk water. Microbial ecology was investigated using 16S rRNA gene amplicon sequencing and shotgun metagenomic analyses were used to investigate the relative abundance of ARGs in a subset of samples. Screening against existing ARG databases enabled the identification of over 800 different known ARGs across 40 sequenced samples. To further characterize the abundance of key ARGs across all samples, quantitative polymerase chain reaction was used to quantify five ARGs that are relevant to human health as known genes conferring resistance among human pathogens (*van*A, *qnr*A, *bla*TEM, *tet*D, and the class 1 integron integrase gene *int*I1) as well as 16S rRNA genes. In addition, Gram-positive and Gram-negative isolates from select water samples were tested for resistance to four antibiotics commonly used in clinical procedures to assess the presence of acute human health risk. Preliminary analysis indicates that total ARG abundance increased during distribution in at least three of the four reclaimed systems. Approximately 25% of *E. coli* and *Enterococci* isolates showed high levels resistance to at least one of the antibiotics tested, but it is unclear at this point if there are differences in levels between reclaimed vs. potable water. This study will help identify which factors are most effective for management of microbial concerns in reclaimed water distribution systems, bearing in mind the relevance of non-potable exposures (e.g., aerosol and skin exposures) to the spread of antibiotic resistance.

ENVR 741

Antibiotic-resistant bacteria and genes in drinking water

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Tap water samples were collected from ten locations across London seasonally, between January and November 2015, and analysed for heterotrophic plate counts (HPCs) and the prevalence of antibiotic-resistant bacteria and genes within that population. Selected resistant colonies were identified using API 20NE system. Samples were also analysed using polymerase chain reaction (PCR) to detect the presence of several antibiotic-resistant genes, with quantitative real-time PCR used to quantify the occurrence of four resistance genes in particular (Mph-A, Bla-Tem, Tet-A and aac(6')-Ib). Several antibiotic-resistant colonies were identified at the species level as *Pseudomonas alcaligenes*, *P. flourescens*, *P. luteola*, *Burkholderia cepacia, Delftia acidovorans, Ochrobactrum anthropi*, and *Aeromonas salmonicida*. Several of these are known to be opportunistic pathogens. The HPC counts in the tap water samples ranged from 3 to $6.2x10^2$ CFU/100ml, with 48.4%±20.0 of the HPCs found to be resistant to trimethoprim, 24.5%±18.07 resistant to erythromycin, 24.1%±10.1 resistant to amoxicillin, 21.6%±7.1 resistant to vancomycin, 4.9%±2.1 resistant to ciprofloxacin and 1.4%±1.0 resistant to tetracycline. The resistance of HPC to tetracycline, erythromycin, amoxicillin trimethoprim and ciprofloxacin was consistently higher during the summer period, however there were no statistically significant differences between seasons ($P<0.05$). Vancomycin resistance was significantly higher during the autumn sampling period. PCR results confirmed the presence of each resistant gene in the samples. The relative abundance of resistance genes were as follow: mpha(A)>tet-A>aac(6')-Ib>bla-tem. In addition to the sampling survey, the effectiveness of free chlorine and monochloramine in reducing resistance genes within a multiple drug-resistant *Escherichia coli* in laboratory-grade water was investigated. Initial results suggest that a free chlorine dose of 5 mg/L with 45 minutes contact time at pH 7.4 and room temperature achieves >90% destruction of tet-A and bla-tem genes, with higher doses required to achieve the same level of gene destruction by monochloramine.

ENVR 742

Antibiotics and antibiotic resistance in surface drinking water sources

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Major gaps still remain in our understanding of potential environmental impacts of antibiotics and antibiotic resistance genes. In an earlier phase of the current study, we evaluated the relative importance of agricultural and treated municipal wastewater sources for contributing to antibiotic concentrations and antibiotic resistance levels in a river ecosystem and found that municipal wastewater inputs were consistently the most significant source of both antibiotics and antibiotic resistance genes. Here, surface drinking water sources and tap water samples were examined. Antibiotic concentrations were quantified using LC-MS/MS with online SPE. Cultivable bacteria resistant to antibiotics from four different classes were quantified on antibiotic-amended solid media. Quantitative polymerase chain reaction (qPCR) was used to quantify six resistance genes in addition to a Class I integron. Although no significant antibiotic concentrations or elevated levels of antibiotic resistance were measured in drinking water sources, antibiotic resistance genes were found in finished tap water.

ENVR 743

Fate, transport, and management of antibiotics and antibiotic resistance genes in the agroecosystem

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Antibiotics are broadly used in the livestock industry to prevent and treat animal diseases. A portion of the antibiotics administered are not absorbed by the animals and are often excreted through urine and feces. When animal wastes are stored in livestock waste management structures (e.g., storage ponds and treatment lagoons) and/or applied to croplands, the antibiotic residuals may exert selective pressure on microbes and cause the emergence of antibiotic resistance in bacteria. This talk will look into the fate, transport, and management of antibiotics and antibiotic resistance genes, the genetic materials that confer antibiotic resistance, in the agroecosystem. Special emphasis will be given to the roles that sediment and soil play in the process. First, the levels of antibiotics and antibiotic resistance genes were measured in both the liquid and the sediment phases of typical livestock waste storage and treatment structures. Efforts were made to identify correlations between the levels of antibiotic resistance genes and the concentrations of antibiotics in the management structures. Second, the distribution of antibiotic resistance genes in intracellular and extracellular DNA was quantified for the sediment samples in the livestock waste management structures. Third, by conducting field-scale rainfall simulation experiments, we compared different manure land application methods on the fate and transport of antibiotics and antibiotic resistance genes in manured soils and in surface runoff. Finally, we investigated the effectiveness of narrow grass hedges as a management practice to control the proliferation of antibiotics and antibiotic resistance genes in agricultural runoffs.

ENVR 744

Antibiotic resistance genes in lake sediments in watersheds impacted by agricultural runoff and by treated municipal wastewater

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Antibiotic resistance, and the infections and diseases associated with antibiotic resistant organisms, is becoming an increasing public health risk. Municipal wastewater treatment plants and animal agriculture are known to be reservoirs for resistance and can have large impacts upon downstream aquatic ecosystems. Quantitative polymerase chain reaction was used to quantify antibiotic resistance genes encoding resistance to: aminoglycosides, β-lactamases, chloramphenicols, fluoroquinolones, macrolides, tetracyclines, sulfonamides, vancomycin, quaternary ammonium compounds, cadmium, chromium, cobalt, mercury, nickel, and zinc, as well as multidrug efflux pumps and integrases of Class 1, 2, and 3 integrons in several sediment cores with varying levels of impact from municipal wastewater treatment discharges and runoff from animal agriculture. Cores were collected from four Minnesota lakes with varying levels of impact from municipal wastewater treatment plants and from animal agriculture operations. The results show that the concentrations of these genes have been increasing over time and that they were present at concentrations two orders of magnitude higher in lakes that were more heavily impacted by wastewater treatment discharges compared to the less impacted lakes. This suggests that wastewater treatment plants and animal agriculture have caused an increase in antibiotic resistance genes in aquatic ecosystems.

ENVR 745

Changes in antibiotic resistance gene abundance during wastewater treatment processes

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The prevalence of antibiotic resistant bacteria in wastewater and biosolids poses human health risks, although several thorough and well-developed methods are applied for treatment of the wastewater. The bacteria that possess antibiotic resistance genes (ARG) are often phylogenetically diverse and can therefore be difficult to identify. Bacteria carrying ARG will typically be removed from the aqueous phase during treatment and will thrive in the biosolids fraction throughout the wastewater treatment process. The increased application of antibiotics for treatment of humans as well as applied in agriculture have increased the proliferation of ARG in wastewater due to an evolutionary biological response to the common use of antibiotics. ARG are rapidly inherited by future generations of bacteria and the possibility of gene survival grows stronger. ARG, if ingested by humans due to land application of biosolids, have the potential to hinder any treatment of disease through antibiotics. The objective of this study, was to investigate the existence of ARG throughout the wastewater treatment processes in biosolids samples from an unnamed wastewater treatment plant by use of molecular approach. Biosolids samples were collected throughout the WWTP and DNA was extracted by a MoBio Soil DNA extraction kit. The ARG were detected via PCR and application of three separate ARG primer sets Qint, int1, and int12 that target the class 1 integron int1, which is an integrase gene with a known sequence coding for antibiotic resistance. The presence of the ARG in individual biosolids samples was determined based on the presence/absence of the corresponding PCR product through DNA gel electrophoresis and UV imaging. The results showed that ARG were observed with the primer sets int1 and int2 throughout the solids phase of the WWTP. Based on the results, it was determined that despite a rigorous treatment method in place to eliminate nutrients and pathogenic bacteria from wastewater, ARG are still present in biosolids during all stages of the treatment process.

ENVR 746

Microbial control with polyvalent phages is significantly enhanced by competitive exclusion by pre-exposed phage-production hosts

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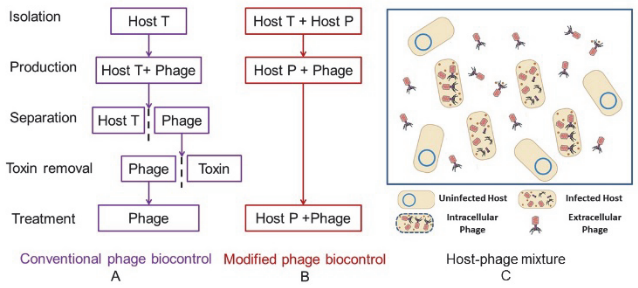
Facing the increasing occurrence of antibiotic-resistant pathogens, the utilization of phages for microbial control is garnering renewed interest. However, the widespread use of phages has been hindered by the rapid development of phage resistance by target bacteria, as well as a reliance on narrow-host-range phages whose production require pathogenic hosts that pose a safety concern. Here we demonstrate that microbial control with polyvalent phages can be significantly enhanced by using coevolved phage-production host for competitive exclusion of the target strain. As a simple example, we exposed *Pseudomonas putida* F1 (host) to polyvalent phage PEf1 and investigated their separate and combined inhibitory effect on β-lactam-resistant *Escherichia coli* NDM-1. Planktonic bacterial challenge tests showed the host-phage mixture (1 pre-exposed *P. putida* and 10 PEf1 per target host) reduced *E. coli* densities by approximately 3.01 orders of magnitude over a 5-day period, compared to 0.54 for PEf1 phage alone. The same strategy (pre-exposed *P. putida* plus PEf1) hindered biofilm formation of *E. coli* by 1.93 orders of magnitude greater than when using PEf1 alone. Overall, this study suggests that pre-exposure of production hosts to polyvalent phages can simplify the phage production process, and enhance long-term suppression of target bacteria, especially when the production hosts are effective competitors.



ENVR 747

Evaluation of various disinfection processes for isolated multidrug resistant bacteria in wastewater treatment plant

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Microbiological quality of water is an important characteristic which relates water quality with epidemiology and human health. In recent years, microbiological quality of water is threatened by the presence and spread of antibiotic resistant bacteria (ARBs) and antibiotic resistant genes (ARGs) in the environment. Large amounts of antibiotics are discharged into municipal wastewater due to incomplete metabolism in humans or disposal of expired antibiotics (Bouki et al., 2013), and as a result, wastewater treatment plants (WWTPs) are favorable reservoirs for proliferation of ARBs. Another concern with respect to the presence of ARBs and ARGs in the wastewater is their effective and efficient removal from sewage, which otherwise finally find their ways into natural environmental compartments. The surfacing spread of ARBs has led to an increasing apprehension about the potential environmental and public health risks. Therefore, further investigation is needed to study the ARBs in WWTPs and their efficient removal mechanisms. In this study, wastewater samples were collected from a local treatment plant before and after UV disinfection to isolate multidrug resistant (MDR) bacterial species. Following incubation of the filtered wastewater samples at 37°C in LB broth containing ampicillin, kanamycin, sulfamethoxazole and tetracycline, 14 different antibiotic resistant bacterial strains were isolated, of which two (*Proteus mirabilis* MDR1 and *Proteus* sp. MDR2) were found to be multidrug resistant with double the MIC_{50} of above antibiotics mixture. In order to estimate the efficiency of disinfection process, various approaches including chlorine, silver nanoparticles (AgNPs), titanium dioxide (TiO_2) nanoparticles, and bacteriophage were carried out on these MDR strains. In addition, disinfection kinetics on these MDRs was studied to understand the optimum CT value for disinfection process. This study provides insight on developing robust approaches to reduce the spread of MDRs in natural ecosystems.

ENVR 748

Estrogen-induced antibiotic resistance

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Drug resistance is a defense mechanism expressed by bacteria to combat intracellular stress caused by toxins, typically antibiotics. These mechanisms include production of enzymes (b-lactamases, for example) and membrane-bound efflux proteins. Here, we show that exposure to low levels (ppt to ppb) of estrogenic emerging environmental contaminants (the plasticizer bisphenol-A (BPA), the detergent byproduct nonylphenol, and the synthetic hormones 17a-ethynylestradiol) induces expression of genes encoding the major multi-drug RND efflux proteins found in *Pseudomonas aeruginosa* (MexAB-OprM), and *E. coli* (AcrAB-TolC), bacteria which have been implicated in antibiotic resistant outbreaks. Efflux genes were differentially expressed in response to low level exposure to estrogen mimics. We concluded that common environmental contaminants cause drug-resistance in pathogenic bacteria beyond that of antibiotics and biocides.

ENVR 749

Strategies to improve triclosan biodegradation in nitrifying activated sludge

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Triclosan is a common antimicrobial agent incorporated in numerous household and personal care products. Environmental occurrence of triclosan has raised a great public health concern because triclosan might promote antibiotic-resistant microorganisms, and be transformed into more toxic compounds like chlorinated dioxins upon exposure to sunlight or UV light. Triclosan is also an endocrine-disrupting compound. Treated wastewater is one major source for environmental triclosan, hence, improving triclosan removal by wastewater treatment processes is the key to minimize the occurrence of triclosan in the environment. In this study, we examined two possible strategies to enhance triclosan removal in nitrifying activated sludge (NAS); these two strategies are increasing ammonia oxidation activity and/or bioaugmenting with a known triclosan degrader *Sphingopyxis* strain KCY1. Ammonia-oxidizing bacteria (AOB) and *Sphingopyxis* strain KCY1, are known to degrade triclosan. AOB play an important role in the first step of nitrification during nitrification of wastewater treatment processes. The strain KCY1 can completely dechlorinate triclosan and remove androgenicity of triclosan. This strain can also degrade triclosan when growing in complex nutrient medium containing with a trace amount of ticlosan. The relationship between the triclosan removal and the changes in strain KCY1, AOB, and amoA gene (encodes the active site of AMO for ammonia oxidation) in the bioreactors were investigated. The quantities of *amoA* gene, AOB and strain KCY1 were also surveyed in selected NAS systems for their triclosan degradation potential. This study offers a now prospect and practical strategy for enhancing triclosan removal in NAS system.

ENVR 758

Mitigation and remediation of organic contaminated soils

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Organic contaminated soils have become a widespread environmental problem, which may lead to a geart threat to quality of agriculture production and human health. Physical, chemical, and biological technologies have been employed for the mitigation and remediation of organic contaminated soils/sites. Mitigation is suggested for slightly-contaminated agricultural soils, and remediation is recommended for heavily-contaminated soils/sites. Consequently, the mitigation and remediation techniques for organic contaminated soils have been developed by regulating the interfacial behavior and bioavailability of organic pollutants.

For assuring the safety of agriculture production in slightly-contaminated soils, transfer and accumulation of organic pollutants need to be mitigated, where the bioavailability of organic pollutants plays a critical role. A speciation scheme for the fractionation of organic pollutants in soils has been designed to conveniently quantify the bioavailable concentrations. In practice, a sequential ultrasonic extraction method is suggested to differentiate organic pollutants into four fractions, including water soluble-, organic acid-soluble-, organically bound-, and residual fractions. The combination of water soluble- and organic acid-soluble fractions corresponds to the bioavailable portions. Furthermore, a new mitigation technique has been developed by utilizing the surfactants or biochar to enhance sorption and fixation ability for organic pollutants to soils. An experiment shows that with this new technique the concentration of polycyclic aromatic hydrocarbons (PAHs) accumulated in plants decreased by two thirds.

For heavily-contaminated soils/sites, surfactant-enhanced bioremediation (SEBR) have been developed. When surfactants are applied to the soils, organic pollutants sorbed on the soils are washed off to the soil solutions, with increased bioavailability. To reveal the remediation mechanism, a pathway linking the gene expression with the physiological phenomena is delineated. Surfactants elevate the expression levels of Δ9 fatty acid desaturase, RHDase and 1H2Nase, then to promote the partition and transmembrane transport and to accelerate the intracellular biodegradation of organic pollutants. To date the SEBR technique has been applied in several remediation projects. The removal rates of PAHs in soils by rhamnolipid-enhanced phytomicrobial remediation increased from ~45% to >70%.

ENVR 759

Biodegradation of 1,4-dioxane in chlorinated solvent mixtures

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Biological degradation of chlorinated solvents and 1,4-dioxane has been previously reported, but the established association of these co-occurring compounds in contaminated environments suggests important considerations for their biodegradation processes. This study investigated the impacts of individual chlorinated solvents and their mixtures on 1,4-dioxane metabolic and cometabolic biodegradation in pure cultures of *Pseudonocardia dioxanivorans* CB1190 and *Mycobacterium austroafricanum* JOB5 as well as in environmental samples. Our results identified that solvents inhibited biodegradation of 1,4-dioxane in the following order: 1,1-DCE > cis-1,2-DCE > TCE > TCA. Inhibition of 1,4-dioxane biodegradation rates by chlorinated solvents was attributed to decreased ATP production and down regulation of dioxane monooxygenase (*dxmB*) and aldehyde dehydrogenase (*aldH*) genes in CB1190 and down regulation of alkane monooxygenase (*alkB2*) in the JOB5 culture. Moreover, increasing concentrations of 1,1-DCE and cis-1,2-DCE increased expression of the *uspA* gene encoding a universal stress protein. *In situ* biological treatment of 1,4-dioxane is being considered for remediating widespread dilute groundwater plumes, so these results have implications for selecting 1,4-dioxane bioremediation strategies where chlorinated solvents are present as co-contaminants.

ENVR 760

Black carbon facilitated dechlorination of DDT and its metabolites in the presence of sulfides

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Although DDT (1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane) has been banned in the United States since 1972, DDT and its metabolites, DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene) and DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene), are often detected in soil and sediments where high concentrations of black carbon resides. Sulfides generated from biological sulfate reduction often co-occur with black carbon at concentrations up to 5 mM in soils and sediments. Sulfides are known to serve as both strong reductants and nucleophiles for contaminant destruction in the aqueous phase. In this study, we evaluated the feasibility of using black carbon to enhance the degradation of DDx (DDT, DDD, and DDE) by sulfides. Our results showed that no transformation of DDx by sulfides occurred in the absence of black carbon. In contrast, 70% of DDT, 40% of DDD, and

30% of DDE were degraded by 5 mM sulfides at pH 7 in 14 days in the presence of black carbon. Chloride was identified to be the product of DDx decay, where the molar ratios of 1:2, 1:1, and 1:1 were found for DDT, DDD, and DDE, respectively. To better understand the reaction mechanisms, further experiments were conducted. Our results suggested that DDT and DDD were reduced by intermediate formed by sulfide sorbed on black carbon; however, DDE degradation was by accepting electron from sulfide through black carbon. This research could lay the groundwork for developing an alternative *in-situ* remediation technique, which not only sequesters contaminants from the aqueous phase but also promotes their destruction under environmentally relevant conditions.

ENVR 761

Enhanced photodegradation of atrazine in the presence of montmorillonite clay and indole-3-acetic acid

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In this study, a new degradation pathway of atrazine was described. Our results show that under simulated sunlight irradiation, the photodegradation of atrazine is significantly enhanced in the presence of natural montmorillonite clay mineral and indole-3-acetic acid (IAA), and the photodegradation process is strongly pH dependent. The reaction is initiated by the adsorption of atrazine and IAA in clay interlayers. Under simulated sunlight (irradiation wavelength >280 nm), the excited IAA generates hydrated electrons, which can quickly react with dissolved oxygen and form hydroxyl radicals. Since proton is necessary to participate in the reaction, the production of hydroxyl radicals is pH dependent and lower pH favors this process in our system. During the reaction, the role of montmorillonite is to provide a matrix with structural negative charges, which can stabilize the radical cation of IAA hence preventing charge recombination. This promotes the release of reactive hydrated electrons and further production of hydroxyl radicals, which can cause the photooxidation of atrazine. *In situ* irradiation time-resolved electron paramagnetic resonance provided direct evidence for the generation of hydroxyl radicals and proved that the presence of natural montmorillonite clay mineral enhances the production of hydroxyl radicals, supporting the proposed degradation mechanism.

ENVR 762

Oxidation of benzo[*a*]pyrene by laccase of *Trametes versicolor* in soil enhanced bound-residue formation and alleviated disturbance to soil bacterial community

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Laccases (EC 1.10.3.2) produced by white-rot fungi are capable of oxidizing benzo[*a*]pyrene rapidly; it was thought that the increase in water solubility resulted from the oxidation may stimulate the sequential benzo[*a*]pyrene mineralization by soil bacteria. However, up to date there was no direct experimental evidence to support this notion. In this study, the effects on mineralization, bound-residue formation and its impact on soil bacterial community composition after ^{14}C-benzo[*a*]pyrene was oxidized by laccase were investigated. The results showed that the metabolites of benzo[*a*]pyrene oxidized by laccase were completely dissipated in soil at the end of incubation, accompanying by trace mineralization (< 0.5%) and substantial bound-residue formation (> 70%). Notably, compared with the parent compound benzo[*a*]pyrene, over 15% of the increase in bound-residue fraction was formed after benzo[*a*]pyrene was transformed by laccase, owing to enhanced chemical binding to humin fraction of soil organic matter (> 10% of increase). During the incubation, the abundance of bacterial 16S rRNA gene and PAHs aromatic ring hydroxylating dioxygenases (PAH-RHDs) gene was not changed remarkable, suggesting that the metabolites of benzo[*a*]pyrene oxidized by laccase have low potential for further utilization and mineralization by soil bacteria. However, the metabolites of benzo[*a*]pyrene oxidized by laccase resulted in a less shift in bacterial community composition, revealing an effect of alleviated disturbance to soil microbial ecology probably resulted from enhanced bound-residue formation. These results suggested that benzo[*a*]pyrene can be detoxified by laccase amendment mainly due to enhanced chemical binding to soil organic matter.

ENVR 763

Adhesion of *Shewanella oneidensis* MR-1 to goethite and its impact on the transformation of enrofloxacin

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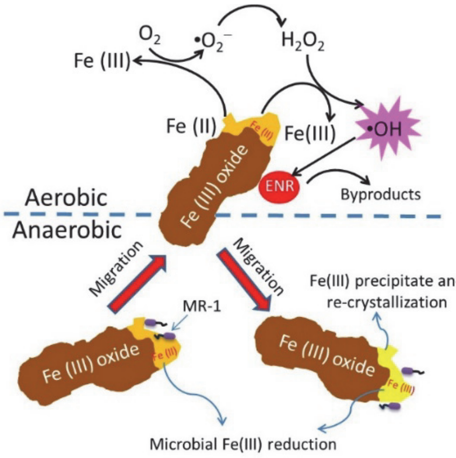
Bacterial adhesion to mineral surfaces is an important but underappreciated process. To decipher the molecular level process and mechanism, the adhesion of *Shewanella oneidensis* MR-1 cells to goethite was investigated using flow-cell attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy coupled with two-dimensional correlation spectroscopy (2D-COS) analysis. The results of FTIR spectra indicate that bacterial phosphate-moieties play an important role in the formation of mono- and bi-dentate inner-sphere complexes, while carboxylic groups on cell surface only have a minor contribution to its adhesion.
On this basis, the rapid aerobic dissipation of enrofloxacin (ENR) was observed by goethite anaerobically reduced by *Shewanella oneidensis* MR-1. The results of spin trap electron spin resonance (ESR) experiments provide evidence that the hydroxyl radicals were generated and involved in the ENR degradation. The formation of hydroxyl radical can be attributed to the effective pathway for electron transfer of Fe(III)/Fe(II) surface complex to surface molecular oxygen. We also investigated the degradation process by using high performance liquid chromatography-quadrupole time-of-flight mass spectrometry on ENR and ENR structural related compounds. Five transformation products of ENR were identified. This study provides an important natural mitigation strategies for in-situ transformation of organic pollutants, since bioreduced goethite can be possible to migrate to the aerobic-anaerobic interfaces. With the hydroxyl radical formation pathway proposed here, the environmental fates of ENR and other antibiotics should be seriously reconsidered.



Schematic diagram of in-situ transformation of enrofloxacin by bioreduced goethite

ENVR 764

Organic pollutant uptake and distribution in plant cuticle: direct observation and diffusion model

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Uptake of organic compounds by plants has been stressed of great importance as the process is involved in territorial and global cycling of organic pollutants and their environmental fate. For the plant pathway, cuticle is believed to be the main interface for the exchange of organic compounds and the first barrier prevents them from uptake into plant organisms. The adsorption and penetration at the cuticle interface was frequent and dynamic. Ascribe to the vertical and lateral heterogeneity of cuticle, the entry of organic pollutants was a speed un-uniform process. During this elaborated process, the role of cuticular components was still a mystery due to the lack of direct measuring method. Moreover, there have been controversies on the role of epicuticular waxes among previous models. Thus it is necessary to revisit the driving force and penetration mechanisms of organic chemicals in plant cuticles. Herein, a three-compartment model was established and in situ method was applied to describe the penetration and distribution characteristics of organic pollutant in plant cuticle. Three main classes of cuticular components (wax, polymeric lipids and polysaccharides) were identified. The preferential barrier effect of CP is highlighted as a superior accumulation reservoir because of its strong sorption capability in a quasi-liquid state. The driving force for the PHE penetration was ascribed to the sharp concentration gradient built up endogenously by cuticular compartments with distinct affinities.

ENVR 765

Comparison of thermal and microwave remediation for a Nigerian oil polluted soil and implications of phytoremediation for photosynthetic efficiency

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For the oil-polluted soil (5% total organic carbon), 78% of the carbon was solvent extractable in dichloromethane/methanol while 95% was thermally labilie being removed by high pressure hydrogen assisted pyrolysis (hydropyrolysis) with the remaining 5% recalcitrant residue being defined as the black or pyrolytic carbon fraction. The biomarker profile of the EOM is typical of Nigerian crude oil from the Niger Delta Region with source inputs were terrigenous from deltaic settings, of Late Upper Cretaceous age and deposited under oxic conditions. Static retorting removed 85% TOC indicating that no solvent extractable remained. This compared with 77% TOC removal by microwave pyrolysis.

An examination of photosynthetic behaviour under hydrocarbon stress was assessed in a 60 day phytoremediation experiment using Mustard. Three spiking levels of 0, 5 and 10% with Nigerian crude oil were used for experiments conducted in triplicate. Also, experiments on the microwave treated soil and its untreated counterpart were introduced conducted for comparison. A decline in the maximum efficiency of Photosystem II photochemistry was observed with increased concentration of hydrocarbon pollution and this fell to zero after 60 days. Complete dryness in the leaves at 10% oil addition may have influenced the optical properties and could be related to observed chlorophyll content. For the measured growth parameters, a 16% increase in produced biomass was observed between the control (0% oil) and the microwave treated soil indicating possible synergistic merits for some remedial technologies.

ENVR 766

Impacts of polycyclic aromatic hydrocarbons (PAHs) emitted by coking industry base on cabbages from neighboring vegetable plots in Shanxi province, north of China

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Dietary intake is one of the main pathways for human exposure to PAHs; it is therefore significant to investigate the impacts of PAHs on agricultural products safety and human health. There were some large coking manufacturers and vegetable planting fields in Shanxi province, north of China. Multimedia samples including ambient air (gaseous and particulate phases), surface soil and cabbage core (edible part) from eight vegetable plots near a local coking industry base were collected in harvest time. Concentration distribution and compositional profile of parent PAHs were determined. The median concentration of atmospheric PAHs near the vegetable plots was up to 301 ng/m^3. Most of air PAHs existed in gaseous phase dominated by 2~3 rings components. Specific ratios of paired isomeric species and principal component analysis preliminarily apportioned the major emission origins of the local PAHs as a mixture of coking, coal/biomass combustion and traffic tail gas. The median concentration of parent PAHs in surface soil from the vegetable plots was 236 ng/g, ranged from 130 to 703 ng/g. Components with 4~6 rings were dominant, and total PAH concentrations in topsoil had a significantly positive correlation with soil organic carbon fractions. The median concentration of parent PAHs in edible part of cabbage was 12.9 ng/g, ranged from 0.9 to 47.6 ng/g. The component profiles in cabbage cores were similar to those in local air with prevailing low molecular weight species. Partial correlation analysis showed contribution from air PAHs transferred into cabbage cores may be greater than that from surface soil PAHs.

ENVR 767

Hexachlorobutadiene (HCBD) in pumpkin seedlings after hydroponic exposure

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Bare researches about the absorption and translocation of Hexachlorobutadiene (HCBD) in plants were carried out. Plants, as the first trophic level in food chains, play an essential role in the biogeochemical cycle of persistent organic chemicals. In this work, the behavior of HCBD in pumpkin seedlings was studied through in-lab hydroponic exposure experiments. Because of the strong tendency of HCBD escaping from water to the air, exposure solution was renewed every day to maintain the exposure concentration. After fifteen days' exposure, pumpkin roots linearly accumulated a great amount of HCBD with concentration of 116286.8 ng/g dry weight (dw). HCBD was taken up by pumpkin roots and translocated to upper shoots at a slight rate. Pumpkin seedlings can also absorb HCBD from the air, which was proved with detection of HCBD in leaves, stems, roots and hydroponic solutions in blank controls at the concentrations range of 3.0-6.6 ng/g, 5.1-14.6 ng/g, 7.0-15.3 ng/g and 0.024-0.049 ng/mL, respectively. No dechlorination products were found. However, other biotransformation products might exist according to the results of mass balance.

ENVR 768

Foliar uptake: An important pathway for the accumulation of Hexabromocyclododecanes in plant leaves

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Based on the previously proposed qualitative frameworks and physicochemical parameters of HBCDs, foliar uptake should be an important pathway for the accumulation of HBCDs in plant aerial parts. Despite its importance, research on the plant uptake of HBCDs is insufficient, with no studies found regarding HBCDs uptake by plant leaves via air. Therefore, a chamber experiment has been designed to distinguish root and foliar uptake pathways of HBCDs in wheat. The EC was composed of a gaseous HBCDs generation system, an air pump, a plant growth chamber (50 cm high, 50 cm diameter, volume 98 L), and an air sampling system (Figure 1). To elucidate which pathway is the predominant route for HBCDs uptake in wheat, we divided potted soils into two groups for each testing EC: one included spiked soil, and the other included unspiked soil. The difference in HBCDs concentrations in the leaf of wheat plants between spiked soil and unspiked soil groups was assumed to be absorbed from soil and subsequently translocated within the plant to the leaf. At the end of the experiment, we calculated the ratios of HBCDs translocation from root to leaf to the total accumulation in wheat leaves (R_t), which ranged in 14.4−29.8% for the three HBCD diastereoisomers, proving that the major portion of the HBCDs in the wheat leaves is directly absorbed from the air. Furthermore, a significant negative linear relationship between their log R_t and log K_{ow} values was found ($R^2 = 0.657$, $p = 0.03$). To our knowledge, the present study is the first report of the relationship between log R_t and log K_{ow} for the three diastereoisomers. This study proved that foliar uptake from air phase is an important pathway for the accumulation of HBCDs in plant aerial parts, and this will increase the exposure of these chemicals to local people through food chain transfer, such as via fruit and vegetable intake.

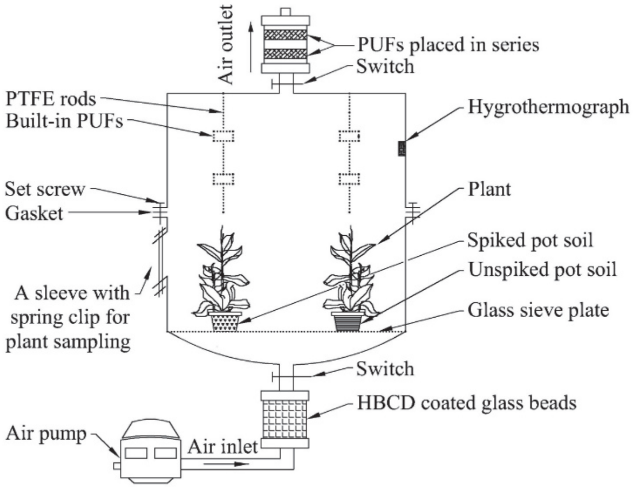

Figure 1. Scheme of the exposure device

ENVR 769

Metachromatic interactions of a dye probe and compounds associated with membrane fouling

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Membrane fouling is one of the central problems in drinking water operations. Model compounds that have been widely used in experiments to explore mechanisms and extent of membrane fouling have been commonly represented by alginate, which is expected to behave similarly to extracellular polymers typical for wastewaters and some drinking water. Yet the intrinsic chemistry of membrane foulants and the representativeness of their properties by alginate or other model compounds remains to be ascertained in much more detail.

This issue was pursued in this study which was based on detailed analysis of interactions of the model compound Toluidine Blue O (TBO) with alginate as well as organic substrates in wastewater or surface water. TBO has been shown to exhibit the consistent and pronounced variation of its absorbance spectra (metachromasy) caused by its aggregation and electrostatically-controlled interaction with charged synthetic and biological polymers. These properties of TBO allow determining whether and how the optical properties of TBO in solution change as a result of its binding with components thought to be representative for membrane fouling processes.

This study quantified interactions between TBO and alginate using the method of differential absorbance and applicable chemometric approaches to ascertain effects of ionic strength and pH on the complexation alginate and TBO. Strong correlations were found to exist between the intensity of the differential spectra or other relevant parameters and the formation of alginate/TBO complexes. The complexation between TBO and alginate was also determined to be strongly affected by ionic strength. Similar effects were observed for wastewater and surface water but more detailed comparison between such effects for environmental systems and, on the other hand, alginate or other model compounds needs to be carried out. These results show that the use of metachromatic probes such as TBO allows exploring properties and model compounds and naturally generated polymers associated with membrane fouling.

ENVR 770

Detection of sartans, related compounds and TPs in real-world aqueous environmental samples using fragment ion search and HRMS

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Polar contaminants like angiotensin II receptor antagonist pharmaceuticals (sartans) are detected in wastewater and surface water as a result of human impact. Like other xenobiotics, they can be metabolised in the body with enzymes such as cytochrome P450 (CYP), UDP-glucuronosyltransferase (UGT), and glutathione S-transferases (GST) which are present in the human liver at high abundance. Once the pharmaceuticals are excreted from the human body both the unchanged fraction of the parent compounds and their human metabolites are discharge into the sewer. Due to biological and/or abiotic processes that the contaminants undergo from the discharge site to the ground or surface water where they are detected, they can be transformed to transformation products (TPs). In a classical approach to identification of the TPs, typically the parent compounds are degraded first at lab-scale and TPs are detected and identified using high resolution mass spectrometry (HRMS). Finally, after identification, a targeted method is developed and they are actually searched for in real aquatic samples in order to be quantified and their concentrations reported.

Here, we propose an alternative approach based on data-dependent fragment ion search where real-world samples are initially screened for plausible TPs, metabolites or related compounds. After the initial screening and based on the results obtained, a set of TP/metabolite-appropriate degradation pathway is performed. For us, the starting point was a suspect screening of a list of all marketed sartans in wastewater effluent and surface water samples. Out of the compounds detected, five of them had an identical core structure, and it was postulated that this sub-structure would fragment identically in all compounds. Following a series of experiments with different MS parameters modified, a list of hit compounds was obtained using Fragment Ion Search (FISh), Mass Frontier software. After the compounds were purchased, a set of biodegradation experiments using activated sludge was performed in order to "source" the detected m/z and compare the possible TPs fragmentation to the one obtained in the bio-reactors. In parallel, a literature search for reported human metabolites was used to complement the identification of compounds detected in cases where no such compound was found to bio-form from the parent compound. Finally, a targeted method was developed for the quantification of these compounds in wastewater and surface water.

ENVR 771

Stable isotope probing for active acidophilic methanotrophs capable of degrading trichloroethylene

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Aerobic and anaerobic biodegradation of common groundwater contaminants, like trichloroethylene (TCE), occur under neutral pH. However, many of acidic aquatic systems (pH < 5.5) in the United State exist and degradation of chlorinated under acidic environment is thus potentially inhibited. While methanotrophs are known to cometabolic TCE using methane monooxygenase (MMO). Little is known about the potential of the acidophilic methanotrophs during TCE biodegradation in acidic aquifers. The goal of this study is to use molecular techniques (stable isotope probing and real-time-t-RFLP) to identify active methanotrophs capable of degrading TCE under acidic conditions. Our preliminary data showed that ^{13}C- methane or ^{12}C- methane were consumed in groundwater microcosms. Biodegradation of TCE was observed after 3 weeks of incubation, with 80% removal of TCE initially spiked. Our on-going research efforts to use ^{13}C-DNA fractions of the samples as templates for identifying active acidophilic methanotrophs and determining microbial community structures. Results of our study will provide a better understanding of the role of acidophilic methantorophs during TCE biodegradation in acidic aquifers.

ENVR 772

In vitro estrogenic activity of endocrine disrupting chemicals mixtures using interaction model

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Exposure to a mixture of endocrine disrupting compounds (EDCs) has been hypothesized to have potential synergistic or antagonistic effect and can cause undesired effects that are not reflected by the individual compound. In this study, the estrogenic activities of eleven EDCs of global environmental concern were systematically investigated using the yeast estrogen screen (YES) method. The contribution of individual chemical to the total endocrine activity of environmentally relevant mixtures was evaluated. For single chemicals, the relative estrogenic potency is in the order of ethinyl estradiol (EE2) > 17β-estradiol (E2) > estrone (E1) > estriol (E3) > octyl phenol (OP) > nonyl phenol (NP) > genistein (GEN) > Bisphenol-A (BPA). n-butyl benzyl phthalate (BBP) and Dibutyl pthalate (DBP) showed only partial agonistic. Bis(2-ethylhexyl) phthalate (DEHP) showed almost no estrogenic activity in the YES assay. For binary mixtures using E2 as standard, E1, E3, EE2, BPA, GEN showed estrogenic effects, while DBP, BBP, DEHP, NP and OP showed anti-estrogenic effects. The full mixture of all these chemicals at an environmentally relevant ratio also showed week estrogenic activity. The mixture data were fitted to an interaction model (IR) that was developed by us previously. Compared with conventional concentration addition (CA) and response addition (RA) models, the IR model has better predictive results in full mixtures containing both estrogens and antiestrogens. Further, EE2 did not have a prominent contribution to the estrogenic activity of the mixture.

ENVR 773

Dioxin-like potencies and concentrations of AhR-active compounds in sediments of Meiliang Bay, Tai Lake, China determined by *in vitro* bioassay and instrumental analysis

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Tai Lake (Ch: *Tai*) is the third largest freshwater lake in China, and Meiliang Bay is a crucial area locating in the northern part of Tai Lake. Meiliang Bay supplies one-third of the drinking water of Wuxi City, which has a population of over 1 million. To better understand the historical deterioration and recent restoration of the ecosystem in Meiliang Bay, extracts of nine surficial sediments were analyzed for their ability to induce dioxin-like potencies *in vitro*. Raw extracts (REs), acid-treated extracts (AEs) and fractionated extracts (FEs) of sediments were examined respectively. The results showed that all of the sediment samples exhibited significant dioxin-like potencies in the H4IIE-*luc* bioassay. REs had 359-1018 pg TCDD-EQ g^{-1} dry mass (dm) derived based on the EC20 (20% of maximal effect). Sediment equivalents in moderately polar (F2) and maximum polarity (F3) fractions were contained 94–260 pg TCDD-EQ and 26–106 pg TCDD-EQ g^{-1} dm, respectively. F2 and F3 fractions were believed to be responsible for the majority of the potency measured in the H4IIE-*luc* bioassay (TCDD-EQ). Four of Nine F1 samples were found cytotoxic or caused morphological changes in H4IIE-*luc* cells and similar toxic effects were also observed in their corresponding REs and AEs, which indicated that the compounds causing cytotoxicity were acid-stable and cannot be removed by Florisil adsorption. 2,3,7,8-tetrachlordibenzo-*p*-dioxin (2,3,7,8-TCDD) equivalencies (TEQs) of dioxin-like PCB congeners (TEQ_{PCB}), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDFs), and polycyclic aromatic hydrocarbons (PAHs) were found to be 0.7-1.6, 2.7 -6.9 and 11.1-22.9 pg g^{-1} dm, respectively, by use of their corresponding H4IIE-*luc* specific relative potencies (RePs),. The total concentrations of TEQs (12.4-30.9 pg g^{-1} dm) in sediment accounted for only a portion of the dioxin-like potencies measured in the HIIE-*luc* bioassay, indicating the presence of unidentified AhR-active compounds. Compared to the results of other studies in Lake Tai, dioxin-like potencies have decreased gradually over the past decade, which indicates that the large national ecological remediation project carried out in Meiliang Bay, Tai Lake has successfully removed some AhR-active pollutants.

ENVR 774

Evaluation of microbial communities in biologically active filters and their effectiveness in treating pharmaceuticals and personal care products

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The microbial community on biologically active filter (BAF) media is believed to play a crucial role in removal of natural organic matter and pharmaceutical and personal care products (PPCPs). Opportunistic pathogens in BAF effluents are a potential issue for drinking water systems. In this study, next generation sequencing (Illumina MiSeq) was used to characterize the microbial communities in the influent, effluent, and media of the bench-scale BAFs. The year long study was designed to evaluate their effectiveness in removing PPCPs. Filter media included granular activated carbon (GAC) and anthracite/sand dual-media collected from two treatment plants. Carbon and nutrient amendments were provided in the source water to enhance the biomass growth. Steady state was reached after 6 months of operation with dissolved organic carbon (DOC) removal ranging from 2% to 10% and 1% to 5% at empty bed contact times (EBCTs) of 18 min and 10 min, respectively. For the GAC BAFs, greater oxygen consumption, increased pH drop, and greater DOC removal per mg of adenosine triphosphate (ATP) were observed indicating increased microbial activity as compared to dual-media BAFs. ATP concentrations observed in the upper portions (37.5 cm from the bottom) of the BAFs ranged from 325 to 800 ng ATP/cm^3 and were as much as four times greater than the middle and lower portions. Sixteen PPCPs were spiked in the source water. Compared to dual-media BAFs, GAC was highly effective at an EBCT of 18 min with or without pre-ozonation with 14 PPCPs removed by greater than 80%. *Proteobacteria* and *Planctomycetes* phylum were dominant in both GAC and dual-media BAF media. In the filter influent and effluent the dominant phylum was *Proteobacteria*. *Legionella pneumophila* was observed to be the most prevalent human potential pathogen in BAF effluents (prevalence below 0.08% compared to the total sequence number), with the lowest prevalence observed when no ozonation pretreatment is applied. This study provides new insights into the BAFs ability to remove PPCPs, associated with the composition of microbial communities.

ENVR 775 - Withdrawn

ENVR 776

Determination of aqueous film forming foams (AFFFs) in the environment using multivariate statistical analysis of liquid chromatography high resolution mass spectrometry (LC/HRMS) data

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AFFFs from different manufacturers consist of varying mixtures of fluorocarbons and hydrocarbons. To track the constituents through various environmental compartments, characterizing the unique, as well as common, components of AFFFs is necessary. For this work, seven AFFFs were analyzed using a QTof based LC/HRMS system in order to obtain comprehensive spectral data for subsequent multivariate analysis. Data was acquired using rapidly alternating high and low collision energy conditions so that precursor and product ions were acquired in a single injection. Samples were diluted in methanol and chromatographic separation performed using an existing liquid chromatography method for the analysis of perfluorinated compounds. Method performance with respect to mass accuracy (<5 ppm mass error), retention time conservation and repeatability of analyte response is particularly important in experiments involving non-targeted analysis. These were assessed using a standard mixture of perfluorinated compounds. Randomized, multiple injections of the seven mixtures were then subjected to a principal component analysis (PCA). Distinctive groupings from PCA plots were observed for three AFFFs in both modes with a cluster of five of the seven in ESI^+ and four of the seven in ESI^-. Pooled samples, which contain all ions used in the multivariate analysis, were clustered appropriately towards the middle of the loadings graph. In order to determine formulation specific markers, group-to-group comparisons were carried out with two foams at a time, resulting in the generation of S-Plots. Investigation of the markers strongly associated with specific groups was performed using structural elucidation software. This resulted in the identification of multiple sulfate, hydrocarbon and fluorinated compounds. Interrogation of the putative markers using trend plots, which represent presence and abundance across injections, yielded additional identifications that were either unique to specific formulations or in other instances common to multiple AFFFs. For those constituents that had a proposed structure, product ion structures were assigned and used as a means to support a potential identification. The utility of both the analytical technique, high resolution LC combined with comprehensive HRMS, and software processing approach, multivariate statistical analysis (PCA), applied to environmental forensic analysis, like transformation product identification, will be discussed.

ENVR 777

Advancements in analysis for emerging organic contaminants in water

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Emerging organic contaminants (EOCs) including pharmaceuticals, hormones, pesticides and others are ubiquitous and have been detected in surface and ground waters around the world. Many of these compounds are recalcitrant and hard to attenuate in conventional water treatment plants while having adverse effects to wildlife at environmentally relevant concentrations. Thus, the monitoring of EOCs for risk assessment and potential regulation in water is of great significance.

When dealing with several different water matrices and with the continual advancement of analytical equipment, it can be a struggle for even experienced analysts to choose the best method of sample clean-up and extraction to get reliable, robust and sensitive data. Traditionally, labor intensive and time-consuming extraction techniques like solid phase extraction (SPE) and liquid-liquid extraction have been used for concentration of samples to achieve required detection limits in the ng/L range. These methods require large amounts of sample (100-2000 mL) and solvents for extraction. Recently, the development of automated online SPE systems have given rise to the possibility of achieving similar detection limits with the use of just a few mL of sample while significantly reducing cost, labor and time of analysis.

On the analytical side, newer mass spectrometers have produced a large increase in sensitivity. This has resulted in large volume injection of the aqueous sample with no or minimal pretreatment to be suggested as a possibility for analysis of these contaminants. In this study, a single LC method for the analysis of several EOCs using on-line SPE, were compared to direct large volume injection and conventional offline SPE followed by LC-MSMS analysis. Factors like method reporting limits and ion suppression were evaluated and compared. Further, the type of water matrix, cost and time of analysis will also be discussed with regards to EOCs. Finally, the use of high resolution accurate mass instruments will be discussed and data provided to act as instruments for screening large numbers of EOCs along with the possibility of identifying transformation and disinfection by products in a single analytical run.

ENVR 787

Influence of soil texture and drought stress on antibiotic uptake into produce

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Increasing stress on freshwater supplies, a result of increasing global population and climate change, has made necessary the reuse of wastewater as a source for irrigation. Wastewater, both municipal and agricultural, is a known reservoir for many emerging contaminants (ECs) including antibiotics. The use of wastewater for irrigation provides a pathway for antibiotics to enter produce. This presentation will focus on variables influencing antibiotic occurrence in soil and uptake into produce when antibiotics are introduced via irrigation water. We will specifically demonstrate the influence of soil texture and drought conditions on antibiotic uptake. The influence of drought conditions was found to be compound specific with increasing drought stress resulting in increased uptake of lincomycin and decreased uptake of oxytetracycline and sulfamethoxazole. To evaluate soil texture, the uptake of three antibiotic compounds (lincomycin, oxytetracycline, and sulfamethoxazole) was monitored in lettuce, *Lactuca sativa,* grown in three soils with different soil texture, all with low soil organic matter (SOM). Uptake as a function of soil texture was also determined to be compound specific.

ENVR 788

Fate and transformation of veterinary antibiotics in soils

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Antibiotics administered to livestock mainly end up in soils via manure land application. It is because antibiotic residues excreted into the manure may not be completely removed during composting processes. Feed containing sulfamethazine (SMZ) plus chlortetracycline (CTC) was fed to three steers at 350 mg of each antibiotic/steer/day. Feed containing 11 mg/kg of tylosin (TYL) was fed to other three steers for 7 days. The manure collected from the treated animals was composited, mixed, and then composted using the FDA recommended static and turned techniques. After 42 days of composting, a microcosm study was set up in which the composted manure was applied to sandy, silty, and silty clay loam soils at agronomic rates to examine the transformation of antibiotics. Manure without being composted was used as controls. This study is unique because most of the published studies often fortify manure with target antibiotics at unrealistically high levels. The soil microcosms were incubated for 120 days with constant soil moisture maintained at 50% field capacity. Samples were sacrificially sampled at 0, 1 ,3, 29, 57, 90 and 120 days for target antibiotic analysis. The transformation of antibiotics largely depends on their initial concentrations. SMZ, TYL, and CTC transformation rates in manure-applied soils were not affected by soils types, with averaged half-lives of 11 days, 1.5, and 15 days, respectively. However, the transformation of SMZ slowed down significantly after 29 days, maintaining at a level of ~41% of its initial concentrations up to 120 days at an average concentration of 15 ng g^{-1} of SMZ for the 3 soils tested. Static and turned compost had no impact on SMZ transformation. Although TYL transformed faster than SMZ in the manure-applied soils, similar to SMZ it could still be detected at 120 days at a level of 6.3% of its initial concentrations with average concentration of 0.70 ng g^{-1} for the 3 soils. On the contrary, TYL were undetectable after 57 days in the compost-applied soils, indicating it pose little risk of accumulating in soils with proper composting. The transformation rates of CTC were much slower than TYL in manure-applied soils with an average half-life of 14.5 days. Similarly, CTC could still be detected in the compost-applied soils at 120 days after treatment at concentrations ranged from 1.79 to 3.29 ng g^{-1}. These findings indicated that feed-based antibiotics could persist in soils at low concentrations for a long period of time.

ENVR 789

Bioavailability of geosorbent-sorbed tetracycline to an *Escherichia coli* bioreporter for expression of antibiotic resistance

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Tetracycline antibiotics are extensively administered as human and veterinary medicine, especially in livestock production. Large portions of tetracycline are eventually disseminated to the environment with soils/sediments as the major reservoirs. It is imperative to understand the mechanistic pathways of bioavailability of soil/sediment-

sorbed tetracycline to microorganisms. In general, most soil bacteria live in the biofilms attached on soil particles where bacteria could get close contact to soil-sorbed tetracycline. In this study, *E. coli* MC4100/pTGM whole-cell bioreporter was used as an effective tool to probe the bioavailability of tetracycline sorbed by geosorbents including clays, soils and carbonaceous geosorbents. In order to achieve close contact with geosorbent surfaces, the bacteria were inoculated on a thin layer of geosorbents under varying relative humidity and/or periods of incubation time. The experimental settings with higher relative humidity manifested the higher antibiotic resistance response emitted from the bioreporter indicating greater bioavailability of geosorbent-sorbed tetracycline. Meanwhile, the extended incubation time could allow better development of biofilms on the sorbent surfaces, and enhance the bioavailability of sorbed tetracycline to the bioreporter for evoking the expression of antibiotic resistance. Overall, water content of geosorbents and attachment of bacteria to solid surfaces affected the bioavailability of sorbed tetracycline, hence altering the selective pressure on the surrounding microbial communities for development and enrichment of antibiotic resistance.

ENVR 790

Phenolic acids alter selective pressure of tetracycline on an *Escherichia coli* for expression of antibiotic resistance by impairing bacterial efflux pump

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The occurrence and proliferation of antibiotic resistance in microbial communities has become a global issue of public health. The presence of trace levels of antibiotics in the environment exerts selective pressure on susceptible microbes for development and enrichment of antibiotic resistance. Environmental conditions may alter the selective pressure of antibiotics on bacteria due to the changes of antibiotic chemistry or bacterial physiology. In this study, we found that some small phenolic acids commonly found in the environment could alter the bioavailability of tetracycline in water hence selective pressure on an *E. coli* bioreporter construct carrying tetracycline resistance genes. In the solution, the presence of phenolic acids was observed to enhance the response of antibiotic resistance genes in an *E.coli* bioreporter, and the magnitude of the enhancement increased with the amount of phenolic acids present in water. The results of increased intercellular concentration of tetracycline in the bacterium suggest that phenolic acids could facilitate bacterial uptake and accumulation of tetracycline. The experimental results of bacterium stained with propidium iodide indicated that phenolic acids did not damage the integrity of bacterial membrane. The increase of bacterial uptake of tetracycline could be attributed to the impairment of bacterial efflux pump activity by phenolic acids.

ENVR 791

Historical trends and spatial distribution of antibiotics in Minnesota lakes and rivers

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Antibiotics are not completely removed by wastewater treatments plants and have been detected in lakes and rivers that receive wastewater and sewage effluent. The presence of antibiotics in aquatic environments, even at relatively low concentrations, is concerning, because they are designed to be effective at small doses and may select for and promote the dispersion of antibiotic resistant genes. The objective of this work was to quantify the current and historical levels of selected human and veterinary antibiotics in Minnesota lake and river sediments. Twenty three antibiotics were selected from the major antibiotic classes (sulfonamides, macrolides, tetracyclines, β-lactams, and fluoroquinolones), as well as a few major degradation products and non-categorized antibiotics. The antibiotics were extracted from the sediment using accelerated solvent extraction and the extract was cleaned prior to analysis via solid phase extraction. Ultra pressure liquid chromatography coupled with electrospray ionization tandem mass spectrometry method was developed to analyze the chemicals. The historical concentrations of several antibiotics - sulfapyridine, sulfamethazine, doxycycline, trimethoprim, lincomycin, and olfaxacin - were quantified in a dated sediment core from Lake Pepin, a natural impoundment of the upper Mississippi River on the Minnesota/Wisconsin border. In general, the antibiotics were first detected in Lake Pepin sediments after the date they were initially manufactured and distributed for human and/or veterinary use. Concentrations rose through the antibiotic era and are present at 0.5 to 20 ng/g in recent sediments.

ENVR 792

Comparing analysis techniques for antibiotic resistance genes (ARG) degradation in UV treatment

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Effluent of municipal wastewater treatment plants (WWTPs) is the greatest contributor to ARG discharge into the environment. In a WWTP, the removal of microbial cells occurs at every stage of treatment including biological treatment, sedimentation, and disinfection. However, eliminating residual genetic products, such as plasmids, is relatively difficult. This can potentially be hazardous, because horizontal gene transferring (HGT) between microorganisms can introduce antibiotic resistance to downstream microbial species. The objective of this study is to look at how a common disinfection process (UV) affects ARG degradation, and how this degradation is related to HGT efficiency. With a bench scale UV treatment model, we compared transformation assays (culture-based) and qPCR (non-culture-based) analysis techniques to assess ARG degradation kinetics. In transformation assays, the pWH1266 plasmids that contain tetracycline (Tet^R) and ampicillin (Ap^R) resistant genes were treated with UV before being co-cultured with *Acinetobactor baylyi* - a soil bacteria strain that naturally picks up extracellular genetic products. By measuring the survival rate of *A. baylyi* on selective plates, we could calculate transformation efficiency and plasmid degradation kinetics. In qPCR, pWH1266 was treated with the same UV disinfection procedure, and the Tet^R and Ap^R genes were amplified with both short (200-nt) and long amplicons (900~1000-nt). While both transformation assays and qPCR results demonstrated first order degradation kinetics of the genes with UV treatment, qPCR showed a significantly slower degradation rate (short amplicon k»-2.1, long amplicon k»-8.6) than transformation assays (k»-44.0). These results suggested that qPCR results are inevitably determined by indirect factors such as amplicon sizes and can be overestimating phenotypic antibiotic resistance if not interpreted correctly. Our model can help provide a link between non-culture-based and culture-based gene analysis techniques, which can potentially assist decision-making in wastewater treatment regulations.

ENVR 793

Kinetics and mechanism of sulfamethoxazole degradation by UV, UV/H2O2, and UV/persulfate (PDS) and influence of bicarbonate

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Sulfamethoxazole (SMX), an important member of the sulfonamide antibacterial class, has been wildly detected in municipal wastewater effluent and surface water bodies. In this study, kinetics and product formation of SMX were investigated during UV, UV/H_2O_2, and UV/PDS processes. The experiments revealed a strong pH dependence of the destruction of SMX by direct photolysis and sulfate radical (SO_4^{*-}). The quantum yield of SMX phototransformation decreased by a factor of seven between the neutral form and the deprotonated form, while the rate constant for the reaction of SO_4^{*-} with SMX increased by seven times. Hydroxyl radical ($^*OH$) exhibited the same reactivity toward these two forms. The structure of reaction products were elucidated for eleven different nominal masses (including constitutional isomers, approximately 13 peaks) by a triple quadrupole TOF mass spectrometer. The yield of hydroxylation product (m/z 270) by $^*OH$ oxidation was much higher than that by SO_4^{*-} oxidation. The electrophilic SO_4^{*-} attacking the amine group formed an N-centered radical as a first intermediate, which was further oxidized to 4-nitro-sulfamethoxazole (m/z 284) and dimer products (m/z 503 and m/z 519). In the presence of bicarbonate, carbonate radical (CO_3^{*-}), the dominant radical during UV/H_2O_2, promoted the oxidation of amine group, but suppressed the formation of hydroxylation product, indicating the similar reaction mechanisms of CO_3^{*-} and SO_4^{*-}.

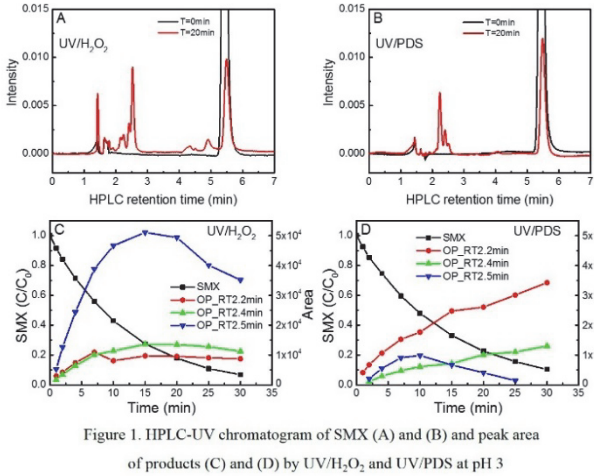

Figure 1. HPLC-UV chromatogram of SMX (A) and (B) and peak area of products (C) and (D) by UV/H_2O_2 and UV/PDS at pH 3

HPLC-UV chromatogram of SMX (A) and (B) and peak area of products (C) and (D) by UV/H2O2 and UV/PDS at pH 3

ENVR 794

Structure-dependent reduction mechanisms of isoxazoles by aqueous Fe^{II}–tiron complex

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This work examines the reduction mechanisms of various substituted isoxazoles (ISXs) in the presence of Fe^{II}–tiron complex. The trend in the reactivity follows: 5-NH_2-ISX >> 3-NH_2-ISX >> 5-O-substituted ISX >> ISX. Electron withdrawing groups at the 3-position significantly enhanced the reactivity. Reduction products of ISXs were identified by HPLC/QToF-MS to be the ring opening analogs. The experimental solvent and iron kinetic isotope effects suggest protonation is not rate-limiting, while Fe(II) is directly involved in the rate-limiting step, which is likely electron transfer. The N–O bond in the ISX ring dissociates after accepting the first electron, and a 6-membered ring formed among the Fe^{II} in Fe^{II}–tiron complex, the N or O atom within the ring, and the 3-N or 5-N substituent. Electron transfer is significantly facilitated within the 6-membered ring, as confirmed by electrochemical cell experiments that separated complexation from electron transfer. Our ongoing work examines molecular descriptors that can be correlated to the reduction reactivity of all ISXs.

ENVR 795

Copper and silver vanquishing of hospital acquired “superbugs”: An economical solution to a major public health problem

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Research on the use of copper alloy surfaces in a hospital environment and research on the antimicrobial uses of silver indicate that incidences of hospital acquired infections can be greatly reduced by the use of permanently antimicrobial surfaces in areas of hospitals that are more likely to have patients exposed to infectious organisms including those that are considered antibiotic-resistant. The use of such surfaces is of no risk to patients and health-care workers, economical, and would use commercially available materials and would provide readily accessible methods to help reduce the seriousness of a major public health problem. The Centers for Disease Control latest figures estimate that four percent of patients acquire such illnesses (two million such occurrences with 23,000 fatalities each year).

These metals, used for thousands of years without any major known resistance by pathogens being developed, break down cell walls, and interfere with microorganism respiration and reproduction.

ENVR 805

Key role played by dissolved black carbon in slow sorption kinetics and sorption hysteresis of hydrophobic organic chemicals to rice-residue-derived biochar

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Due to the extraordinarily strong adsorption affinity, environmental black carbon (soot, biochar, char, and charcoal) pronouncedly affects the fate and transport of hydrophobic organic contaminants. This study revealed an important and previously unrecognized role of dissolved black carbon (operationally defined as components with sizes less than 0.45 μm) in the nonideal adsorption (e.g., slow sorption kinetics and irreversible sorption-desorption/hysteresis) of organic contaminants to bulk black carbon. Batch sorption experiments were carried out for three adsorbate molecules (nitrobenzene, naphthalene, and atrazine) with varying molecular size to rice-residue-derived biochar. After rinsing the raw biochar with deionized water or 0.5 M NaOH to remove the component of dissolved black carbon (accounting for 0.61% and 9.04% of the total organic carbon, respectively), adsorption of the test adsorbates to the residual biochar was increased slightly to moderately, and the adsorption increase positively correlated with adsorbate molecular size (atrazine > naphthalene > nitrobenzene). Removal of dissolved black carbon also facilitated adsorption kinetics, and in the meantime attenuated the degree of sorption hysteresis when compared with the raw biochar. The observations were accounted for by the enhanced exposure of adsorbent micropores originally occupied by dissolved black carbon. To be consistent with this hypothesis, adsorption kinetics to template-synthesized mesoporous carbon (CMK3), which was used as a model adsorbent, was impeded while the degree of sorption hysteresis was greatly increased when the adsorbent pore spaces were invaded by surface-grated alkylamino groups and upon further loading of humic acid.

ENVR 806

Molecular fractionation of dissolved organic matter induced by adsorption on soil minerals and soil inorganic components

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DOM is an important labile organic-carbon pool. Studies have demonstrated the importance of minerals, especially iron oxyhydroxides, for stabilization and preservation of labile organic matter in soils and sediments. The present work investigated the molecular scale fractionation of labile DOM by adsorption on different types of iron oxyhydroxides using electrospray ionization coupled with Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS). The results demonstrated that poorly crystalline ferrihydrite exhibited higher affinity to DOM and induced more pronounced molecular fractionation of DOM than did goethite or lepidocrocite. Double bond equivalence (DBE) and the oxygen number distribution were considered as valuable parameters to evaluate the fractionation behavior. High molecular weight compounds and compounds rich in oxygen or high unsaturation including polycyclic aromatic, polyphenols and carboxylic rich compounds had higher affinity to ferrihydrite. On the opposite, low molecular weight compounds and compounds with low unsaturation and low oxygen (mainly as alcohols and ethers) were preferential maintain in water. Molecular fractionation of DOM in soil inorganic components sequentially separated from a krasnozem soil was further investigated using the same method. The results indicated that Fe and Al oxides in the soil played a very important role in molecular fractionation of DOM at the soil-water surface. This process will influence the molecular composition of organic matter in both the aquatic and soil environments, and further influence the interfacial behavior of environmental contaminants.

ENVR 807

Dynamic changes in the sorption capacity of biochar-amended soils: A field study

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Due to its high sorption capacity for organic contaminants, the amendment of biochars to soil is advocated as a novel remediation strategy for contaminated soils. However, it is not clear how long this extraordinary sorption affinity could be maintained after biochars enter and interact with soil. In this study, a commercial biochar was applied to an agricultural soil and the sorption of atrazine and phenanthrene on biochar amended soils with different aging times (0 to 2 years) were investigated. The fresh amendment of biochar into soil led to an obvious enhancement on the sorption coefficients (K_d) of atrazine (by 3.71 times at $C_e = 0.01\ S_w$) and phenanthrene (by 1.33 times at $C_e = 0.01\ S_w$) compared with the bare soils without biochar. Along with aging, the properties of biochar changed with surface area increasing first and then decreasing. Correspondingly, the sorption of atrazine and phenanthrene on the biochar amended soils first increased and then markedly decreased with aging time. Based on the changing trend of the K_d values with aging time, it could be predicted that the sorption capacity of biochar amended soils will decrease to the level of the bare soil after 2.5 years. Several lab experiments were conducted to verify the findings in field and to explain the micro-level mechanisms of aging effects. The results showed that the aging effects varied with the properties of biochar and soil, which could be ascribed to the degradation of labile moiety of biochar and the interactions between biochar and soil constituents, such as organic matter and inorganic minerals. Our results provide important scientific bases for the development of biochar remediation technology.

ENVR 808

Facilitated transport of phenanthrene and oxytetracycline by oxidized-multiwalled carbon nanotubes in soil columns

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In this study, the facilitated transport of phenanthrene and oxytetracycline by oxidized-multiwalled carbon nanotubes (o-MWCNTs) in soil were systematically investigated. The result showed that o-MWCNTs could significantly enhance the mobility of both phenanthrene and oxytetracycline (OTC) in soil columns. O-MWCNTs played as a vehicle for phenanthrene transportation in soil. The main migration form of phenanthrene was o-MWCNTs-bound phenanthrene. However, the main migration form of OTC was dissolved OTC. The adsorption of OTC on soils was inhibited in the presence of o-MWCNTs, which was responsible for the enhancement of OTC migration. Our findings indicate that o-MWCNTs can greatly enhance the mobility of organic contaminants of low mobility in the subsurface environment. Such effects should be taken into account when assessing the potential environmental risks of engineered carbonaceous nanomaterials.

ENVR 809

Adsorption, mobility, and bioaccessibility of PBDEs: Roles of heavy metals, natural organic matter, and fertilizers

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We conducted twenty-cycle batch loading tests to study the adsorption of BDEs on an e-waste site soil under the influence of heavy metal, NOM and fertilizer. The adsorption of BDE-47 on the soil was less than BDE-28 and BDE-99. Only BDE-47 adsorption was enhanced by heavy metals (Cu, Zn, Pb, Cd), which may be related to surface bonding angle. However, metals adsorption was increased in the presence of BDEs, which may result from change of SOM macromolecular structure and addition of -COOH and –OH groups. The addition of peat-derived DOM selectively increased BDE-47 adsorption, while commercial fertilizer had no significant influence on BDEs adsorption. The mobility of BDEs was double in simulated acid rain than water leaching tests. The bioaccessibility of BDEs varied with BDEs concentrations and presence of metals, DOM and fertilizer. Hence, the fate of BDEs correlates to its distribution equilibrium in the soil-water system.

ENVR 810

Polychlorinated biphenyls in agricultural soils from the Yangtze River Delta of China: Contamination characteristics, combined ecological effects, and human health risks

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Polychlorinated biphenyls (PCBs) are a well-known class of persistent organic pollutants, and have led to ubiquitous contamination of the environment. Combined pollution (including PCBs, OCPs, PAEs and PBDEs) could result in drastic changes in microbial community composition, and affect the ecosystem function of soil. The potential risks of combined pollution to human health have been not well estimated. This work revealed the concentration, composition and spatial distributions of PCBs in soils of the YRD region, south China. The ecological effect and human health risk of combined pollution in soils were also assessed to provide a basis for assessing soil quality due to rapid economic development in the last three decades. The concentrations of total PCBs in the YRD agricultural soils ranged from <1.0 to 130 ng/g, with a mean of 20.2 ng/g, and a detection rate of 77%, in the soil samples indicating that PCBs were widespread pollutants in the YRD. The major PCB homologue group residing in the topsoil was tetra-PCB, followed by tri-PCB and penta-PCB, which were different from the compositions in the global background soil, indicating that local sources are potentially the main sources of PCBs. The higher PCBs concentrations were observed in the 0–30 cm surface section relative to the subsurface soils. The concentrations of PCBs were much higher in Jiangsu Province than those in Zhejiang Province and Shanghai Municipality, probably due to the use of PCBs-containing materials.
The results showed that the 11 selected environmental factors together could explain 12.73% of the observed variation in the microbial community composition. Other factors, such as soil type and land management practice might have higher impacts on soil microbial communities. The subset of selected soil physiochemical parameters together explained more variation than the combination of selected organic pollutants in soil. Human health risks of combined pollution in soils were estimated. PCBs present higher carcinogenic and non-cancer risks than OCPs, PAEs, and PBDEs. The high risks were mainly derived from PCB-126, 81 and 169. DEHP, α-HCH, γ-HCH, and BDE-47 also showed relatively high risks. Although an individual pollutant may not impose health risk to human, a long-term chronic exposure to a mixture of pollutants potentially causes serious adverse effects.

ENVR 811

Effects of environmental organic matters on the distribution of bisphenol A in soil-water interface

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Bisphenol A (BPA) is widely used by the industry to make plastics and epoxy resins. Due to the structural similarity to human estrogen, it can interfere with normal hormonal processes and therefore is recognized as a postulated endocrine-disrupting chemical. BPA is an organic compound having two phenolic functional groups and having a low to moderate degree of mobility in soil. Several approaches including adsorption and partition models have been developed to estimate the distribution of BPA and to predict its mobility in the soil-water interface. In this work, we collected and analyzed environmental samples including surface water, sediment/soil, and soil pore water from two rivers (Yanshuei river and Jishuei river in Tainan, Taiwan) to test the hypothesis that the amount of organic content is, in general, proportional to the concentration of BPA in both liquid and solid phases. An accelerated solvent extractor was used to extract BPA from sediment/soil samples and the quantification of BPA was conducted by HPLC with a fluorescence detector. Excitation-emission matrix (EEM) fluorescence spectroscopy was used to characterize dissolved organic matter (DOM) in water samples. A stable and high recovery rate (between 74%-124 %) from 37 soil/sediment samples was obtained. It was observed that the BPA concentration in the pore water (ranging from 0.01 to 3.57 μg/L) was generally greater than that in surface water (ranging from 0.01 to 1.04 μg/L). Positive correlation was found between BPA concentration and organic carbon content in both liquid phase and solid phase. The BPA concentration in Yanshuei river samples, of which average organic content in sediment was 1.16%, was generally higher than that in Jishuei river samples of which average organic content in sediment was 0.57%. However, the soil-water distribution coefficient K_d was 27.91 L/kg for Yanshuei river and 38.1 L/kg for Jishuei river, suggesting that BPA tended to accumulate in solid phase in the Jishuei river area. EEM analysis results showed there were more microbial by-product-like substances in Yanshuei river samples which helped BPA stay in the liquid phase. Our results showed that the environmental samples exhibited a much higher K_d than the values predicted by adsorption isotherms in the referenced literature whose samples were prepared in the laboratory.

ENVR 812

Phthalate ester contamination in facility agriculture and cumulative health risk assessment

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A total of 120 soil samples and 120 vegetable samples from facility agriculture farms distributed in 7 provinces were collected in this study. The concentrations of 6 major phthalate esters (PAEs) were analyzed with GC-MS. The results showed > 95% of soil samples and vegetable samples were contaminated with PAE, but the total concentrations of PAEs were under the environmental standards. Among the total PAEs, DnBP and DEHP were two abundant chemicals, which indicated that plastic film was the main source of PAEs in facility agriculture. Human health cumulative risk assessment were also conducted in this study. The daily average intake values of DMP, DEP, DnBP, DiBP, BBP and DEHP were 0.32, 0.84, 1.8, 0.8, 0.8 and 2.3 μg kg^{-1} d^{-1} for 6-13 yr children and 0.22, 0.45, 1.3, 0.6, 0.48 and 1.8 μg kg^{-1} d^{-1} for adults, respectively. The values of hazard index (*HI*) and carcinogenic risk (*CR*) were < 0.21 and $< 1.7 \times 10^{-5}$ for 6-13 yr children and < 0.11 and $< 6.7\times 10^{-6}$ for adults, respectively. The results showed vegetables from facility agriculture were still safe to eat. Considering other sources of PAEs in atmosphere, toys, personal care products, PAEs in agriculture soils and vegetables post a potential health threat to consumers. A proper action should be executive to prevent PAEs contamination in facility agriculture.

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ABSTRACTS

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