AGRO DIVISION

AGRO 1

Introduction, past, present and future of INSTAR summits

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Following the International Congress of Entomology (ICE) in 2016, a half day workshop was held at the Emerging Pathogens Institute (EPI) on the University of Florida campus. It was organized by Professor Jeff Bloomquist, along with the assistance of many members of his research group and the staff of the EPI, and given the moniker INSTAR, a contraction of INSecticides and TARgets. Particular thanks are due to Dr. Jennina Taylor-Wells, who took the original draft of the Workshop minutes, and also the interest and financial support for the workshop provided by Dr. Glenn Morris, Director of the EPI, who also arranged for the EPI to serve as the Workshop venue. There were no set objectives for the Workshop, and it was used as an opportunity to introduce the EPI to first time visitors, to talk about experiences from the ICE, and to discuss advances in the field of insecticide toxicology and discovery, with a unique opportunity to exchange ideas between industrial and academic scientists from all over the world. The INSTAR workshop was well received by the participants and stimulated discussions with officers of the American Chemical Society (ACS), Division of Agrochemicals, and those of the Entomological Society of America (ESA). A planning meeting was held at the ESA national meeting in Denver, Colorado, in November 2017. In attendance were Drs. John Clark, Kun Yan Zhu, Troy Anderson, Daniel Swale, and Jeff Bloomquist. It was decided to propose to the ACS and ESA an annual INSTAR workshop or summit, to be held in yearly rotation at the national meeting of these two scientific societies. The activities of the original Workshop dovetail well with this first follow-up Summit at the 2018 national ACS meeting, on new and improved methods of chemical insect control and resistance management.

AGRO 2

Current status of new insecticide chemistry, targets, mode of action and the future

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New insecticidal chemistries with new or underutilized modes of action are needed to provide food and fiber for an expanding global population, to address changing regulatory requirements, and to deal with the continued evolution of resistance to available commercial products. The past two decades have seen significant changes to the Agrochemical Industry, including the globalization of agrochemical discovery with an associated number of new insecticides with new modes of action (MoAs), and potential new options on the horizon. This talk will focus on how the discovery of insecticides with new MoAs has evolved, and it will provide a window to future directions.

AGRO 3

Perspectives on the identification and development of new insecticide targets

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The discovery and development of new active ingredients to control arthropod populations and circumvent the inevitable evolution of insecticide resistance has been of consistent interest to the field of insecticide science. However, despite the significant interest in the identification of novel targets and chemicals, the diversity of the insecticide arsenal and novel target sites has remained relatively limited for the past two decades. The Insecticide Resistance Action Committee (IRAC) only recognizes approximately 25 different modes of action, or exploited insecticide target sites, with the extreme majority of these targeting neural proteins. This number is dramatically lower than the number of exploited human drug targets, which range between 200-400 depending upon classification. After the sequencing of the human genome, Hopkins and Groom analyzed the Investigational Drugs and Pharmaprojects databases and identified 399 current drug targets based on known ligands with drug-like properties. They also estimated that approximately 5-10% of the genes from the human genome are pharmacologically tractable and represent exploitable targets, which is approximately 1500-3000 genes representing putative targets. Considering this, it is reasonable to speculate the arthropod genome comprises significantly more tractable target sites than the 25 that are currently exploited. The large-scale sequencing of vector genomes has intensified in recent years, and the rise of insect genomics has enabled the discovery of insecticide targets via functional characterization of proteins that are essential to survival or other functions. The significant advancements in data mining, bioinformatic tools, and recent initiatives (i.e., i5K) will benefit insecticide development in a similar manner to human drug development. Mechanisms for understanding the identity of targets encoded by the arthropod genome and the allocation of resources within academic and industrial research will be discussed.

AGRO 4

Navigating the global regulatory landscape for crop protection products: Lessons learned and opportunities for the future

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Since publication of the 2007 NAS report on "Toxicity Testing in the 21st Century: A Vision and a Strategy," there have been significant multi-sector efforts to develop models and platforms aligned to turning this vision into a reality. What once seemed like an apparent mirage grows closer to reality as the research community explores combination *in silico* and *in vitro* approaches to advance our toxicity testing methodologies. As efforts have been underway to develop tools for prioritization of chemicals or *in vitro* acute alternative approaches as part of regulatory toxicology assessments, parallel efforts within industry have been underway to design predictive toxicology platforms to develop products of the future with more favorable environmental and human health profiles. Layered on top of these challenges is the global regulatory landscape, which is rapidly evolving, often in divergent ways, and can lead to throwing out the proverbial 'baby with the bathwater.' In this talk, we will provide case studies of the challenges that can arise when developing *de novo* approaches to novel molecule design, including interdependencies that must be balanced between key molecule characteristics. Discussion of validation of assays across chemistries with different physical chemical domains, main drivers for molecule selection, prioritization and weighting of end points, and case examples of various *in silico* and *in vitro* tools utilized within the crop protection product development field will be the focus of this presentation. Finally, a proposal will be presented on how to integrate various data streams into a semi-quantitative

assessment scheme for the purposes of molecule design with an eye to providing science-driven decision making to global regulatory agencies and stakeholders.

AGRO 5

Pesticide regulation and trade: National and international considerations

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In the United States, agricultural exports account for over thirty percent of gross farm income. While farmers rely on export markets for their livelihood, an increasing number of non-tariff measures imposed by U.S. trading partners threaten to undermine their ability to export. One growing area of concern is a shift occurring in some countries to establish pesticide maximum residue levels based on hazard rather than based on full risk assessments. The shift to hazard-based regulation is likely to lead to substances that could be used safely to increase crop yield being removed from the market, without offering any increased protection for consumers. Global cooperation is critical to ensure that the core principles of science and risk-based decision-making established in international organizations such as the Codex Alimentarius are maintained, and that the results of hazard assessments are not misused to create consumer fear and mistrust of regulatory decision-making. We need to work together to ensure that the shift toward hazard-based regulation is not normalized internationally.

AGRO 6

Government of Canada 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. 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 regard 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 participates in the Industry-Government Working Group on Pesticides to promote increased international coordination and collaboration, supporting aligned approaches to pesticide regulations and addressing market access issues. To facilitate international standard-setting, the Canadian Government funded the JMPR Training session in November 2017 to increase the capacity of the FAO Panel of Experts required to support the extraordinary session of the JMPR, scheduled for May 2019. Moving forward, PMRA will continue participating in 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 7

Global outlook on MRL harmonization: U.S. trade and international capacity building

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This presentation will explore: 1) The main trade challenges and opportunities for specialty and row crops from USDA's Foreign Agricultural Service. This will include an update on recent trade policy issues and trends, as well as a forward-looking analysis of what trade flows and priorities will be over the next few years. 2) A description of the recent work taking place in APEC on MRL harmonization, including a detailed description of the October U.S.-led workshop on MRL trade barriers and the forthcoming study commissioned by FAS to evaluate these barriers empirically. The presentation will also connect the recent efforts in APEC to ongoing FAS-led capacity-building projects, largely conducted through IR4, in many developing APEC economies to establish MRLs by conducting field trails and building human capital.

AGRO 8

Challenges Pacific Northwest tree fruit producers have meeting MRL requirements when exporting fruit around the world

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The Northwest Horticultural Council (NHC) represents apple, cherry, and pear growers, packers, and shippers in Idaho, Oregon, and Washington. NHC members grow, pack, and ship approximately 77% of the fresh apples, 78% of the fresh cherries, and 84% of the fresh pears, produced in the United States. Approximately 30% of the total fresh crop from this region is exported to over 60 countries around the world. It is vitally important that growers have safe and effective pesticide tools available to produce these crops. The absence of coordinated information regarding pesticide application strategies to meet MRL requirements across all destination markets has created orchard management and marketing challenges for the industry and market penetration challenges for the crop protection chemical registrants when new active ingredients are introduced. Furthermore, national authorities appear to be moving away from the reliance on the international standards established by Codex and are developing their own regulatory systems. This has presented additional challenges to fruit producers in an already complicated system of MRL compliance. Additionally, the increasing use of biopesticides and other chemicals used in organic fruit production, which have exemptions from the requirement of a tolerance in the United States but there is no equivalent program in some countries, presents a new set of challenges for producers. A discussion of grower responses to the challenges and ideas for ways to improve decision-making will be presented.

AGRO 9

Import pesticide tolerance pilot project

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The United States Environmental Protection Agency establishes tolerances for pesticide residue levels in foods and feeds. Typically, tolerances are associated with the registration of a pesticide product for use in the U.S. With the increasing global economy for agricultural commodities and the use of pesticide residue tolerances (or maximum residue levels, MRLs) as food-safety trade standards, the lack of such tolerances can present a trade barrier. Recognizing that

pesticide use in an exporting country is generally supported by some form of residue chemistry data generation and review, the Office of Pesticide Programs undertook a pilot project to assess the feasibility and limitations of using data reviews by national registration authorities, the European Food Safety Authority (EFSA), or the Joint Meeting on Pesticide Residues (JMPR) to support the establishment of pesticide residue tolerances without a U.S. registration (i.e., import tolerances). This talk discusses the project, the outcomes to date, and next steps to facilitate a streamlined U.S. EPA business practice for establishing import tolerances.

AGRO 10

Progress on global crop grouping for extrapolation of pesticide residue studies and outcomes from the third Global Minor Use Summit

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Grouping of crops based on taxonomic and/or agronomic similarity is a practical solution for the establishment of Maximum Residue Limits (MRLs) for similar crops and has been a major asset in addressing minor use and specialty crop needs. Data from representative commodities are extrapolated to the entire group of related crops, including minor crops that might not be supported otherwise. The Global Minor Use Summits continue to recommend that Codex serve as a model for crop grouping where the Codex principles for the selection of representative commodities be used for extrapolation to commodity groups. With a great deal of effort, the Codex Committee on Pesticide Residues has adopted these principles along with updated fruit, vegetable and legume plant commodity type groups. The Codex updates should serve as a valuable tool not only for JMPR reviewers but also for data generators who use Codex. As other countries revise or create a crop grouping system, they are encouraged to adopt the new Codex principles and crop groups to the greatest extent possible, rather than creating a unique system.

This paper will also provide outcomes from the Third minor use summit that took place in 2017, to include topics such as considerations for a global list of exempt compounds (biopesticides), use of APEC-like procedures to expedite import tolerances, and considerations for a global MRL database. A brief report on a survey to define minor uses and progress on several global capacity building residue projects.

AGRO 11

Establishing Import MRLs: South Korea and Taiwan

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South Korea and Taiwan are two very important markets for U.S. agricultural specialty crops. Both the Ministry of Food and Drug Safety, South Korea (MFDS) and the Taiwan Food and Drug Administration (TFDA) have regulations for establishing import MRLs. In 2017 an intensive effort to learn of these regulations and procedures was launched in the form of in-country workshops. The results of the workshops were documented in guidance documents and meeting summaries to faciliatate the application for the import MRLs in both countries. An analysis of the MRLs established to date will demonstrate the progress made, the gaps yet to be filled, and the level of harmonization achieved.

AGRO 12

Import MRLs in Japan: Snapshot of the positive list sytem

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A large quantity of agricultural products is imported to Japan from the U.S. and Canada (*e.g.*, approximately US $18.5 billion in 2015), and Japan has been an important market for North American businesses. In light of abundant foods coming from overseas, the Ministry of Health, Labour and Welfare (MHLW) decided to introduce the positive list system in 2006 and consequently, obtaining import MRLs in Japan became critical for North American businesses. Since then, many import MRLs have been set by the MHLW, based on Codex MRLs, U.S. Tolerances, E.U. MRLs, and MRLs in other export countries. The presentation will include how the positive list system has worked in Japan, and also touch on recent updates and trends on data requirements, submission timing, MRL calculation, and reviews regarding import MRLs in Japan. Additionally, Japan started to set ARfD and do acute dietary risk assessment in 2014, and ADI/ARfD and dietary risk assessment in Japan will be covered in the presentation as well.

AGRO 13

Extractability of adsorbed organic chemicals using cations

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The extractability of pesticides and other organic chemicals, which may find their way onto soil, has been of great interest. Extractability of any test substance in Environmental Fate Testing is a necessary objective in order to measure the parent test substance and its degradates to describe the degradation pathway of the test substance. The goal of this experiment was to utilize different cations in an attempt to increase extractability of organic compounds that have adsorbed to soil. Different cations were tested with various oxidation states including K^+, Na^+, NH_4^+, Mg^{2+}, Fe^{2+}, and Al^{3+}. Cations that are commonly found in the environment (Na^+, Ca^{2+}, Mg^{2+}, K^+) are known to influence the adsorption of organic chemicals to soil. Six ^{14}C-radiolabled organic chemicals (Acetaminophen, Daidzein, TPCK, Atrazine, N-Isopropylpipecolic acid, and N,N-Diethyl-3-methylbenzamide) and four different soils with varying characteristics were used. Results indicated that extraction solvents containing monovalent cations (K^+, Na^+, NH_4^+) were effective at increasing the extractability in comparison to 4:1 acetonitrile:water alone. The addition of cations to an extraction scheme can be a useful tool to enhance the recovery of adsorbed residues onto soil.

AGRO 14

What is extractability? Are non-extractable residues in our food supply?

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Incurred pesticide residues are residues that are present after exposure under *in vivo* conditions such as crops grown under production conditions or livestock under animal husbandry conditions. Formation of incurred residues generally cannot be duplicated in a laboratory setting as they are the result of biological and environmental interactions with the pesticide and the organism. Several mechanisms may be involved in the generation of incurred residues. These include chemical modification of the pesticide to form conjugates or other metabolites, chemical binding to various plant or animal components, and non-specific physical entrapment. In many

cases these incurred residues are not extractable by simple solvent extractions. Plant and animal metabolism studies required by regulatory authorities worldwide are designed to identify these various residues. Radiolabeled materials are employed so material balance can be monitored and the route of degradation can be determined. Based on these metabolism studies, the total toxic residue (TTR) is established. The TTR is composed of the parent pesticide and metabolites of toxicological concern. Analytical methods are then developed to measure all components of the TTR which are ultimately used for tolerance enforcement. The tolerance enforcement methods must be validated by analyzing actual metabolism samples and showing concurrence with the metabolism study results (radiovalidation). The method must account for ≥ 70% of the TTR in the aged samples, as identified in the metabolism study. The resulting enforcement method thus accounts for incurred residues if present. This presentation will discuss the importance of metabolism studies for determining incurred and non-extractable residues, leading to subsequent tolerance enforcement method development and radiovalidation. The importance of these methods as they relate to pesticide screening for food safety will be discussed.

AGRO 15

Benzobicyclon hydrolysate sorption coefficients in soils used for rice production

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Benzobicyclon (BZB) is a pro-herbicide, soon expected to be commercially available for rice (*Oryza sativa* L.) production in Arkansas. In the environment, BZB undergoes hydrolysis to form the herbicidally active metabolite benzobicyclon hydrolysate (BH). The objective of this study was to determine the soil sorption coefficient (K_D) of BH through batch-equilibration, soil adsorption experiments using 10 soils commonly used for rice production in Arkansas. The batch-equilibration experiments used a soil:solution ratio of 1:1 with a 0.01 *M* $CaCl_2$ solution, air-dried soil, and an equilibration time of 16 hours. High performance liquid chromatography with diode array detection was used to determine the aqueous-phase equilibrium concentration of BH at a wavelength of 287 nm. Soil sorption coefficients were correlated with soil properties (sand, silt, and clay fractions, organic matter content, pH, and electrical conductivity) using regression to determine soil property influence on the soil sorption coefficients. As expected, preliminary results indicate greater K_D values in finer-textured soils, such as clays, than in coarser-textured soils, such as silt loams. Soil sorption processes control whether the herbicide is sorbed to the solid soil phase or dissolved in the aqueous phase, and has implications for compound transport throughout the environment. Understanding the environmental fate and transport of BH will be essential for making efficient agronomic use of BZB herbicides and for managing BZB herbicides to prevent negative environmental impacts.

AGRO 16

Environmental fate and impact assessment of thiobencarb application in California rice fields using RICEWQ

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Thiobencarb is a commonly used herbicide in Northern California rice fields. Released paddy water containing thiobencarb may pose ecological risks to non-targeted organisms. In this research, the RICEWQ model was employed to assess the environmental fate and impacts of thiobencarb in the Colusa Basin. RICEWQ was first equilibrium tested and then calibrated at the field level. Different water/pesticide management practices for water use and thiobencarb use were compared. The model predicted the thiobencarb concentrations from rice fields for multiple years in the entire Colusa Basin, using input information from the California Pesticide Use Reporting database. The temporal/spatial distribution of thiobencarb was evaluated, followed by assessment of ecological impacts on various non-target organisms. Our research indicated that RICEWQ was able to correctly reflect the initial partitioning of thiobencarb in both paddy water and soil phases. The dynamics of thiobencarb were also well captured by the model at field level after calibration. Mandatory water holding plays a critical role in reducing thiobencarb in released paddy water. A thirty-day holding time can reduce thiobencarb concentration by 64%, when compared to a 6-day holding time. The geospatial pattern of exposure in the study domain indicates the differing extents of pollutant level and its distribution over space. "Risk zones" for different species could be identified based on the geospatial pattern of thiobencarb exposure. The same concentration of thiobencarb may impose different levels of risk to various non-target species due to their susceptibility differences.

AGRO 17

Accurately evaluating the photolytic fate of agrochemicals in natural waters

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Pesticides are important for increased crop productivity, but their regular application onto agricultural lands makes them of particular interest to those studying the environmental fate of anthropogenic chemicals. Indeed, pesticides are ubiquitously detected in natural waters as well as in water supplies, which suggests we may have an incomplete understanding of some pesticides' transport and degradation. In this research, the photochemical fate of fungicides, which accounts for approximately 50% of pesticide usage in Europe and 10% in the U.S., was investigated. Eighteen commonly used fungicides were selected, and their susceptibility to both direct and indirect photolysis was quantified including reaction with photochemically produced reactive intermediates (e.g., triplet state dissolved organic matter, singlet oxygen, hydroxyl radical). The comprehensive photochemical half-lives of these chemicals were compared to the values reported in the registration literature. The results of this study show the importance of a complete characterization of the photolytic degradation of chemical if the environmental fate of these

chemicals is to be understood. In particular, the fungicides fludioxonil and fenhexamid are highlighted as case studies to demonstrate the importance of understanding the photolytic fate of agrochemicals. Additionally, a protocol of photochemical experiments was devised in order to accurately and quickly assess both direct and indirect photolysis.

AGRO 18

Transformation products of 2,4-D sunlight photolysis in simulated leaf surface systems

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Pesticides are commonly applied on foliage post-emergence and can form dry deposits on the cuticular wax of crop leaves. Although leaf surfaces can be the first reaction environment for these pesticides after application, their transformation on these lipophilic surfaces is typically not considered in current fate and transport model. Some pesticides were recently reported to undergo enhanced photolysis on leaf surfaces, but several high-use, albeit less photo-labile, pesticides have not been evaluated. Previously, we showed that the sunlight photolysis of six phenoxyacetic acid herbicides (i.e., 2,4-D and its structural analogues) proceeded faster in non-polar solvents (e.g., *n*-heptane) than in polar solvents (e.g., methanol). Photolysis of these compounds on paraffin wax surface proceeded at similar rates as that in non-polar solvents, but 3-9 times slower than that on quartz. In this work, we focused on elucidating the mechanisms for the photolysis of 2,4-D herbicides. Measured pseudo-first-order photolysis rate constants followed the order: quartz > wax ≈ *n*-heptane > 2-propanol > water > methanol ≈ acetonitrile. The quantum yields followed the same order with similar magnitude of relative difference. Quantum yields for 2,4-D herbicides on quartz ranged from 0.03-0.12, which are much higher than that in organic solvents. Product analyses show that photoreductive dechlorination accounted for all parent compound decay in organic solvents. Therefore, this postulated radical pathway is likely favored in non-polar environments. On surfaces, however, products of photoreductive dechlorination only accounted for less than 60% of the parent compound decay. The rapid photolysis on surface was likely attributable to differences in reaction mechanisms unique to surfaces. The estimated photolysis half-life of 2,4-D herbicides on wax was 0.4-2.2 d, within the range of their half-lives for biodegradation, the previously considered major dissipation pathway.

AGRO 19

Web-based access to experimental and predicted data for environmental fate, transport and toxicity data

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The EPA CompTox Chemistry Dashboard provides access to data associated with ~760,000 chemical substances. The available data includes experimental and predicted physicochemical properties, environmental fate and transport data, *in vivo* and *in* silico toxicity data, *in vitro* bioassay data, exposure data and a variety of other types of information. The data are under continuous expansion and curation and the experimental data have been used to develop QSAR and QSPR models. A number of these models are available via a web interface so that users can submit a chemical structure and predict properties in real time. The dashboard also provides access to pre-compiled chemical lists and categories, including pesticides, and chemicals detected in the environment via non-targeted mass spectrometry analysis. The data are searchable using chemical identifiers (systematic names, trade names, CAS Registry Numbers), by structure, mass and formula. Batch searches allow for data associated with thousands of chemicals to be obtained in a few seconds, with just a few button clicks, and downloaded to the desktop. This presentation will provide an overview of the Dashboard and its applications to accessing source data associated with agriculturally related chemicals.

AGRO 20

Framework for tiered endangered species assessments

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Ensuring that threatened and endangered species populations in the United States remain viable is an important factor for maintaining biodiversity. While habitat loss is the primary risk, endangered species risk assessments (ESRA) should be designed to identify cases in which exposure to pesticides under realistic conditions could potentially increase risk. In a 2013 report, "Assessing Risks to Endangered and Threatened Species from Pesticides," the National Academy of Sciences (NAS) provided guidance for ESRA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) with direct consideration of the Endangered Species Act (ESA) Section 7 requirement for federal actions. The NAS panel recommended refined approaches (*e.g.*, probabilistic methods, population modeling, spatially explicit exposure estimates) when conducting assessments. In response to the NAS report, EPA, the Services (Fish and Wildlife Service and National Marine Fisheries Service), and USDA released an implementation plan ('Interagency Interim Approach') for ESRA. This approach was applied in the development of the 2017 organophosphate biological evaluations (BEs), which were the basis for the subsequent NMFS Biological Opinion. Unfortunately, these BEs (and BOs) illustrated that the interagency interim approach is inefficient, does not follow a tiered approach, does not apply best available information, is not scientifically transparent, will result in unacceptable delays and unpredictability in regulatory decisions, and require large increases in federal resources, with uncertain benefit to listed species and their critical habitats. This presentation describes a framework for conducting efficient ESRA using a scientifically defensible and rational approach adapted from the NAS panel report recommendations, the Overview of the Endangered Species Ecological Risk Assessment Process in the Office of Pesticide Programs, the 1998 EPA Risk Assessment Framework, and lessons learned from the OP case studies. The framework describes a tiered approach using the best available information on pesticide use, exposure estimates, ecotoxicology endpoints, and endangered species data.

AGRO 21

Estimation of annual agricultural pesticide use

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A method was developed to calculate annual county-level pesticide use for selected herbicides, insecticides, and fungicides applied to agricultural crops grown in the conterminous United States. Pesticide-use data compiled by proprietary surveys of farm operations located within Crop Reporting Districts (CRDs) were used in conjunction with annual harvested-crop acreage reported by the U.S. Department of Agriculture National Agricultural Statistics Service to calculate use rates per harvested crop acre, or an 'estimated pesticide use' (EPest) rate, for each crop by year. Pesticide-use data were not available for all CRDs and years. When data were unavailable for a CRD in a particular year, EPest extrapolated rates were calculated from adjoining or nearby CRDs to ensure that pesticide use was estimated for all counties that reported harvested-crop acreage. EPest rates were applied to county harvested-crop acreage differently to obtain EPest-low and EPest-high estimates of pesticide-use for counties and states, with the exception of use estimates for California, which were taken from annual Department of Pesticide Regulation Pesticide Use Reports. EPest values from this study are suitable for making national, regional, and watershed assessments of annual pesticide use. Although estimates are provided by county to facilitate estimation of watershed pesticide use for a wide variety of watersheds, there is a greater degree of uncertainty in individual county-level estimates when compared to CRD or state-level estimates because (1) EPest crop-use rates were developed on the basis of pesticide use on harvested acres in multi-county areas (CRDs) and then allocated to county harvested cropland; (2) pesticide-by-crop use rates were not available for all CRDs in the conterminous United States, and extrapolation methods were used to estimate pesticide use for some counties; and (3) it is possible that surveyed pesticide-by-crop use rates do not reflect all agricultural use on all crops grown.

AGRO 22

Incorporating usage data to identify areas where pesticide exposure to listed species is most likely to occur

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The United States (U.S.) Environmental Protection Agency registers uses of pesticides in the U.S. and its territories. As such, a national level registration of a pesticide may have potential exposure to large portions of the ranges of endangered and threatened species. For registrations that apply to agricultural uses (*e.g.,* corn, apples), the U.S. Department of Agriculture's cropland data layer (CDL) can be used to identify potential pesticide use sites in the 48 conterminous states. Additional data are also available to identify where a pesticide has been applied (termed "usage" data). Combining usage data with CDL landcovers can help identify areas where pesticide applications are most likely to occur. Subsequently, likely use sites can be compared with listed species ranges to determine the most likely proportion of the species range or population that may be exposed. One major challenge to this approach involves fitting data from different scales. Combining these data requires some assumptions related to distributions of sites where the pesticide is applied relative to the species range. This presentation will discuss some of the benefits and challenges of incorporating usage data into risk assessments and some options to overcome the challenges.

AGRO 23

FESTF Gopher: Improving data management, accessibility, and use

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The FIFRA Endangered Species Task Force (FESTF) has contributed to the U.S. Environmental Protection Agency's (USEPA) endangered species and pesticide data development process for nearly 20 years. Throughout this time, FESTF's efforts have been focused on aggregating, analyzing, and providing data related to endangered species and pesticides use areas to the USEPA to inform their assessments. Such data is informative for Ecological risk assessments of endangered species, and efforts to improve management and accessibility to these aggregated datasets markedly increases their value. To manage and deliver these data, FESTF designed an Information Management System (FESTF IMS) in 2002. Improvements in data availability and advancements in technologies and data processing lead to the need to update and expand the capabilities of FESTF IMS, and consequently FESTF created an enhanced service tool – Gopher. Gopher integrates the best available datasets related to endangered species and pesticide assessments into a single tool that allows users of various levels of expertise to spatially interact with datasets using different data analytics tools. This presentation will: 1) provide a general overview highlighting the functionality of Gopher, 2) describe the value of Gopher for informing and facilitating preparation of USEPA's endangered species pesticide assessments, and 3) discuss potential future applications of Gopher for capturing voluntary measures as a mechanism to better inform the interface of pesticide use and endangered species data.

AGRO 24

Tools for evaluating indirect effects of pesticides for informed management decisions

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Based on recommendations from the National Academy of Science (NAS) panel on endangered species risk assessment approaches and methodologies, EPA, FWS, NMFS, and USDA established joint interim approaches. As part of the interim approaches, EPA performs an assessment, termed a Biological Evaluation (BE), to determine whether individuals of a listed species have the potential to be exposed to and affected by a pesticide. Since pesticides may have the potential to affect a listed species through direct exposure or indirectly through effects on other species in the community, EPA evaluates direct and indirect effects in the BE in order to determine if the pesticide's registered use will have "no effect," "may affect, but is not likely to adversely affect," or "may affect and is likely to adversely affect" the species or designated critical habitat. In response to NAS panel questions, EPA clarified "may affect" determinations would only be reached for indirect effects if biological information for a listed species establishes a relationship between the affected taxa and listed species. The NAS panel recommended limiting the evaluation to easily quantifiable indirect effects that might affect an organism's survival and reproductive success. However, the 2016 BEs for three organophosphate insecticides did not adequately consider species relationships or effect magnitude, which resulted in "may affect "conclusions for nearly all plants and fish based on indirect effects despite the lack of obligate relationships for many of these species. A realistic and

practical assessment of the potential for indirect effects is critical to informing effective management decisions. Therefore, we evaluate options for incorporating major components and linkages of species and habitat to assess indirect effects of pesticides on listed species in an adequate and efficient manner, which can be incorporated in future BEs, using case studies with high profile species.

AGRO 25

Weight-of-evidence pesticide assessments for threatened and endangered species to inform management decisions

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Under the Endangered Species Act, the U.S. Environmental Protection Agency (EPA) is required to assess potential risks to threatened and endangered (listed) species when registering pesticides. In cases where the pesticide "may affect" one or more listed species, the EPA must consult with the Services (i.e., National Marine Fisheries Service [NMFS], Fish and Wildlife Service [FWS]). Recent endangered species assessments for chlorpyrifos, diazinon, and malathion by EPA and NMFS included a weight-of-evidence (WOE) approach to make their "may affect/no effect" and "jeopardy/no jeopardy" conclusions. The Agencies' WOE approach was troubling in several respects. First, the lines of evidence given by far the greatest weight in the assessments were slight variations on the same theme, i.e., comparison of modeled exposure estimates to highly correlated endpoints. These "lines of evidence" are really just one line of evidence with multiple measures of effect. As pointed out by Glenn Suter and Susan Cormier in a 2011 publication, if an assessor has ten toxicity tests and one field observational study, the assessor would combine the toxicity tests into one line of evidence, weight it, and combine it with the weighted field observational study line of evidence. Second, the Agencies only superficially considered other lines of evidence, e.g., water quality monitoring studies, mesocosm and field toxicity tests, *in situ* tests, field observation studies, incident reports, listed species population trends, etc. Finally, the scoring system used by the Agencies to arrive at their risk conclusions was simplistic, qualitative and relied heavily on professional judgment. In this presentation, a WOE approach and rubric for weighting and combining lines of evidence is proposed for endangered species risk assessments involving pesticides. The WOE approach and rubric are simply adaptations of what has been commonly used elsewhere since the early 1990s.

AGRO 26

Voluntary conservation: Benefit and cost considerations for stewardship programs

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The adoption of conservation practices within or adjacent to farmland potentially offers opportunities for growers to be directly involved in conserving and improving species habitat, while continuing to have access to a range of chemistries for pest control. The benefits from conservation practices such as riparian forest buffers and herbaceous cover are numerous, including reduced soil erosion and pesticide drift, improved water quality, and increased wildlife habitat. However, there are costs incurred by growers that may limit adoption. While federal and state agencies, local municipalities, private industry, and non-governmental organizations provide some financial and technical assistance, there are considerations that must be evaluated to have a successful stewardship program with long-term adoption. Growers must have a clear understanding of the benefits, which accrue in both the short and long-term, and costs, which do not begin nor end with installation costs. Consideration must be given to how the practice impacts their production methods, the appropriate location and scale of the practice, and required maintenance. Once conservation practices are adopted, documenting the benefits and impact on species as compared to the cost is a logical next step; however, much of this information is known only by the grower and funding organization. Being able to identify the location and scale of conservation practices throughout the U.S. would provide an opportunity for a clearer understanding of how conservation practices fit together across the landscape. This presentation will outline 1) benefit and cost considerations for stewardship programs aimed at conserving and improving species habitat and 2) the need for a clearer understanding of where conservation practices are installed and the associated benefits.

AGRO 27

Creating environmentally resilient agriculture landscapes using precision agriculture technology: An economic perspective

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Agricultural landscapes face increased societal pressure to meet global food demands while simultaneously providing ecosystem services. Increased use of agricultural chemicals is expected in order to meet global yield objectives and reduce risk. However, increased use of agro-chemicals poses significant risk to ecosystem function and sustainability. Natural plant communities, as a component of agricultural landscapes, can mitigate this risk and support ecosystem function by providing essential ecosystem services with broad societal value (i.e., pollination, beneficial insects, wildlife populations). Sustainability of global agricultural systems will require greater focus on strategic integration of conservation practices into production agricultural systems to protect and enhance ecosystem services and crop production. However, balancing these objectives creates challenges for producers as the allocation of land to non-crop uses (e.g., natural plant communities) entails economic opportunity costs for producers. Agricultural producers will implement conservation actions provided the economic incentives are equal to or greater than traditional farming. Therefore, it is essential for natural resource professionals to help producers identify and understand the economic opportunities of conservation implementation. Precision agriculture technology provides a unique framework for identifying economic and conservation opportunities in production agriculture. By using precision agriculture in a conservation framework, natural resource professionals can demonstrate the overlap between conservation eligibility and economic opportunity. I illustrate the application of this technology to create natural plant communities in production agricultural landscapes that increase field-level profitability, wildlife populations, and ecosystem services. This approach could easily be used to increase the use, efficiency, and profitability of vegetation filter strips to reduce pesticide movement in the environment.

AGRO 28

Using publicly available data and quantitative models of uncertainty to characterize composition of consumer products in a simulation model of chemical exposure

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EPA is developing a probabilistic software tool (the Human Exposure Model, HEM) to determine human exposures to chemicals from the use of certain consumer products. A challenge for this project is that composition of many products is considered confidential. To address this, EPA has established: (1) a set of 241 Product Use Categories (PUCs) that are based on the purpose of the product and the

products' exposure-related characteristics; and, (2) a database (Chemical Product Database or CPDat) of publicly available composition data organized by PUC. The composition data comes from two sources, material safety data sheets (MSDSs) and lists of ingredients disclosed by the manufacturer. Each source reports useful, but incomplete composition data. MSDSs often report ingredients' weight fractions (WFs) as ranges. Ingredient lists generally do not report WFs, but ingredients are typically listed in descending order of WF. When modeling product use by individuals, HEM assigns corresponding product compositions using a two-stage process. First, a composition record of a product from the same PUC is selected from CPDat. Second, probabilistic models estimate distributions of WF values for each reported chemical in the product that are consistent with the available data and have an equal probability of occurring. When ranges of WFs for a product are reported in an MSDS, the model assumes a uniform distribution across these ranges. WFs for products with ingredients list data are determined using a predictive approach based on the length of the list and the rank of each chemical. HEM samples from these distributions to assign a WF for each chemical in each product. The values of WF are then used with the chemicals' physicochemical properties and product-specific exposure scenarios to estimate exposure for each product ingredient.

AGRO 29

Leveraging chemistry data to improve exposure analyses using the EPA's CompTox Chemistry Dashboard

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High resolution mass spectrometry (HRMS) and non-targeted analysis (NTA) are improving the means by which exposure analyses can be conducted by advancing the identification of emerging contaminants in environmental and biological matrices. Confident identification in NTA requires cohesive workflows that combine high performance instrumental analysis with database searching, *in silico* predictions, and refined data processing. It is recognized that chemical metadata significantly improves identification of candidate compounds in NTA as part of identification workflows with or without spectral matching. The US EPA's CompTox Chemistry Dashboard is a comprehensive chemistry resource and web application containing data for ~760,000 substances. The goal of this research is to identify chemistry data available via the Chemistry Dashboard to improve the identification of unknowns in NTA. Multiple data streams have been investigated to provide evidence to support 'spectral agnostic' identification. Metadata such as chemical consumer product occurrence, chemical functional use, likelihood of occurrence in environmental media, PubMed literature refence counts, and PubChem data source counts were investigated individually and collectively as part of a scoring-based identification scheme. Functionality to access these data and conduct searches of unknowns was developed, optimized, and surfaced via the CompTox Chemistry Dashboard, enabling a freely available software tool for structure identification and NTA. *This abstract does not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

AGRO 30

Spatial and temporal modeling of potential residential bystander exposures associated with the use of agricultural chemicals

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Primary (particulate) drift has been a subject of evaluation by regulatory agencies due to offsite movement of agriculture use pesticides that may have the potential to adversely affect ecosystems and human health. Similarly, methods have been developed to address fumigant pesticide transport that occurs by vaporization, often termed as secondary drift. Advanced fumigant drift modeling requires a geospatial, temporal/calendar-based human bystander exposure construct to characterize concentrations of vapor in ambient air as a function of distance from multiple and intermittent application events during seasonal use. A stochastic, data-informed (e.g., product use, time-activity, meteorology) spatial and temporal analysis is presented for 1,3-dichloropropene (1,3-D is used as a pre-plant nematicide in numerous economically important crops) demonstrating advances in dose-response and exposure assessment, including: 1) characterization of a toxicological threshold; 2) the use of residency duration and mobility data in communities with high demand for 1,3-D; 3) air modeling data using a validated air dispersion model (SOFEA) that provides detailed information on spatial and temporal variation in air concentrations of 1,3-D; 4) the use of age-cohort (life stage-specific) physiological factors; and 5) predictions of potential human health risk from possible long-term exposures.

AGRO 31

Integrating pharmacokinetic considerations with dose-response data to support risk-based chemical safety assessment

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Risk-based chemical safety assessment involves first determining the concentration that is sufficient to produce biological effects in an organism, as determined using dose-response analysis. If dose-response analysis involves *in vivo* animal tests, the point of departure (PoD) found for the test species needs to be extrapolated to that for humans. If dose-response analysis involves *in vitro* systems, an internal concentration that is equivalent to the *in vitro* PoD should be converted to an external concentration, which is compared to concentrations that are estimated to be encountered by the organism from all sources of exposure. Both scenarios require the need to account for a chemical's pharmacokinetic (PK) properties. We present, in this talk, a tiered approach for integrating such PK considerations into chemical safety assessment based on data availability and study purposes. This tiered approach ranges from qualitative chemical structure-based queries to identify those chemicals possessing the necessary PK properties that allow for reaching a biological target, to quantitative modeling of absorption, distribution, metabolism, and excretion (ADME) processes. By integrating PK characteristics of chemicals with dose-response data, chemicals that are incapable of reaching or perturbing a given biological target, or those that may generate active metabolic products, can be identified to improve *in vitro*-based chemical safety assessments. Simulation of ADME processes in the presence of available *in*

vivo time-concentration data can allow for more informed extrapolation from dose-response analysis conducted for a given test species to another species or life-stage of interest. We will also discuss how uncertainty in PK properties can influence the applicability of this tiered approach in assessing chemical safety.

AGRO 32

Building a more relevant bridge: Interspecies extrapolation based on real-world exposure conditions

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Human risk assessment traditionally relies on extrapolation from rodent toxicity data, requiring dosimetric adjustments and UFs to account for species differences. Dosimetric adjustments and interspecies extrapolation can be improved by taking into account human exposure conditions. Laryngeal toxicity is observed in rats exposed nose-only to ≥ 0.5 mg/L 2,4-D aerosol (MMAD = 2.3 µm, GSD = 1.7). This is a common response to inhaled irritant chemicals in rodents and heightened sensitivity relative to other species is well documented. Therefore, reduction of the interspecies UF to 1X is justified. Dosimetry addressing relative regional aerosol deposition further improves interspecies extrapolation. US EPA previously modeled tracheobronchial (TB) deposition of 2,4-D using RDDR, yielding a rat:human ratio of 1.49. To better address laryngeal exposure, the same conditions were modeled in MPPD to obtain more detailed data on fractional deposition throughout the TB region. Deposition in the trachea was used as a best approximation of the larynx, yielding a rat:human ratio of 3.24, better representing the higher toxicokinetic sensitivity of the rat to laryngeal portal of entry toxicity. Consideration of particle size distributions relevant to human field aerosol exposures further refines this approach. Case studies were developed based on 2,4-D application aerially or with a groundboom sprayer. Particle size distribution for the applicator or bystander breathing zone was modeled with AGDISP, simulating drift of a medium or coarse to very coarse quality spray from the nozzle to the edge of the field. This gives a worst-case exposure for the applicator, who would be closer to the nozzle and therefore exposed to larger particles. A lognormal distribution was fit to the inhalable (<100 µm) portion of the distributions using Crystal Ball, and fractional deposition was modeled in MPPD (MMD = 22.94-23.72 µm, GSD = 1.52-1.66). The rat:human fractional deposition ratio at the trachea using study conditions for rats and field conditions for humans is >2700. While this value can be applied as a conversion factor to calculate a human equivalent concentration, this large ratio suggests that laryngeal portal of entry effects are not human relevant under these conditions. For portal of entry effects from aerosol exposure, species sensitivity to a given concentration depends not only on physiological differences, but on differences between test conditions and real world exposures.

AGRO 33

Current status of insecticide resistance in insect vectors

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The Insecticide Resistance Action Committee, IRAC, works as a specialist technical group of the agrochemical industry association CropLife International. IRAC was formed in 1984 to provide a coordinated industry response to the development of resistance in insect and mite pests and was reformed in 2006 in response to the growing problem of insecticide resistance in public health pests. IRAC acts as a conduit between industry and interested third parties, providing technical advice and producing educational material. Effective resistance management requires sound understanding of the vector's biology, and effective control methods as well as knowledge of detection, monitoring and consequences of resistance. One of the challenges in both designing and implementing resistance management programmes for vector control remains the paucity of data directly linking increasing cases of vector-borne diseases to the presence and frequency of resistance. The case of malaria exemplifies this. Since 2000, the global reduction in malaria cases has been largely attributed to vector control with long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). These gains are threatened by the emerging and rapid spread of insecticide resistance. In 2017, the World Malaria Report highlighted that the declining trend in malaria cases (and deaths) has stalled and even reversed in some regions, yet implementation of new insecticides, tools and policies, and resistance management programmes in malaria endemic countries remains slow. Much effort is going into the development of new insecticides and formulations to enable sustainable, cost effective, vector control, but comprehensive pro-active resistance management strategies are needed to accompany these new tools as they are developed and as soon as they are launched in order to effectively manage the lifetime of these valuable new products.

AGRO 34

One health approach to resistance management

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The One Health Strategy focuses on the collaborative, holistic surveillance of human, animal, and environment health as a global response to the threat of infectious diseases. The honey bee plays an economically vital role in global agriculture as a pollinator of a wide variety of food and fiber crops that are needed to satisfy the needs of human and animal health. The loss of honey bees is a major environment health challenge that demands attention from the scientific community. There are numerous environment stressors that negatively impact the health and survival of honey bees, although a growing consensus identifies the high levels of parasites and pathogens, especially arthropod-borne viruses, are among the most significant threats to the health of these pollinators. A common approach to arthropod-borne virus management is the use of synthetic neurotoxicants alone or in combination with organic acids and botanical oils to reduce ectoparasitic mite infestations. These conventional acaricides not only have adverse health effects on honey bees, but widespread acaricide resistance limits their use to reduce mite infestations and their transmission of viruses to honey bees. The development of acaricide resistance is an evolutionary phenomenon that requires appropriate and comprehensive monitoring and management strategies within an integrated vector management framework, and similar to that of One Health. Here, I will discuss (i) novel acaricide resistance surveillance and reporting tools, (ii) alternative interventions to mitigate acaricide resistance evolution and preserve the efficacy of existing acaricides, (iii) exploration of next generation acaricide modes of action and their applications, and (iv) building stakeholder partnerships that bring together people to work locally, nationally, and globally as a collaborative One Health Response Team actively engaged in reducing arthropod-borne diseases to honey bees and improving the health and protection of these important pollinators.

AGRO 35

Insecticide resistance and management of malaria vectors

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Insecticides are the cornerstone of insect transmitted disease control and largely responsible for the reduction in malaria transmission by Anopheline mosquitoes in Africa this century. Transcriptional profiling, population genomic, biochemical and transgenic approaches have been applied to determine the causes of insecticide in malaria and other tropical disease vectors. Great strides have been made in identifying target genes and networks associated with insecticide metabolism in mosquitoes. This is feeding the development of new vector control tools to monitor and predict insecticide resistance liabilities. Most recently, results from the Ag1000g project highlight extreme genetic diversity in *An. gambiae* (a variant allele every 2.2 bases). This focusses our attention on the production of tools capable of functional differentiation of resistance. This presentation explores recent developments and their potential impact on tropical disease control.

AGRO 36

Changes in neuronal signaling and cell stress response pathways are associated with a multigenic response of *Drosophila melanogaster* to DDT selection

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The adaptation of insect populations to insecticidal control is a continual threat to human health and sustainable agricultural practices, but many complex genomic mechanisms involved in this adaptation remain poorly understood. This study applied a systems approach to investigate the interconnections between structural and functional variance in response to dichlorodiphenyltrichloroethane (DDT) within the Drosophila melanogasterstrain 91-R. Directional selection in 6 selective sweeps coincided with constitutive gene expression differences in DDT resistant flies, including the most highly upregulated transcript, Unc-115b, which plays a role in axon guidance, and the most highly downregulated transcript, the angiopoietin-like CG31832, which is involved in directing vascular branching and dendrite outgrowth but likely may be under trans-regulatory control. Direct functions and protein–protein interactions mediated by differentially expressed transcripts control changes in cell migration, signal transduction, and gene regulatory cascades that impact the nervous system. Although changes to cellular stress response pathways involve 8 different cytochrome P450s, stress response, and apoptosis is controlled by a multifacetted regulatory mechanism. These data demonstrate that DDT selection in 91-R may have resulted in genome-wide adaptations that impact genetic and signal transduction pathways that converge to modify stress response, cell survival, and neurological functions. This study implicates the involvement of a multigenic mechanism in the adaptation to a chemical insecticide, which impacts interconnected regulatory cascades. We propose that DDT selection within 91-R might act systemically, wherein pathway interactions function to reinforce the epistatic effects of individual adaptive changes on an additive or nonadditive basis.

AGRO 37

Uncertainties maximum residue levels create for the global movement of grains and oilseeds

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The global movement of grains and oilseeds has become increasingly more important to meet the world's food security needs for a growing population, moving goods from areas of surplus into areas of deficit. These commodities move through a sophisticated system of international trade to provide a safe, high volume, affordable, competitive, timely, predictable, responsive, and resilient supply. Regulatory approaches and policies around the world for crop protection products continue to put pressure on farmers who use these tools to combat a variety of production challenges. Continued use and future innovation in crop protection products depends on trade enabling and consistent regulatory outcomes and endpoints. Global disorganization in the establishment and enforcement of MRLs to manage crop protection products is impacting all U.S. agricultural sectors with increasing intensity. Grains and oilseeds have found growing complexities in trade of crops cultivated, stored, and shipped with crop protection products. The success of U.S. agriculture is greatly dependent on the market access hurdles exacerbated by global policies and regulations which are misaligned or are not risk and science-based. Missing and misaligned Maximum Residue Levels (MRLs) create significant uncertainty for the global grain trade. Zero or near-zero default tolerances, the application of different levels of MRLs in exporting and importing countries, or missing MRLs resulting from misaligned policies impede trade, food security, and innovation. Regulatory coherence across exporting and importing countries is key to supporting grain trade for food, feed, and processing and needs to result in manageable MRLs. Both government and the private sector have a role in developing and implementing trade-enabling solutions, which can reduce the risk for market access challenges and also position U.S. grains and oilseeds to capture future opportunities.

AGRO 38

Effect of the hazard-based cut-off criteria on agriculture exports to the European Union

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In 2009, the European Union revised its regulation of crop protection products, adopting a "hazard-based" approach to the approval of active substances under Regulation 1107/2009. This Regulation establishes hazard-based "cut-offs" for certain categories of substances, including carcinogens, mutagens, or reproductive toxicants ("CMR"), as well as substances that are persistent in the environment. In addition, active substances deemed to have endocrine disrupting properties are subject to a hazard-based cut-off, although EU criteria for identifying substances as endocrine disruptors have not yet been adopted. Under Regulation 1107/2009, active substances meeting the criteria for any of these categories will be cut off from the European market based solely on the health or environment hazard they pose, without a risk assessment that considers levels of exposure. Regulation 1107/2009 requires that active substances used in crop protection products be assessed for potential hazard each time the substance is subject to an approval or renewal of approval at the EU level. If deemed to belong to one of the cut-off categories, the EU Maximum Residue Levels (MRLs) are likely to be revoked as the substance is withdrawn from the market. This creates the potential for numerous plant protection products to be withdrawn from usage in EU member states and associated MRLs to fall to the default level

of 0.01 ppm. This presentation will analyze how the EU's cut-off criteria policy will affect exports of commodities to the EU. It will identify which regions and crops are potentially most affected and provide specific examples of where MRL revocations have already affected trade. The overall potential costs of the hazard based approach will also be discussed.

AGRO 39

New tools for finding potential solutions for differential MRLs and for growers' needs in the area of pests and diseases

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Homologa contains detailed information about registered crop protection products and its MRLs for about 70 countries. New software has been developed that allows for cross-analysis of data from several sources and content like product-registration, MRLs, Global Needs, Trade import/export statistics, and residue statistics. The presentation will show some examples: 1) Explaining differences in MRLs between countries in looking at the use pattern of crop protection products; 2) Finding 'minor use solutions' with different criteria, like available ai in same country but for another crop or another country; 3) Available solutions with lower tox-class; 4) Available solutions with specific type of mode of action or product-class; 5) Help to set priorities for the establishment of MRLs or for finding Minor Use solutions with a cross-analysis of crop areas grown and crop-export values; 6) Help to find alternative solutions for active ingredients that risk being banned in import countries; 7) Solutions for Needs from Global Needs list that a manufacturer has available in its product range.

AGRO 40

Pesticides residue regulations governing U.S. commodity imports

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Agricultural commodities, both domestic and imported, may occasionally contain very low residual levels of agricultural chemicals that do not pose a risk to consumers. These chemicals have been applied to protect the growing crop and/or as a post-harvest protectant for transport and storage. As analytical detection methods become more precise, lower levels of these chemicals can be detected in food products. In situations where there is no EPA-defined tolerance or tolerance exemption, the imported commodities are considered adulterated by the FDA. When this occurs, the agricultural commodities are often destroyed, adding to the approximately 60 billion tons of food that is wasted each year in the U.S. and resulting in financial and environmental costs that are eventually passed on to the consumer. In this study we report the current volume of commodities imported into the U.S., pesticides residue violations, and possible opportunities.

AGRO 41

IESTI update: How a review of dietary exposure methodologies can best support global MRLs

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There is an ongoing discussion regarding proposed changes to the general International Estimate of Short-Term Intake (IESTI) equation used by many regulatory bodies including the specific IESTI version used by Joint Meeting on Pesticide Residues (JMPR). The IESTI equation provides an estimate of acute dietary exposure to pesticides based on 97.5th percentile large portion consumption data and is used to support the establishment of MRL values (the maximum legally allowed residue on food or feed). The IESTI equation is a deterministic model where both the food consumption and the residue concentration are represented by a single numerical value. The current IESTI output is known to be conservative, but its relationship to real world exposure has not been systematically explored. A benchmarking exercise was proposed at the 49th Codex Committee on Pesticide Residues (CCPR) meeting to compare current and proposed IESTI outcomes to probabilistic distributions of actual dietary exposures based on dietary monitoring data to assess real world exposures. This talk provides perspective on the progress of international discussions around IESTI occurring this year.

AGRO 42

Benchmarking proposed changes to the international estimated short-term intake (IESTI) model for acute exposure to pesticides

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In late 2015, changes to the current IESTI calculations were proposed in a joint FAO/WHO workshop. Subsequent discussions at the Codex Committee on Pesticide Residues (CCPR) and an associated electronic working group focused on whether change is needed and potential impacts of proposed changes. No decision has been made on final proposed equations, but the appropriate level of conservatism has been a focus of discussion. The 2015 proposed equations included: use of the MRL in place of residue trial data, removal of consideration of unit weight, introduction of a new conversion factor accounting for metabolites not included in the MRL, and use of large portion consumption data on a body weight rather than per person basis. Preliminary assessments have projected an increase in calculated dietary intakes, despite statements *that changes to the IESTI should not lead to substantial changes in the level of conservativeness* (CX/PR 17/49/12; discussion paper from IESTI eWG at 49th CCPR meeting). The ultimate impact on MRLs critical for the free global trade of food remains unclear. Based on these events, it has become apparent that benchmarking the conservatisms in the IESTI approach will be necessary to better understand the need for any changes and the implications of those changes. This presentation will describe benchmarking case studies using probabilistic dietary modeling (using the CARES-NG US dietary model) and pesticide monitoring data from the USDA Pesticide Data Program (PDP) to address conservatisms in the current and proposed IESTI approaches.

AGRO 43

APEC tools for import maximum residue limits (MRLs)

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The Food Safety Cooperation Forum of the Asia Pacific Economic Cooperation (APEC) is developing two implementation tools to facilitate international harmonization of import Maximum Residue Limits (MRLs) for pesticides. The first tool is a database that will capture the processes used for regulatory establishment of import MRLs in APEC nations, along with contact points in the corresponding regulatory agencies. The second tool is a proposed template to be used by importers to expedite import MRL applications in the APEC region. The process began at a "First Expert Workshop" in Haikou China, April 4-5, 2018. The tools are being developed through an Electronic Working Group.

AGRO 44

Postharvest fumigants: Global MRL progress & challenges

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We report on regulatory challenges associated with the use of postharvest fumigants in the global marketplace. While postharvest fumigation is principally relied upon to guarantee food security, particularly of quarantine pests, the regulatory infrastructure for the adoption of new postharvest fumigants, as well as new uses, has not kept pace with conventional preharvest pesticides. We detail technical aspects that render postharvest fumigants unique from a regulatory perspective, as well as key challenges for horticultural and stored product sectors.

AGRO 45

Preliminary assessment of residual herbicide concentrations in tailwater recovery systems

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Tailwater recovery systems recycle irrigation water using a network of edge-of-field ditches and storage reservoirs. Tailwater recovery has the potential to reduce agricultural groundwater use in Arkansas critical groundwater depletion zones and intercept downstream export of agrochemicals, but residual herbicides in these systems could lead to cross-crop contamination through irrigation water and yield loss in sensitive crops. Further, during the non-growing season, tailwater may serve as source water for artificial groundwater recharge if compliant with environmental and human health standards. Therefore, residual herbicide concentrations in these systems must be assessed. This study monitored concentrations of a suite of herbicides in seven tailwater recovery systems in the Cache Critical Groundwater Area in Northeast Arkansas. Grab samples were collected weekly from ditches and reservoirs during the growing season (April-August) and biweekly thereafter. Samples were processed by filtration through 0.45 µm membrane filters and concentration using solid phase extraction on a Strata-X polymer sorbent. Target herbicides were 2,4-D, clomazone, dicamba, glyphosate metolachlor, propanil, and quinclorac and were selected based on grower application information. All analytes, except glyphosate, were measured using high performance liquid chromatography with diode array detection with a C18 column and an acetonitrile : 0.1% H3PO4 (aq) gradient mobile phase. Glyphosate was measured with enzyme-linked immunosorbent assay. We will report on year one findings and explore preliminary spatial and temporal trends in herbicide concentrations in the tailwater systems.

AGRO 46

Off-site transport of pesticides with runoff from golf course fairway turf: An evaluation of creeping bentgrass versus a fine fescue mixture

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Stormwater runoff can transport pesticides from managed turfgrass to surrounding surface waters, raising concern for non-target organisms. Environmental stewardship includes looking for new approaches to reduce adverse environmental impacts of current practices. One strategy is to replace traditional turfgrass with low-maintenance turfgrass. Fescue grasses have been shown to provide characteristics desirable for golf course fairways. However, it is unknown what influence this change will have on the off-site transport of pesticides with storm runoff. Therefore, studies comparing runoff from plots planted in creeping bentgrass or a fine fescue mixture were conducted. Shoot density, thatch depth and soil moisture were the most important factors related to runoff volume. Concentrations of pesticides and the overall mass of pesticides transported off-site with runoff from the different turfgrasses will be discussed. Results of this research contribute to the understanding of pesticide transport with runoff and provide golf course superintendants with information for management decisions.

AGRO 47

How can risk management practices be considered in regulatory risk assessments: Reducing pesticide transport via surface run-off and soil erosion?

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On sloped agricultural fields, water and sediment can be transported downhill as run-off and erosion. This process produces loss of valuable soil mineral mass as well as the transport of plant protection products (PPP) into adjacent surface water bodies. In European and US risk assessment for the registration of PPP, runoff and erosion is numerically calculated with the simulation model PRZM which uses the USDA runoff curve number (CN) concept for the water movement and a modification of the Universal Soil Loss equation (MUSLE), i.e., MUSS, for an estimation of soil erosion. Results from run-off field trials can be used to estimate the effect of dedicated management practices such as micro-dams between the ridges of potato fields or conservation tillage or erosion ploughing in maize on model input parameters. Application of different cultivation techniques and tillage techniques for example showed a consistent decrease of CN, runoff, erosion, and PPP transport (Table 1). The results presented here support the approach to quantitatively consider in-field risk mitigation measures in the context of regulatory surface water exposure calculations, as proposed by the MAgPIE workshop. Based on these data, a robust case can be made to quantitatively consider innovative run-off mitigation for risk assessment purposes by, e.g., lowering CN in the exposure scenarios. In other situations, mitigation effectiveness parameters derived from field experiments may be more appropriate.

	Potatoes	Maize
	5 studies (microdams)	2 studies (each with 3 diff. techniques)
CN change	– 24% (± 12%), 10th percentile: – 15%	– 4% (± 0.4%); sim. results for all techniques
Runoff change	– 86% (± 7%), 10th percentile: – 80%	overall: – 52% (tillage tech.: – 51%; others: –55%)
Erosion change	– 90% (± 5%), 10th percentile: – 86%	overall: – 76% (tillage tech.: – 71%; others: – 84%)
PPP change	– 88% (± 4%), 10th percentile: – 85%	overall: – 55% (tillage tech.: – 51%; others: – 62%)

Table 1: Effects of micro-dams in potato cropping and distinct strategies in maize cultivation on curve numbers (CN; means), runoff quantities, erosion, and plant protection product (PPP) loads (percental means)

AGRO 48

Summer fertigation of dairy slurry reduces subsurface drainage nitrate losses compared to fall injection

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Leaching of nitrate (NO_3-N) from manure-applied cropping systems can represent a substantial route of nitrogen loss to the environment for dairy farms, particularly in fields with artificial subsurface drainage. In this on-farm study, we used a Before/After analysis to assess the effectiveness of summer fertigation with reduced manure rates versus fall injection of dairy slurry in terms of subsequent corn silage yield, corn N uptake, soil NO_3-N distribution, and NO_3-N losses in subsurface tile drainage from a 65-ha field in Minnesota, USA. Here, we present the results for NO_3-N losses. Weekly flow-weighted mean NO_3-N concentration in tile drainage was lower during fertigation (47.7 mg L^{-1}) than injection (56.8 mg L^{-1}), although mean weekly drainage depth was greater during fertigation (2.3 versus 1.1 mm). This resulted in similar weekly loads between periods (mean of 0.96 kg NO_3-N ha^{-1}), but average annual loads were lower during fertigation (47.1 versus 55.5 kg NO_3-N ha^{-1} yr^{-1}). For non-snowmelt flow, relationships between drainage and NO_3-N load showed log–log slopes of near 1.0 for injection and 0.97 for fertigation, indicating that concentrations were diluted with increased flows during fertigation, but not during injection. Differing intercepts indicated a treatment effect of fertigation, independent of flow effects. The intercepts corresponded to loads of 16.0 kg NO_3-N ha^{-1} for injection and 12.2 kg NO_3-N ha^{-1} for fertigation, a reduction of 24% at 10 mm flow depth. Results suggest that summer fertigation with lower overall N application rates is a viable method for reducing drainage NO_3-N losses without impacting yield of irrigated silage corn in the U.S. Midwest.

AGRO 49

Soil carryover residue modeling to support safe product use to rotational crops

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Herbicide applications may have the potential to result in carryover residues in soil that can injure rotational crops. This presentation describes national-scale soil carryover residue modeling from herbicide applications made in cereal crops to support safe plant-back intervals for rotational crops. A conceptual field dissipation model was developed based on laboratory and field environmental fate datasets. The model, calibrated and validated with data from field carryover trials, was simulated to predict spatial and temporal soil residues in cereal crop growing areas of the United States. Crop-specific residue thresholds derived from laboratory bioassays and field studies were used to evaluate the potential carryover effect on rotational crops. Crop-specific rotational intervals were determined based on the predicted soil residues and associated thresholds. Uncertainties in modeling results due to limited soil residue sampling time points were addressed by implementing a conservative modeling approach and taking into account bioassay and field injury information. The presentation will conclude with recommended crop rotational planting intervals following an herbicide application to cereal crops to minimize potential carryover effects.

AGRO 50

Refined land cover for improving the confidence of pesticide risk assessments

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Today, field-scale crop location data are available at higher spatial resolution and classification accuracy than ever before. These data represent "best available scientific data" and can improve the confidence of human health and ecological risk assessments by providing more realistic, site-specific exposure estimates. For example, state-wide agricultural-focused land cover on an annual basis with 56 crop classes and a spatial resolution of ~2 meters is now available in California. Given that California ranks first in total crop receipts in the U.S. and bounds nearly 20% of all federally listed species, increased spatial land use data resolution has the potential for measurable impact in risk assessment. Other novel opportunities exist for incorporating better data by cooperating with specialty crop grower groups who often have field surveys locating their member's farms (*e.g.,* Florida green beans). High-confidence data can be used to improve Percent Crop Area (PCA) adjustment factors required for estimating drinking water concentrations in human health risk assessments. From a FIFRA/ESA perspective, it is proving difficult to make determinations for many crop-species combinations with any degree of confidence using the spatial information presently available. Although inconvenient, there is arguably a need to incorporate better data as it becomes available.

To demonstrate the impact of the newly-available California land use data, a case study was conducted to contrast the effects of refined crop cover data on spatial proximity to non-target terrestrial species' habitat. From an aquatic perspective, watershed scale (HUC-12) PCAs calculated using the EPA crop use sites, the 2014 CWS PCA guidance document approach, and the CA DWR land cover will be covered. These results can be used to gauge the impact that higher spatial resolution and classification accuracy can have on pesticide risk assessments relying on crop location information.

AGRO 51

Ecoregion similarities of field trials – Comparison of field degradation data of some pesticides from New Zealand, Chile, and Europe

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Pesticide terrestrial field dissipation (TFD) studies are conducted in North America and/or Europe to fulfill data requirements on persistence, dissipation, degradation rates, formation and decline of transformation products,

volatilization, plant uptake, leaching, etc. in the field under actual use conditions. In the European regulatory framework, the degradation rates (DegT50) from TFD studies are seen to be more realistic for exposure modeling than from laboratory studies and can originate from "any" site with similar soil and climatic conditions. In an experimental and GIS/modeling feasibility study, we investigated if TFD-studies conducted in the Southern hemisphere (i.e., New Zealand or Chile) may deliver similar endpoints (degradation rates in soil, DegT50) than those from from the Northern hemisphere (Europe or NAFTA region). An OECD Ecoregion similarity model was used for analysing the representativeness of field trials conducted in NAFTA vs. Europe and the Southern hemisphere. As the current ENASGIPS method only allows NAFTA/Europe comparisons we extended the concept to other regions using ArcGIS and available geodata from the 3 regions. TFD trials were conducted according to OECD 232 (DegT50 module, bare soil covered with sand) with 3 different pesticides having moderate DegT50 at 3 sites in New Zealand and 3 sites in Chile, having no historic use of these pesticides. All pesticides were applied in commercial formulations as a tank mix together in the same spraying on the same field plots at the same time. The sites had soil types ranging from sands, loamy sands, to loams. In New Zealand the sites were located on the Northern Island having an average annual air temperature of ~12-13 °C and an average cumulative annual rainfall of ~780- 970 mm. In Chile the sites were located in the Región del Bío-Bío, east of Concepción having an average annual air temperature of ~14 °C and an average cumulative annual rainfall of ~800-900 mm. The Field DegT50 for the 3 compounds in New Zealand and Chile are normalized to reference conditions (20 °C, moisture pF2) during kinetic analysis according to FOCUS kinetics, considering soil characteristics and actual local weather data. They are then compared with normalized DegT50 values of TFD studies with the same compounds in Europe. The normalized SFO DegT50 of all compounds in the "Southside" trials in New Zealand and in Chile as well as in Europe were found to be in the same range.

AGRO 52

Proactive conservation facilitated through section 7(a)(1) of the Endangered Species Act

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Section 7(a)(1) of the Endangered Species Act (ESA) stipulates that Federal agencies shall utilize their authorities to further the conservation of listed species. This section of the ESA is an under-utilized tool which promotes species conservation and recovery in a proactive, flexible manner. In addition, section 7(a)(1) can account for the voluntary, beneficial actions undertaken by communities, applicants, or other partners and stakeholders which lead to the recovery of listed species. The U.S. Fish and Wildlife Service will describe examples of existing 7(a)(1) consultations and discuss a nationwide 7(a)(1) program currently under development which specifically encourages active participation of local communities and citizens in the conservation process. Opportunities for how a similar strategy could be utilized to complement pesticide consultations will also be discussed.

AGRO 53

Leveraging national compensatory mitigation conservation offset strategies to pro-actively address endangered species section 7 authorized take of residual, unavoidable impacts permitted within national scale pesticide biological opinions

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The release of the three organophosphate (and pending carbamate) national scale endangered species assessments have presented new challenges to the USEPA, NMFS, and USFWS. The biological evaluations (BEs) have identified many species and/or critical habitats as "may affect, likely to adversely affect" (LAA) which lead to extensive and costly Biological Opinions (BiOPs). The NMFS opinions indicated jeopardy and adverse modification and therefore the production of Recommended Prudent Alternatives (RPAs) which were designed to broadly reduce pesticide loadings with either significant use restrictions or significant drift and runoff controls. The extensive and complex burden on growers will result in significant impact on use of these important crop protection products, with unclear benefit to the specific listed species. Industry and the evaluating agencies are entrusted to both protect species populations while also support sound scientific decisions regarding federal actions related to crop protection chemical use in U.S. agriculture. In some cases, localized use restrictions and buffers may offer adequate protections for a specific species population. In other cases, conservation offsets, of a similar spatial and temporal nature to the authorized take may provide for the agricultural use of crop protection products while improving the viability of the listed species. This talk will focus on the transfer of extensive experience in leveraging national compensatory mitigation strategies (Clean Water Act Section 404, Endangered Species Act Section 7) to mitigate the potential effect of an action and therefore providing both the ability to proceed with the action, providing an offset to address the authorized take provided to the applicant and associated parties, and working toward the species protection and recovery goal. The national application footprint of crop protection products offers risk assessment challenges, but solutions may be found and offramps to avoid expensive and potentially ineffective risk assessment refinements if all parties involved use creativity and tested approaches to holistically integrate the risk assessment findings and recovery plan options. The effect is to better leverage both the ESA and EPA authorization processes, resulting in improved endangered species viabilities (less listings, increased recoveries) and national scale pesticide risk assessments that are more practically linked to the landscape.

AGRO 54

Addressing agricultural pollutants in the Little Arkansas River using best management practices

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The Little Arkansas River Watershed (HUC 11030012) is located in south central Kansas, where the Little Arkansas River empties into the Arkansas River at Wichita. It covers over 1400 square miles with 478 stream miles and 88 acres of lakes. Cropland is the major land use in the watershed, and production of corn, soybeans, wheat, and grain sorghum contributes to water quality impairments within the watershed. Atrazine and sediment were identified as major pollutants from agriculture in the Little Arkansas River Watershed, and the City of Wichita spent considerable funds and time filtering water from the river to remove atrazine.

The City of Wichita, along with Kansas State University Research and Extension (KSRE), developed a payment program to incentivize growers to adopt best management practices reducing the amount of atrazine that reaches the Littler Arkansas River. This presentation will highlight the success and future of this extension work as well as the history and implementation of atrazine.

AGRO 55

Wisconsin "Healthy Grown" Program: Research, innovation and implementation of high-bar, whole-farm production systems with certification for potatoes, carrots, and onions

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The single resource maximum sustained yield management model, for commodities like corn or natural resources like deer, usually pits benefits accrued for one resource against costs compiled in other resources that are produced by the same land area. Through development of the Healthy Grown Standard (HG) we have created a process that concomitantly produces meadowlarks, pollinators, sustainable aquifers, clean water, and carbon banks along with potatoes and other crops. While farm producers need to grow to a more complicated, whole-farm standard, natural resource managers must also develop tools and means that help producers achieve these universal goals. Most pressing conservation problems can only be solved by land-based solutions upon which agriculture, worldwide, also depends. To certify potatoes, carrots or onions, HG growers must document sustainable activities to an independent certifier for validation that include 1) an assessment document for the farm, 2) an IPM Planning Protocol Form, and 3) ecological restoration plans for non-production lands. Future modules include carbon storage and perhaps social guidelines. Since establishment in 1996, HG farms have reduced potential pesticide risk, increased participatory practice points and implemented prairie, ecosystem restoration. Future metrics of progress need greater flexibility, lower management costs, and better spatial records. UW-Madison faculty, a representative commodity group, individual farms, and conservation NGO's collaborate to improve the standard over time. Food production under HG is more expensive than conventional agriculture but currently no premiums have offset these costs. Instead, producers have gained market share and more control over their business models in the face of increasing complexity of the regulatory environment. Yet participation by leaders alone is insufficient to achieve the scale of adoption necessary to solve land-based environmental problems such as climate change. To succeed, market forces must encourage broader participation by growers and consumers must pay for some added costs of production.

AGRO 56

Providing habitat for pollinators and the monarch butterfly *(Danaus plexippus*) using in-field and edge of field conservation practices

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The placement of conservation practices within and adjacent to row-cropped fields can provide potential net agricultural conservation benefits. Native prairie vegetation, such as prairie strips, not only reduces soil erosion and retains nutrients but also provides habitat for wildlife, including pollinators and monarchs. In Iowa, the STRIPS (Science-based Trials of Row crops Integrated with Prairie Strips) project is focused on evaluating the impact of prairie strips on crop yields, sedimentation, nutrient management, and biodiversity at the field and watershed level. Current research includes how the placement of habitat may influence non-target impacts of pesticides and the potential for monarch habitat to be planted within 38m of agricultural fields. This presentation will address the context of and progress on research with in-field conservation habitat (STRIPS) and edge of field habitat conservation practices as it relates to pollinators and the monarch butterfly, including preliminary insights from monarch research.

AGRO 57

Rusty-patched bumble bee habitat restoration in Northeast Iowa: Meeting multiple conservation objectives in a working landscape

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The rusty-patched bumble bee (*Bombus affinis*; 'RPBB') was a common and widespread species in eastern North America before an abrupt loss of more than 90% of its range in the late 1990s, leading to recent endangered species designation by the US Fish and Wildlife Service. Remaining populations are restricted to agricultural landscapes of the Midwestern U.S., where ongoing threats of habitat loss may be compounded by a dearth of conserved lands managed to meet RPBB habitat needs. Other at-risk animal species such as the monarch butterfly (*Danaus plexippus*) occupy the same flower-rich habitats favored by this bee, suggesting that stewardship projects in these areas could meet multiple overlapping conservation goals simultaneously. In pilot work in eastern Iowa, we addressed this need using a combination of species distribution modeling, native seed mix development, field inventory, and engagement with private landowners. We assembled a database of ~10,000 RPBB occurrence records from museum holdings and citizen science inventories, using it to construct species distribution models, characterize seasonal patterns of bee activity, and identify plant species that serve as nectar and pollen resources. We then obtained occurrence records for RPBB host plants and analyzed their flower phenology in the study region. In partnership with the Iowa Monarch Conservation Consortium we designed native seed mixes to meet the nectar, pollen, and larval host plant needs of RPBB and monarch butterflies, and are working with landowners and other stakeholders to restore flowering habitat in the vicinity of USFWS RPBB Priority Zones. We plan to measure the value of these plantings to the target species as well as other pollinators in field surveys. Our work serves as a model for science-based endangered species stewardship integrating efforts across private, industry, and government stakeholders that could be applied in a variety of additional contexts.

AGRO 58

Evaluation of applied, cross-sector vegetative best management practices in rights-of-way on pollinators

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Transmission and pipeline rights-of-way (ROWs) in the U.S. are estimated to occupy approximately 21 million acres, roughly equivalent to the average harvested corn acres in Missouri and Illinois combined. Additionally, there are nearly 4 million miles of roadside ROW and 233 thousand miles of railroad ROW in the U.S. These ROWs dissect agricultural land, urban areas, and natural areas like forests and grasslands. Managed ROWs represent an opportunity to provide habitats for numerous species of plants and animals, including pollinators that provide critical ecosystem services for farms, natural areas, and private homeowners. Strategic creation of pollinator habitat in ROW with respect to cross-sector land management offers large potential benefits to agricultural production, conservation, education, and research. In this work, we hypothesize that the success of managed ROWs to provide quality pollinator habitat is based on a combination of integrated vegetation management (IVM) practices within ROWs, physical habitat characteristics, surrounding land use composition specific to geography/ecoregion characteristics, and pollinators of interest. We evaluate an experimental design aimed at identifying the effects of different IVM practices in ROWs on pollinators. We rely on variabilities in IVM practices and physical characteristics of ROWs reported in literature and other sources to statistically determine which design elements are important to discern potential effects on pollinator habitat quality. The findings will promote informed evaluation of conservation management strategies. Appropriate statistical and field study designs aimed to characterize pollinator success based on IVM practices would allow ROW managers to gain quantitative information to better understand where and when to establish IVMs across spatially explicit, complex, and diverse landscapes. Additionally, agricultural land managers may benefit from implementation of certain IVM practices in ROWs that will positively impact crop production while promoting conservation of pollinators and other species.

AGRO 59

Discussion session: Reflection on the day's information

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This final session of the day will engage speakers and the audience in reflections on the presentations made and will address any further questions that arise collectively based on the session presentations. Speakers will be asked to sit as a panel to exchange ideas between them as well as questions or comments from the audience.

AGRO 60

Multi-stakeholder collaborations to advance non-animal approaches for testing agrochemical formulations

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The EPA Office of Pesticide Programs has formally committed to advancing the use and acceptance of robust twenty-first century toxicity testing approaches that are more efficient, less expensive, and use fewer animals than traditional methods (http://bit.ly/2nyJx7k). These approaches will enhance the quality of EPA's risk assessments and risk management decisions and better protect human health and the environment. A near-term goal was established to replace the "6-pack" of animal tests (acute oral, dermal, and inhalation systemic toxicity, skin irritation, skin sensitization, and eye irritation) for agrochemical formulations. Many companies and non-governmental organizations share EPA's goal to replace animal tests for agrochemical products and thus multi-stakeholder collaborations have formed to work collaboratively towards this mutual goal. This presentation will discuss ongoing collaborative projects and progress made to replace the "6-pack" for testing agrochemical formulations, including gathering existing data to use for model development and validation as well as the use of waivers, *in silico* and *in vitro* models, and the GHS additivity equation.

AGRO 61

Predictive toxicological approaches: Development, challenges, and applications

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The development of useful *ex vivo* methods for predicting *in vivo* responses to a chemical exposure is a challenging activity. Once established, these methods allow for the reduction of animal use, and can increase the efficiency of discovering new compounds with desirable efficacy and safety profiles. The importance of translational relationships between predictive approaches and organismic level outcomes, and the differing requirements on this relationship for different phases of research and development will be discussed. Examples from both the Agrochemical and Pharmaceutical industries will illustrate the development and application of *in vitro* methods, expression profiling approaches, pharmacology screening, and *in silico* models to predicting important toxicological outcomes.

AGRO 62

How a problem formulation process helped refine inhalation risk assessment for plant protection products

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This presentation gives an overview of a case study that used a Problem Formulation (PF) framework developed by Syngenta to guide a team through to a decision. The fundamentals of the frame work have been adapted from scientific activities such as environmental and human health risk assessments. The PF framework is a simple iterative process with three stages namely, framing the problem, exploring the problem and mapping the approach. In order to refine the short-term and intermediate term inhalation risk assessment, characterization of particle size distribution of inhalable particles from typical crop protection product applications is needed. This along with inhalation exposure dosimetry models, would provide precise estimation of inhalation exposure to pesticide handlers. A simple refinement option often suggested was to adjust the standard Unit Exposure value by the fraction of inhalable aerosol particle from typical agricultural nozzles. The lack of direct evidence that this refinement is health protective has resulted in the US EPA rejecting this approach for informing risk assessments. Following the problem formulation framework within Syngenta and then with key scientists at the US EPA enabled us to frame the specific problem we needed to address. This approach led to another review of the scientific challenge, clarified the problem that was to be addressed,

resulting in a specific problem statement. This also helped align the approach with the all stakeholders. By exploring the problem, a conceptual model was developed that helped identify information and data gaps and a set of testable hypotheses. Novel *in vitro* methods to derive human-relevant Points of Departure (PoD) and a Computational Fluid Dynamics (CFD) model for precise deposition estimation in the human respiratory tract were used in developing the Source to Outcome approach for inhalation risk assessment. Using the final stage, mapping the approach, different pieces of technical information were acquired in order to derive an accurate Human Exposure Concentration (HEC), which was then used in the inhalation risk assessment for applicators, mixer/loaders and residential pesticide handlers. This PF framework was easy to use, transparent, provided focus, and enabled the development of a chronic inhalation risk assessment without performing an animal study.

AGRO 63

Using high-throughput pharmacokinetic simulation and *in silico* property estimates to anticipate mammalian toxicity

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Discovering and developing new agrochemicals that work well under field conditions is difficult, but the need to satisfy regulatory requirements regarding their safety while minimizing animal testing can be even more challenging. One way to address the challenges involved is to use *in silico* modeling tools to identify potential problems early on and use the information obtained to guide compound design, development, and testing. The tools needed to reliably estimate physical properties like solubility, pKa, size, and lipophilicity from molecular structure alone are now widely available and are used extensively. Individual property predictions, however, do not take into account the interplay between the properties – e.g., the trade-off between acidity and lipophilicity as regards permeability or metabolism. Physiologically-based pharmacokinetic (PBPK) simulation provides a rigorous mechanistic framework for modeling the interplay among properties. It is widely used late in pharmaceutical development, and efforts are now underway to extend its use to discovery and early development. Whereas pharmaceutical researchers typically seek to maximize absorption and minimize metabolism, however, agrochemical development usually focuses on reducing exposure by minimizing absorption and maximizing metabolism. What the two fields share is a drive to make sure that any metabolites that accumulate are innocuous. This talk will demonstrate how the use of in silico predictions in lieu of some or all measured properties can generate exposure estimates early in development and improve exposure estimates later.

AGRO 64

Integration of toxicokinetics in agrochemical toxicity testing

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Integrating toxicokinetics (TK) into guideline toxicity studies without use of additional animals, provides valuable data on systemic exposure levels and dose response analysis for observed toxicity. Guidance documents, *e.g.,* OECD 116 highlight the importance of TK for dose selection. Increasingly, TK data are being used to provide insights on mode-of-action (MoA), study design, and *in vitro* to *in vivo* extrapolation for human health risk assessments to chemicals. Described here are two case studies with the herbicides, Halauxifen-methyl and Florpyrauxifen-benzyl, highlighting the use of TK approaches in exploring the MoA and dose selections, respectively. In the MoA case study, Halauxifen-methyl is rapidly hydrolyzed in rodent liver to a single primary metabolite, Halauxifen-acid, and induces rodent liver effects via nuclear receptor (NR) activation. Halauxifen-acid does not activate NR and *in vitro* assays in rat and human blood, liver S9, and gastric fluid evaluated species differences in hydrolysis rates. TK and hydrolysis data were incorporated into a PBPK model for rat and human systemic exposure to Halauxifen-methyl, and supported non-human relevance of the Halauxifen-methyl liver MoA. In the Kinetically derived Maximum Dose (KMD) case study, florpyrauxifen-benzyl is hydrolyzed to a single primary metabolite, XDE-848 acid, which displays sub-linear kinetics based on integrated TK from 28- to 90-day toxicity studies. Interestingly, these studies indicated no toxicity up to the limit dose (1000 mg/kg/day) and the TK data provided strong justification for use of a KMD approach on the OECD 453 and 416 studies in the rat, and 1-year chronic toxicity study in the dog. A high dose of 300 mg/kg/day was chosen in contrast to traditional toxicity testing paradigms using a maximum tolerated dose approach selecting the limit dose in the absence of toxicity. Taken together, integration of TK into guideline toxicity studies can increase understanding of the intrinsic properties and generate data that is more relevant to human health risk assessments.

AGRO 65

Regulatory perspective: Human health risk assessment for pest control products and reduction in animal use

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There is widespread agreement that a reduction in the number of animals used and the refinement of testing to reduce suffering should be important goals in the development and implementation of testing methods that avoid the use of live animals. The Guiding principles for more ethical use of animals in testing are the Three Rs (3Rs), namely, reduce, refine and replace animal testing. These principles are now followed in many regulatory agencies worldwide, including Health Canada. Over the past few years, Health Canada's Pest Management Regulatory Agency (PMRA) has continued to significantly contribute to and/or collaborate on the advancement of various initiatives intended to reduce the use of animals without compromising the underlying health risk assessment for pest control products (pesticides). Like the US Environmental Protection Agency (EPA), PMRA has focused its efforts from two perspectives: hazard and exposure.

From the exposure lens, the Agency has successfully registered several products whereby the toxicology requirements have taken into consideration the negligible exposure from the proposed use. The Agency has also attempted to better communicate its approach to risk assessment, which includes problem formulation, in recent regulatory directives such as the proposed policy for the cumulative risk assessment (CRA) framework. From a hazard perspective, the Agency has played a key role in the development of several PMRA, NAFTA and OECD technical guidelines designed to further reduce the need for animal studies. In 2017, the Agency also published its retrospective analysis of the added value of 1-year dog studies in pesticide human health risk assessments in a peer-reviewed journal. The US-Canada Regulatory Cooperation Council initiative has also provided an opportunity for the PMRA and the US EPA to collaborate on an Integrated Approach to Testing and Assessment work plan. While animal studies will continue to play a key role in the human health risk assessment for pesticides, collectively, the momentum from all the previous and ongoing initiatives is also providing opportunities for discussing and proposing approaches to address the

challenges associated with the regulatory acceptance of alternative approaches to animal studies.

AGRO 66

Inadvertent residues: Food handling uses & emerging regulations

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Official monitoring reports from major countries like the USA and Europe typically show less than 5% incidence of food standards violations (e.g., Maximum Residue Level - MRL< or Import Tolerances - IT). About 1% are real MRL/IT violations, while the rest are actually triggered by lack of standards. This may be caused by foreign uses missing IT in importing countries, or by domestic and foreign non-agricultural food handling uses (e.g., fumigations of warehouses food processing facilities, ships, and other transportation means). There are two precedents of regulations trying to give solutions to this type of residue, often called as "inadvertent": 1) US EPA is the only agency that has been setting tolerances (MRL) on food handling uses for several pesticides; 2) Australia and New Zealand are now setting MRL on "other foods, except animal commodities." These are great, successful examples of setting additional food standards (MRL) based on real data (measured residues, as opposed to uniform, default levels) and the subsequent risk assessment to further enable trade. This presentation includes details about how these two procedures have been working and examples that would hopefully serve as evidence and encouragement to other regions to address missing MRL and replace default-MRL by new regulation for food handling, or other food uses than the uses of pesticides on agricultural crops. By contrast, another example will also be presented to demonstrate how addressing missing MRL with default levels has brought Europe to having more than 80% of the existing MRL listed at the analytical method's default level. This has created a lot of havoc for agricultural producers that are trying to comply with multiple food standards, including uniform levels that are not based on real uses. This is a trade impediment, triggered by non-tariff barriers and it is our duty as scientists to continue supporting scientific evidence over politicized views.

AGRO 67

***In vitro* studies with human intestinal epithelial cell line monolayers for protein hazard characterization**

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Evaluating the safety of newly expressed proteins in genetically modified (GM) crops is conducted prior to commercialization to determine whether they could present a hazard upon consumption. A multicomponent, weight of evidence approach has been applied to individual proteins that has often included acute oral toxicology studies. Based on resources required to produce and purify the proteins, the number of animals necessary for these studies and the fact that no evidence of hazard has been observed for any of the proteins tested to date, it is questionable whether acute toxicology studies should be conducted for all proteins. This article reviews the chronology of the acute toxicology study from its origins into application for hazard assessment and classification of individual substances including proteins expressed in GM crops. It further proposes that a physiologic approach using cultured intestinal epithelial cell (IEC) line monolayers as an *in vitro* model of the gastrointestinal system provides results relevant to the hazard characterization of proteins when necessary. Benefits of this approach would include reduced quantities of proteins for testing and minimization or elimination of animal studies while maintaining confidence in the safety assessment process.

AGRO 68

My 33 years trying to understand P450 endowed herbicide resistance in multi-resistant Lolium

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In 1985, with my appointment at the University of Adelaide, I commenced a research program in the very early days of herbicide resistance. At that time in Australia, herbicide resistance was an academic curiosity only. Herbicide resistance was known on only two fields across the nation. Heap & Knight (1982) had published the first case of a Lolium population resistant to the ACCase herbicide diclofop-methyl. Amazingly, the diclofop resistant Lolium was cross-resistant to the new, first ALS herbicide chlorsulfuron. We believed that this cross-resistance must be non-target-site based and hypothesised that cross-resistance across dissimilar herbicides might be due to enhanced rates of metabolism of diclofop and chlorsulfuron, and potentially other herbicides. Using 14-C labelled herbicides we showed enhanced rates of herbicide metabolism in resistant Lolium and in some cases reversal of resistance *in vivo* with known P450 inhibitors. In the 1980s and 1990s, such resistance exploded in Lolium across Australia, becoming a major real-world problem. At that time very little was known about plant P450s, and despite great effort we could not isolate active herbicide-metabolising P450 microsomes from Lolium. Over many years we could not advance our understanding, until the recent genomics revolution provided new and powerful tools. Over the past few years, in collaboration with the laboratory of Dr. Roland Beffa at Bayer, Frankfurt, we have been able to identify specific P450 genes from herbicide resistant Lolium that endow the capacity for enhanced rates of metabolism of diclofop, chlorsulfuron and other metabolisable herbicides. After 33 years we now have concrete evidence that P450's can endow non target site herbicide resistance in many resistant Lolium populations. In this presentation, I will outline the highlights of this 33 year research journey. This journey would would not have been possible without excellent colleagues, including Drs. J. Holtum, J. Christopher, M. Burnet, C. Preston, D. Werck-Reichert, Q. Yu, H. Han, R. Beffa, and others. The journey would not have been possible without pivotal funding from the Grains R & D Corporation and the Australian Research Council.

AGRO 69

The evolution and management of non-target site resistance

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Many of the guiding principles for the evolution and management of herbicide resistance were founded on an assumption of single gene traits, inherited in Mendelian fashion and exemplified by target site resistance. For many weed species and for many resistance traits, it is increasingly apparent that non-target site resistance (NTSR), and in particular, herbicide metabolism, is the dominant mechanism of herbicide resistance. Whilst in most cases, the precise genetic basis for NTSR remains unresolved, it is clear that NTSR traits are polygenic, being inherited in a quantitative fashion, and leading to complex patterns of cross-resistance. NTSR traits do not evolve via the selection of rare mutations in herbicide target genes, but rather through the selection and recombination of standing genetic variation in defence- and metabolism-related pathways in weed populations. The underlying genetic basis and evolutionary dynamics of NTSR traits call into question a number of the resistance management guidelines that were established for single-gene

resistance traits. NTSR traits, selected from standing genetic variation, may be preferentially selected by low herbicide doses. Where these traits lead to broad cross-resistance patterns, underpinned by a suite of plant defence genes, such as cytochrome P450s, it may even be the case that herbicide rotations and herbicide mixtures are not able to slow selection for resistance. Here, I will present results from a range of experimental, modelling, and epidemiological studies which demonstrate that the increasing importance of NTSR mechanisms should cause us to re-evaluate the influence of herbicide dose, herbicide mixtures and rotations, stacked herbicide resistance traits, and fitness costs on the evolution and management of herbicide resistance. In the face of NTSR mechanisms that confer broad patterns of herbicide resistance, it is ever more important to recognise the need for weed management strategies that integrate chemical, cultural, physical, biological and ecological approaches.

AGRO 70

Fighting weed resistance - how Steve Powles helped us get innovation back on track

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When Steve Powles visited us for the first time in 2009, the most immediate impact that he had on Bayer was to help come to the realization that herbicide resistance had turned the corner from being a nuisance to a real threat to farmers. He was constantly reminding us of the impact weed resistance was having in Australia and beyond, and telling us to keep innovating in herbicides to provide diverse weed control solutions for the future. His stellar reputation, diverse, innovative and productive research program, numerous publications, and especially his ability to deliver his message personally in a convincing manner backed with sound facts were instrumental. After all, he is also a farmer. His message: that the resistance phenomena playing out at the time in the Americas and elsewhere represented opportunities for our company, if we seized upon the moment and did a few things in addition to the obvious. His contacts with the Grains Research and Development Corporation (GRDC) of Australia led to Bayer instituting the Herbicide Innovation Partnership. Their support has increased the number of researchers looking for new herbicides and new modes of action in Frankfurt. Bayer also paid attention to his recommendation to increase non-chemical measures in our integrated weed management programs in order to better protect their sustainability. In 2014, the Weed Resistance Competence Center (WRCC) was inaugurated with the mission to investigate resistance evolution and mechanisms, develop tailored solutions and communicate the value of implementing truly integrated weed management systems. We have also benefitted from our research partnership with Steve and his program at Western Australia University. This work has contributed to his long-term quest for identifying the genes responsible for the enhanced metabolism of certain herbicides that confer resistance in certain weed populations. Our cooperative work resulted in the identification of a particular cytochrome P450 that confers metabolic resistance to both diclofop-methyl and chlorsulfuron in *Lolium rigidum*. At long last his quest has borne the results for which he has been searching for a long time. Along the way he has developed into a steady collaborator and challenger for Bayer and made many acquaintances, and yes, friends, at Bayer. We are honored to be able to participate in this tribute to this great man and good friend.

AGRO 71

Role of xenobiotic detoxification in non-target site herbicide resistance in weeds

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Non-target site herbicide resistance (NTSR) is now common in many problem weeds and can lead to a loss of selective control with multiple chemistries. Using blackgrass (*Alopecurus myosuroides*) that have evolved multiple NTSR populations in Northern Europe as a case study, we are investigating the underpinning mechanisms of this class of resistance. While NTSR can potentially occur through several routes, enhanced herbicide detoxification is invariably cited as the major cause of resistance. NTSR is commonly associated with an elevated expression of detoxifying enzymes including cytochromes P450 (CYPs), glutathione transferases (GSTs), glycosyltransferases (UGTs) and ATP-binding cassette (ABC) transport proteins. However, in many cases, these large changes in gene expression only result in minor differences in the overall metabolism of herbicides. Furthermore, recent studies in different populations of blackgrass are revealing significant differences in the relative expression of CYPs, UGTs, GSTs and ABC proteins linked to NTSR, with their inter-associations suggesting coupling of functions. A unifying feature of the regulation of these detoxification systems in NTSR blackgrass is the associated elevated expression of a single phi(F) class AmGSTF1 that directly correlates with the activity of CYPs involved in herbicide metabolism. Based on this biomarker, we have developed a molecular diagnostic assay for AmGSTF1 that is predictive of NTSR. In parallel, the molecular biology, cellular expression and ligand associations by which AmGSTF1 regulates detoxification are currently being investigated to reveal similarities to the activity of the distantly related pi (P) class HsGSTP1 implicated in multiple drug resistance (MDR) in human tumours. These studies indicate that herbicide detoxification is just part of the NTSR phenotype in blackgrass and that additional protective resistance mechanisms are being elicited. Intriguingly, NTSR is under-pinned by very different changes in the expression of cellular proteins to that determined when blackgrass are exposed to biotic and abiotic stress. Instead, the NTSR proteome shows many similarities to that determined in MDR tumours. These suggest that NTSR is underpinned by a group of very specific stress-responses that may be of universal importance in protecting cells from chemical injury and these are now forming the basis of comparative studies in other problem resistant species.

AGRO 72

What roles for metabolism-based resistance to pre-emergent herbicides in *Lolium rigidum*

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Lolium rigidum is a ubiquitous weed of cropping systems in southern Australia. It has evolved resistance to numerous herbicides used for its control. Field surveys indicate widespread resistance to post-emergent ACCase- and AHAS-inhibiting herbicides across southern Australia. As a consequence, farming systems now rely on pre-emergent herbicides from just three modes of action for control of this weed in most crops. These are the microtubule elongation inhibitors trifluralin and propyzamide, the lipid synthesis inhibitors triallate and prosulfocarb, and the very long chain fatty acid biosynthesis inhibitors pyroxasulfone and metazachlor. Resistance has evolved to all of these herbicides

in recent years. In particular, one population of *L. rigidum* has been identified that has resistance to all of these herbicides. Susceptible *L. rigidum* can be protected from triallate and prosulfocarb by P450 inhibitors, but these have no effect on the resistant population. This suggests resistance to these herbicides is the result of a failure to activate these herbicides to their sulfoxide forms. In contrast, P450 inhibitors synergise trifluralin and other pre-emergent herbicides in both susceptible and resistant populations, indicative of P450-based metabolism as the resistance mechanism. Investigations of trifluralin resistance in *L. rigidum* show that the gene(s) responsible for resistance to trifluralin are different to those responsible for resistance to the post-emergent ACCase-inhibiting herbicides. *L. rigidum* has the ability to accumulate numerous non-target site resistance mechanisms in addition to target site resistance making long-term control of this weed with herbicides particularly problematic.

AGRO 73

Genomics to characterize Cyt P450 function in herbicide metabolic resistance: A review of recent works

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Cost effective Integrated Weed Management (IWM) involves combinations of non-chemical and chemical technologies adapted to the crops, the weed diversity, and environmental factors. When low diversity is present in the cropping system, including herbicide use, weed-herbicide-resistance can evolve with the results of poor weed control and significant crop losses. Herbicide-resistance has spread worldwide and concerns a high number of weed species. It is of particular concern in certain locations in cereal, soybean, maize, cotton, and rice cropping systems. Among several resistance mechanisms, herbicide detoxification (EMR, enhanced metabolic resistance) can confer resistance to a broad spectrum of chemical classes representing one or several modes of action. Molecular elements involved in herbicide detoxification are still poorly characterized and understood. Recent data using RNA-Seq transcriptome analyses to identify genes conferring EMR resistant populations in rye-grass and Amaranthus populations resistant to different herbicides will be summarized and compared. Among genes overexpressed in the herbicide-resistant plants compared to the sensitive plants, several, including CytP450s, GSTs, and GTs, were validated by genetics (co-segregation with the resistant phenotype), and functionally, by biochemical activity on the herbicide compounds. For some of the characterized genes, structure activity was performed on a range of herbicides representing several chemical classes. In addition, data on protein modelling and herbicide docking in the active site of detoxification enzymes will be presented. Not all overexpressed genes co-segregating with the resistance phenotype were found to be able to detoxify the herbicide(s) showing poor activity on a given resistant population. Possible resistance evolution mechanisms will be discussed in comparison to resistance evolution in other organisms, in particular, in insects resistant to insecticides.

AGRO 74

Differences in P450-mediated metabolic resistance mechanisms to triketone and pyrazole HPPD-inhibiting herbicides in *Amaranthus tuberculatus*

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Waterhemp [*Amaranthus tuberculatus* (Moq) Sauer] is a problematic dicot weed severely affecting corn, soybean, and cotton production in the United States. Previous research reported resistance to 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides in a waterhemp population from Illinois (named SIR), which is also multiple resistant to *s*-triazines and certain ALS inhibitors via rapid metabolism. Experiments were conducted with the objective of determining and comparing underlying resistance mechanism(s) to the triketone mesotrione and pyrazole topramezone, two HPPD-inhibiting herbicides having distinct chemical structures, in SIR as well as a metabolic HPPD-resistant waterhemp population from Nebraska (named NEB). The major difference between these HPPD-resistant populations is that SIR had been exposed to triketones and topramezone, while NEB had only been exposed to triketones in the field. Whole-plant dose-response studies indicated both populations were resistant POST to mesotrione and topramezone, although SIR displayed a higher level of resistance to both herbicides than NEB. Physiological studies with whole plants demonstrated elevated rates of mesotrione metabolism in both populations, which correlated with whole-plant R/S ratios. Biochemical studies using excised leaves and whole-plant methods indicated that an elevated rate of oxidative metabolism confers topramezone resistance in SIR relative to two sensitive populations. However, the metabolic route of topramezone determined in SIR is different than rapid *N*-demethylation and formation of a benzoic acid derivative that occurs in tolerant maize within 48 hours after treatment (HAT). Structural identification of the two main topramezone metabolites by LC-MS formed within 48 HAT in SIR strongly indicate ring hydroxylation reactions, presumably catalyzed by cytochrome P450(s). However, it is not known if these ring hydroxylations are performed by P450(s) that also catalyze *N*-dealkylation reactions in plants. These findings indicate waterhemp populations possess multiple genes encoding diverse metabolic enzymes that confer complex, herbicide-dependent, cross- or multiple resistance patterns, which may be influenced by prior field-use histories.

AGRO 75

Measuring spray drift from aerial application using horizontal and vertical collectors in a field experiment

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The aim of this study was to compare methods for measuring spray drift from aerial application using horizontal and vertical collectors in a field experiment. Two treatments (fine and medium sprays, according to the ASABE S572.1 standard) were applied with four replications each using a fixed wing aircraft (Embraer 201-A Ipanema) equipped with CP-03 nozzles. A spray solution, including an SC insecticide formulation and a dye tracer, was used, diluted to 20 L/ha. Drift was evaluated downwind from the applied area, using both horizontal collectors (glass slides) and vertical collectors (nylon strings), positioned at distances up to 500 meters downwind from the field edge. The total area sprayed for each replication was around 20 ha. The drift index for each replication was determined based on spectrophotometry data and adjusted curves that were correlated based on the Pearson Coefficient at a 5% probability. Results based on both collectors showed that up to 380 m from the sprayed field, the fine droplets applications gave more drift than medium droplets applications. However, from 380 m until 500 m downwind from the applied area, there was an inversion on this result, based on the data from the horizontal collectors. In this case, the drift index showed more drift for the medium droplet compared to fine spray applications. This happened because the fine droplets remained suspended in the air (as airborne drift) for longer distances and the horizontal collectors were unable to collect them in these conditions. The

vertical collectors were able capture the airborne drift from both the fine and medium droplets on longer distances, showing no inversion between fine and medium sprays as observed for horizontal collectors.

AGRO 76

Estimating appropriate buffer distances to mitigate environmental risk of spray drift using field data and computer automation

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Pesticide spray applications can cause unintended off-site deposition in non-target water bodies through aerial drift. Setting aside no-spray buffers during pesticide applications can reduce off-site drift deposition and mitigate potential environmental exposure. In the absence of empirical data, quantifying the mass of off-site pesticide drift requires the use of readily available, yet not necessarily representative, distance from edge of the field – mass deposition curves. Furthermore, estimating spray buffer distances that result in pesticide concentrations that meet accepted risk quotients can require tedious iterations of multiple model simulations. This paper will discuss a methodology that integrates field spray drift deposition sampling, statistical analysis, and environmental modeling to efficiently estimate required spray buffer distances. First, spray drift samples are collected from an experimental trial at given distances from the edge of the field. The fraction of applied active ingredient deposited by aerial drift is then calculated for all samples. Using custom computer scripts, the field data is then processed to 1) graph and fit a series of non-linear regressions and generalized linear models, 2) calculate goodness of fit statistics to evaluate the quality of the resulting statistical models, 3) extract model parameters and 4) calculate the pond-integrated drift fraction of applied active ingredient for a given spray buffer. These results are then combined with programmatic batch simulations of the Pesticide Root Zone (PRZM) and the Variable Volume Water (VVWM) models to calculate expected environmental concentrations of the active ingredient for multiple crops, chemical use patterns, and spray buffer combinations. This process allows efficient determination of appropriate aerial spray buffer distances. A case study with a new active ingredient will be used in this paper to illustrate the aerial spray buffer distance calculation method described.

AGRO 77

Using AGDISP to assess bystander exposure to pesticide spray drift: A California example

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Pesticides applied via aircraft may drift off-site resulting in exposures to bystanders that reside downwind of the treated field. Various factors may affect off-site drift, such as aircraft type, application rate, wind speed, etc. Accurate assessment of pesticide spray drift potential is fundamental to evaluating bystander exposure risk and to developing mitigation measures. AGDISP is a first-principles mathematical model that is used by regulatory agencies including the U.S. Environmental Protection Agency (U.S. EPA) and the California Department of Pesticide Regulation (DPR) to estimate spray drift. This presentation demonstrates DPR's current method of using AGDISP to calculate residential bystander pesticide exposure. The Pesticide Use Reporting and California Product/Label databases were used to identify products registered in California and the use patterns (e.g., crop, application rate, acreage, etc.), and the California Irrigation Management Information System was used to summarize weather conditions in possible use areas. Various sets of AGDISP input values that represent pesticide use and weather conditions in California were then modeled and screened for the scenario(s) with the worst drift potential. Raw AGDISP outputs, including pesticide deposition at different downwind distances and air concentrations at different heights, were processed to comply with exposure assessment requirements. Residential bystander exposure was assessed following U.S. EPA's latest standard operating procedure for residential pesticide exposure assessment. As pesticides are often applied at different rates or used with other compounds, the effects of different tank-mix properties on pesticide drift potential were also analyzed. Results show that even at the same application rate, pesticide drift potential was still affected by tank-mix solvent volume and other non-volatile contents (adjuvants or other pesticides). The effects also varied with different downwind distances. For instance, increased solvent volume decreased pesticide ground deposition within downwind 100 feet, but increased the deposition farther downwind. Thus individual AGDISP runs of specific application conditions are needed for accurate drift exposure assessment.

AGRO 78

Influence of operational and environmental conditions on spray deposition, uniformity and transport with remotely-piloted aerial spray systems (RASS)

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Irrespective of previous resistance, Remotely-Piloted Aerial Spray Systems (RASS) are currently in use in the USA. There is, however, a paucity information about the distribution of that spray in swath and the potential for off target losses. The flow field around an aerial vehicle is one of the most important factors affecting spray distribution. The flow field and resultant spray deposition will vary as the operational parameters and the environmental inputs change. Experiments have been and are currently being conducted to measure the spray distribution under an array of different biotic and abiotic conditions. The abiotic inputs were forward speed and altitude whilst the biotic were atmospheric conditions. Although it is understood that nozzle type and placement is important at this stage, we are working with commercial systems as they stand. Wind speed, direction, temperature, and humidity measures were taken as a record of meteorological inputs. The flow field and spray deposition were measured in swath via wireless air velocity sensors, Mylar cards and Water Sensitive Papers placed perpendicular to the line of travel. Downwind deposition and flux sensors were placed downwind as a measure of drift. This presentation will discuss the results of these experiments and maybe implications for future research and sprayer optimization.

AGRO 79

Computational fluid dynamics modelling for plant canopy interception of pesticide spray droplets

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Accurately quantifying the magnitude and subsequent implications of off-target transport of pesticides via spray drift

is critical to estimating exposure for ecological risk assessment and inferring potential spray drift impact to adjacent crops. Past work with this aim has included field, wind tunnel, and laboratory studies examining the contribution of environmental conditions, spray equipment, and spray mix (active ingredient and/or formulation) on the potential extent of off-target transport. However, there is limited research focused on better understanding factors affecting deposition on a more finite spatial scale such as the physical properties of intercepting objects that may affect directly adjacent airflow and the trajectory of the spray particles entrained. Regulatory risk assessments typically assume 100% drift interception and do not account for the unique physical properties of individual plant species/types. Improved modelling of plant canopy interaction with pesticide particles entrained in airflow has the potential to improve scoping and design of non-target spray drift studies and to inform the risk assessment process. The aim of this work is to improve the understanding of pesticide spray drift particle interactions with plant canopies and the relative capture efficiency of different canopies. This presentation demonstrates an application of ANSYS CFX (a computational fluid dynamics (CFD) modelling tool) to estimate the capture efficiency of various plants receiving pesticide spray drift from applications made in a wind tunnel. The CFD model includes realistic, high-resolution, 3D plant geometry generated using photogrammetry freeware. The method for creating a robust model is the topic of a companion poster. Model-predicted plant canopy efficiencies are compared to measured canopy interception obtained in wind tunnel experiments.

AGRO 80

Relative importance of droplet drift versus vapor drift in terms of deposition

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Pesticides applied to bare ground and crops can create drift as liquid droplets and/or as vapor. While the chemical compound involved with drift may be the same, the pathways for liquid versus vapor drift are quite different. Liquid drift is often dominated by gravitational settling in the near-field of the larger droplets in the distribution. Further downwind, however, the relative magnitude of vapor drift and smaller droplets may become greater. This paper describes the key factors that affect the deposition of vapor and droplet drift including the gravitational terms and the terms not dominated by gravitational settling, such as the aerodynamic and surface deposition resistance terms associated with dry deposition. A key question is addressed, i.e., how important is each form of drift as a function of downwind distance? This paper summarizes key terms in the analysis of deposition, including: (a) estimated the distribution of liquid droplets during the application, (b) using the dispersion model AERMOD to compute both airborne concentrations and deposition rates, (c) the relative importance of wet and dry deposition terms, and (d) refining the treatment of the deposition term for a crop in the field as compared to the standard AERMOD treatment basing deposition velocity on the clipped grass surface representative of a typical airport meteorological data set. Normalized case study examples will be used to evaluate airborne concentrations and deposition rates as a function of downwind distance of a hypothetical semi-volatile chemical for a bare ground and crop example based on coarse and very coarse nozzles.

AGRO 81

Early phase metabolism studies to identify compounds that could be toxic to bees, Phillip Cassidy and Shari Long, Exponent, Inc.

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The toxicity of pesticides and other chemicals to bees is under intense scrutiny. Bee populations worldwide have shown a decline and one of many contributors to the decline has been considered to be pesticides and their toxicity to bee populations. In 2012, the first OPPTS Guidelines for assessing acute bee toxicity were introduced as a FIFRA requirement for registration. If potential toxicity has been demonstrated from the results of the five tier I studies and tier I risk assessment, then very costly tier II studies may need to be conducted. The guidelines for these tier ll studies are still in development, but are required for compounds showing potential toxicity to bees unless mitigation or other actions waive the need for these studies. It is apparent that the colony collapse disorder has not diminished, and the potential effect of compounds on bee populations should be an important consideration toward registration of compounds. This presentation assesses the mechanisms known that contribute to the toxic response in bees in order to propose how early phase metabolism studies could be conducted to determine a compound's propensity to be toxic to bees. Such knowledge would certainly be important to know sooner rather than later in the product development and registration pathway

AGRO 82

Approaches of leveraging *In Vitro* metabolism assays to support animal nature of residue studies and safety assessment of agrochemicals

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Animal nature of residue studies are required for the regulatory registration of agrochemicals, and the information derived from these studies plays a critical role in consumer and environmental safety assessment. However, these animal metabolism studies conducted *in vivo* can be costly and time-consuming, and usually encounter challenges in sample analysis and metabolite identification (MetID) due to the low levels of residues and the complexity of sample matrices. *In vitro* metabolism assays using liver cell fractions (e.g., microsome, S9 and hepatocytes) can efficiently mimic *in vivo* metabolism in hours and greatly benefit the *in vivo* animal metabolism studies in a variety of perspectives. This presentation will discuss the advantages of the *in vitro* assays and their correlation to *in vivo* metabolism as well as how *in vitro* information can facilitate MetID *in vivo* using ^{14}C labeled agrochemicals as case studies. Furthermore, an *in vitro* comparative metabolism study between fish and livestock (hen, goat, and swine) will be presented to evaluate the differential metabolism in fish relative to livestock. This information can be used to help address emerging regulatory questions about residue levels in fish edible tissues and related safety assessment.

AGRO 83

***In-vitro* biotransformation of an avicide**

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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 causing damage to crops. The avicide causes damage to the proximal convoluted tubule in kidneys of exposed birds. The exact mechanism for this damage is not

understood. Experiments were conducted with liver and kidney cellular preparations which led to the identification of two metabolites of the parent compound. Additionally, formation of a reactive metabolite was observed in the microsomal fractions of both tissues. These results point towards the parent compound as the likely source of kidney damage observed in exposed birds. One of the metabolites (an acetylated compound) has the potential to be developed as a new avicide.

AGRO 84

Synthesis, biological evaluation, and enzymatic activity of the endophenazines and analogues

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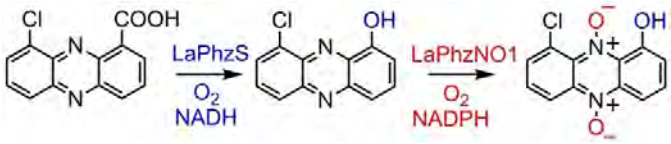
The phenazines, secondary metabolites isolated mainly from bacteria, are heterocycles with a plethora of biological activities. Although they have potential application in the treatment of many diseases, their most intriguing activity is related to their antimicrobial action. In this talk, I will report the practical synthesis of some phenazines including endophenazine A and endophenazine G, and some of their analogues. I will also discuss their biological activity against MRSA, E. Coli and other bacteria, cell toxicity against mammalian cells, and some preliminary studies to understand their mechanism of action. The obtained results allowed my research group to develop structure activity relationships (SAR) that are being used to generate more potent analogues. In addition, the stability of the most active compounds towards human hepatocytes will be presented. Finally, I will present the chemical transformation the phenazines can undergo when treated with some bacterial enzymes and what are the structural parameters that determine the metabolic susceptibility.



AGRO 85

Stability and biological activities of pharmaceuticals and personal care products in open water bodies: Roles of environmental factors

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The growing medical and personal needs of human populations have escalated release of pharmaceuticals and personal care products into surface waters. This work investigates abiotic degradation pathways of a particular PPCP, ibuprofen, in the presence of a major mineral component of sedimentation (kaolinite clay), as well as the health effects of the primary compound and its degradation products. Results from these studies showed that the rate and extent of ibuprofen degradation is greatly influenced by environmental factors, including the presence of sedimentation particles and solar radiation. In the absence of solar radiation, the dominant reaction mechanism was observed to be the adsorption of ibuprofen onto sedimentation particle surface where surface silanol groups play a key role. In contrast, under solar radiation and in the presence of clay particles, ibuprofen breaks down to several fractions. The decay rates were at least 6-fold higher for irradiated samples compared to those of dark conditions. Biological activities of primary ibuprofen and its secondary residues were tested on three microorganisms: *Bacillus megaterium*, *Pseudoaltermonas atlantica*; and algae from the *Chlorella* genus. The results from the biological assays show that primary PPCP is more toxic than the mixture of secondary products. Overall, however, biological assays carried out using only 4-acetylbenzoic acid, the most abundant secondary product, show a higher toxic effect on algae compared to its parent compound.

AGRO 86

Machine learning models for the prediction of xeniobiotic metabolism

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The ability to understand and predict the metabolic stability of xenobiotics is of key importance to a host of different industries. This contribution will start with a brief overview of the scope and limitations of current *in silico* models for the prediction of (i) interactions of xenobiotics with metabolizing enzymes, (ii) sites of metabolism (i.e., atom positions where metabolic reactions are initiated) and (iii) likely metabolites. We will then report on our current efforts in exploring various machine learning strategies and data sources to develop more accurate models with a broader applicability domain. In particular, we will report on the further development of FAst MEtabolizer (FAME), a random forest-based predictor of sites of metabolism in small molecules that obtains high robustness by using a small set of circular atom descriptors. We will also present a new metabolite structure predictor that makes use of predictions of metabolically labile atom positions by FAME to significantly reduce the false-positive prediction rates, which pose a big challenge to current *in silico* approaches. In addition, we will report on the development of a new model for predicting the interaction of xenobiotics with metabolizing enzymes trained on a large curated dataset compiled from several public and proprietary sources.

AGRO 87

Structure-stability relationships of tetrahydroisoquinoline-containing CXCR4 antagonists and lipid prodrugs of tenofovir in liver microsomes

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CXCR4 is a 7-transmembrane chemokine receptor that is upregulated in > 48 different cancer types, resulting in tumor cell proliferation, immunosuppressive leukocyte infiltration, and cancer metastasis. Despite the development of potent CXCR4 antagonists for cancer treatment, current inhibitors suffer from poor pharmacokinetic properties, including metabolic stability. To address these limitations, novel tetrahydroisoquinoline-containing CXCR4 antagonists were designed, synthesized, and evaluated with an emphasis on optimizing pharmacokinetic parameters while maintaining on-target potency. The metabolic stability for each of these compounds was measured in liver microsomes, and metabolite identification was performed on a subset of these molecules. Structure-stability relationships stemming from these results are currently guiding our efforts towards novel CXCR4 antagonists with robust metabolic stability profiles. Another ongoing project in our group focuses on optimizing the metabolic stability of novel lipid prodrugs of tenofovir, an FDA-approved nucleoside phosphonate inhibitor of HIV reverse transcriptase. While current lipid prodrugs of tenofovir and similar nucleoside phosphonates dramatically improve oral bioavailability, the concentration of active tenofovir diphosphate in HIV-infected cells remains limited by CYP450-mediated ω-oxidation. Accordingly, novel lipid prodrugs of tenofovir were designed, synthesized, and evaluated with a focus on reducing the rate of ω-oxidation. Several of these derivatives demonstrated enhanced metabolic stability in human liver microsomes, as compared to the parent prodrug. Structure-stability relationships resulting from these efforts will be discussed.

AGRO 88

Kinetic evaluation of environmental fate studies

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Environmental Exposure Assessment depends on environmental fate properties of an agrochemical, including sorption and degradation behaviors. Therefore, kinetics evaluation of environmental fate studies is very critical to exposure and risk assessment. The PESTDF tool is used to implement the NAFTA guidance to characterize degradation rates in environmental media for regulatory purposes in the U.S. and in Canada. While the NAFTA guidance provides a general procedure for kinetic analysis, it does not consider specific study conditions (such as microbial activity) which can significantly impact degradation behavior. Therefore, the resulting half-life may not reflect real degradation rates, especially for studies conducted under older guidelines. There are also examples where the derived half-lives following the guidance are not supported by the studies because of conservative assumptions incorporated in the kinetic analysis. Case studies will be presented to demonstrate that study data must be evaluated for its suitability in the kinetic analysis.

AGRO 89

Modeling chemical partitioning at the water-sediment interface

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The varying composition of bed sediments, combined with hydrodynamic and biological perturbations, have created challenges in modeling the partitioning of chemicals at the water-sediment interface in natural waters. A variety of approaches have been developed to predict chemical mass balance between the water column and benthic sediment. These approaches often involve some empirically-derived component to account for the many physical, chemical, biological, and temporarily varying processes that may affect chemical exchanges between water and sediment. This presentation looks at the different deterministic and empirical approaches, and commonly used assumptions, in several water quality models used in regulatory risk assessments and the establishments of TMDLs. Results of several approaches are compared for a variety of water depths, water chemistries, and hydraulic conditions.

AGRO 90

Analysis of spatial data to reduce the uncertainty of pesticide spray drift contributions to aquatic exposure at the watershed scale

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Off-site transport of pesticides from treated agricultural fields to receiving water bodies from spray drift can be an important potential pathway for aquatic exposure. Current methods used in regulatory exposure modeling are based on a simplistic conceptual model of a single treated field adjacent to a static receiving water body. New challenges in ecological risk assessment, including the evaluation of endangered species, require understanding exposure in more complex water body and landscape configurations at the watershed scale. In these more complex environmental conditions, the potential for spray drift exposure is largely driven by the spatial characteristics of the agricultural landscape relative to the receiving water system. This paper will focus on a methodology that takes advantage of nationally available Cropland Data Layer and NHDPlus watershed/hydrography data to reduce the uncertainty in potential drift contributions to aquatic exposure in flowing water bodies. The methodology considers individual crop years independently to minimize the potential for over-representing crop extents while accounting for the year-to-year variability in cropping practice. Using a raster-based spatial analysis approach, the proximity from individual water body grid cells to the nearest treated crop of interest is assessed over the entire extent of the flowing water body and connected impoundments. These proximity distances are translated to drift fractions using drift versus distance curves applicable to the application method for each crop of interest. A drift deposition mass at each grid cell can then be calculated based on the pesticide application rate and drift fraction, then summed to represent the entire water body. Receiving body width at the sub-grid cell scale is determined through estimation channel width versus streamflow relationships using average annual flow estimates from the NHDPlus dataset. The approach developed can be combined with stream connectivity data to make refined estimates of drift-based exposure in headwater and downstream catchments.

AGRO 91

Pesticides in flooded applications model (PFAM) ecological modeling sensitivity and the impact of a receiving water body on ecological estimated environmental concentrations

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The Pesticide in Flooded Application Model (PFAM) is used to estimate surface water concentrations primarily for pesticide applications to rice paddies. PFAM (version 2.0) has the potential to assess pesticide concentrations in rice paddy water and a receiving water body. However, the Environmental Fate and Effects Division in the EPA currently uses only the in-paddy concentration from the PFAM model for ecological risk assessments. A receiving waterbody such as a canal would be appropriate as a representative aquatic environment for ecological risk assessment of species (e.g., fish) not found in a typical U.S. rice paddy. An assessment was performed using a hypothetical pesticide to conduct PFAM ecological sensitivity runs. The "ECO CA Winter No Turnover" and "ECO MS Winter No Turnover" scenarios were used in the modeling exercise. The simulations were performed with a single application per year on a standard 10-ha paddy. Pesticide concentrations in the paddy were compared with concentrations in two receiving waterbodies (canal and pond). The presentation will show the impact on the estimated environmental concentration (EEC) due to changes in the baseflow, surrounding watershed size and curve number, holding periods, and drift factors. Concentrations in the pond waterbody and canal were significantly lower than the in-paddy concentrations. This presentation highlights refinement options for appropriate aquatic environments that may receive outflow from a rice paddy.

AGRO 92

Assessing the impact of distributional analysis in drinking water exposure assessments

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Improving the representation of estimated drinking water concentrations (EDWC) in dietary risk assessments will afford a more realistic characterization of potential risk in regulatory human health risk assessments for crop protection products. Per regulatory guidance, EDWCs used in the dietary human health assessment represent the 90th percentile estimate of yearly maximum 1-day average and the annual average EDWCs for acute exposure and chronic exposure durations, respectively. This work proposes to use the daily EDWCs provided from the 30-year simulation of each model run (>10,000 daily EDWC values) instead of a single EDWC value. The challenge is to use 10,000+ daily EDWC values in the probabilistic dietary assessment that is typically conducted with 1000 iterations using the Dietary Exposure Evaluation Model (DEEM). Using all the 10,000 EDWC directly in a 1000 iteration DEEM assessment would result in under-sampling of the EDWC values. To address this challenge, a representative sample with 500 data points was created using an optimum allocation scheme. The approach generated a data set which is more easily manageable and its size comparable to the food residue data sets; therefore, it is more appropriate for a probabilistic dietary assessment using DEEM. This presentation will discuss the sampling methodology used to generate the representative samples from the daily EDWC values. The impact of using these optimized distributions on the upper percentiles of drinking water exposure estimates will also be presented.

AGRO 93

Probabilistic dietary assessment technique for refining combined milk residues resulting from livestock dietary burden sources with milk residues resulting from insecticide-impregnated ear tags to mitigate potential acute dietary exposures

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The conventional practice for the estimation of residues of insecticides in the milk of dairy cattle relies upon dietary burden calculations coupled with milk transfer factors often obtained from livestock feeding studies. Additional exposure pathways for dairy livestock commonly include animal health treatments using sprays, dips, or ear tags impregnated with insecticides to prevent stresses to the animals from biting insects. When combining these pathways, the easiest and most conservative approach is to combine maximum estimated milk residues arising from dietary sources with maximum milk residues arising from animal health sources. Human acute dietary assessments conducted from these maximum combined milk residue point estimates will be overly conservative and will present an overestimate of exposure. This presentation demonstrates a simple technique to better estimate potential human dietary exposures via milk residues from multiple pathways through the use of a probabilistic approach. This technique for combining estimated milk residues from livestock dietary burden sources with actual ear tag magnitude-of-residue data for use in acute dietary human exposure assessments is still adequately conservative and is suitable for use in regulatory assessments in support of U.S. EPA pesticide registrations.

AGRO 94

Evaluation of potential safety hazards associated with the Suzuki-Miyaura cross-coupling of aryl bromides with vinylboron species

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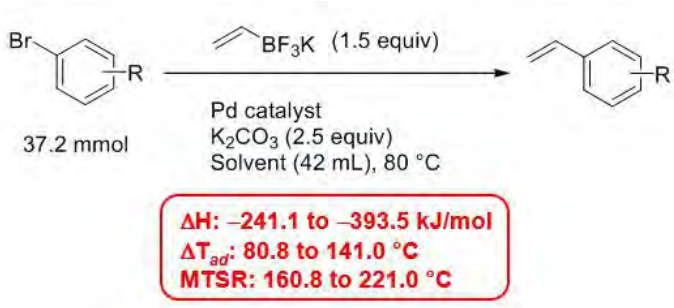
The potential safety hazards associated with the Suzuki-Miyaura cross-coupling of aryl bromides with vinylboron species were evaluated. An unexpected exotherm was observed upon the addition of catalytic 1,1'-bis(diphenylphosphino)ferrocene palladium(II) dichloride [$Pd(dppf)Cl_2$] to a mixture of 1-bromo-3-(trifluoromethyl)benzene with potassium vinyltrifluoroborate in the presence of potassium carbonate (K_2CO_3) in 9:1 dimethyl sulfoxide (DMSO)/water at 80 °C. Further investigations indicated that the exotherm was consistently higher in the studied aqueous systems compared to anhydrous conditions. Although under anhydrous conditions the exotherms were comparable among the studied cases, the rate of exotherm was highly dependent on the choice of aryl electrophile, solvent, base, catalyst, as well as vinylboron species. In many of the studied cases the maximum temperature of a synthesis reaction (MTSR) was considerably higher than the boiling point of the solvent and/or the onset temperature of the DMSO decomposition, indicating that in the absence of active cooling the system could quickly exceed the boiling point of the solvent or trigger the decomposition of the reaction mixture to result in a runaway reaction.



AGRO 95

New catalytic reactions for agroscience

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Our group has developed a series of catalytic reactions that are valuable for the synthesis of agrochemicals on both discovery and process scales. These reactions include coupling of aryl halides with amines and enolates, as well as C-H bond functionalization reactions. This presentation will focus on contemporary coupling reactions with unusual classes of amines and enolates, including those of small rings and fluorinated nucleophiles, and on C-H bond functionalization reactions that lead to the installation of small substituents, such as methyl groups and partially fluorinated methyl groups.

AGRO 96

Process route scouting of X087, a picolinamide fungicide

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X087 is an experimental picolinamide fungicide inspired by the natural product UK-2A. This structurally complex molecule represents a unique opportunity for early stage process research. Route scouting efforts from our team led to several scalable synthetic routes, which enabled the delivery of field samples and facilitated discovery SAR studies. An overview of these efforts will be presented, and key advances will be highlighted.

AGRO 97

Development of fluorination reactions: Collaboration between the University of Michigan and The Dow Chemical Company

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Fluorinated molecules are becoming more and more prevalent in agrochemicals, yet incorporating fluorine into organic compounds is still viewed as a major challenge. Common issues with fluorination are use of expensive fluorinating reagents, harsh reaction conditions, and limited substrate scope. A collaboration between the University of Michigan and The Dow Chemical Company was started several years ago to address these challenges associated with fluorination. This presentation will discuss the results achieved through this collaboration; the main focus will be on the efficient fluorination of aryl and heteroaryl compounds, a common motif found in many recent argochemical actives.

AGRO 98

A novel enzymatic process to produce active L-glufosinate from inactive D-glufosinate

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Glufosinate is a broad spectrum, amino acid herbicide that is an increasingly important tool for growers facing the challenges of weeds resistant to other herbicides. Currently marketed glufosinate products are racemic mixtures of the active L-glufosinate isomer and the inactive D-glufosinate isomer. It is estimated that in 2012, in the United States alone, more than 5 million pounds of non-herbicidal D-glufosinate were purchased and applied, representing a significant cost and environmental burden. This burden continues to increase as use of racemic glufosinate grows. AgriMetis has developed and patented a novel enzymatic process that converts D-glufosinate into L-glufosinate, resulting in an L-glufosinate product (Glu-L™) with an enantiomeric excess greater than 98%.

In AgriMetis' novel enzymatic approach, a D-amino acid oxidase (DAAO) is combined with a transaminase (TA) to perform deracemization of glufosinate. The first reaction, catalyzed by an evolved DAAO, specifically converts D-glufosinate to 4-(hydroxymethylphosphinyl)-2-oxobutanoic acid (PPO), an intermediate without chirality. The second reaction, catalyzed by a TA, specifically converts PPO to L-glufosinate. Both reactions can occur simultaneously, or in separate steps and reactors. The reactions occur under mild conditions, in aqueous solutions at moderate temperatures, and can reach completion within 10 hours. The enzymes are immobilized, which allows for their recovery and reuse in ten or more reactions. Product isolation from the completed reaction mass is also performed under mild conditions. Data will be presented showing the improvements to the DAAO enzyme to improve its efficiency, optimization and consequences of enzyme immobilization, process conditions, and scale up of this technology to pilot scale. AgriMetis continues to drive its Glu-L technology toward commercialization so that it can deliver to growers a lower cost solution to herbicide-resistant weeds.

AGRO 99

Metabolic degradation of glyphosate and glyphosate tolerance and resistance

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Glyphosate is a slow-acting herbicide that is metabolically degraded to aminomethylphosphonic acid (AMPA) and glyoxylate in many plant species. AMPA is much less phytotoxic than glyphosate, and glyoxylate is not phytotoxic. So, even a moderate rate of metabolic degradation of this herbicide should impart tolerance or resistance. Considering the base line metabolism in some weed species and the massive selection pressure on weeds in glyphosate-resistant (GR) crops, one would assume that that selection pressure would select for weed biotypes with greater rates of metabolic degradation. But, there are few data to indicate that glyphosate metabolism relates to the low levels of tolerance of some species, and there are no confirmed studies that show that enhanced glyphosate metabolism has been selected for in GR weeds. The enzyme that degrades glyphosate to AMPA (glyphosate oxidoreductase, GOX) has not been isolated from plants, but the gene that encodes GOX has been isolated from a soil microbe (*Acrhromobacter* sp.) and, at one time, used as a transgene to help provide some of the glyphosate resistance in GR canola. Many soil microbes can metabolize glyphosate with GOX or a C-P lyase. The fact that selection pressure with glyphosate has apparently not led to horizontal gene transfer of genes for either enzyme to

weeds argues against horizontal gene transfer being a common phenomenon. The role of endophytes in glyphosate degradation in plants is unexplored.

AGRO 100

Gene expression hotspots in herbicide-resistant waterhemp (*Amaranthus tuberculatus*)

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In the last decade, waterhemp (*Amaranthus tuberculatus*) has evolved resistance to 2,4-D and HPPD inhibitors in multiple states across the midwestern United States. Two populations resistant to both chemistries, one from Nebraska (NEB) and one from Illinois (CHR), were studied using an RNA-seq approach to identify the genes responsible for resistance. In this study, cDNA libraries were generated and sequenced for eight replicates of herbicide-resistant (HR) and herbicide-sensitive (HS) plants from each of the two populations (32 total plants sequenced). Using both a waterhemp transcriptome assembly and a high-quality grain amaranth (*A. hypochondriacus*) genome as references, differential gene expression analysis was conducted to identify genes that were significantly over- or under-expressed in HR compared to HS. When these differentially expressed genes (DEGs) were mapped back to the grain amaranth genome, physical clustering of the DEGs was apparent at gene expression "hotspots" along several of the 16 grain amaranth scaffolds. Interestingly, mapping of known metabolic genes (cytochrome P450s, glutathione-S-transferases, glucosyltransferases, and ABC transporters) to the *A. hypochondriacus* genome also showed clustering of these genes at several of these hotspot regions. It may be that the evolutionary forces acting on weedy populations - especially the continual selection provided by herbicides - lead to the formation of genetic islands of metabolism-based resistance genes, akin to the 'domestication islands' of crops and 'pathogenicity islands' of bacteria. Furthermore, SNP calling on all 32 samples to look for statistically significant resistant-specific variants and subsequent mapping of these variants also found similar patterns of clustering, with allele-specific regions coinciding with the hotspot regions. This allele-specificity at the hotspot regions not only allows for the development of allele-specific assays to diagnose resistance problems in fields, but also provides insights into the regulation of these regions. Further work has begun to identify any potential cis-acting regulators or epigenetic variation leading to this localized difference in expression between R and S plants. These allele-specific expression hotspots are a potentially useful tool in future RNA-seq studies to narrow down the regions of true regulatory control leading to resistance, and will likely provide insights into the evolution of herbicide resistance in weeds.

AGRO 101

Mechanism of multiple-herbicide resistance in *Echinochloa phyllopogon*

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Multiple-herbicide resistant *Echinochloa phyllopogon* has been found in California paddy fields. The resistant line exhibits resistance to herbicides from at least five modes of action including acetolactate synthase (ALS) inhibitors and acetyl-CoA carboxylase (ACCase) inhibitors. Previously, we identified two ALS-herbicide-metabolizing cytochrome P450 genes (*CYP81A12* and *CYP81A21*) whose overexpressions are associated with the ALS inhibitor resistance. Meanwhile, the genes involved in the resistance to ACCase inhibitors remain to be identified. To better understand resistance mechanism to ACCase inhibitors, we examined susceptibility of the resistant line to four ACCase inhibitors from three chemical classes such as fenoxaprop-P-ethyl and diclofop-methyl (FOPs), tralkoxydim (DIM) and pinoxaden (DEN). The resistant line exhibited reduced susceptibility to all the four herbicides compared to the susceptible line. Rice transformation of *CYP81A12* or *CYP81A21* was performed to evaluate its possible involvement in the reduced susceptibility to ACCase inhibitors. Transgenic rice expressing either of the genes grew vigorously in media containing diclofop-methyl, tralkoxydim or pinoxaden while the growth of wild type rice was severely suppressed. Interestingly, fenoxaprop-P-ethyl resistance was not observed in the P450 expressing rice. The results strongly suggest that the overexpressions of the P450 genes cause metabolic cross-resistance to ALS inhibitors and some ACCase inhibitors, diclofop-methyl, tralkoxydim and pinoxaden, while other mechanisms are suggested for fenoxaprop-P-ethyl resistance.

AGRO 102

Multiple herbicide resistance in Iowa waterhemp is the norm: Implications of multiple resistances on fitness, resistance mechanisms and future management

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A survey of more than 900 Iowa waterhemp (*Amaranthus tuberculatus*) populations demonstrated that multiple herbicide resistances is the norm; evolved resistance to three herbicide groups was found in 69% of the populations and resistance to four and five herbicide groups was found in 15% and 3% of the populations screened. These estimates are predictive at the 95% confidence interval. Representative 3-way, 4-way, 5-way herbicide-resistant and a herbicide-susceptible waterhemp populations were included in herbicide-free field experiments to determine if multiple herbicide-resistant waterhemp incurred a fitness penalty attributable to the mutation(s) that conferred herbicide resistance. Relative growth rate, flowering, biomass and seed production were measured. No significant differences between the multiple herbicide-resistant waterhemp populations and the herbicide-susceptible population were observed, and this supports the conclusion that multiple herbicide-resistant waterhemp are not at an ecological disadvantage. A population of HPPD-resistant and an HPPD-sensitive waterhemp population were studied to assess the mechanism by which resistance occurred. Our results indicated that the response of HPPD-resistant and susceptible waterhemp populations to mesotrione were very rapid and measureable as soon as three hours after treatment. Furthermore, little overlap was found among the differentially expressed transcripts expressed by each genotype. We also identified the possibility of overlapping gene networks in response to other herbicides. The results of these studies indicate that multiple herbicide resistances in waterhemp is likely to increase in frequency given the lack of fitness penalty found in the resistant populations and the fact that the resistances may be the result of non-target site mechanisms. Clearly, the management of waterhemp will be increasingly difficult given the frequency of multiple herbicide resistances and the lack of herbicides with novel mechanisms of action to which no resistances have evolved.

AGRO 103

Identification of genes involved in metabolism-based tembotrione resistance in Palmer amaranth (*Amaranthus palmeri*)

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Over the past decades, hundreds of weed species have evolved resistance to numerous herbicide modes of action, reducing the number of herbicides available for successful chemical weed control in several agricultural cropping systems. Among the most troublesome weed species in the U.S. is Palmer amaranth (*Amaranthus palmeri*), which has already evolved resistance to five different modes of action including 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibitors. The mechanism of resistance to HPPD inhibitors in one population of this species has previously been confirmed to be enhanced metabolism. Metabolite studies with the HPPD-inhibitor tembotrione revealed that resistant and susceptible plants form the same metabolites. However, resistant plants are able to hydroxylate tembotrione faster than susceptible plants, a process typically catalyzed by cytochrome P450 monooxygenase enzymes. Therefore, an RNA-Sequencing experiment focused on the identification of differentially expressed *CYP* genes using F_2 individuals from three separate crosses sampled before, six hours, and twelve hours after treatment with tembotrione. The experiment identified two genes with the putative annotations *CYP72A219* and *CYP81E8* as candidate genes for metabolic resistance in *A. palmeri*. The contigs were constitutively upregulated in resistant individuals, and their expression increased after treatment in both resistant and susceptible individuals. The expression patterns varied between the three different crosses. Several glucosyltransferases, a multicopper oxidase, and a glutathione-S-transferase were additional metabolic genes identified to be constitutively upregulated in resistant plants. Furthermore, eight *CYP* genes were identified as upregulated after treatment with tembotrione in either susceptible plants, resistant plants, or both. Further research aims to validate the involvement of the candidate genes in HPPD-inhibitor resistance in *A. palmeri*. Gaining knowledge about the connection between specific *CYP*s and herbicides they confer resistance to is crucial to predict compound liabilities, improve the design of herbicides, and allow for better diagnostics and management of metabolic resistance in the field.

AGRO 104

2,4-D metabolic resistance occurs via a P450-mediated hydroxylation reaction in waterhemp (*Amaranthus tuberculatus*)

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Auxinic herbicides have been applied for more than 70 years for broadleaf weed control, and just a few cases of resistance have been reported. 2,4-D detoxification in dicotyledonous species usually occurs by amino-acid conjugation, while in monocotyledon species it occurs by ring hydroxylation. In this study, a broadleaf species *Amaranthus tuberculatus* (waterhemp) resistant to 2,4-D was found to rapidly metabolize the herbicide, forming six different compounds in the resistant (R) individuals, and just one main metabolite in the susceptible (S) individuals. To characterize the chemical structure of the main metabolites produced in R and S plants, we purified and analyzed them using NMR and mass spectrometry. The main metabolite produced by susceptible plants corresponded to an amino acid conjugate, 2,4-D-Aspartate. Surprisingly, resistant plants showed indications of 2,4-D ring hydroxylation followed by sugar conjugation. That is the first case showing that a broadleaf weed is able to metabolize 2,4-D by pathways previously only known in monocotyledon 2,4-D tolerant species. As hydroxylation reactions are usually catalyzed by cytochrome 450 enzymes, this work opens new possibilities to elucidate mechanisms of metabolic herbicide resistance.

AGRO 105

Cytochrome P450s and multiple resistance in *Amaranthus palmeri* and *Echinochloa colona*

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Cases of multiple resistance are increasing in many weed species, among which are *A. palmeri* and *E. colona*. Multiple resistance is due to the accumulation of mutations in different target-sites, a combination of target-site (TSR) and non-target site resistance (NTSR) mechanisms, multiple NTSR mechanisms affecting different herbicide groups, or one NTSR mechanism affecting multiple herbicides. Cytochrome P450s (cytP450) are responsible for many herbicide detoxification processes in plants and are responsible for several cases of NTSR. Experiments on *A. palmeri* indicated that CYP 81B is associated with resistance to sulfonylureas. The majority of ALS-resistant *A. palmeri* populations harbor TSR mechanisms only, but some comprise both TSR- and NTSR-plants. Many ALS-resistant populations are also resistant to glyphosate and PPO-inhibitors. CYP72A219 is associated with elevated tolerance to glufosinate in *A. palmeri* with multiple resistance to glyphosate and ALS herbicides. In an obligate outcrossing species, resistance mechanisms that are non-lethal can easily combine within a population or plant, resulting in higher level of resistance to a broad range of herbicides. CYP709B2 is associated with high resistance to propanil in *E. colona* with multiple resistance to quinclorac. The CYP72A subfamily may also be involved in multiple resistance to propanil and quinclorac in *E. colona*. Despite being primarily selfing, the high ploidy of *E. colona* allows for the accumulation of multiple NTSR genes, which would complement the activity of cytP450s, also resulting in broad resistance to herbicides.

AGRO 106

Pyroxasulfone resistance mediated by enhanced metabolism in *Lolium rigidum*

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The evolution of resistant weed populations in response to intensive herbicide selection pressure is a global issue. We investigated metabolic resistance to the pre-emergence herbicide pyroxasulfone, recently introduced in Australia to provide control of *Lolium rigidum* populations resistant to multiple post-emergence herbicide modes of action. Pyroxasulfone resistance was based on enhanced metabolism

in a *L. rigidum* population recurrently selected with pyroxasulfone. A QTL for pyroxasulfone resistance was mapped on *Lolium* linkage group five. Approximately 88% of parental [^{14}C]-labelled pyroxasulfone was metabolized in resistant plants 24 hr after the herbicide treatment, compared to 54% in susceptible plants. Pyroxasulfone metabolites were formed via a glutathione conjugation pathway in pyroxasulfone-resistant *L. rigidum* plants. Constitutive higher expression, from six to nine-fold, was found for two candidate resistance-endowing *GST* genes. Rapid detoxification of pyroxasulfone is likely to involve a glutathione conjugation pathway in which *GST* over-expression produces glutathione-pyroxasulfone metabolites in resistant *L. rigidum* plants. Future research will determine whether the candidate *GST* genes directly conjugate glutathione to pyroxasulfone, and whether cross-resistance patterns involve *GST* or other genes.

AGRO 107

Consensus ranking and fragmentation prediction for identification of unknowns in high resolution mass spectrometry

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High resolution mass spectrometry (HRMS) and non-targeted analysis (NTA) are advancing the identification of emerging contaminants in environmental and agricultural matrices. However, confidence in structure identification of unknowns in NTA presents challenges to analytical chemists. Structure identification requires integration of complementary data types such as reference databases, fragmentation prediction tools, and retention time prediction models. The goal of this research is to optimize and implement structure identification functionality within the US EPA's CompTox Chemistry Dashboard, an open chemistry resource and web application containing data for ~760,000 substances. Rank-ordering the number of sources associated with chemical records within the Dashboard (Data Source Ranking) improves the identification of unknowns by bringing the most likely candidate structures to the top of a search results list. Incorporating additional data streams contained within the database underlying the Dashboard further enhances identifications. Integrating tandem mass spectrometry (MS/MS) data into NTA workflows enables spectral match scores and increases confidence in structural assignments. We have generated and stored predicted MS/MS fragmentation spectra for the entirety of the Chemistry Dashboard using the *in silico* prediction tool CFM-ID. Predicted fragmentation data was incorporated into the identification workflow by matching to observed MS/MS data, and results were implemented as both a scoring term and as a candidate threshold cutoff. Combining these steps within an open chemistry resource provides a freely available software tool for structure identification and NTA.

AGRO 108

Analysis of anionic phosphorus species and isotope dilution measurement of phosphate in surface water samples

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Phosphate, an important nutrient, is the most frequent form of phosphorus present in surface waters. It has been predominantly measured by colorimetry following exactly or with minor modifications the method published by Murphy and Riley in 1962. While a relatively easy and fast analysis, measurement at low concentrations are known to be biased due to easy absorption of the phosphate ions to glassware from both samples and calibration standards. The reliable analysis of phosphate at trace levels remains challenging using existent methods. This is the first report of using an optimized isotope dilution method for unbiased analysis of phosphate in surface water samples. Pre-concentration, matrix elimination and separation were achieved using two-dimensional ion chromatography (IC) coupled to a QExactive mass spectrometer (MS). The sulfate ions, normally present in surface water at much higher concentrations than phosphate (20 mg/L vs. 20 µg/L) were removed using first IC column and the high resolution MS can differentiate between the remaining HSO_4^- ions (m/z=96.95956) and $H_2PO_4^-$ (m/z=96.96907). The internal standard was spiked in the field to compensate for the phosphate loss during storage and also to eliminate the instrumental variability during analysis. Other anionic P species were simultaneously monitored, including 4 inorganic condensed P species, 3 organic condensed phosphates (AMP, ADP and ATP) and 9 sugar phosphate compounds. The method was tested by analyzing over 1000 samples collected from the Georgian Bay and Lake St. Clair (ON, Canada) during late spring, summer, and fall seasons. The estimated detection limit for phosphate is 0.1 µg/L as P.

AGRO 109

High resolution mass spectrometry applications in the identification of polar environmental metabolites to support development of new agricultural products

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Our objective is to develop new agrochemical products with favorable environmental profiles. In order to achieve this goal, a series of environmental studies are conducted early in the discovery process to generate preliminary data on biotransformation pathways and rate of metabolism in soils, plants, and various *in vitro* cell models. These systems can generate multiple trace level metabolites that must be identified to recognize and eliminate actives which could potentially transform into persistent or toxic metabolites in the environment. Many of the metabolites which are generated in these studies are small polar substances that present several analytical challenges. Recent developments in high resolution mass spectrometry, combined with techniques including ion mobility, SFC, CE, and nano separations, have been applied to these complex problems. We will present selected examples where these approaches have proven essential in the identification and quantitation of metabolites in environmental systems. Examples will show the analysis of challeging environmental samples on several modern LC/MS systems capable of accurate MS and MS/MS measurements including the; Fusion Q-OT-IT (Thermo), 5600 Q-TOF (Sciex), and 6560 Q-TOF (Agilent) coupled with separation techniques including SFC, ion mobility, and capillary electrophoresis. In early stage discovery, this work is generally performed prior to the availability of a radiolabel. The detection of the resulting xenobiotic metabolites is often aided by the use of data reduction methods including mass defect filtering (MDF), isotope filtering, sample/control comparison, and screening for predicted metabolites. These approaches may be additionally enhanced through the incorporation of stable-label isotopes (^{13}C, ^{15}N, or deuterium), which can provide a unique isotopic fingerprint to be monitored in the presence of complex matrices. Data are processed using software

packages that can filter for the unique isotopic pattern of the applied parent material, which increases the flexibility, accuracy, and efficiency of our metabolite ID workflows.

AGRO 110

Targeted and untargeted metabolomics to resolve bitter off-taste challenges in carrots (*Daucus carota* L.)

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Besides its unrivalled color, fresh cultivated carrots (*Daucus carota* L.), which ranks with a current annual world production of more than 30 million tons among the top ten vegetable crops, are favored by the consumer for their typical sweet flavor. Unfortunately, the attractive sensory quality of carrots is hindered by a sporadic bitter off-taste which is often the reason for consumer complaints and therefore a major problem for vegetable processors. This off-flavor is induced by abiotic and biotic stress factors during harvesting, transportation, storage, and processing. Although recent application of a Sensomics approach gave first insight into individual bitter tasting phytochemicals in carrots, their up-regulation during abiotic stress as well as the quantitative trait loci (QTL) controlling their biosynthesis pathways still remains elusive. In order to gain a more comprehensive knowledge on the chemical mechanisms involved in taste changes of cultivated carrots in response to abiotic stress factors, like water or post-harvest mechanical (transport-) stress as well as biotic factors such as *Atrenaria dauci* infestination, different stressed and non-stressed carrot genotypes were comparatively screened by application of a fast and robust high-throughput UPLC-TOF-MS metabolic profiling analysis. Software-assisted marker molecule selection in stressed carrots, followed by preparative chromatographic purification (MPLC and prep. HPLC) revealed the chemical structures of bitter tasting phytochemicals by means of LC-MS, LC-MS/MS, and 1/2D-NMR experiments. Accurate quantitation of these target molecules by means of UPLC-MS/MS_{MRM}-ECHO techniques in carrots before and after abiotic stress challenge of the same genotypes as well as in crossing lines revealed for the first time novel insights into the stress-induced metabolic response of distinct carrot genotypes. By narrowing down the correlated genomic regions using QTL results, catalogs of potential candidate genes can be set up. Such lists may be used to detect existing lines with mutation in the corresponding genes. These results might help to navigate breeding programs and to optimize post-harvest treatment of carrots from producer through processor to consumer towards high quality carrot products.

AGRO 111

Comprehensive pesticide analysis by SWATH® and MRM-HR acquisition using the SCIEX X500R QTOF high resolution accurate mass spectrometer

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Traditional triple quadrupole mass spectrometry techniques are very capable of analyzing compounds with high sensitivity, but high resolution accurate mass spectrometry techniques are useful when the target analyte list is not well established (i.e., non-target acquisition). Further, non-target acquisition allows for the retrospective mining of previous data to investigate "novel" compounds. SWATH™ acquisition is a non-target analysis technique that obtains MS/MS product scans on all precursor ions, and the product scans can be compared to established MS/MS libraries for compounds identification or confirmation. In contrast, MRM-HR may be a more sensitive acquisition technique due to the ability to use optimized collision energies. Samples were analyzed using the SCIEX Exion LC system that was coupled to the X500R QTOF High Resolution Accurate Mass Spectrometer. A simple SWATH™ acquisition method was built spanning precursor masses from 100-1000 Da. The TOFMSMS scans were divided into 16 windows of varying size with narrow windows to minimize the MS/MS pattern complexity. The total cycle time was 1.0 sec which ensured sufficient data points across the peak and strong MS/MS for library matching. During data processing a target XIC components list of 275 compounds was prepared and retention times were verified by comparison to an authentic standard. MS/MS patterns were compared to the SCIEX HR pesticide library and NIST MS/MS library. In addition, a scheduled MRM-HR method was built. The SWATH™ acquisition technique allowed for the unbiased analysis of the 275 pesticide compounds while simultaneously collecting MS/MS scans for compound confirmation. Quantitation was performed on the TOFMS precursor ion, or MS/MS fragment ion. For example, analysis of imidacloprid in raisin extract showed that the fragment XIC has a "clean" background, as compared to the TOFMS XIC, and thus uncompromised quantitation. The MS/MS library matching showed that the NIST library contained an additional ~20 compounds that were not present in the SCIEX HR Pesticide library. The scheduled MRM-HR acquisition method could be advantageous when higher levels of sensitivity are required. Finally, the two developed acquisition techniques were applied to the analysis of 8 fruit & vegetable extracts. The methods were capable to identifying a range of pesticides, including several at levels <10 ng/g.

AGRO 112

Method development for analysis of picloram in compost

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Herbicides play a critical role in controlling undesired weed growth and are widely used in agricultural applications including pasture lands and against invasive species. Composting has been advocated as means to not only recycle waste, but also to capture carbon, reduce emission of greenhouse gases, improve soil quality and structure, reduce soil erosion, reduce chemical inputs, reduce requirement for landfills, and create jobs. Currently, cut grass hay is one of the common sources of compost feedstock. Herbicides present in cut grass hay may not be completely removed in the composting process, resulting in the presence of trace level of residues in the finished composts. In order to detect low level of herbicide residues in compost, an innovative analytical method was developed using picloram as an example. The primary issue with compost as a matrix when analysing for picloram in particular and herbicides in general is the matrix interference. Matrix interference may prevent accurate quantitation of picloram in compost, especially at very low levels (low ppb). We will discuss the sample clean-up options that were evaluated, and share information on the linearity, accuracy, and precision of the final method. In brief, we were able to obtain a lower limit of quantitation (LOQ) of 1 ppb with a linearity range from 1 ppb to 100 ppb of picloram in compost.

AGRO 113

Use of modern MS techniques and informatics to support agricultural research and a pragmatic approach to contaminant screening

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Agrochemical companies, environmental regulatory authorities and food companies are under pressure to not only thoroughly research new agrochemicals but also to develop screening methods capable of detecting a broad spectrum of environmental contaminants and transformations in as few analytical runs as possible. Tandem quadrupoles (MSMS) are the current gold standard detectors used in routine screening methodologies. However, to facilitate an expanded scope of screening or compound research, many labs have focused on High Resolution Mass Spectrometry (HRMS) for obvious reasons, since modern non-targeted, data independent, analyses offer a high level of selectivity through the acquisition of full spectra with accurate mass and isotopic information for both precursor and product ions in a single run. Informatics is now playing a major role, not just in data collection but in the processing, interpretation and elucidation of this information rich data to enhance, streamline, and simplify the research and routine arenas.

Hardware advances now allow the use of several ionization techniques in addition to the use of different chromatographic techniques (LC and GC) on the same Mass Spectrometer, which drives down the cost of instrument ownership whilst maximizing compound coverage and versatility. Using relevant example data, we will demonstrate the ability to utilize several chromatographic, ionization and orthogonal separation (Ion Mobility) techniques to collect comprehensive, information rich datasets using a Data Independent Acquisition (DIA) mode on the same mass spectrometer. We will focus on the benefits of a modern Scientific Information System utilizing apex peak picking and componentization algorithms to process raw data which facilitates data interrogation and elucidation and allows a user to perform a targeted, suspect, unknown, and metabolite identification with a single software package and more importantly, a single data processing step.

AGRO 114

Combining sample clean-up techniques and high resolution LC-MS, with software manipulation, for metabolite identification in support of agrochemical product development

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The rapid and robust identification of agrochemical metabolites from animal, plant and e-fate studies is an ongoing challenge in new product development. The challenge is often exacerbated by the trend in lower application rates coupled with complex matrices. The application of high resolution LC-MS and LC-MS/MS remain the primary tool in industry for this purpose. Despite the welcome developments in instrument sensitivity, resolution and dynamic range, this does not necessarily guarantee the analyst a useable dataset. The importance of a good quality sample should remain paramount, especially with the associated cost of state of the art LC-MS instrumentation and the time pressures which exist in regulatory development. Fortunately, there are now more robust and powerful software packages which can assist the analytical chemist in mining meaningful data from the analysis of complex samples. This can lead to more rapid identification of unknown metabolites, which would otherwise remain extremely difficult to find and elucidate using classical sample clean-up techniques alone.

This presentation will highlight the power of combining creative sample clean-up techniques in the laboratory *together with* quick and simple-to-apply software algorithms for refinement of high resolution MS datasets, beyond what is possible using these techniques on their own.

AGRO 115

Challenges and approaches on the conduct of aqueous photolysis studies: Case study for a low solubility compound producing volatile organics and polar unknown degradates

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A guideline aqueous photolysis study seeks to characterize the phototransformation of chemical pollutants in surface water through kinetics and degradation pathways under abiotic conditions and direct irradiation. Pesticides with extremely low solubility in water (ppb range) present unique challenges when designing a successful aqueous photolysis study. A case study is presented which will outline different approaches for study design (including vessel construction, traps for volatiles, use of co-solvent, etc.) and sampling techniques to overcome mass balance challenges, as well as concentration techniques required for adequate chromatographic analysis. Additionally, approaches to characterization and identification of polar and/or volatile degradates when working in extremely low concentrations will also be discussed.

AGRO 116

Study design and conduct of surface water mineralization in either dark or diffuse light with optional inclusion of sediment

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The primary objective of an aerobic mineralization in surface water study (OECD guideline 309) is to determine the biodegradation rate of a test substance in aerobic natural water, but it also seeks to obtain information of primary degradation and formation of transformation products other than CO_2. There are several approaches to study design of aerobic mineralization guideline studies, to fit the expected environmental conditions based on use patterns. We will discuss different study conditions (source of test systems, pelagic vs. addition of sediment, temperature, dark vs. diffused light, etc.), designs (subsampling, individual samples, agitation, concentration of test substance, etc.) and some of the challenges encountered during the conduct of these guideline studies, and our approach to solving these issues.

AGRO 117

Designing experiments to support USDA National Conservation Practice Standards and air quality guidelines

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USDA-Natural Resources Conservation Service (NRCS) has encouraged the use of vegetative environmental buffers (VEBs) as a cost-efficient, best management practice for managing poultry house air pollutant emissions, including particulate matter (PM), ammonia, and volatile organic compounds (VOCs). In preliminary, controlled-emission studies, results indicated that VEBs could capture PM and some ammonia. However, these studies were conducted using VEB components and did not provide a sufficient quantitative assessment of functioning VEBs to support VEB design criteria development and conservation practice standard updates. Therefore, in this study, field experiments were designed and conducted in multiple sampling campaigns at a commercial poultry house equipped with an established VEB under realistic conditions, i.e., during a normal flock growth period. Sampling towers with multiple sampling cross bars were deployed at several distances from the tunnel fans (the emission source) to provide optimal emission measurements. Three different sampling techniques were employed (low-volume PM samplers, ammonia Radiello™ cartridges, and amber glass canisters) to capture the major air pollutants. All the sampling techniques were synchronized during each experimental period to provide a more-complete dataset. A small-scale Gaussian Plume air-dispersion model was modified and validated using data from a sampling campaign at a poultry house without a VEB. The model was used to estimate the concentration profiles for the VEB-equipped house under the same meteorological conditions during the sampling period assuming that the VEB was not installed. These model estimates served as the controls and were used to assess the performance of the VEB. Results from this study will be used to assist in evaluation and modification of the NRCS National Conservation Practice Standards for buffer systems.

AGRO 118

Describing aged sorption behavior of pesticide in soil field dissipation studies via inverse modeling

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The aged sorption behavior of a pesticide in sandy soil was examined in two field dissipation studies. In these studies, soil samples collected from 0-5 cm horizon were first extracted by 24-hour equilibration in 0.01 M $CaCl_2$ and then by organic solvent extraction. The 0-5 cm soil data were fit to the two-site aged sorption model to determine the parameters of equilibrium or aged sorption (if significant). The $DegT_{50,EQL}$ from 0-5 cm soil also accounts for leaching loss and thus does not represent degradation rate in soil. However, the other 0-5 cm soil aged parameters K_{fom}, K_{des}, and F_{ne}, can be used to determine the actual $DegT_{50,EQL}$ by fitting the 0-90 cm soil depth-specific concentrations to PEARL 4.4.4 via inverse modeling with PEST. At Step 1, the normalized (20°C and pF 2) total soil profile $DegT_{50}$ values were calculated by the FOCUS kinetics guidance and then used with the 0-5 cm K_{fom} to examine if soil profile distribution of pesticide can be fit by equilibrium sorption. If the fit is acceptable, aged sorption is considered insignificant. If not, at Step 2, inverse modeling was further conducted with 0-5 cm soil K_{fom}, K_{des} *and* F_{ne} to optimize $DegT_{50,EQL}$ or only 0-5 cm soil K_{fom} to optimize $DegT_{50,EQL}$, K_{des} *and* F_{ne}. The results indicate that aged sorption in the German site is significant, while not in the California site due to extremely low organic matter. The optimized equilibrium or aged sorption parameters provide excellent fits to soil profile distribution of pesticide in both field studies and are statistically robust for use in leaching risk assessment.

AGRO 119

Guideline adsorption/desorption study design and approaches to adsorption coefficient determination

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Adsorption/desorption studies are used to evaluate the sorption behavior of chemical compounds in environmental air, water, and soil (or sediment) phases, and must be performed for pesticide registration (and recently, pharmaceutical or industrial chemical applications) or to satisfy standard information requests. The soil adsorption coefficient of a compound is a vital component when assessing its environmental risk and predicting its distribution and leaching potential. The U.S. EPA and EU OECD regulatory agencies have developed guidelines for the conduct of adsorption/desorption studies. The presentation will address study designs and the best approaches to determining adsorption coefficients according to the relevant guidelines. Factors such as which guideline to follow, soil selection and application to the different study tiers, sample vessel types, solid-liquid phase separation, determining the equilibration period, correcting for compound degradation, adsorption coefficient calculation, and when to use an isotherm model will be discussed.

AGRO 120

Enhanced laboratory techniques for the evaluation of persistence

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Standardized biodegradation tests following established guidelines like those published by the EPA and the OECD have been used for several decades now to determine the fate of chemicals and pesticides in the environment. Biodegradation guidelines often start with a tiered approach to evaluate the biodegradability and/or persistence of a chemical. These guidelines are focused on a specific aspect of the natural or manmade environment, whether it is a pond or stream or a wastewater treatment facility. Chemicals that are found to be resistant to degradation under a narrow set of laboratory parameters may be found to be degradable under the right conditions.

In this presentation, techniques for enhancing the chances for chemical degradation to occur are investigated. These techniques will include the various ways of introducing the test substance into the test system, varying the inoculum, extending the exposure, and modifying the testing conditions to include the simulation of sunlight.

AGRO 121

Transformation of organic chemicals in aquatic sediment systems (OECD 308) under simulated natural sunlight

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Chemicals that are directly applied to surface water or that are likely to reach the aqueous environment by routes such as run-off, drift, and agricultural effluents, are required to pass environmental risk assessments. As an essential part of tiered testing strategies, the OECD 308 Guideline defines laboratory testing to assess aerobic and anaerobic transformation of organic chemicals in aquatic sediment systems in the dark. In order to further simulate environmental conditions, and ascertain half-lives or disappearance times (DT50, DT75, DT90) for test compounds while being exposed to irradiation, requests for including natural sunlight in OECD 308 study designs have been emerging. Innovative Environmental Services (IES) Ltd, Switzerland, as an independent GLP-certified contract research organization, brings ten years of experience with aerobic aquatic sediment studies under simulated natural sunlight conditions, performed for a variety of recurring clients. The laboratory set-up used by IES Ltd for irradiated OECD 308 studies will be elaborated upon in detail, and potential advantages and disadvantages of including simulated natural sunlight in the test design will be explained. In addition, the need for a new or adapted test guideline will be discussed.

AGRO 122

Understanding the behavior of herbicide residues in composts with small scale composting and bioassay tests

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Composts are products from controlled aerobic, biological decomposition of biodegradable materials. They are typically utilized in agriculture and horticulture to provide plant nutrients. When agricultural commodities or animal manures that contain herbicide residues are used as feedstock in the composting process, the herbicide residues may not be totally degraded and end up in the field when utilized as soil amendments and top dressing (mulching) treatments. A small scale composting system followed by bioassay testing is a study designed that assists us to better understand the behavior of herbicide residues in the composting process and the potential residues impact on plant growth when composts are used. Two types of composting systems: vegetative matter (i.e. fresh cut grass), or animal manure as feedstock materials are compared. Duplicate reactors in each system with and without herbicide residues are monitored through a 90 day composting process to investigate the herbicide degradation kinetics. Negative control composts, which are composting products without herbicide residues, are utilized to predetermine the maximum amount of finished compost that can be used in a soil amendment without negatively impacting plant health. Based on the predetermined maximum compost mixing ratio, a bioassay with four sensitive plant species are tested using finished composts with and without herbicide residues in soil amendment and top dressing treatments. The bioassay test results will reveal potential phytotoxicity effects from residual herbicide remaining in the finished compost.

AGRO 123

Mode of action of insecticides and repellents

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Chemical insecticides and repellents play a dominant role in protection of crops, livestock, homes, pets, and humans from arthropod pests and disease vectors. Since the advent of widespread use of synthetic organic and microbially-derived insecticides in the 1940s, insecticide resistance and increasingly stringent regulations have fostered a continuing demand for new modes of action, which the chemical industry has been able to deliver at a steady pace. In the early 1980s, companies began hiring biologists to study these new modes of action, and I am fortunate to have been among them and to have had the opportunity to study successive generations of novel insecticides. I will briefly summarize this work, which includes studies on the mode of action of pyrethroids, fiproles and neonicotinoids on already known targets, as well as the identification of the novel insecticidal modes of action of sodium channel blockers (indoxacarb and metaflumizone), nicotinic receptor allosteric modulators (spinosyns) and chordotonal organ TRPV channel modulators (pymetrozine, pyrifluquinazon and afidopyropen). Innovation in repellents has been comparatively less remarkable, with the market still dominated by DEET, the first widely used repellent, which was introduced more than seventy years ago. The mode of action of DEET is still not understood, but it is widely thought to involve disruption of olfaction. In the second part of this talk, I will describe the discovery of a novel sensory modality that is used by ticks in host-finding, and its disruption by low concentrations of DEET, picaridin, and other repellents. This new insight into host-finding by ectoparasites the mode of action of repellents could facilitate future discovery of novel repellents.

AGRO 124

Vapor phase repellents: New methods, chemistry, and mechanisms of action

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The basic amines, 1-methylpiperazine and 1-methylpyrrolidine interfere with *Aedes aegypti* host-seeking behavior through a possible anosmic effect. This study investigated the effects of these compounds, along with triethylamine (an active ingredient in FlynapÔ anesthetic). Glass tube vapor phase assays of 1-methylpiperazine and 1-methylpyrrolidine on *Ae. aegypti* adult females showed initial repellency, followed by narcosis, knockdown, and paralysis. Under identical conditions, triethylamine showed quick knockdown without repellency, and the knockdown was reversible, unlike that of the other two amines. Electroantennographic (EAG) study showed significant responses of *Ae. aegypti* to the three basic amines; moreover, triethylamine increased the EAG response of background air (blank). I also studied the EAG responses of repellent/attractant standards (including VUAA-1, reported to be an Orco channel agonist) and a series of fluorinated phenylamide repellents. EAG responses of the pyrethroid-resistant Puerto Rico strain were significantly reduced for DEET, IR3535, and 2-undecanone, while no difference was observed for 1-octen-3-ol. These results corroborated our vapor phase repellency data for DEET, IR3535 and 2-undecanone, which showed significant cross resistance in the pyrethroid-resistant Puerto Rico strain. Among the experimental phenylamide repellents, only one showed a

significantly greater EAG response than DEET. VUAA-1 gave no EAG response, but it significantly decreased the EAG response of the blank by 40%. Because a recent patent claimed thermal volatilization of VUAA-1 initiated an EAG response, we devised an induction coil heating system that induced vaporization of chemicals within seconds and without changing the overall air delivery temperature. Statistically significant increases in EAG responses were recorded for DEET and 1-octen-3-ol, at a temperature of 118 °C. These findings provide insights into the mode of action of basic amines and spatial repellents on mosquito behavior, and the induction heating system has the potential improve testing of rare and low volatility compounds with the EAG technique.

AGRO 125

Discovery of chemicals that mediate mosquito host-seeking and biting behavior

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Mosquitoes locate and obtain bloodmeals from suitable hosts by using various host location cues, perhaps the strongest of which are the volatile chemicals emanated by the host. These biting flies demonstrate a selection preference for different species of host, e.g., human versus avian, as well as for hosts within the same species, e.g., different humans. Semiochemicals from humans and other animals have been identified and studied to determine how they mediate mosquito-host seeking and feeding. Some of these chemicals function as kairomones, which help mosquitoes find their preferred host. Others of these function as allomones, which can either repel the mosquitoes or result in anosmia, whereby the behavior impact on mosquitoes is that they are seemingly unable to detect and orient towards the source of host kairomones. While there are a number of naturally produced allomones, the most effective repellents (those that protect at nearly 100% efficacy and for the longest durations) are synthetic and have been designed structurally based upon trends observed in structure-activity relationships. This presentation will cover the chemistry the surrounds the behavior of mosquitoes, specifically on those compounds that function as kairomones (attractants) or allomones (repellents and inhibitors) for mosquitoes.

AGRO 126

Next-gen biorational spatial repellents

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Among possible new tools to use against vectors, spatial repellents represent a category of tools that provide additional protection, especially against mosquitoes. In yards, parks, campgrounds, horse stables, and livestock facilities, spatial repellents can deter flies and mosquitoes from entering the treated area. Many personal or local uses of spatial repellents involve burning of a pyrethroid coil or otherwise emitting a volatile pyrethroid insecticide. Emitting oil of citronella is the primary alternative to the pyrethroids. Our lab has synthesized a series of biorational derivatives of natural terpenes to create repellents that provide physicochemical and biological properties that are improvements over citronella and other terpene repellents. Pyrethroid spatial repellents work well in some cases, but they are less effective against pyrethroid-resistant strains of mosquitoes. Some pyrethroid spatial repellents cause mosquito knockdown and mortality, which adds to their current efficacy, but potentially contributes to evolving of pyrethroid-resistance in a mosquito population. The mechanism of repellent action for terpenes is likely different from that of pyrethroids. The advanced terpenes could be valuable spatial repellents when used in conjunction with other more traditional tools.

AGRO 127

Toxicity and physiological actions of fatty acids and related potassium channel blockers to mosquitoes

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This study tested a series of potassium channel blockers, including 1-((2-chlorophenyl)diphenylmethyl)-1*H*-pyrazole (TRAM-34), 11-dansylaminoundecanoic acid (DAUDA), and 5-hydroxydecanoic acid (5-HDC), because their effects have not been explored on insect voltage-gated Kv2 channels. We evaluated the action of these compounds in patch clamp recordings of engineered HEK cells expressing *Anopheles gambiae* Kv2.1 channels. They were also tested for insecticidal effects through topical application to adult mosquitoes (*Anopheles gambiae* and *Aedes aegypti*), for paralysis in a headless larvae assay with 4th instar *Anopheles gambiae*, as well as in central nervous system (CNS) recordings from 3rd instar larvae of *Drosophila melanogaster*. Patch clamp studies revealed that fatty acid compounds without functional groups in the alkyl chain (*e.g.*, decanoate, DAUDA) yielded a more potent blocking action on Kv2.1 currents than substituted fatty acids (*e.g.*, 5-HDC). Also, in comparison to 2-methoxy-*N*-((1-phenylcyclopentyl)methyl)benzamide (2S-65465), a known Kv2 channel blocker (IC_{50} = 100 nM), decanoate and DAUDA were 6-fold and 12-fold less active, respectively, as blockers of potassium current. TRAM-34 was the least potent inhibitor tested in patch clamp studies (IC_{50} = 30 µM). When tested on *D. melanogaster* CNS, the compounds typically gave an initial increase in firing rate, followed by a decrease, both effects at micromolar concentrations. Paralysis obtained from headless larvae assays had the same potency ranking as that obtained through patch-clamp recordings, indicating a possible connection between channel block and whole organism toxicity. Moreover, when DAUDA and decanoate were tested on adult mosquitoes, they were not as lethal as 2S-65465. As expected from its low activity on Kv2 channels, *in vitro*, TRAM-34 showed little toxicity in any whole insect screen. In order to possibly improve the insecticidal activity of the fatty acids, we are currently working on derivatized analogs (*e.g.*, methyl esters) that could better penetrate the cuticular barrier.

AGRO 128

Evaluating the mode of action of neonicotinoid insecticides and sulfoximine derivatives on *Ixodes ricinus* nicotinic acetylcholine receptors

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Ticks and tick-borne diseases have a major impact on human and animal health worldwide. Nicotinic acetylcholine receptors are targets of highly effective insecticides including neonicotinoids and the recently introduced sulfoximine compounds. Because ticks are resistant against chemical acaricides, we have evaluated the mode of action of several compounds on ticks *Ixodes ricinus* nicotinic acetylcholine receptors. To test this, we developed a new approach using tick membranes from the synganglion and found that it may provide a basis for further studies of the interaction between new acaricides and tick nicotinic acetylcholine receptors.

AGRO 129

Designing "smarter" insecticides for vector control

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The discovery of "smarter" insecticides that are pest selective and environmentally benign is a high priority for the continued control of vector-borne diseases. To meet this challenge, our program is pursuing rational design of small molecule insecticides that operate via unique modes of action in Aedes, Anopheles and Culex species. First, we have discovered new chemical series that selectively disrupt novel targets - mosquito G protein-coupled receptors (GPCRs) - and are lethal to the insect. Second, we have re-designed whole organism screening platforms to take advantage of new imaging technologies and identify novel, non-lethal chemistries that disrupt flavivirus transmission by the mosquito. The pros and cons of the target-based and empirical screening strategies will be presented, and prospects for the development of new and safer insecticide classes will be presented

AGRO 130

Commercialization of natural products from discovery via microbial fermentation processes

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At Dow AgroSciences LLC, natural products are discovered and commercialized through synthetic chemistry, as well as microbial fermentation processes. One of the key responsibilities of the Bioengineering and Bioprocessing R&D (BBRD) team is to screen novel microbial strains for the production of natural products (insecticides, herbicides, and fungicides). The fermentation productivity of compounds produced by the native strain could never compete with synthetic chemistry. However, advanced technologies in bioengineering and bioprocessing enable productivity improvement of the native strain, thereby creating commercial feasibility. An integrated workflow for rapid commercialization involves screening microbes capable of synthesizing actives at higher rates, as well as optimization of fermentation processes, to achieve high titers with compliance to product quality specifications. Improved strains are created through random mutagenesis and selection, as well as through targeted engineering. High-throughput screening technologies (HTPs) are used to rapidly screen through thousands of strains to identify the true top performers. The productivity of new strains is amplified through streamlined process optimization of the media composition and process conditions (temperature, pressure, pH, agitation, and aeration rates). This presentation will summarize current approaches for strain and fermentation development that lead to rapid commercialization of novel natural products with agriculturally relevant activity.

AGRO 131

Use of green chemistry principles in the design of crop protection processes and products

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Green Chemistry principles are especially important in agriculture due to the sheer size of the food value chain. Unlike the relatively small volumes of active pharmaceutical ingredients, typical annual manufacturing volumes for crop protection active ingredients are hundreds to thousands of metric tons. The manufacturing footprints of crop protection active ingredients can be reduced by applying the 12 Principles of Green Chemistry which are closely aligned with general best practices for process development. This talk will describe selected examples of the use of these principles in the synthesis of crop protection active ingredients. In addition, extension of these principles to the design of crop protection formulations will be described.

AGRO 132

Selective liquid phase hydrogenation of *p*-hydroxybenzyl cyanide over a supported Pd catalyst

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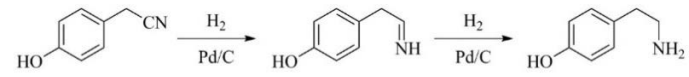
Primary amines are of significant importance in the chemical industry with many products requiring the use of these compounds in their production. Although there are several possible routes for these species to be synthesised, it is the heterogeneously catalysed hydrogenation of nitriles that is most commonly used in agrichemical production chains where this research finds application. Nevertheless, as a consequence of imine derived chemistry, selectivity is a major issue associated with these reactions. Against this background the catalytic hydrogenation of nitriles by a 5% Pd/C catalyst to yield primary amines of relevance to the manufacture of agrichemicals has been explored. In this instance *p*-hydroxybenzyl cyanide has been selected as a model substrate suitable to probe this catalytic process and allow valuable insight applicable to more complex systems to be gleaned. Initially very poor, the selectivity towards the desired product was improved through application of an acid additive and the tuning of operational parameters linked to the hydrogen availability in the system. The completely selective production of tyramine has now been achieved as confirmed by both high performance liquid chromatography analysis and proton nuclear magnetic resonance spectroscopy. Moreover, also considered was the potential for a detrimental hydrogenolysis step - cleavage of the amine functionality. Although not active in this particular study, advantageous information regarding this process was obtained.



Hydroxybenzyl Cyanide Hydroxybenzyl Imine Tyramine

AGRO 133

Simple and highly effective mono-ligated arylpalladacycle complexes for Suzuki cross coupling reactions

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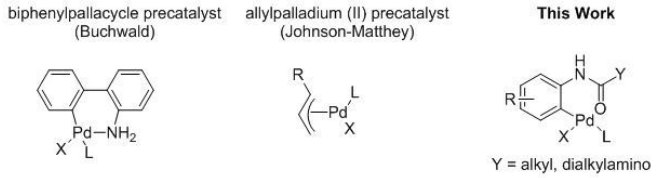
The Palladium catalyzed reaction of aryl halide (iodide, bromide, chloride), and aryl pseudohalides (e.g., triflate, tosylate, mesylate) with various substrates is a general method employed for the formation of C-C, C-N, C-O bonds, which plays an important role in synthesis of fine chemicals, agricultural and pharmaceutical products, and advanced materials. The reactivity of the palladium catalyst is greatly influenced by its structural features and the number of associated ligands to the metal. Mono-ligated palladium complexes, bearing one bulky and electron-rich ligand, have been demonstrated to be highly effective catalysts/precatalysts. Among such examples are Buchwald's

biphenylpalladacycle precatalysts and Johnson-Matthey's allylpalladium (II) complexes. Here we report a type of very simple, and highly efficient mono-ligated arylpalladacyle precatalysts (Figure below). In Suzuki reactions these precatalysts exhibit mild reaction conditions, low catalyst loadings, short reaction times, and provide high yields. Their ease to prepare, stability to air and moisture could make them desirable for applications in large-scale industrial processes.



AGRO 134

Development of scalable Sn-catalyzed regioselective allylation of 1-methyl-L-α-rhamnopyranoside

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A robust selective allylation of 1-methyl-L-α-rhamnose was developed using di-*n*-butyltin oxide (nBu$_2$SnO) as the catalyst and proton sponge as the base. The optimized condition afforded the 3-*O*-allylated rhamnose in excellent regioselectivity (>20 : 1) and isolated yield on 50 g scale. A scalable isolation/purification process was developed which afforded the desired product in 72% yield with 1530 ppm of Sn. Further scavenger treatment successfully reduced the residual Sn to <20 ppm. This catalytic system also showed outstanding potential in selective benzylation of the same substrate.

AGRO 135

Synthetic studies toward ryanodol, ryanodine, and related insecticidal natural products

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(+)-Ryanodine was first identified in 1948 by Folkers and coworkers as part of an effort to identify the insecticidal constituents of *Ryania speciosa* Vahl, a shrub native to Central and South America. In fact, the crude extract from the shrub was marketed as Ryanex, a non-specific insecticide. However, this product was later removed from the market, and efforts to make improved ryanodine-derived insecticides have been thwarted by challenging synthetic chemistry. In this seminar, we will describe a synthetic strategy that provides access to (+)-ryanodine and the related natural product (+)-20-deoxyspiganthine in 18 and 19 steps, respectively. A key feature of this strategy is the reductive cyclization of an epoxide intermediate that possesses the critical pyrrole-2-carboxylate ester. We will also describe recent efforts to prepare the antifeedant isoryanoid natural product perseanol.

AGRO 136

Award Address (ACS Award in Industrial Chemistry sponsored by the ACS Division of Industrial and Engineering Chemistry). Strategies in the discovery of new insecticides and nematicides: A career perspective

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The discovery and development of new insecticides and nematicides is essential for protection of crops worldwide. Key elements of the discovery and development process include a robust strategy for the identification of new molecular starting points coupled with a carefully managed optimization process that identifies compounds with the most preferred combination of attributes of biological efficacy, and mammalian and environmental safety. An overview of these strategies from early lead identification through the optimization process will be presented.

AGRO 137

Discovery and development of Simparica® (Sarolaner): A novel companion animal isoxazoline parasiticide

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A novel isoxazoline, sarolaner, was identified during a lead optimization search for an orally-active compound with efficacy against fleas and ticks in dogs. The molecule has unique structural features important for its potency and pharmacokinetic properties, including spiroazetidine and sulfone moieties. The flea and tick activity resides in the chirally pure S-enantiomer. Sarolaner potently inhibits flea and tick GABA-elicited currents, without blocking GABA-induced currents in human GABACls (α1β2γ2 subtype). *In vitro* sarolaner demonstrated an LD_{80} of 0.3 µg/mL against *C. felis* and an LD_{100} of 0.003 µg/mL against *O. turicata*. Development studies demonstrated efficacy of sarolaner at 2 mg/kg in an oral chewable tablet formulation against fleas (*C. felis*) and multiple species of ticks (*Rhipicephalus sanguineus*, *Dermacentor variabilis* , *Amblyomma americanum* and *A. maculatum*) for 35 days. Studies also showed that sarolaner acted quickly, killing fleas within 3 hours and ticks within 8 hours.

The presentation will focus initially on the medicinal chemistry efforts towards the discovery of Sarolaner, and in the second part will describe some of the work that went on to develop the manufacturing process of the lead molecule.

AGRO 138

Optimization of mesoionic pyrido[1,2-a]pyrimidinone insecticide and discovery of dicloromezotiaz

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Recently, a novel class of mesoionic pyrido[1,2-a]pyrimidinones has been discovered with exceptional insecticidal activity controlling a number of insect species. In this presentation, we will present the part of the optimization program that led to the identification of dicloromezotiaz as a potent insecticide to control a broad range of lepidopteran species. Our efforts in discovery, synthesis, structure-activity

relationship elucidation, and biological activity evaluation are also presented.

AGRO 139

Novel insecticidal bifenazate derivatives

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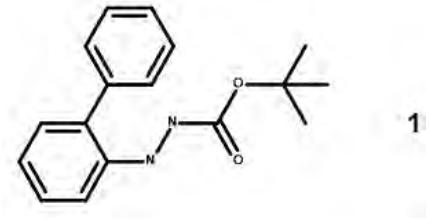
Bifenazate is a hydrazine carbazate acaricide discovered in 1990 by Uniroyal Chemical. The compound itself doesn't show insecticidal activity, but the derivative **1** described by Dekeyser et al. was reported to control delphacids and mites. Triggered by a random screening hit we revisited the bifenazate chemistry with the goal to find analogs with improved insecticidal potency and better breadth of spectrum. A comprehensive analysis of the structure-activity relationships will be shown.



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AGRO 140

Fungal and plant phytotoxins as tool for legume crops protection

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Food legumes are annual and multifunctional crops with extraordinary historical importance for the agriculture and the environment of the Mediterranean basin. Their production is decreasing in most of the Mediterranean farming systems for the low and irregular yield as a consequence of biotic and abiotic stresses.

Among the main biotic constraints are necrotrophic fungi, including essentially *Ascochyta* and *Botrytis* species producing phytotoxins, belonging to different classes of natural compounds, involved in the development of disease symptoms. Foliar diseases caused by biotrophic pathogens, such as rusts and powdery mildews, are other limiting factors in legume production worldwide. Parasitic weeds and aphids also cause significant yield and quality reduction. In this communication the chemical and biological characterization of the phytotoxins produced by fungal pathogens of legumes will be illustrated as well as their potential for crop protection based on their antagonistic activity to develop new ecofriendly fungicides. Phytotoxins could also be used to select resistant varieties of host plants and for their antifeedant activity. Furthermore, the ability of some fungal, plant and plant root exudate metabolites as stimulants and/or inhibitors of parasitic seed germination and in the induction of haustorial development will be discussed.

AGRO 141

Antibacterial metabolites from *Alternaria alternate* ZHJG5, an endophytic fungus in *Cercis chinensis*

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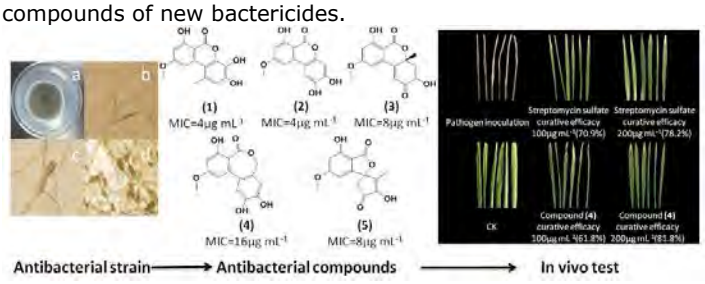
Plant diseases such as bacterial blight of rice caused by *Xanthomonas oryzae* pv*. oryzae* (*Xoo*) result in immense crop as well as economic losses worldwide. However, sustained use of the commercial bactericides such as streptomycin causes serious drug-resistance, which reveals the urgent need for highly effective and environment-friendly new bactericides. Screening of microbial natural products with antibacterial activity is an effective approach for the discovery of novel pesticides. Continuing our work on the characterization of structurally novel and/or biologically active metabolites from endophyte cultures, an endophytic fungus *Alternaria alternate* ZHJG5 residing in *Cercis chinensis* showed potent antibacterial activity against *Xoo*. Under the bioassay-guided fractionation, five metabolites including one new compound were isolated, with their structures identified by spectroscopic analyses and X-ray crystal diffraction. Compounds 4-hydroxyalternariol-9-methyl ether (**1**), altenuisol (**2**), dehydroaltenusin (**3**), alterlactone (**4**) and new compound iso-talaroflavone (**5**) exhibited antibacterial inhibitory activity against *X*oo with minimum inhibitory concentration (MIC) values of 4, 4, 8, 16 and 8 μg mL^{-1}, respectively. *In vivo*, the result demonstrated that alterlactone (**4**) could effectively control the rice bacterial leaf blight with curative efficacy of 81.8% at 200 μg mL^{-1}, comparable to that of streptomycin sulfate (78.2%). In this study, the metabolites from *A. alternate* ZHJG5 are promising antibacterial compounds that could be used as lead compounds of new bactericides.



AGRO 142

Role of a multiactive bio-organic substance on protection and yield of rice crop

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This paper studies the effect of a multi activity bio-organic granular substance applied to soil for rice crop. It is seen to enhance healthy growth and protect rice plants, although climate changes influence efficacy and net yield. Pest incidence and microbial properties of the soil along with its fertility have been assessed. It has been observed that rice and straw yields increase substantially on adding the bio-organic granules to the soil, and effective dose needed varies according to season. The rise in yield could be attributed to the multifunctional behaviour of the as-prepared granular formulation named G5 made up of a unique combination of five different bio-organic ingredients (seaweed extracts, amino acid blend, herbal and seed extract, humic acid and anti root rot substance consisting of calotropis and whole aloe) adsorbed on a suitable clay substrate. The studies on growth of the crop as a result of action of biostimulants shows decreased attack by pathogen antagonists due to the presence of a natural plant pest control extract. Further,

lower doses of G5 correlated with less incidence of pests like stem borer, gall midge, whorl maggot, and leaf miner in one particular season (Kharif) as against higher doses of G5 required during the wet season (Rabi) as observed in India.

AGRO 143

A new furanocoumarin from leaves of *Amyris elemifera* with antifungal and phytotoxic activities

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Plants have evolved to survive in the biosphere by producing secondary metabolites to compete with other fungi, plants, and insects. Thus, these secondary metabolites can have various biological activities such as antifungal, insecticidal, and phytotoxic activities. The plants in the Rutaceae family are especially enriched in these compounds. We investigated *Amyris elemifera*, a plant in the Rutaceae family in search of such compounds. Chromatographic fractionation of the ethyl acetate extract of the leaves of *A. elemifera* afforded a novel furanocoumarin, 2-(9-((3-methylbut-2-en-1-yl)oxy)-7-oxo-2,3-dihydro-7H-furo[3,2-g]chromen-2-yl)propan-2-yl acetate with phytotoxic and antifungal activities. In the seedling development bioassay for evaluating phytotoxic activity, this compound was more active against the monocot *Agrostis stolonifera* than the dicot *Lactuca sativa*. TLC bioautography revealed antifungal activity of this compound against *Colletotrichum* species. This novel compound is the major constituent in the ethyl acetate extract of the leaves. Isolation of the active metabolite, elucidation of structure and biological activities, and synthesis of analogs will be discussed.

AGRO 144

Mode of action of spliceostatin C, a potent herbicidal compound from a microbe

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A herbicide with a new mode of actions (MOAs) has not been introduced for about 30 years. During this time, resistance has evolved at a steady pace to the herbicides with the ca. 25 old MOAs, creating a strong need for herbicides with new MOAs. Spliceostatin C, a compound from the soil microbe *Burkholderia rinojensis*, is a very effective herbicide at low doses with several important weed species such as *Amaranthus* spp. A related compound spliceostatin A, also from a *Burkholderia* species acts as a spliceosome inhibitor. Herboxidiene, produced by *Streptomyces chromofuscus*, is a selective herbicide, and recent studies have shown it to also disrupt the mRNA splicing process in animal cells. In this report, Arabidopsis was used to investigate the phytotoxic properties and possible modes of action of spliceostatin C and herboxidiene in plant cells. Both spliceostatin C and herboxidiene caused leaf bleaching and significantly inhibited leaf and root growth. Dose-response assays showed that the half maximal inhibitory concentration (IC_{50}) of spliceostatin C and herboxidiene were 2.2 and 0.37 µM, respectively. At IC_{80}, spliceostatin C (15.2 µM) and herboxidiene (1.72 µM) disrupted gravitropism and inhibited lateral branching in roots. To study the inhibition of spliceosomal catalysis, twenty genes including intronless genes, regulation factors, genes encoding multiple transcripts, and stably expressed genes were selected for gene expression analyses using semi-quantitative reverse transcription-polymerase chain reaction. Seven-day old *Arabidopsis* seedlings were exposed to spliceostatin C or herboxidiene at the IC_{50} and IC_{80} concentrations. Spliceostatin C and herboxidiene generated slightly different gene expression patterns. The expression of most of these genes after exposure to these compounds was significantly changed. However, in several cases, the transcripts affected by both inhibitors, exhibited additional and longer transcripts, either by alternative intron splicing or by alternative 5' or 3' splicing. Although the number of transcripts stayed the same, their level of expression as affected by herboxidiene differed from those affected by spliceostatin C. Further study is needed to determine factors responsible for the differences. The results strongly support the view that spliceostatin C has a new MOA, spliceosome inhibition.

AGRO 145

Mosquitocidal constituents from plant pathogenic fungi

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Phyto-pathogenic fungi produce metabolites that are toxic to host plants. These fungi have also evolved to survive in their ecological niche by producing secondary metabolites to compete with other fungi, plants, and insects. These toxins can have various biological activities such as insecticidal, phytotoxic, and antifungal activities. As part of ongoing research efforts at the USDA with the DoD under the Deployed War Fighter Protection (DWFP) program, we have investigated some plant pathogenic fungi in search of natural products that can be used as mosquitoidal compounds or compounds that can be used as lead compounds in designing such compounds. Ascochyta rabiei was isolated from infected leaves of Ipomea aquatica, also known as water spinach. The PDB culture filtrate of A. rabiei showed adult mosquitocidal activity and larvicide activity. Two closely related solanopyrones were isolated as active metabolites. Phomalactone isolated from Nigrospora spherica, a plant pathogenic fungus isolated from Zinnia elegans and Hydrangea macrophylla, was found to be mosquitocidal. From the culture broth of Diaporthe eres isolated from infected Hedera helix leaves, an isocoumarin was isolated and found to have mosquito larvicide activities. Based on this molecule, several analogs of isocoumarins were synthesized with higher mosquitocidal activity. Isolation of active metabolites and synthesis of analogs will be discussed.

AGRO 146

Discovery and development of phytochemical phytotoxins for weed management

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Billions of tons of agricultural production are lost annually due to weeds. Herbicides are the most important method of weed management, as this technology has been much more effective and economical than previous weed management approaches. After more than 70 years of the dominance of synthetic herbicides for weed control, evolved resistance to herbicides has become a major problem. New herbicide modes of action (MOAs) are badly needed for resistance management, but a MOA has not been introduced for about 30 years. Natural phytotoxins are a source of compounds with new MOAs, which has fueled interest in the discovery of new natural phytotoxins. Plants constitute a source of novel and structurally diverse phytotoxic compounds to be explored in searching for effective and environmentally safe bioherbicides. Our successes in using a bioassay-directed approach towards the discovery of new bioherbicides and new phytotoxic compounds from plants will be highlighted with

recent examples including systematic investigations of *Ammi visnaga* (L.) Lam. and *Eragrostis plana* (Nees).

AGRO 147

Use of acaricides for integrated management of the black-legged tick: Current science and new opportunities

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The black-legged tick, *Ixodes scapularis*, is the vector of the bacterium *Borrelia burgdorferi* that causes Lyme disease (LD). LD is the most important vector-borne disease affecting humans in the United States. Over 30,000 LD cases are reported to the Centers for Disease Control and Prevention (CDC) each year, but a recent CDC study estimated the actual number to be at 300,000 annually. Reducing the risk of tick bite and LD to humans relies on successful control of the blacklegged tick populations in the environment. Due to the tick's complex life cycle and multiple hosts involved, the control of blacklegged tick remains a huge challenge to public health. In addition to spray treatment of tick habitats with synthetic pyrethroid pesticides, host-targeted control techniques, particularly the '4-Poster' deer bait and treatment station and rodent bait box, have been developed in the past decades. In recent years, various biopesticides have been developed and tested against the blacklegged tick and other tick species. Three major integrated tick management (ITM) projects have been initiated in the United States during the past two years to evaluate the synergism of combining two or more tick control measures / pesticides to achieve areawide suppression of tick populations. This presentation summarizes pesticides currently being used for tick control, analyzes problems encountered, and highlights the needs and opportunities for new chemistry.

AGRO 148

Inward Rectifier Potassium (Kir) Channels: An emerging target for the control of tick populations and tick-vectored pathogens

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The salivary gland is critical to the biological success of ticks since it performs key roles in blood feeding and osmoregulation, leading us to speculate that the tick salivary gland represents a target tissue for preventing tick feeding. Since, potassium ion channels are a foundational component of physiological mechanisms, we hypothesized inward rectifier potassium (Kir) channels are critical for tick salivary gland function and that pharmacological modulation of these channels will prevent blood ingestion and induce mortality. To test this hypothesis, we first employed *ex vivo* assays to quantify changes in secretory activity of the isolated tick salivary gland after exposure to structurally diverse modulators of Kir channels. Kir modulators were shown to dramatically reduce the secretory activity of the isolated salivary gland with IC_{50} values in the low- to mid-micromolar range. Next, the elemental composition of secreted saliva was analyzed, and results suggest that Kir modulators increased the concentration of Na^+, K^+, and Cl^- ions in the secreted saliva by 8- to 15-fold when compared to saliva from control treatments, suggesting Kir channels are important for proper ion secretion and osmoregulation. Lastly, *in vivo* feeding assays were performed to test the hypothesis that reduced secretory activity of the salivary gland will reduce blood ingestion and that altered osmoregulation will induce mortality due to an inability to deal with the cation rich mammalian blood. Indeed, exposure to Kir channel modulators reduced the ingested volume by up to 15-fold. Further, the rate of mortality for ticks that fed on blood treated with Kir channel modulators was significantly increased with ET_{50} values of <12 hours compared to 4.75 days for treated and control ticks, respectfully. Our data suggest Kir channels are critical for salivary gland function of *Amblyomma americanum* and are promising target sites for the development of novel acaricides.

AGRO 149

Genomics and reverse vaccinology research for the integrated use of anti-tick vaccines to manage ticks and tick-borne diseases

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Vaccines against ticks represent a tool that can be used with other technologies in integrated tick management strategies to mitigate the burden of tick-borne diseases on human and animal populations. Advances in nucleic acid and protein research enabled the production of recombinant tick molecules that can be formulated with adjuvants to maximize immunoprotection of the host against infestation and the transmission of tick-borne pathogens. Several candidate antigens were identified through our anti-tick vaccine discovery research program by adapting the concept of reverse vaccinology. This anti-tick vaccine research supports efforts by the Cattle Fever Tick Eradication Program (CFTEP) to keep the U.S. free of the cattle fever ticks (CFT) *Rhipicephalus microplus* and *R. annulatus*, which are vectors of the pathogens causing bovine babesiosis and anaplasmosis. Sequencing the genome of *R. microplus* facilitated this process. A successful public-private partnership delivered a safe and efficacious vaccine based on the Bm86 antigen that is being used by the CFTEP to immunize bovines. This Bm86-based vaccine also enhanced the effectiveness of integrated interventions to manage the problem with CFT affecting livestock in Puerto Rico. Because of the host immune response to vaccination, the Bm86 antigen also reduces the risk for bovine babesiosis when the entire cattle herd is immunized. Test results with the aquaporin 1 of *R. microplus* warranted further testing for its ability to protect hosts other than cattle from tick infestation. Preliminary findings with the Rm86TX antigen suggest cross-protection of white-tailed against other disease vectors. Our interdisciplinary research continues to identify an antigen, or antigens to be used in a polyvalent vaccine, that affords higher efficacy against *R. microplus* causing outbreaks in the U.S. Interest in the use of anti-tick vaccines to manage the risk for transmission of zoonotic tick-borne diseases will be discussed.

AGRO 150

Evolution of insecticide resistance is unpredictable: Lessons learned from the *Drosophila* Genetic Reference Panel

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Insecticides are widely used to control agricultural and structural pests, as well as insect vectors of disease. In response to the strong selection pressure exerted by insecticides, resistance has evolved in multiple species against numerous insecticides. This can lead to increased application rates and even control failures; impacting both crop production and control of human (and animal) diseases. Thus, understanding the genetic basis underpinning the evolution of resistance to insecticides is of fundamental

importance. Using first a Genome Wide Association Study (GWAS) and then a nested GWAS approach with the *Drosophila* Genetic Reference Panel (DGRP), we were able to identify and validate a set of genes involved in the resistance to insecticides belonging to widely used classes: organophosphates (OPs, parathion) and pyrethroids (deltamethrin). The major effect genes were found under selection, and we identified traces of soft sweep around their loci. Importantly, the alleles of the major effect genes we identified were not a particularity of the DGRP lines, but they were found in two other wild-caught *D. melanogaster* populations. Our study, therefore, reveals the specific and conserved mechanisms of resistance to various insecticides. Using a "nested GWAS" (using the lines that did not carry the alleles responsible for the major effects) allowed us to identify and validate the lesser contribution of other genes in the genome. Altogether, our data reveal that the widely used OP and pyrethroid insecticides imposed a strong selection on natural insect populations from which we clearly identified the genetic basis of resistance.

AGRO 151

Transcript expression changes of cytochrome P450 and ABC transporters in *Aedes aegypti* due to age, sex, and pyrethroid-resistance status

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Aedes aegypti is a vector of flaviviruses impacting humans. Insecticide resistance makes mosquito control difficult, but understanding resistance mechanisms can help improve management practices. The expression of cytochrome P450 (CYP9J) genes is increased in pyrethroid-resistant *A. aegypti*, potentially inducing fitness costs. We hypothesized that ABC transporters genes were repressed in compensation, as ABC transporters were involved in pyrethroid metabolism. Additionally, the age of mosquitoes used for bioassays varies between 1 and 7 days old, and females are the focus. However, males are also resistant and understanding expression of these genes in both sexes at all ages could be beneficial. The expression of selected CYP9J genes and ABC transporters was assessed for the resistant "Puerto Rico" strain of *A. aegypti* compared to the susceptible "Rockefeller" strain between sexes and age groups by quantitative-PCR, focusing on 7 CYP9Js and 6 ABC transporters. Low variation in ABC transporter expression was detected between strains, but it was influenced by age in both sexes and between sexes. The expression of ABCB4 in males was higher in "Rockefeller" until 5-7 days old, whereas in females, it was higher at 5-7 days old. CYP9J10, CYP9J19 and CYP9J28, were higher in "Puerto Rico" than in "Rockefeller" in females of all ages, while CYP9J24 expression was higher in "Puerto Rico" at 3-5 days old. In males, all the CYP9J genes were overly expressed in "Puerto Rico", regardless of age. The CYP9J28 gene was absent from the "Rockefeller" strain, while highly expressed in "Puerto Rico" males and females of all ages, indicating that this gene may be essential in pyrethroid resistance. The significant effect of age on gene expression shows that inconsistencies in evaluating susceptibility in a strain based on one age group can occur. Information about males is scarce, however, there are intrinsic differences between sexes in gene expression.

AGRO 152

Overcoming insecticide resistance: Inhibiting ABC transporters as a means to increase insecticide efficacy

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Mosquitoes affect millions of people worldwide because of their ability to vector disease. There are two general approaches to reduce the risk of mosquito-borne diseases including personal protective measures and area-wide chemical interventions. The mosquito central nervous system is a proven target site for high efficacy insecticides; however, widespread resistance and inefficient target-site delivery limits the use of these insecticides to reduce the risk of mosquito-vectored diseases. Thus, insecticide resistance and target-site delivery are serious public health challenges that warrant the development of improved chemical interventions for mosquitoes. The ATP-binding cassette (ABC) transporters are integral membrane proteins at the mosquito blood-brain barrier interface that act as molecular "vacuum cleaners" to traffic out insecticides and reduce the intracellular delivery of these chemistries to their intended target sites. The data presented here will provide evidence of a blood-brain barrier obstacle for the intracellular delivery of insecticides to the mosquito central nervous system. A series of chemodulators will be reported to enhance the target-site activity and toxicity of insecticides to susceptible and resistant mosquitoes as will the intracellular target-site delivery of these chemistries via increased ABC transporter-ATPase hydrolysis and gene expression. The sequence-specific gene silencing of ABC transporters, with RNA interference, will show improved efficacy of the insecticides to the mosquitoes. A functional ABC transporter cell line has been developed and will be discussed not only as a tool to identify conventional insecticides as substrates for ABC transporters in mosquitoes, but as an innovative approach to screen drug libraries for ABC transporter inhibitors that can serve as alternative chemical interventions for these vectors of disease.

AGRO 153

Characterizing permethrin and etofenprox resistance in two laboratory strains of *Anopheles gambiae*

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The African malaria mosquito, *Anopheles gambiae*, is the primary vector of malaria in sub-Saharan Africa, and is responsible for the disproportionately high burden of global malaria. Pyrethroid insecticides are commonly used to control mosquito populations, and therefore disrupt the transmission of mosquito-borne diseases. However, the widespread use of chemical insecticides has resulted in resistance. Two *An. gambiae* laboratory strains, G3 (pyrethroid-susceptible) and Akron-kdr (pyrethroid-resistant), are commonly used to evaluate the efficacy of synthetic and natural insecticides. The presented research will focus on investigating the level of resistance to permethrin and etofenprox by comparing resistance ratios (RR) of three life stages (first instar, fourth instar, and adult female mosquitoes) of these two strains. First instar Akron-kdr larvae displayed moderate resistance to permethrin (RR=5) along with etofenprox (RR=3.4). Fourth instar larvae of the Akron-kdr strain displayed stronger resistance to permethrin (RR=108) that was 3-fold higher than resistance observed with Etofenprox (RR=38). Topical application of adult female mosquitoes (Akron-kdr) with permethrin (RR=14.1) was 3-fold more resistant compared to etofenprox (RR=4.3). Pretreatment (4 hr) of adult female Akron-kdr mosquitoes with piperonyl butoxide (PBO; 100 ng) had modest effects on the synergism of permethrin (synergistic ratio (SR)=4.9) and etofenprox (SR=2.7). Pretreatment (4 hr) with 100 ng of diethyl maleate (DEM) synergized permethrin (SR=8.7), but it had no synergistic effect with etofenprox (SR=1.3) in Akron-kdr adult female mosquitos. The results of this study indicate that the *An. gambiae* Akron-kdr strain displays less resistance to etofenprox, in the laboratory, compared to permethrin. Further, our studies indicate that metabolism, in addition to

previously identified target site modification of the voltage-sensitive sodium channel (kdr), results in mosquito resistance to permethrin.

AGRO 154

Joint reviews of new pesticide active ingredients: A historical perspective

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Over the past two decades national pesticide regulatory authorities have been addressing coordination of and cooperation on reviews of pesticide active ingredients. Much work has taken place on standardizing data requirements, dossier and study review formats, coordinating review schedules and discussions on hazard endpoint selection and final registration decisions. The joint review effort has progressed on regional levels and to some degree globally over the last several years. The importance of newer chemistries approved in various countries in the same timeframe with similar uses and regulatory decisions has risen as global trade of agricultural commodities and consumer demand for a safe, affordable food supply has increased. The history of the joint review programs including an overview of the successes, benefits and issues will be presented with a look toward encouraging the program in the future.

AGRO 155

Trends in agrochemical product introduction

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In recent years the relative rate of new agrochemical active ingredient introductions has declined in comparison to previous time periods. The key factors influencing the rate of new product introductions has historically centered on cost; government regulations; weed, pest and disease resistance; and market opportunity. This review will examine these key factors in a more recent context, to identify how they now influence new active ingredient introductions in today's market. While innovative systems utilizing combinatorial chemistry and high-speed biological screening have been developed, the overall costs associated with discovering and developing a new agrochemical active ingredient have steadily increased when viewed in a historical context. A major factor behind this trend has been the increasing number of regulatory tests required by government agencies before the new product is deemed safe and can be commercially launched. New chemistry targeted at key metabolic sites has been increasingly used to address resistance issues in weeds, insects, and disease-causing pathogens. Disease resistance is an important factor in major crops grown in Asia and Europe, while herbicide resistance has become particularly significant in the Americas. The review will also examine market sectors to determine where the impact of new regulations is most likely to be felt, while also looking at the opportunities that could arise in these sectors. Against a background of industry consolidation and potential changes in the regulatory situation, potential future changes in the rate of new product introduction as well as trends in new product chemistry will be outlined.

AGRO 156

Industry 10-year retrospective view of joint reviews (2008-2017) for conventional active ingredients

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In 2016 and on behalf of the Industry Working Group (IWG), a retrospective analysis of Joint Reviews (global or NAFTA) for conventional active ingredients was completed. This analysis included 12 new active ingredients submitted by multiple registrants during the period of 2008-2013 and reviewed by a range of 3-5 regulatory agencies. In this analysis, the US EPA and Canadian PMRA decision timelines were quite similar across the 12 active ingredients, and those active ingredients identified as Reduced Risk had shorter review timelines as compared to non-Reduced Risk. The previous retrospective analysis will be updated to include information related to joint reviews completed through 2017. A summary of the results from the 10-year analysis will be shared including decision timelines, factors potentially impacting timelines, as well as recommendations to improve the process. In addition to this broader industry perspective, further insights specific to Syngenta new active ingredients evaluated as part of a joint review will also be shared.

AGRO 157

Flupyradifurone (SiVanto): A registrant's experience with benefits for MRL harmonization through pesticide global joint review

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Flupyradifurone (SiVanto), an insecticide developed by Bayer Crop Science, was registered by US EPA in January 2015 under the Reduced Risk program. This presentation describes the global joint review process of the chemical, involving 4 countries (Australia, Canada, Mexico and the U.S.) and 2 observers (Brazil and China), highlighting successful strategies, challenges encountered, and suggestions for future endeavors. Throughout its development and registration, Bayer worked in a close partnership with IR-4 and PMC (the US and Canadian specialty crop organizations, respectively) to both support the needs of specialty crop growers and to participate in an IR-4 global residue project in blueberry. Opportunities to proactively accelerate MRL availability and harmonization to enable trade were explored and effectively implemented, including the U.S. import tolerance for coffee from outside the U.S. as well as MRLs in Canada for treated commodities imported from the U.S. This effort also led to a successful establishment of Japanese import MRLs (harmonized with the U.S. tolerances) within 11 months of U.S. approval.

AGRO 158

Global joint reviews: An Isoclast (sulfoxaflor) and Zorvec (oxathiapiprolin) perspective

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Global Joint Reviews were established under the auspices of the OECD to ensure new active ingredients were reviewed using consistent OECD data requirements. The sharing of the data evaluation among the participant agencies was aimed at reducing the overall resources required by each agency, resulting in shorter and more predictable timelines. This paper reviews the global joint reviews for Isoclast™ (sulfoxaflor) and Zorvec™ (oxathiapiprolin) from an industry perspective, highlighting what worked well and what

improvements could be made to the global joint review process. A unique aspect of the Isoclast review program to be considered was the parallel evaluation of a global residue dossier by the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) in support of harmonized recommendations for Codex MRLs.

AGRO 159

Global joint reviews: BASF success stories and key benefits

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Pesticide Registrants and Authorities have utilized the Global Joint Review (GJR) process for more than a decade. With a single, comprehensive registration data package submitted simultaneously to multiple authorities, the GJR process provides the opportunity for 1) significant resource savings by Authorities through shared registration review activities; 2) timely establishment of globally consistent MRLs necessary for import/export of the treated agricultural commodities; 3) predictable and timely registrations through consistent use and assessment of registration data; and 4) open, transparent communication between the Authorities. A successful GJR benefits the Authorities, the Registrant, and most importantly the Grower, who gains timely access to an innovative crop protection product with broad uses for both domestic consumption and export.

BASF Corporation has successfully participated in many GJRs over the past decade. This presentation will review many of the benefits of the GJR process and key issues encountered during BASF's GJR experiences.

AGRO 160

Recent experience of a registrant with joint review of new agrochemicals

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Ishihara Sangyo Kaisha Ltd. (ISK) has been active in the discovery, development, and registration of new agrochemicals for over 60 years. Within the past 3 years, ISK Biosciences has been one of the most prolific registrants of new agrochemicals in North America, having registered two new fungicides and a new herbicide through joint review between US EPA and Canada PMRA. Additionally, a new diamide insecticide, cyclapryn™ was registered through joint review between Canada, Australia, and the USA this past year. In many of these registration actions, Mexico has been an observer country. The registrant experience with joint review with respect to harmonization of endpoints in assessment and MRLs, are presented, as well as examples where differences of opinion between regulators were identified and resolved. The perceived benefit of joint review from a registrant perspective, given the recent experience with these registration actions, is provided as a basis for further discussion.

AGRO 161

Classification and modelling of non-extractable residues (NER) formation from pesticides in soil

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This presentation provides a comprehensive overview about the formation of non-extractable residues (NER) from organic pesticides and contaminants in soil and tries classifying the different types. Anthropogenic organic chemicals are deliberately (*e.g.*, pesticides) or unintentionally (*e.g.*, polyaromatic hydrocarbons [PAH], chlorinated solvents, pharmaceuticals) released in major amounts to nearly all compartments of the environment. Soils and sediments as complex matrices provide a wide variety of binding sites and are the major sinks for these compounds. Many of the xenobiotics entering soil undergo turnover processes and can be volatilised, leached to the groundwater, degraded by microorganisms, or taken up and enriched by living organisms. Xenobiotic NER may be derived from parent compounds and primary metabolites that are sequestered (sorbed or entrapped) within the soil organic matter (type I) or can be covalently bound (type II, Fig. 1). Both types may pose a considerable environmental risk of potential release. However, NER resulting from elevated biodegradation, which means the conversion of carbon (or nitrogen) from the compounds into microbial biomass molecules during microbial degradation (type III, bioNER), do not pose any risk (Fig. 1, 2). Experimental and analytical approaches to clearly distinguish between the types are provided and a model to prospectively estimate their fate in soil is proposed.

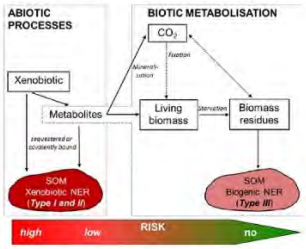

Fig. 1: Different paths of NER formation in soil

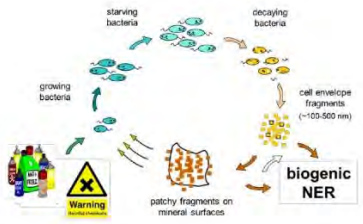

Fig. 2: Incorporation of pollutant-derived C into microbial biomass and soil organic matter

AGRO 162

Formation and stability of non-extractable residues (NER) of phenolic emerging pollutants in soil

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The major fate of most phenolic emerging pollutants, such as nonylphenol, bisphenol S, bisphenol A, and tetrabromobisphenol A in soil is formation of non-extractable residues (NER). We used ^{14}C-tracer to study NER formation dynamics of these pollutants in soil, in the presence and absence of earthworms or plants, the mechanisms for NER formation, and stability of NER in soil. NER formation of most phenolic pollutants was rapid in soil and closely dependent on soil microbial activity. Physical entrapment, which was quantitatively characterized by a silylation procedure, contributed significantly to NER formation. Ester- and ether-linkages contributed to binding of both parent compounds and their metabolites (some of which were not identified in free form in the soil). The presence of plants and earthworms decreased NER formation. NER that were formed under anoxic

conditions were released and became bioavailable when the soil was exposed to air. During incubation in soil, alkaline-releasable NER could be significantly transformed into alkaline-stable part. Mineralization of NER was significant and was not affected by amendment of plant exudates. The results suggested that Type I NER may play crucial roles in NER formation and should be characterized for environmental risk assessment of these phenolic emerging pollutants.

AGRO 163

Correlation between solvent extractability and bioavailability of benzo(a)pyrene in 19 soils measured in juvenile swine

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Oral bioavailability of benzo[a]pyrene (B[a]P) in 19 soils was measured using a juvenile swine model. These soils include 12 surface samples and 7 subsurface samples collected from 12 sites in Australia covering major soil types. The B[a]P-spiked soils (50 mg/kg) were aged for 90 days at 60% water holding capacity. Following ageing, the soils were air-dried, homogenized, and then dosed to juvenile swine at a rate of 1.25 g/kg body weight with food in triplicates. Bioavailability of B[a]P was measured by the area under the plasma concentration of B[a]P curve (AUC) over 24 h after dosing. Freshly spiked silica sand was used as a reference material to calibrate dose-responses and calculate the relative bioavailability (RB). Two solvent extraction methods were used to measure the B[a]P extractability (i) an exhaustive extraction using dichloromethane and acetone (DCM/Ace, v: v = 1:1) facilitated by sonication and (ii) a mild extraction using butanol (BuOH) vortex for 50 seconds. The relative bioavailability of B[a]P in soils were generally below 60% in swine, ranging from 21.1 ± 1.2% to 57.3 ± 8.6%, except for three soils, in which RB was not significantly different, and therefore high, from silica sand RB. The *in vivo* RB of B[a]P in soils showed no clear correlation with any simple soil parameter except for pore diameter (positively correlated, $r = 0.53$, $p < 0.05$) when the three soils with high RB were excluded from regression. An index of carbon fraction considered recalcitrant as protected by fine particles in soil, fine particle associated carbon (FPAC; (Silt + Clay)/TOC), showed a strong negative correlation with RB in most of the surface soils ($R^2 = 0.87$, $p < 0.001$). The pore size fraction < 6 nm also showed a strong negative correlation with RB in most of the surface soils ($R^2 = 0.86$, $p < 0.001$). Most of the outliers were from subsurface soils low in organic carbon demonstrating the influence of FPAC on bioavailability. The deviated actual data is likely related to the surface charges of the soils, where the nature of the soil mineral/carbon, pH and ions are all important. Extractability of B[a]P using DCM/Ace and BuOH ranged from 10.8 ± 0.8% to 75.2 ± 0.1% and 4.2 ± 0.2% to 56.7 ± 1.4% respectively. Despite the variation in RB, there was a strongly significant positive correlation between the DCM/Ace extractability and RB of B[a]P in all soils ($R^2 = 0.54$, $p < 0.001$, $n = 19$), indicating it is a good indicator for the potential risk of human exposure.

AGRO 164

Nature and bioavailability of non-extractable soil residues of the herbicide cloransulam-methyl

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Cloransulam-methyl (CM) is an herbicide which undergoes decreased extraction recovery during soil incubation, with up to 76% of applied material remaining as non-extractable residues (NER) after one year. Herein, experiments were performed to examine the nature and plant toxicity of CM NER, to determine whether CM carbon can be assimilated by soil microorganisms, and whether the compound may be predisposed toward oxidative couple reactions, such as those mediated by peroxidases. Reactions of ^{14}C-cloransulam-methyl with dissolved soil organic carbon in the presence of peroxidase produced radiolabeled high molecular weight residues (> 5 kDa), suggesting oxidative coupling may be possible in soil. Exhaustive extraction of aged residues recovered 7-21% of the applied radiocarbon as metabolites, with recovery being inversely correlated with soil organic carbon content. Accumulation of NER, as well as formation of metabolites diminished in sterile controls. A portion (up to 19%) of the non-extractable soil residue was associated with high molecular weight (>5 kDa) material, and up to 6.5% of the non-extractable residue was in the form of amino acids, with nearly six times as much amino acid carbon recovered from the phenyl versus the pyrimidine ring, the latter exhibiting significantly greater mineralization. Movement of metabolites into biologically inaccessible soil pools (via biological and non-biological mechanisms), may explain limited mineralization of the herbicide, despite a short half-life and susceptibility to microbial transformation. The presence of NER was not associated with plant injury in a corn plant bioassay after extractable CM had been removed. Results suggest that the phenyl ring system may be more prone toward coupling with soil organic matter or incorporation into microbial biomass, whereas the pyrimidine ring may be converted to energy or used as a nitrogen source. Non-extractable residues of CM appeared not to cause plant injury within the context of the experiment.

AGRO 165

Not extractable residues (NER): How extractable are they?

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Non extractable residues (NER), so called "bound residues" of plant protection products, are formed, e.g., in soil as a result of degradation processes. Due to their inherent nature, analysis and further assessments of bound residues are challenging. Therefore, an estimation of the generation and behaviour of NERs is mainly done indirectly. In order to evaluate the microbial impact on the formation of NER in addition to the microbial degradation experiments, sterile control experiments are conducted to proove whether the formation of NERs is only due to adsorption processes, or due to microbial degradation processes. If the formation is due to microbial degradation processes, the nature of the NERs is of course different than the nature of the original parent compound, and the release of NER is very slow in time and strongly correlated to the degradation processes of the matrix (*e.g.,* soil). New investigations in the elucidation of the nature of NER have been published (Possberg et al. 2016, Nowak et. al. 2011) and evolved a new classification approach for NER (Kaestner et al. 2014):
NER type I: Entrapped, strongly adsorbed, no covalent binding
NER type II: Covalently bound not extractable residues
NER type III: Biogenic bound residues

Both evaluations mainly relayed on a digestion step of the soil using 6 N hydrochloric acid for 22h at 110° C following clean up procedures and derivatization for amino acids before analysing with radio thin-layer chromatography, followed by bio-imaging and definitive identification by GC-MS and LC-MS/MS or with isotope ratio determination with GC-MS-MS. We investigated the method of Possberg, et al. As a main result, 16% of the generated NER could be assigned to amino acids and therefore clearly indicate an extensive microbial degradation of bromoxynil before incorporated into biomass of soil as amino acids. Amino acids identified were phenylalanine, valine, leucine and isoleucine, proline, alanine, and glutamine. However, nearly 75% of the soil bound NERs stuck in the soil and could not be made extractable or available even with soil destructing methods like extraction with 6 M HCl at 110° C. This strongly indicates that under natural environmental conditions, even suggesting a real harsh climate change, those bound residues will not be liberated or are extractable from the matrix. Therefore, a future risk of those bound residues could not be foreseen, and therefore NER is regarded as a "safe sink."

AGRO 166

Remobilisation of 'non-extractable' Benzo[a]pyrene residues in contrasting Australian soils

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The environmental and health risks associated with 'non-extractable' residues (NERs) of polycyclic aromatic hydrocarbons (PAHs) in soils and their potential for remobilisation remain largely unexplored. These represent considerable uncertainties constraining risk-based approaches to managing contaminated lands. Using sequential extraction, we investigated the time-dependent remobilisation of benzo[a]pyrene (B[a]P) NERs and associated kinetics after re-equilibration periods of 30 d in four artificially-spiked soils which had been aged for up to 200 days. Benzo[a]pyrene NERs, following exhaustive solvent extraction of soils, were remobilised after re-equilibration based on increase in cumulative B[a]P extractability after re-equilibration, as well as the decrease in the absolute amounts recovered by methanolic saponification of NER. The kinetics of remobilised B[a]P in soils throughout aging followed a first-order decrease, and modelled rates were similar to rates reported of slowly desorbing pools (10^{-3}). Soils with larger clay contents exhibited more delayed NER remobilisation compared to sandier soils. Estimated half-lives of B[a]P in soils may span months or years. Amounts of B[a]P remobilised were significantly influenced ($p < 0.05$) by aging and soil properties. Specifically, butanol-remobilised B[a]P in soils spiked at 10 mg/kg and 50 mg/kg B[a]P ranged from 0.15 to 0.39 mg/kg and 0.67 to 2.30 mg/kg respectively after 200 d of aging. Overall, the remobilised B[a]P concentrations were lower than the health investigation levels (3 mg/kg) for residential land uses in Australia, implying that health risks associated with B[a]P NERs in the aged soils may be minimal and acceptable. To the best of our knowledge, this is the first report of the time-dependent remobilisation of B[a]P NERs in aged soils with associated aging kinetics and may be useful for risk-based contaminated land management.

AGRO 167

Characterization of non-extractable residues in faoxadone degradation via kinetics modelling

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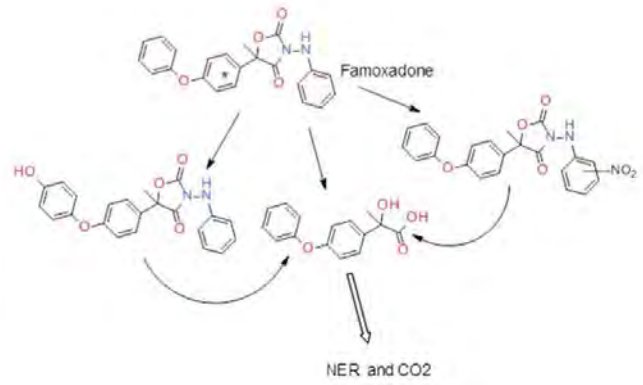
Famoxadone degrades readily in soil and sediment systems. A common characteristic in most systems is the formation of a large amount of $^{14}CO_2$, as well as an abundant amount of non-extractable residue [NER]. Even though several metabolites are also formed, the metabolites tend to be present in small amounts and are often short lived. We have demonstrated that the formation of NER can be explained to be originating from the metabolites and kinetics fitting justifies the formation of NER from metabolites. Utility of such a kinetics modelling approach to shed light on the formation of NER will be discussed.



AGRO 168

Synthetic studies towards complex natural products

Thomas J. Maimone*, maimone@berkeley.edu.* Dept of Chemistry, University of California-Berkeley, Berkeley, California, United States

Concise total syntheses of biologically active natural products will be disclosed and the enabling synthetic tactics and methods discussed. Emphasis will be placed on the total synthesis of complex terpene-derived metabolites. The utilizations of oxidative synthetic processes, as well as new methods for complex polycycle synthesis, will be showcased. Efforts to uncover the biological targets of prepared natural products will also be highlighted.

AGRO 169

Design and explore sulfur containing heterocyclic insecticides

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A new class of sulfur containing heterocyclic compounds has been widely explored as new insecticides in recent years. Oxazosulfyl, the first common name published in Nov. 2017 in this area from Sumitomo, was developed as potential new insecticide for crop protection industry. Here we will report the design and discovery of novel chemo types of sulfur containing heterocycles for crop protection utility as insecticides. The chemical synthesis, biological activity, structure-activity relationships and mode of action studies will be discussed in detail during this presentation.

AGRO 170

Discovery of insecticidal 3-aminopyridyl ureas

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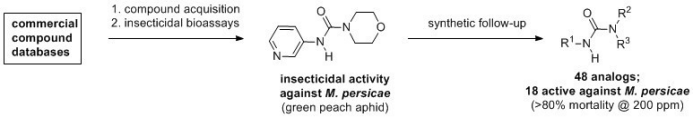
Commercial compound databases represent rich sources of potential starting points for pharmaceutical and agrochemical product development. Routine screening of compounds acquired from these sources led to the identification of a 3-aminopyridyl urea with activity against the sap-feeding insect pest *Myzus persicae* (green peach aphid). A series of ureas based on the structure of this intial hit were synthesized and screened for insecticidal activity. While a number of these compounds also showed activity on *M. persicae*, activity on other important sap-feeding agrochemical pests was lacking. Nevertheless, this work demonstrates the viability of commercial compound acquisition as a strategy for identifying actionable agrochemical hits.

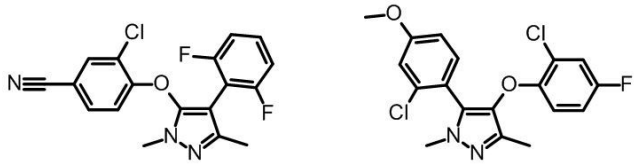


AGRO 171

New 5-phenoxypyrazoles and 4-phenoxypyrazoles as fungicides

Jeffrey K. Long[1]*, Jeffrey.Long@fmc.com, Michael J. Mahaffey*[1]*, Andrew Taggi*[2]*.* (1) Stine Reseach Center, FMC Corporation, Newark, Delaware, United States (2) Discovery Chemistry, DuPont Crop Protection, Newark, Delaware, United States

5-Phenoxypyrazoles have been discovered that modulate the vinblastine-binding site on beta-tubulin in fungi and provide effective control of pathogens of interest for crop protection. Structurally-related 4-phenoxypyrazoles were also prepared and found to be active. The synthesis and biological activity of these compounds will be presented.



AGRO 172

Modular approach to macrocyclic fungicides

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The natural product UK-2A is the precursor of the picolinamide fungicide fenpicoxamid and also the inspiration for the design of macrolides with more desirable molecule attributes. Replacement of one of the macrocyclic esters in UK-2A with an ether linkage revealed a molecule with enhanced whole plant translation of its *in vitro* activity. Further modifications of the macrocyclic ring via systematic assembly of key synthetic inputs resulted in analogs with a broad spectrum of whole plant activity against agriculturally important pathogens. The synthetic assembly of these molecules and their bioefficacy will be the subject of this presentation.

AGRO 173

N-linked azoles as design elements in bioactive molecules

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N-linked azoles have a rich history as isosteric replacements for a variety of aromatics in both crop protection and medicinal chemistry. They can stand in for either meta- or para-substituted benzenes and heterocycles depending upon the situation. When substituted on methylenes, they can be effective replacements for benzylic and phenoxy residues. More recently trifluoromethylazoles have been used as lipophilic electron withdrawing aromatic substituents. Additionally, N-linked azoles possess interesting chemical properties, they can be both leaving groups and activate proximal leaving groups for displacement. Selected examples from the past 30 years of research across the industry will be highlighted.

AGRO 174

Chemical mediators of multitrophic interactions for biorational pest management

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The chemical ecology of insects, plants, nematodes, and pathogens is closely intertwined. Transitions within and between nematode life stages are mediated by chemical communication among nematodes and between nematodes and their environment. This communication can be exploited to enhance infection of insect parasitic (entomopathogenic) nematodes for biological control. Furthermore, plant pathogens modify the physiological status of their host plant. Specifically, plant pathogens can modify volatiles emitted by their hosts qualitatively and quantitatively. In the case of plant pathogens vectored by insects, these odor changes of infected host plants may have consequences on vector behavior. For example, *Candidatus* Liberibacter asiaticus, a fastidious, phloem-limited bacterium responsible for causing huanglongbing disease of citrus, induced release of a specific volatile chemical, methyl salicylate (MeSA), which increased attractiveness of infected plants to its insect vector, *Diaphorina citri*, and caused vectors to initially prefer infected plants. This volatile can be used to manipulate vector behavior in order to reduce host finding. In another example, we investigated the redbay ambrosia beetle, *Xyleborus glabratus*. It is the vector of a symbiotic fungus, *Raffaelea lauricola* that causes laurel wilt, a highly lethal disease to members of the Lauraceae. Infection of swamp bay with the phytopathogenic fungus, *R. lauricola*, induced profound changes in the odors emitted by the swamp bay leaves. Beetle repellence was associated with peaks of MeSA. Identification of a salicylic acid pathway stimulant in Lauraceae to increase the release of MeSA in leaf volatiles could lead to a management oportunity. The concept is to 'prime' trees to become naturally repellent to *X. glabratus* by activating their defenses prior to initial fungal inoculation by *X. glabratus*. Such management methods require further investigation and would consist of applying fundamental knowledge of pathogen-induced changes in plants that affect vector behavior for practical management of lethal phytopathogens.

AGRO 175

Combination of host and fungal volatiles provides improved detection of *Euwallacea* nr. *fornicatus* in Florida

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Invasive ambrosia beetles in the *Euwallacea* near *fornicatus* complex vector a fungal pathogen that causes *Fusarium* dieback disease, a serious threat to avocado (*Persea americana*) and many forest trees in the USA and other countries. These small wood-boring beetles do not feed on wood, but construct galleries in which they cultivate symbiotic fungi, the sole food source for larvae and adults. In susceptible hosts, the fungi destroy functional xylem surrounding the gallery, resulting in branch wilt and dieback, and even tree mortality with heavy infestations. There has been an increase in avocado consumption lately due to its high nutritional value and many health benefits. However, with the threat posed by *Fusarium* dieback, there is an urgent need for effective, environmentally safe tools for control of the vectors. In this study, we quantified the chemical composition and emissions from two lures currently used for *E.* nr. *fornicatus*, and evaluated lure efficacy in Florida avocado groves. Treatments included quercivorol (*p*-menth-2-en-1-ol isomers), an essential oil enriched in α-copaene, a combination of these two lures, and an unbaited control. Lures were analyzed by GC-FID and GC-MS systems, and volatile emissions from lures field-aged for 12 weeks were quantified by headspace collections on Super-Q adsorbent. Twelve sesquiterpenoids were detected from the α-copaene lure and four monoterpenoids were detected from the quercivorol lure. In addition, enantiomeric distribution of α-copaene and *p*-menth-2-en-1-ol isomers were determined, and (-)-α-copaene was found to be the predominant isomer in the α-copaene lure and four diastereomers were determined in order of (1*S*, 4*S*)-, (1*R*, 4*R*)-*cis*- *p*-menth-2-en-1-ol and (1*S*, 4*R*)-, (1*R*, 4*S*)-*trans*- *p*-menth-2-en-1-ol in the quercivorol lure. Results indicated that the combination of α-copaene (a host volatile) and *p*-menth-2-en-1-ol (a fungal volatile) provides an effective lure for sensitive detection of *E.* nr. *fornicatus* in Florida, with field longevity of 3 months.

AGRO 176

Microbiome as novel target for the biocontrol of invasive fruit flies

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Invasive fruit flies are a global threat to the agricultural industry with estimated annual losses exceeding 1.5 billion dollars. Particularly, the drosophilid flies *Drosophila suzukii* (also known as the spotted winged *Drosophila*, SWD) have become established in the U.S. and are major concerns for growers and associated stakeholders. Current controls rely on the use of insecticides. However, because the larvae feed inside fruits, insecticides are only effective against the adults and thus have limited efficacy. Novel methods for control are needed and the microbiome offers some promising opportunities.

Drosophilid flies are frequently associated with symbiotic bacteria and yeast in the gut, some of which can also be found on their host plants (Wong et al., 2013 and unpublished data). We are interested in the extent to which fly-associated microbiome impacts fruit fly nutrition and foraging. Through culturing, we have isolated multiple bacterial and yeast species from SWD, as well as from their target berry crops collected from the field in Florida. By experimentally manipulating the microbiome of SWD, we found that the microbiome is essential for SWD survival and development on fruits. Using food choice trap assays, we also discovered the fruit microbiome promotes attraction to the SWD. By elucidating the precise nutritional functions of the SWD microbiome and the attraction cues from the fruit microbiome, our work is facilitating the development of innovative approaches for SWD control that can be applied broadly to other invasive fruit flies. These include: disrupting symbiotic microbial functions, altering fruit-SWD communications mediated by the microbes, and exploiting microbial-based products to make better insect attractants and traps.

AGRO 177

Associations between *Drosophila suzukii* and fungal microbes

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The invasive pestiferous vinegar fly, *Drosophila suzukii*, interacts with a diverse microbial community in raspberry fruit, including yeasts and fruit rot pathogens. Yeast microbes provide a critical source of dietary protein and impact various aspects of fitness and behavior for many *Drosophila* species. In contrast, relationships between *Drosophila* and plant-pathogenic fungi have been less intensively studied and are generally considered antagonistic. To better understand *D. suzukii*'s interactions with yeast and plant pathogenic microbes, we surveyed larval gut microbes using culture-based methods for larvae collected from raspberry fields. We isolated several yeast fungi (primarily *Hanseniaspora* spp.) as well as *Cladosporium*, *Botrytis,* and other non-yeast fungi from larval frass, suggesting that *D. suzukii* larvae encounter and feed upon these fungi. Subsequent field surveys confirmed that *D. suzukii* larvae occurred in raspberry fruit affected by *Botrytis* and *Cladosporium* fruit rots. Using field-isolated microbes, we conducted laboratory studies to evaluate potential vectoring relationships between *D. suzukii* and *Botrytis* or *Cladosporium.* Both yeast and fruit rot fungi may also impact *D. suzukii*'s fitness and behavior, with specific effects varying between fungal species. Understanding these insect–fungal interactions may have important implications for pest and pathogen management in primocane raspberries.

AGRO 178

The relationship between diapause preparation and diapause length: A possible target for European corn borer management

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The European corn borer (*Ostrinia nubilalis*) invaded the United States in the 1940s and quickly became a primary corn pest capable of decimating crops. Conventional pesticides and biological control efforts have been used in management of this pest to varying effect. If these pests respond as predicted to anthropogenic changes in their environment with increased growth, development, and reproduction rates, new management approaches will be necessary. Resolving the relationship between the diapause strategy adopted by these pests to survive winter and the

environmental resources on which they depend could uncover possible targets for future pest management strategies. We hypothesized that lipid accumulation in preparation for diapause significantly influences diapause duration. To understand this relationship, we characterized lipid content prior to diapause in two strains of European corn borer that differ only in diapause length. European corn borers with a shorter diapause period stored significantly less lipids in preparation for diapause in relation to larvae with longer diapause length. This work is fundamental to understanding the metabolic relationship between nutrients and diapause could lead to innovations in managing this pest.

AGRO 179

Identification, synthesis and field activity of sex pheromone of the Tecia solanivora Polvony (Lepidoctera: Gelechiidae), an invasive pest of potatoes

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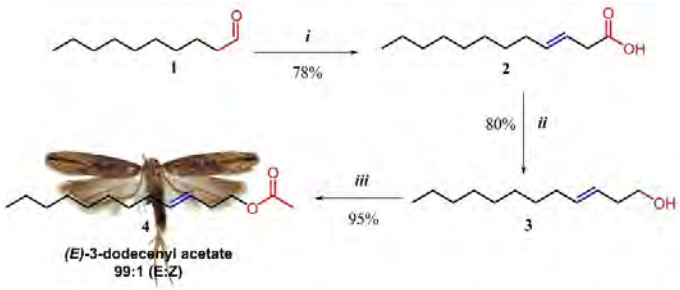
In this work, after identification of the sex pheromone of *Tecia solanivora* (known as Guatemalan potato moth) by GC-FID and GC-MS, an efficient three-step synthesis of *(E)*-3-dodecenyl acetate, major component of the sex pheromone of *T. solanivora*, and its *Z* isomer byproduct was conducted. The synthesis route comprises the stereoselective formation of the *E*3 double bond by a modified Knoevenagel condensation, followed by a reduction of the carboxylic acid and finally the ester formation by acylation of the *(E)*-3-dodecenol intermediate, with an overall yield of 59%. The field activity using three different polymers impregnated with the sex pheromone as diffusers, showed that mostly male moths are attracted by the pheromone components, attraction highly dependent on the type of diffuser polymer. Although the pheromone synthesis has already been reported and there are commercially available diffusers, this work contributes with a complete and unequivocal characterization which has not been reported before. And additionally, the field test compares the diffusers developed in this work against commercial diffusers, showing better results for the novel diffusers of this work.



Synthesis of biologically active natural compounds of *Tecia solanivora* Polvony.

Field trap for *T. solanivora* using poliethylene as material diffuser

AGRO 180

Synthesis of a range of carbohydrate natural based volatile organic compound analogues and the evaluation of their pesticide activity

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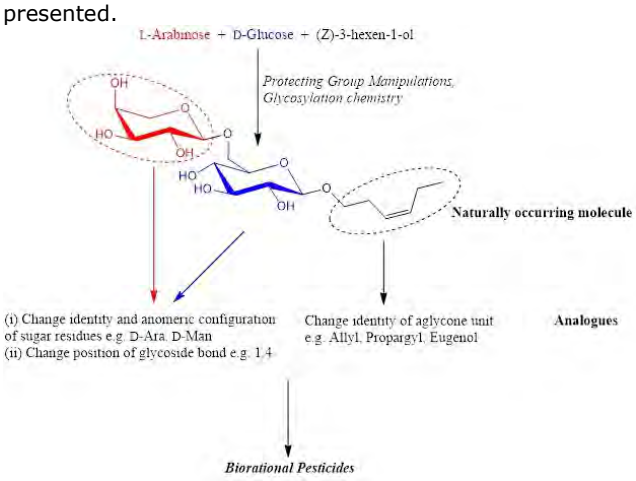
Most synthetic pesticides show varying levels of toxicity and long impacts on humans and the wider environment. The most common classes, organochlorides, organophosphates, and carbamates, exhibit adverse effects on the central nervous system. Despite these unfavourable occurences, synthetic pesticides are seemingly unavoidable in modern agricultural practices. Current focus has been extended on the discovery and use of pesticides that are either naturally occurring or can be easily degraded to non-toxic components in the environment. Volatile organic compounds isolated from two species of plants, *Camellia sinensis* and *Solanum lycopersicum*, have been shown to play a critical role in the plants' defence; deterring insect and herbivore attack in both species. Among the VOCs identified, were disaccharides conjugated to naturally occurring volatiles such as (Z)-3-hexen-1-ol and geraniol. These compounds are particularly attractive since they present as water-soluble, biodegradable, biorational pesticides that can be chemically synthesized in large yields. In this body of work, the native disaccharides were chemically synthesized, along with a range of analogues in which the following were changed: (i) the aglycone, (ii) sugar moiety and (iii) the stereochemistry at the anomeric centre (s). The results of these syntheses as well as the promising results from their pesticide assays will be presented.



AGRO 181

Development of lures for blueberry maggot (*Rhagoletis mendax*)

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We report progress toward the development of postharvest treatments for control of blueberry maggot (*Rhagoletis mendax*) (BBM), a pest that restricts the domestic and global trade of blueberries from the Eastern United States. We detail the challenges associated with a singular reliance on postharvest treatments, while highlighting the contribution that an effective trapping and monitoring program could make towards negotiating quarantine regulations. We hypothesize regarding the commercial development of attractants for BBM, a highly monophagus species, using volatiles released by its host. Such volatile attractants would likely be technically and practically amenable to aqueous formulation, creating a series of challenges for commercialization in trapping systems. Important factors of such lure design, including aqueous equilibria, Henry's Law partitioning, and kinetics of volatile emission will be examined. Results from laboratory, mesocosm, and field trials are briefed, as are elements of the contemporary blueberry pest-control "system." This work adds to our knowledge of natural host-pest signaling pathways and how they must be exploited, particularly in cases such as for BBM, where low pest prevalence could minimize the quarantine requirements of standalone postharvest treatments.

AGRO 182

To bee collect or not to bee collect: Efficiency and efficacy in commodity collections for bee residue studies

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Measurements of residues in pollen and nectar are used to establish exposure levels of bees to pesticides and to refine risk assessments in tier 2 studies. The US EPA *Guidance on Exposure and Effects Testing for Assessing Risks to Bees* provides on outline of factors to consider when designing field residue studies. However, this document does not cover all the variables, and there are a multitude of factors, which influence the outcome in these studies. From the study director's perspective, success in a field residue program can be defined as providing all samples of sufficient size for residue analysis, with GLP generated documentation, from a "worst case" proposed or labelled use scenario of the product, resulting in a coherent dataset, with minimal deviations, none of which impact the integrity of the study. From the selection of the crop variety, its maintenance, and bloom timing, to the application method, bee colony attributes, and sample collection technique, many decisions impact the propensity of a field trial to be successful (i.e., to provide sufficient, representative matrices for analysis). Here we present considerations and recommendations gleaned from nearly 100 bee matrix residue trials conducted in the previous 3 years in the USA. Initial decisions on whether to hand collect or bee collect pollen and nectar, to efficiency in forager bee sample collection, the use of pollen traps and water provisioning, to sample sizes, and replication, we will propose a toolkit that will help standardize the methodology used to collect samples from a variety of crops and improve the quality of residue data used to assess the exposure of test chemicals to bees.

AGRO 183

Measuring multiple matrices to determine wild bee exposure to pesticides in an intensively managed agricultural landscape

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To better understand the exposure of wild bees to pesticides in an agricultural landscape, samples were collected from fields in northern California. Hedgerows are known to provide habitat for wild bees, but these bees may also be exposed to pesticides from nearby agricultural fields. The current study targeted eight hedgerow sites located in an intensively managed agricultural landscape that includes almonds, (wine) grapes, rice, tomatoes, and walnuts. In addition to collecting both wild bees and honeybees, soil, flowers, and silicone passive sampling devices (PSD; staked near the hedgerows to sample the air) were also included. Sampling was conducted from April to June, 2016, to coincide with peak bloom and bee activity for comparison with pesticide use records from the surrounding landscape. Samples were analyzed for >150 pesticides and degradates using similar extraction clean-up steps for the various matrices and analysis with both gas and liquid chromatography-tandem mass spectrometry. Overall, 38 pesticides were detected in all matrices (10 insecticides and degradates, 12 fungicides, 15 herbicides and degradates, and 1 plant growth regulator). The number and type of pesticides detected varied by matrix; 24 compounds were detected in the PSDs, 24 in soil, 15 in flowers, 18 in native bees, and 10 in honeybees. These results can help determine which matrices are best at estimating wild bee pesticide exposure and which pesticides should be targeted for future effects work.

AGRO 184

Understanding the impact of pesticide exposure on honey bee immunity

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The honey bee (*Apis mellifera*) plays an economically vital role in global agriculture as a pollinator of a wide variety of crops, in addition to being valued for the honey and other natural products that it provides. Declines in the numbers of both managed and wild pollinators have increased public awareness of bee health issues and prompted researchers to intensify efforts to understand the forces driving these declines. While there exist a variety of factors that negatively impact the health and survival of both managed and wild bee populations, there is a growing consensus that high levels of parasites and pathogens, especially viruses, are among the most significant threats to pollinator health. Furthermore, research continues to accumulate which describes the complex and largely harmful interactions that exist between pesticide exposure and bee immunity. Unfortunately, both managed and wild bees are subject to routine exposure to agricultural pesticides, while managed colonies face additional exposure to beekeeper-applied apicultural pesticides. Pesticide risk assessment has often focused on acute toxicity without taking into account the more subtle effects resulting from sublethal exposures, which can negatively impact insect immunity, detoxification, and behavior. One of the longstanding challenges associated with bee research is the lack of tools and standardized approaches for the study of host/pathogen interactions in bees, in particular interactions between viruses and the antiviral immune response of bees. This presentation will focus on a recently developed model virus system for screening individual level effects on bee

immunity and some of the findings from ongoing work detailing interaction effects between pesticide exposure and the honey bee antiviral immune response. More importantly, this work will also explore the connection between insect cardiac function and disease tolerance, as well as the impact that pesticides and other stressors can have on this relationship.

AGRO 185

Honey bee toxicity of residues on foliage (RT_{25}) study: Issues and possible improvements

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Residual toxicity data for bees are generated through the Toxicity of Residues on Foliage Test (OCSPP Guideline 850.3030) and are referred to as RT_{25} data. The RT_{25} is intended to be a measure of the time that a pesticide product is expected to cause contact toxicity to bees in the field when sprayed at the maximum application rate. The RT_{25} information has been considered useful to growers and beekeepers as it can help identify the optimal timing to reduce bee exposure from a foliar pesticide application and ensure bee safety. However, inconsistency and variability in RT_{25} data has been observed, raising concerns about the reliability and usefulness of these data. The Pollinator Research Task Force (PRTF), in collaboration with the USEPA, has taken on the task to review the current test design and work with different stakeholders to improve the method, and ensure reliability and predictive nature of RT_{25} data. This is one of several types of research currently being conducted by PRTF to improve pollinator risk assessment. After the initial review, the PRTF believes that the major sources of variability in RT_{25} data are inherent in the test design. The current test guideline does not adequately specify various test parameters (e.g., test cage size, crop and treated foliage weathering conditions), leaving room for interpretation. As a result, different study parameters are used by different laboratories. Therefore, the initial PRTF efforts would focus on "standardization" of the test guideline. Through these efforts, we hope to improve the consistency of the RT_{25} data and address the uncertainty regarding the extent to which test results can be relied upon by the EPA, growers and beekeepers to reduce the frequency and magnitude of pesticide exposure to bees.

AGRO 186

Laboratory challenges associated with small sample size and matrix suppression in nectar and pollen analysis

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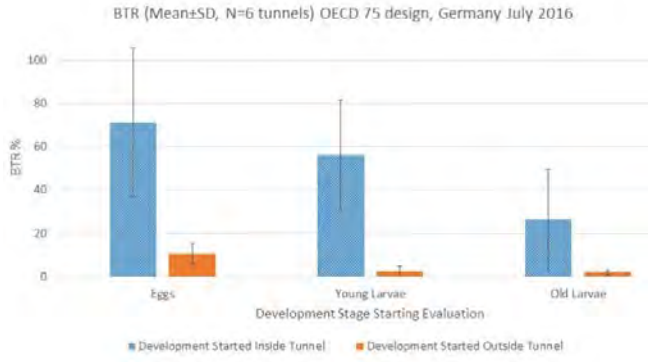
Pollinator health studies involve the collection and analysis of pollen and nectar from target agricultural crops to determine the magnitude of residues. Common to these sample matrices are the extremely small sample sizes. Pollen and nectar samples collected from plants are commonly less than 100 mg in mass, and the entire field sample is typically extracted. Study requirements for limit of quantitation and detection are also very low (<1 ppb). Small sample size and low LOQ/LOD requirements present challenges in the analytical laboratory. However, these matrices lend themselves well to the application of high-throughput sample preparation techniques. This presentation will detail the evolution of the method procedures that were developed to increase the number of samples processed in a day and the modifications to accurately analyze samples as small as 5 mg. Data for a representative neonicotinoid insecticide, thiamethoxam, and its major metabolite clothianidin will be presented. Utilization of these techniques will help laboratories meet the throughput requirements for the ever increasing number of pollinator health studies to be conducted in the near future.

AGRO 187

Monitoring brood development in honeybee colonies: The right, the wrong, and the optimum

Vincent J. Kramer*, VJKramer@mac.com.* Research and Development, Dow AgroSciences, Westfield, Indiana, United States

It is often desirable to those who study honeybees to track the development of the brood, that is, the progress of development from egg to larvae to pupae and finally to adult emergence, as a sign of colony health. Because brood development is dependent upon the many and various functions properly performed by different castes of bees—nectar and pollen foraging, food processing, royal jelly production, feeding, cleaning, capping, and thermal homeostasis—it is thought that brood development is especially sensitive to external stressors and the monitoring thereof can serve as an important indicator of their potential effects on long term colony health. Although the developmental progression of the reproductive castes (queens, drones) is essential to the production of new queens and new colonies, it is the development of the worker caste that is important for colony strength and growth long term. However, monitoring the developmental progression of worker brood in controlled experimental conditions in the field has proven to be extremely challenging, probably because the measurement techniques employed (so far) negatively and unpredictably affect that which is being measured. This presentation reviews untreated control and reference item data on brood development collected in recent studies conducted under the guidance of the Organization for Economic Cooperation and Development Document No. 75 (OECD 75) for consideration in pesticide registration actions. Data on the negative effects of tunnel confinement on brood development are presented, showing that confinement itself adversely affects brood development and release from tunnel confinement quickly returns colonies to normal brood rearing. Importantly, the exposure to toxic reference items in the tunnel confinement period is readily detected in the brood development period following exposure, indicating that the monitoring of brood development during tunnel confinement is unnecessary, and is actually detrimental, to the purpose of the study which is detection of long term effects on colony health.



AGRO 188

How pesticides move through honey bee hives

***Allen Olmstead**, allen.olmstead@bayer.com.* Bayer, Hillsborough, North Carolina, United States

Higher tier honey bee effects studies generally consider the colony as a super-organism with effects tied to either controlled application rates (tunnel studies) or concentrations of added food items (colony feeding studies). The distribution of residues of test material in various hive components is not well characterized or understood either spatially or temporally. Improved understanding of the fate of test material within hives may lead to a better understanding and design of higher tier studies. This talk will present conceptual models of how contaminants move through the various components of the hive, drawing from residue results from previous semi-field regulatory studies and experiments with dyed feeding solutions. Generally, nectar incoming into a hive is mixed relatively rapidly between frames leading to consistent exposure over a long period of time. Pollen, however, does not appear to be mixed leading to a patchy distribution within the hive. Examples of how these models could influence study design and interpretation will be given and expanded to other areas of pollinator risk assessment.

AGRO 189

Determination of cyhalothrin insecticide residues in pollinator matrixes of soybean

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Crop residue analytical methods have been routinely modified over the past several years to determine neonicotinoid insecticide residues at parts-per-billion (ppb) concentrations in pollinator-relevant matrixes such as pollen and nectar. More recently, interest has increased in employing crop residue methods for determination of other compound classes in these matrixes. With the knowledge obtained from analyzing neonicotinoids, techniques have matured for dealing with common problems such as small sample size, lack of extract dilution scale, and observed matrix effects, all at desired limits of quantitation at or near sub-ppb levels. The described analytical procedures were verified in our laboratory for use to determine residues of the pyrethroid insecticide cyhalothrin (CGA337745) in varieties of soybean (*Glycine max*) leaves, flowers, anthers, and nectar. Due to the physiology of soybean plants and low pollen production, anthers of the soybean flowers were sampled and analyzed as a surrogate for pollen. An overview of the crop analytical method and applied modifications, method verification performance results, and descriptions of the challenges encountered in different matrixes will be presented and discussed.

AGRO 190

Australia's experiences in global joint reviews (GJRs) of pesticides

***Jason Lutze**, jason.lutze@apvma.gov.au, Alan Norden.* Australian Pesticides and Veterinary Medicines Authority (APVMA), Kingston, Australian Capital Territory, Australia

Australia has participated in several Global Joint Reviews (GJRs) over the last decade. Australia is a relatively small market for agricultural and veterinary chemicals, and efforts at reducing regulatory burden and providing incentives for bringing new chemistry and uses to the Australian market is a key focus of the Australian government. The recent release of guidance on the use of international data and participation in joint reviews is core to bringing new active constituents and new uses to Australian producers. However, often not all uses that are included in the joint review submission for other markets are bought to Australia, and those in the submission are sometimes proposed for the Australian market with formulations and use patterns that can differ to the other joint review partners. Australian participation in second entry submissions which often follow the original joint review has also not been realised. This creates difficulties in MRL harmonization and is also a missed opportunity for Australian producers who may wish to access the uses that have been considered in the original or second entry submissions. This often results in applications from producers of minor crops for legal off-label approval of these uses soon after registration of the product, and based on uses obtained in other countries, usually those involved in the original joint review. An analysis of a number of these reviews will be discussed, and the additional data, if any, that may have been required by the regulator to register those uses in Australia will be explored. Australia, Canada, and New Zealand have also recently collaborated on a tripartite review of a veterinary medicine. The application involved the addition of a new use in a new food producing species to an already approved product. This was a successful review despite the differences in residues methodologies normally applied by the participant regulators. This assessment will be discussed, and the process used contrasted with that of OECD joint reviews for pesticides

AGRO 191

20 years of North American collaboration – Lessons learned and future directions

***Michael Goodis**[1], goodis.michael@epa.gov, Peter Brander[2].* (1) Office of Pesticide Programs, US Environmental Protection Agency, Washington, District of Columbia, United States (2) Pest Management Regulatory Agency Health Canada, Ottawa, Ontario, Canada

Mr. Peter Brander, Chief Registrar and Director General of the Pest Management Regulatory Agency, Health Canada, and Mr. Michael Goodis, Director of the Registration Division, Office of Pesticide Programs, USEPA, will present an overview of the joint review process for new pesticide active ingredients and will discuss the objectives of joint reviews. They will be providing information on the lessons learned after almost 20 years of international partnership, including highlighting success, discussing challenges, and enumerating benefits gained from participation in the joint review program. Building on knowledge gained, the presentation will also explore future directions for this important program given both countries' continued commitment to collaboration. Mr. Brander and Mr. Goodis will outline a joint review pilot project which is just beginning, which seeks to tailor the joint review process to the specific chemical under review and capitalize on efficiencies gained through collaboration, while streamlining the process to make it more efficient, flexible, and effective.

AGRO 192

Global joint reviews: Considerations and advances for minor uses

***Daniel Kunkel**, Kunkel@aesop.rutgers.edu, Jerry Baron.* IR-4 Program, Rutgers University, Princeton, New Jersey, United States

The IR-4 Project has assisted growers for many years in gaining access to plant protection products. In this endeavor, IR-4 focuses on products that are highly effective in integrated pest management (IPM) systems that also provide a higher level of safety to applicators, consumers, and the environment. IR-4 has had to build many partnerships, reinforced with confidence, in order to have access to the latest plant protection technology that meet the above criteria. These partnerships have led to IR-4's participation in several global joint reviews and well in the structured routine

process for Minor Use Joint reviews in North America. This paper will share some of IR-4s participation in the global joint review process with products such as chlorantraniliprole, flupyradiflurone, oxathiapiprolin, and detail the formalized minor use joint review process in North America. It will also provide a historical perspective where these efforts have contributed to the Crop Protection Industry moving from relatively few crops being submitted in their phase one submissions, to including a much larger number of crops, including many specialty crops. As well, secondary submissions are including larger number of crops. This makes the process efficient while addressing specialty crop grower needs at the same time.

AGRO 193

Europe, Africa, and Asia: Regional policy challenges impacting joint submissions

Jose Joao Carvalho[1]*, jcarvalho@knoell.com, Kerstin Fullner*[2]*, Piyatida Pukclai*[3]*, Rejane de Moraes*[4]*.* (1) Dr. Knoell Consult GmbH, Berlin, Germany (2) knoell UK, Cardiff, United Kingdom (3) knoell Thai, Chiang Mai, Thailand (4) knoell USA, Garnet Valley, Pennsylvania, United States

Global reviews have been in the wish list of the international regulatory community for many years. But have we been considering all the details and looking at the broader implications of having a global review? Did we consider the existing regional and national blockage to such reviews? This talk will explore regional and national differences on critical issues such as hazard identification/quantification, risk identification associated with the use, agricultural practices, and approaches to risk assessment and risk management. Different regions look at the very same hazard, and the probability of that hazard being transformed into a risk, very differently. Why do different regions have such different perception of risk? What is behind these differences? Such differences in perception are at the heart of policy-making and lead to different legislations for the regulation of pesticides/plant protection products (i.e., different guidance, standards for testing, end-points, MRL and other trigger values). Understanding the differences and motivations leading to national policy-making may help set the way forward to more consistent reviews and eventually achieving an agreement on how a global submission should look like. Agreement on a single/similar data package should be the first step toward regulatory rationalization in plant protection, yet a better understanding of national policies needs to be built to achieve this goal. Examples of different policies – and their impact on join submissions – will be presented, covering the EU and some of its member states, briefly on Russia, South East Asia, South Africa and Northern Africa; and finally China where significant developments in policy-making for pesticide registration are happening right now.

AGRO 194

UK experience on joint reviews

Darren Flynn*, darren.flynn@hse.gov.uk, Christine Snaith.* Health and Safety Executive (HSE), York, United Kingdom

UK's Health and Safety Exective (HSE) will present views on what works well and what needs to be improved, in the following areas: 1) EU review programme and new active substances – under Directive 91/414 then the renewal programme under Regulation 1107/2009 – numbers, timelines and co-operation between countries; 2) Novel approaches to meet the needs of industry - phased submissions, the co-rapporteur approach and being flexible from peer review to partial evaluations; and 3) Mutual recognition and related processes for product authorisations – how the problems of process and lack of harmonised approaches are tackled. Based on HSE's experience, recommendations to assist with the GJR process will be discussed.

AGRO 195

Plant protection products: Is Europe really interested in global work sharing?

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Despite the resource issues experienced by plant protection product (PPP) regulatory authorities worldwide, Europe is isolated with its hazard based policies, leaving it difficult to have any meaningful work sharing arrangements. This is further complicated with involvement of the European Food Safety Authority (EFSA) in its role in the peer review of active substance evaluation, whereby there are often divergence of opinions between EFSA and the authority responsible for the active substance review. With the review of current EU pesticide approval processes, will this create a renewed opportunity for global work sharing or will it serve to further differentiate Europe from other OECD partners. What does this mean for pesticide approvals in Europe? What will this mean for crop protection in Europe at farm level? And lastly, are there likely to be any implications on world trade of commodities?

AGRO 196

Post-market re-evaluation of agricultural chemicals: Challenges and opportunities for international worksharing

Richard Aucoin*, richard.aucoin@canada.ca.* Health Canada, Pest Management Regulatory Agency, Ottawa, Ontario, Canada

Canada has benefitted from international joint reviews and worksharing in the evaluation of new agricultural chemicals - in terms of efficiency, alignment of policies and approaches, similar time access to new technologies, international standard setting reducing trade irritants, and contributing to and using the best science available globally. An emerging challenge facing Canada in terms of pesticide regulation is the statutory obligation to reassess older chemicals on a cyclical basis (i.e., every 15 years). Unlike pre-market approval processes which benefit from a high level of globally aligned, prescribed data and information requirements (at least among OECD countries), post market re-evaluations face often unique challenges. These include managing a high volume of often contradictory evidence, the demand from stakeholders for a high level of engagement and risk communications, and the economic implications that are inherent in regulatory decisions involving potentially high value agricultural chemicals. Building on lessons learned from international joint reviews for new agricultural chemicals, there is also an opportunity to explore postmarket opportunities for greater alignment and worksharing.

AGRO 197

Harmonization of maximum residue limits of pesticides among ASEAN countries

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Codex MRL facilitates international trade globally; however, within ASEAN (Association of South East Asia Nations) region, ASEAN MRLs have been established as part of ASEAN Member Countries' initiative to facilitate trades within ASEAN region, in the case where Codex MRLs are not available for certain commodities. The Expert Working Group on Harmonisation of Maximum Residue Limit of Pesticides among ASEAN Countries is the technical working group that evaluates and endorses ASEAN MRLs for further approval at the higher levels of ASEAN meetings. The establishment of ASEAN MRLs is based on ASEAN harmonization principles and consensus of member

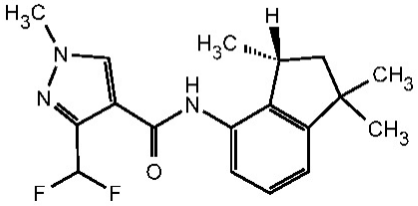
countries. There are currently more than 850 ASEAN MRLs since the first Expert Working Group meeting in the late 1990s. ASEAN MRL complements Codex MRL in trade facilitation within ASEAN region.

AGRO 198

Transformation of chiral fungicide Inpyrfluxam to stereoisomeric metabolites in confined rotational crops

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

Inpyrfluxam, 3-(difluoromethyl)-1-methyl-*N*-[(3*R*)-1,1,3-trimethyl-2,3-dihydro-1*H*-inden-4-yl]-1*H*-pyrazole-4-carboxamide, is a fungicide to control fungal pathogens in various agriculturally important crops. Inpyrfluxam has one asymmetric carbon atom at 3 position in the cyclopentyl ring, which gives rise to 2 isomers, R and S. The 3R-isomer is the active form of the fungicide and represents about 97% of the total Inpyrfluxam in commercial formulations. A confined rotational crops study was conducted with the ^{14}C-Inpyrfluxam, separately labeled in the phenyl and the pyrazolyl rings. The metabolites of ^{14}C-Inpyrfluxam produced in the rotational crops of sorghum, lettuce, and radish included several des-methylation and oxidation products in both free and conjugated forms. These metabolites existed in mixtures of various enantiomeric and diastereomeric forms, which were separated, characterized, and quantitated using various analytical techniques. The qualitative and quantitative results of these isomeric metabolites in rotational crops at various planting intervals will be discussed.



AGRO 199

Application of chromatographic technologies in support of agrochemical research and development

***Paul Rodwell**, paul.rodwell@sial.com.* MilliporeSigma, Bellefonte, Pennsylvania, United States

The development of chiral pesticides requires a detailed analysis of differences in stereoselectivity in their biological effects, toxicity and environmental fate. Analytical support for reaction monitoring of chiral synthetic routes, characterisation of final product, and allied studies can be challenging and may involve the utilisation and combination of several analytical technologies; separation science plays an important role. This presentation will give an overview of the application of separation technologies in support of agrochemical research and development and include method development strategies for general analysis, enantiomer isolation and LC/MS analysis. Applications utilising GC, HPLC and SFC will be shown.

AGRO 200

Enantioselectivity in environmental processing and ecotoxicology of chiral pesticides

***Weiping Liu**, wliu@zju.edu.cn.* College of Environmental and Resource Sciences, Zhejiang University, Hangzhou, Zhejiang, China

The enantioselective environmental processing and toxicity of chiral pollutants are becoming more important. As the industry develops, increasing numbers of chiral chemicals will be introduced into the market, potentially posing toxic effects on non-target organisms. Chiral chemicals, including synthetic pyrethroids, organophosphates, acylanilides, phenoxypropanoic acid, imidazolinones and DDT, HCH, often behave enantioselectively during agricultural use. These compounds also pose unpredictable enantioselective ecological threats to non-target organisms and/or humans, affecting the food chain and entire ecosystems. Thus, it is necessary to explore the enantioselective environmental processing and toxicology of these chiral contaminates during environmental risk assessment. The enantioselective environmental process and toxicology of chiral pollutants, especially the findings obtained from studies conducted in our laboratory during the past 10 years, is introduced.

AGRO 201

Separations of chiral molecules in support of process chemistry and formulations research

***Daniel Knueppel**, danielisaiahknueppel@gmail.com, Jens Richards.* Dow AgroSciences LLC, Indianapolis, Indiana, United States

A surge in the number of chiral crop protection active ingredients under development has resulted in an increase in the amount of chiral intermediates produced during process chemistry research. Once synthesized, chiral active ingredients need to be formulated to produce commercial products. This presentation will highlight the capabilities that were established to address the increasing analytical method development needs for chiral active ingredients. Creative solutions for analytical-scale isolations, as well as critical problem-solving during formulations research, will be discussed.

AGRO 202

Chiral analysis of pesticides using SFC-MS and 2D LC-MS

***Guannan Li**, guannan.li@agilent.com, Lisa Zang, Yanan Yang.* Agilent Technologies, Santa Clara, California, United States

Chiral pesticides may show stereoselective bioactivity, toxicology or degradation behaviors. To effectively conduct the safety evaluation and risk assessment of chiral pesticides, chiral separation is often required. Supercritical fluid chromatography (SFC) can provide fast chiral separations and has been considered as an alternative technique to high-performance liquid chromatography (HPLC). Here, we present chiral separations using SFC for pesticide analysis. As the chiral center number of the compound increases, the chiral separation can get really challenging. Two approaches can be considered to resolve the challenging chiral separations. The first is coupling achiral and chiral columns in series using SFC. 2-dimensional LC coupling reversed phase LC and normal phase LC is the other approach.

AGRO 203

Environmental transformation of the chiral agrochemical Mandestrobin

***Kirk Gohre**[1], kgohr@valent.com, Jeane C. Aston[2], Joel J. Maurer[1], John Whitby[1], Tasha Nguyen[3], Scott H. Jackson[4], Mahbubul A. Jalal[1].* (1) Valent USA Corporation, Dublin, California, United States (2) Valent USA Corporation, Fremont, California, United States (4) Regulatory Science Strategy, Valent USA, Dublin, California, United States

Mandestrobin is a novel strobilurin fungicide registered for the control of a variety of fungal pathogens on canola, grape, strawberry and turf. The registered product is formulated as a 50:50 mixture of *R/S* stereoisomers. The chiral center of mandestrobin helps define its efficacy and the degradation potential in the environment. Environmental fate studies were designed using three different radiolabeled compounds of mandestrobin - [benzyl-^{14}C]S-2167 (mandestrobin *R*-isomer,

RB), [benzyl-^{14}C]S-2354 (mandestrobin *S*-isomer, SB), and [phenoxy-^{14}C]S-2167 (mandestrobin *R*-isomer, RP) to investigate the effect of chirality on degradation in the environment. Chiral HPLC analysis confirmed that specific degradation products were stereospecific (i.e., only a *S*-isomer degradate was formed). This suggests that the reaction mechanism involves a reaction intermediate that cannot rotate (chiral enzymatic reaction). The effect of chirality on rate of degradation and the formation of unique degradates will be discussed. The experimental conduct and results from several environmental fate studies, including aerobic soil, anaerobic soil, aerobic aquatic, and anaerobic aquatic studies will be presented.

AGRO 204

Application of SFC-MS to chiral agricultural active ingredients

***Jens Richards**, jensrichards@live.com, Daniel Knueppel, Jeffrie A. Godbey, Chengli Zu.* Dow AgroSciences LLC, Indianapolis, Indiana, United States

An increase in efficacy of crop protection active ingredients, which has resulted in lower use rates of these molecules, compounded with a higher quantity of chiral molecules under development for agricultural use has increased the complexity of analytical method development. Supercritical fluid chromatography (SFC) has been shown to be an excellent tool for separation of chiral molecules, while the need for higher sensitivity may necessitate the use of mass spectrometry (MS) detection. This presentation will highlight optimization of parameters for separation of chiral agricultural active ingredients by SFC as well as showcase efforts to improve the MS detection using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) to show the benefits and limitations of this hyphenated technique.

AGRO 205

Chiral chromatography of pesticides with SFC and SFC-MS

***J P. Preston**, jpreston@phenomenex.com, Seyed Sadjadi.* Phenomenex, Torrance, California, United States

Enantiomers are mirror image isomers of the same compound. It is widely understood that life science chemical interactions can have a significant molecular chirality dependence. Compounds with this special category of isomers have many identical physical and chemical properties. Separating and quantifying enantiomers is one of the most challenging analytical tasks. The active ingredients in many modern pesticides have chiral structures. Often there are dramatic differences between these enantiomers with respect to efficacy and toxicity. From a manufacturing perspective, there are many compelling reasons to know the chiral composition of a pesticide. This type of analysis can be accomplished with typical analytical techniques since there is a significant amount of sample available. However, many chiral compounds can convert from one form to the other by chemical means. If this conversion occurs in the environment, trace analysis will be necessary to monitor these materials. Very sensitive detection techniques such as triple quad mass spectrometry will be required. Supercritical fluid chromatography (SFC) is a powerful technique for the chromatographic separation of chiral compounds and it can be coupled to a mass spectrometer for detection. The work presented will use a variety of modern chiral pesticides as examples to describe a general approach for chiral SFC method development. Topics will include the selection of eluent components, screening of chiral chromatography columns, and detector selection. Examples will be given that utilize UV detection, single quad MS detection and triple quad MS-MS detection.

AGRO 206

Physicochemical property guidelines for modern agrochemicals

***Yu Zhang**, yzhang9@dow.com, Beth A. Lorsbach, Castetter Scott.* Discovery Chemistry, Dow AgroSciences, Indianapolis, Indiana, United States

The relentless need for the discovery and development of new agrochemicals continues due to driving forces such as loss of existing products through the development of resistance, the necessity for products with more favorable environmental and toxicological profiles, shifting pest spectra, and the changing agricultural needs and practices of the farming community. These new challenges underscore the demand for novel, high quality starting points to accelerate the discovery of new agrochemicals that address market challenges. This talk will discuss the efforts to identify the optimum ranges of physicochemical properties of agrochemicals through analysis of modern commercial products. Specifically, we will review literature studies examining physicochemical property effects and analyze the properties typical of successful fungicides, herbicides, and insecticides (chewing and sap-feeding pests). This analysis delivers a new set of physicochemical property guidelines for each discipline, as well as a building block classes. These new guidelines should aid in the discovery of next generation agrochemicals.

AGRO 207

Synthesis and biological activity of 1,2,4-Triazoles as broad spectrum herbicides

***Paula L. Sharpe**[1], Paula.Sharpe@fmc.com, Thomas M. Stevenson[1], Matthew J. Campbell[1], Teodorica Cenizal[1], Cheryl Liberato[2], Earl Reed[1].* (1) FMC Agricultural Solutions, Newark, Delaware, United States (2) Discovery Chemistry, FMC, Newark, Delaware, United States

Over the past 20 years a variety of benzyl and phenoxy substituted heterocyclic phytoene desaturase inhibitors have been found to exhibit high levels of herbicidal activity. We have recently found that two different isomers of benzylic substituted 1,2,4-triazoles show excellent levels of herbicidal activity. In this talk we will describe the syntheses, herbicidal activity, and structure-activity relationships of this new sub-class of phytoene desaturase inhibitors.

AGRO 208

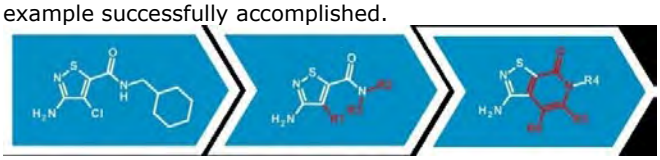
Chemistry behind the aminoisothiazoles: A new class of herbicides

***Stefan Lehr**[1], stefan.lehr@bayer.com, David Bernier[1,2], Thomas Droege[1,3], Marc Mosrin[1,3], Jullien Rey[1], Joerg Tiebes[1].* (1) Bayer AG - Crop Science Division, Frankfurt, Germany (2) Bayer SAS, Lyon, France (3) Bayer AG - Crop Science Division, Monheim, Germany

The economic relevance of resistance building among agronomically important weeds against multiple herbicide classes has become widely accepted during the last five to ten years. In consideration of this fact, it is generally understood that new chemistries addressing unprecedented modes of herbicide action are urgently needed for the development of future weed control solutions. As part of our early phase program, the aminoisothiazoles, a new class of herbicides displaying a new mode of action, were discovered and provided interesting biological efficacy.

Several new chemistries were developed in order to fully explore this class[1),2),3),4)]. For example, despite being unprecedently reported in the literature, the C-4 functionalization of 3-aminoisothiazole derivatives was done using halogen-magnesium exchange and quenching with various electrophiles. Several non-commercial amines were also prepared to broaden the scope of the amide part at the

C-5 position of the isothiazole. To access such amines, selective chlorination or decarboxylative fluorination were for example successfully accomplished.



AGRO 209

Post-emergence dicot weed control using a novel chemical cluster with a new mode of action

***Danny Geerdink**, danny.geerdink@basf.com.* BASF SE, Ludwigshafen, Germany

With expanding resistance of weeds against herbicides from various HRAC classes, the development of active ingredients having a new mode of action is highly desirable. To fill the current void, a new compound class with a previously unknown mode of action was identified. Synthetic access to a broad variety of these isoxazolopyridines is possible using efficient, classical chemistry, leading to a very good understanding of the SAR. Several compounds show excellent cross-spectrum post-emergence broadleaf weed control and selectivity toward monocot crops. The origin of selectivity toward monocots was investigated and can be explained by differential ADM effects between mono- and dicots. In dicots, an excellent phloem mobility was observed, whereas the active ingredients showed only limited translocation in grasses. Extensive mode of action studies revealed that the isoxazolopyridines target a carbohydrate metabolism pathway, a previously unknown herbicidal target. Combined with the high in planta metabolic stability of the xenobiotics, this compound class offers great potential to manage resistance.

AGRO 210

Rational design of agrochemicals: Extending the toolset beyond crystal structures

***Daniel Kloer**, Daniel.kloer@syngenta.com.* Syngenta, Bracknell, United Kingdom

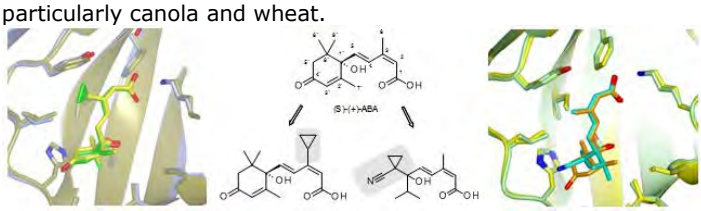
HPPD is the enzyme targeted by a range of commercial herbicides, including Mesotrione, Isoxaflutole, Tembotrione, and Bicyclopyrone. This talk will describe how understanding of the target protein structure has led to the discovery of a new class of highly potent HPPD herbicides by rational design. Looking beyond classical structure-based approaches, we will also discuss computational methods for predicting protein-ligand binding affinity, and how these can be applied in today's crop protection research.

AGRO 211

Quantum of solace for plants: Exploring unprecedented variations of plant hormone Abscisic Acid to identify new lead structures against drought stress in crops

***Hendrik Helmke**[1], hendrik.Helmke@bayer.com, Jens Frackenpohl[2], Jana Franke[1], Joerg Freigang[1], Gudrun Lange[3].* (1) Bayer AG - Crop Science Division, Frankfurt am Main, Germany (2) Weed Control Chemistry, Bayer AG - Crop Science Division, Frankfurt, Germany (3) Crop Science Division, Bayer AG, Frankfurt, Germany

Abiotic stress adversely affects crop production in various parts of the world, decreasing average yields for most of the crops significantly. Among various abiotic stresses affecting agricultural production, drought stress is considered to be the main source of crop losses. Several strategies have been investigated for reducing the impact of drought on crop yield, such as exploiting beneficial effects of crop protection agents, developing drought tolerant crops through transgenic approaches or breeding, but also exploring novel chemical entities inspired by naturally occurring plant hormones. Analogues of plant hormone abscisic acid (ABA) bearing yet unexplored head group and side chain motifs have been prepared within this study based on a combination of structure-based design, *in vivo* hits and agrochemical experience. It could thus be explored how modifying key parts of ABA influenced receptor affinity and *in vivo* efficacy against drought stress. In line with X-ray crystallography studies and molecular modeling novel ABA-derivatives with small alkyl, cycloalkyl or haloalkyl substituents in its terpenoid side chain showed strong effects *in vitro* and *in vivo* against drought stress in selected crops. Furthermore, cyano-cycloalkyl groups proved to be suitable re-placements of the cyclohexanone moiety leading to ABA analogues with strong activity *in vivo*. The versatile synthesis of these novel ABA analogues proceeded *via* Stille or Sonogashira couplings as the key steps enabling us to carry out in-depth SAR studies. In summary, combining novel cyano-cycloalkyl headgroups with novel substituents in the terpenoid side chain afforded the most promising effects against drought stress in crops, particularly canola and wheat.

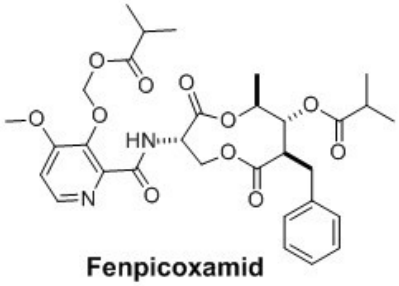


AGRO 212

Preparation of fenpicoxamid standards to support registration studies

***Pete Johnson**[1], pljohnson@dow.com, Lawrence Creemer[2], Kevin G. Meyer[2], Ron Ross[2].* (1) Process Chemistry, Dow AgroSciences, Indianapolis, Indiana, United States (2) Dow AgroSciences, Indianapolis, Indiana, United States

Inatreq™ active is an innovative new fungicide under development by Dow AgroSciences for the control of key diseases in cereals. The new molecule, ISO common name Fenpicoxamid, is of natural origin, derived from the microbial fermentation product, UK-2A. Inatreq is being evaluated globally in all major cereal fungicide markets and will provide farmers a powerful new fungicide with a favorable environmental profile. To support analytical method development, metabolism, and environmental fate studies for registration of Fenpicoxamid, isotopically labeled and unlabeled standards were prepared. The synthesis of these standards will be discussed.



AGRO 213

Development of host marking pheromones for the control of fruit flies in Africa: The *icipe* experience

Baldwyn Torto[3]*, btorto@icipe.org, Xavier Cheseto*[3]*, Donald Kachigamba*[4]*, Sunday Ekesi*[3]*, Mary Ndung'u*[3]*, Peter E. Teal*[1]*, John J. Beck*[2]*.* (1) USDA ARS, Gainesville, Florida, United States (2) Chemistry Research Unit, USDA-ARS, CMAVE, Gainesville, Florida, United States (3) International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya (4) Department of Agricultural Research Services, Bvumbwe Research Station, Limbe, Malawi

Fruit flies (Diptera: Tephritidae) are notorious pests of horticultural crops, causing significant economic losses, especially in the tropics. Controlling fruit flies relies on an integrated approach through application of a variety of techniques including fruit bagging, field sanitation, use of host plant resistant varieties, and soft insecticides. Additional eco-friendly options involve the use of traps baited with protein and semiochemical lures; however, this approach targets mainly males. To target females requires exploitation of their host marking behavior. Ovipositing females of certain fruit fly species use to mark their oviposition sites with a host marking pheromone (HMP), to deter other females from overexploiting these sites. This suggests that HMPs if identified can be exploited for the control of females. Previous work has identified HMPs for ovipositing females of the cherry fruit fly, *Rhagoletis cerasi* and the Mexican fruit fly *Anastraphe ludens*. However, few are known for African indigenous fruit flies. This presentation will highlight the identification of the ubiquitous antioxidant tripeptide glutathione and amino acid glutamic acid as HMPs for the economically important African indigenous fruit fly species, *Ceratitis coysra*, and *C. rosa* and *C. fasciventris*, respectively and their implications for use in their management.

AGRO 214

Agricultural ecology: Systems to solutions

John J. Beck*, john.beck@ars.usda.gov, Caitlin C. Rering.* Chemistry Research Unit, USDA-ARS, CMAVE, Gainesville, Florida, United States

Since the end of the millennium there has been a sustained increase in the use of natural products to delineate important plant-insect, plant-microbe, and plant-plant interactions. While each of these binary systems involves complex chemical and biochemical processes between the two organisms, the progression of increasing complexity toward a ternary system (i.e., plant-insect-microbe) presents higher-order complexity for potential ecological questions about these systems. Accordingly, it will be important that we take into consideration the utilization of new experimental designs, readiness to observe unanticipated results, and the ability to interpret intricate resultant data. This challenge of planning, performing, and interpreting a plant's defensive response to multiple biotic stressors will be even greater if abiotic stressors (i.e., temperature or water) are factored into the system. We will discuss some important interaction considerations, and how we as interdisciplinary scientists can contribute our expertise toward the extraction and interpretation of chemical information exchanged between agricultural commodities and their associated pests. A holistic and thoughtful approach to plant-insect-microbe interactions can lead to a better understanding of agricultural ecology, in turn leading to practical and viable solutions to agricultural problems.

AGRO 215

Attract and kill bait for controlling the small hive beetle, *Aethina tumida* (Coleoptera: Nitidulidae)

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

This research investigates an Attract and Kill bait for in-hive control of the small hive beetle, *Aethina tumida*. The control method employs attracting the beetles to an in-hive trap with a feeding attractant/stimulant where a toxicant is delivered by consuming an edible bait. This research was designed to determine if the diet would be consumed by the beetle, what is the lethal dose with the lowest amount of toxicant and the effects the bait would have on the honey bee. The formulation of bait is highly attractive, readily consumed, and extremely successful in producing high mortality in adult beetles. In laboratory trials the trap containing the Attract and Kill system reduced the beetle population to zero within a few days. The development of a small hive beetle trapping system is essential for the control of this devastating pest. This technology will give our beekeepers control of this pest species that is affecting honey bee survival throughout the world.

AGRO 216

Solventless sampling and GC/MS analyses: A comparative study of three volatile collection techniques

Hans T. Alborn*, hans.alborn@ars.usda.gov, Robert Bruton, Nausheena Baig, John J. Beck.* Chemistry Research Unit, USDA-ARS, CMAVE, Gainesville, Florida, United States

Many interactions in natural systems are governed by odors. In our studies of semiochemical-based plant, insect, and microorganism communications we have routinely utilized dynamic collections on adsorption polymers (i.e., Super Q) followed by solvent extraction, or static collection on solid phase microextraction fibers (SPME). However, we have had a continual need for new or improved methods to collect and identify semiochemicals that, in addition to having high sensitivity, are simple and reliable. Ideally, these methods should be solvent free, allowing for shorter sampling times and free of solvent peaks that might exclude detection of potentially important highly volatile compounds. A crucial step toward these goals was the development of a simple thermal desorption system as an alternative to Super Q collection, and more recently, the addition of a new needle trap technique that combines dynamic sampling with the ease of a SPME-like injection technique. Although these solventless techniques all are powerful, it is not always clear under what situation which method would be preferred over another, and in what way their inherent strengths and weaknesses might affect the analysis results. Discussed will be a comparison of Tenax/thermal desorption, SPME, and needle trap collections where all techniques were used to collect a volatile blend of known composition and concentration. We investigated the quantitative and qualitative effect of factors like equilibrium and sampling time as well as how the results were affected by factors such as sorbent capacity and sampling breakthrough. Furthermore, we explored how the results might also be affected by the desorption technique including the use of pulse desorption and/or cold trap focusing. The presented results will be used as a guide for technique selection for our group, as well as our chemical ecology colleagues.

AGRO 217

Competition between nectar specialist and generalist microorganisms: Effects on metabolite emission and pollinator acceptance

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Visitor-mediated pollination is necessary or beneficial for the majority of the world's food crops, and nectar-inhabiting microorganisms, commonly reaching high cell densities (>10^6 cells μL^{-1}), but low species richness (i.e. 1-2 strains per flower), contribute to floral scent and influence acceptance of flowers. Competition between nectar microbes has been evaluated to some extent, but volatile emission of microbial consortia and subsequent impacts on pollinator acceptance of nectars is not known. A frequently observed and broadly distributed nectar-specialist yeast, *Metschnikowia reukaufii*, has been found to attract or not repel pollinators, unlike many nectar microorganisms. The volatile blend produced by *M. reukaufii*, which includes a rich mix of fermentation volatiles (e.g., ethanol, 3-methyl-1-butanol) and notably, honey bee pheromones (e.g., isoamyl acetate, 1-hexanol) and floral volatiles (e.g., 2-phenylethanol, ethyl acetate), is thought to play an important role in mediating this response. To examine whether *M. reukaufii* produces its signature volatile blend under competitive pressure, the yeast was simultaneously introduced to synthetic nectar with a single generalist bacteria competitor (either *Asaia astilbes* or *Neokomagataea*) at identical cell densities. Metabolites (volatile and non-volatile) were screened over a 48 h duration. Microbe survival, growth and biofilm formation was evaluated. Honey bee acceptance of dual and solo-inoculated synthetic nectars was also compared among strains. Not unexpectedly, all microorganisms coexisted and grew during the short incubation period. Volatile production in nectar differed significantly when *M. reukaufii* was grown in the presence of competitors. Results will be described.

AGRO 218

The impact of flooding on the chemical defenses of maize against the insect pest fall armyworm

Anna Block*, anna.block@ars.usda.gov, Shawn A. Christensen, Charles Hunter.* Chemistry Research Unit, CMAVE-ARS-USDA, Gainesville, Florida, United States

Maize (*Zea mays*) is an important agricultural crop plant in many parts of the world and its successful cultivation requires the ability to overcome both biotic and abiotic stresses including insect pests and diseases as well as drought, flooding, and extremes of temperature. Often these stresses don't occur in isolation, and the plant is required to sense, adapt, and prioritize its responses to deal with combined problems. In this study we assess the effect of the abiotic stress of flooding on the ability of maize to respond to attack by fall armyworm. We show that flooding impacts both the stress signaling responses of maize and its ability to produce endogenous chemical defenses. These changes lead to altered growth of fall armyworm on maize plants subjected to flooding stress and indicate probable changes are required to pest management strategies during periods of flooding.

AGRO 219

Diverse environmental stimuli result in differential regulation of plant-produced natural product defenses in maize

Shawn A. Christensen[1]*, shawn.christensen@ars.usda.gov, Eric Schmelz*[2]*, James Sims*[3]*, Alisa Huffaker*[2]*, Denis Willett*[1]*, Anna Block*[1]*, Charles Hunter*[1]*, Hans T. Alborn*[1]*.* (1) Chemistry Research Unit, CMAVE-ARS-USDA, Gainesville, Florida, United States (2) Section of Cell and Developmental Biology, UC-San Deigo, La Jolla, California, United States (3) Department of Environmental Systems Science, ETH Zurich, Zurich, Switzerland

Maize (*Zea mays*) is a key agricultural commodity throughout the world. While advances in maize breeding and biotechnology have resulted in unprecedented yields, diverse forms of biotic and abiotic stress continue to result in billion-dollar losses in maize production annually. With the objective to elucidate maize defense mechanisms and enhance resistance to biotic/abiotic stress, we investigated the protective roles of inducible phytohormone and terpenoid defenses in response to pathogen attack then examined the impact of environmental stress on these elicited responses. Evaluation of diterpenoids (kauralexins), sesquiterpenoids (zealexins), and other defense metabolites in commercial hybrids revealed distinct defense accumulation patterns in pathogen-infected tissues and led to the identification of a novel plant-produced natural product sesquiterpene keto acid, termed zealexin A4 (ZA4). ZA4 and kauralexin diacids demonstrated potent antimicrobial action against fungal pathogens, and an overall negative correlation was observed between total analyzed terpenoids and fungal growth. Statistical analyses highlighted kauralexins as the most potent defense antimicrobials, and genetic analysis of the *ent*-copalyl diphosphate synthase Anther ear 2 (*An2*) lacking kauralexin biosynthetic capacity displayed increased susceptibility to pathogen attack. Examining the effects of different abiotic stressors on fungal-elicited responses demonstrated diverse impacts on plant defense. Both elevated CO_2 and heat stress caused significantly reduced levels of inducible defenses to maize pathogens. However, drought stress prior to fungal inoculation elicited heightened defenses to fungal attack, suggesting that resistance mechanisms are potentiated in plants exposed to drought conditions prior to infection. Collectively, these results describe the complex nature of maize-microbe interactions under diverse forms of abiotic stress and highlight the challenges of improving agro-economics for crops under changing environmental conditions.

AGRO 220

Method development for complex agricultural formulations containing multiple active ingredients

Mary D. Evenson*, mdevenson@dow.com.* Analytical R&D, Dow AgroSciences, Zionsville, Indiana, United States

Agricultural formulations containing multiple actives at various concentrations present unique challenges for analytical method development. With the increase in efficacy of new active ingredients, the loading of actives in formulations can be very low with the concentrations of inert ingredients and surfactants high, which further adds to the complexity of the requisite method. This presentation will highlight the use of software to facilitate method development using HPLC/UV and HPLC/MS for screening column chemistry, temperature, and solvent gradients. Specific application to a complex formulation will be showcased to outline the amount of problem-solving that is required for developing successful analytical methods for agricultural formulations.

AGRO 221

Method development for relevant impurities in agricultural formulated products

***Todd Kajdan**, twkajdan@dow.com.* Dow AgroSciences, Indianapolis, Indiana, United States

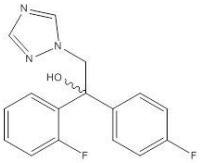
The manufacturing impurity profiles of crop protection active ingredients are specified as part of the registration process of the active substance. Some active ingredients contain relevant impurities that are of toxicological or ecotoxicological / environmental concern. European Union regulations require that if a relevant impurity is observed and declared on the specification, then a method must be available to analyze for this impurity within commercial formulated products relative to its concentration in the active substance. With potential low concentrations (ppb range) of these relevant impurities in formulated products that have complex matrices, it can create significant challenges for analytical method development. The development of various relevant impurity methods and the challenges encountered will be discussed.

AGRO 222

Identification of closely related structural and stereoisomeric trace impurity species, via the isolation and purification of these impurities using chiral preparative SFC, allowing for 2D NMR structural studies

***John P. McCauley**, john.mccauley9@verizon.net, Marian Twohig.* Applications, Waters, Folcroft, Pennsylvania, United States

Examples using the fungicides Flutriafol and Propiconazole, compound preparations having unknown trace impurities, including closely related synthetic impurities as well as structural, regional, and also stereoisomers were studied using chiral preparative SFC. LC/MS tools alone have great power in assigning formula and structure, but such information is often insufficient in the case of closely related species and isomers as the formulae may be isobaric, and the MS fragmentations are also often quite similar. In the case of truly new unknown compounds, the answers will also not be found in MS libraries. In these cases, a full structural assignment needs to be carried out on isolated materials, which can be obtained using preparative chiral SFC techniques. The pure materials garnered in this manner are appropriate for the 1D and 2D NMR studies that can unambiguously give full structures for each of the isomers. Various 2D NMR techniques, which are best applied to isolated milligram amounts of the purified unknown impurities, are capable of giving suitable structural assignments. With the correct structure in hand, larger amounts can be obtained through synthesis. Absolute configurations can also be assigned using other techniques such as VCD and X-Ray. Trace impurities in synthetic products that interact with human end users or may have an undesirable environmental fate are regulated by various government agencies such as the FDA, the USDA, and the EPA. As a result, impurity separations and their structural identification is an important research area in many different industries, including pharmaceuticals, agrochemicals, foods, cosmetics, and consumer products. Unknown impurities present in amounts greater than the regulatory limits may be required to undergo additional toxicology studies. For this reason, there is great motivation to identify all of the trace impurities, which are then able to be categorized as known impurities. Known impurities have higher limit thresholds for triggering required further studies.



AGRO 223

Quantitation of a minor impurity in Inatreq™ active (Fenpicoxamid) using two-dimensional liquid chromatography

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Inatreq™ active, a novel fungicide produced in one synthetic step from the natural product UK-2A, is under development by Dow AgroSciences. Detailed LC-MS-based examination of one batch of UK-2A revealed the presence of a structural analog at a concentration of approximately 0.1 weight percent. An analytical method was needed to estimate the concentration of the impurity in process solutions and in the final active, fenpicoxamid, so that a specification could be set on the permissible concentration of the impurity in UK-2A. The impurity in fenpicoxamid co-eluted with two other impurities of similar structure in the complex mixture of more than twenty structurally related minor compounds when analyzed using the validated one-dimensional LC method. Therefore, two dimensional liquid chromatography with UV detection was used to measure the new impurity where the validated conditions were used as the first dimension and a second reversed phase separation was used to resolve the impurity of interest.

AGRO 224

Unifying, informatics-based approach to life cycle management of impurity data

***Joseph DiMartino**, joe.dimartino@acdlabs.com, Andrew Anderson, Sanjivanit K. Bhal, Graham McGibbon.* Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, Ontario, Canada

The historic overlap between pharmaceutical and agrochemical sectors has led to the adoption of technologies, initially designed for pharma/biotech, in agrochemical R&D. Impurity analysis is an example of scientific endeavor that is common to both industries. While heavily regulated in pharmaceutical R&D for patient safety, the potential effects of impurities in agrochemicals—environmental pollution and health effects in animals and humans—make it a key step in the registration of new chemical entities. Global regulatory authorities continue to push *Quality-by-Design* (QbD) in pharmaceutical development to support risk management. While it affords many important long-term benefits, these expectations are having a dramatic impact on product development groups and their supporting corporate informatics infrastructure. Informatics platform innovation—particularly to support reduction of data abstraction, data assembly, and human data preparation—is necessary to mitigate risk and facilitate regulatory adherence. Informatics software for impurity/contaminant control should optimally provide users with the ability to construct 'process maps' which allow for visual comparison of molecular composition across unit operations. The platform should also allow the user to visualize the wide variety of related spectroscopic and chromatographic data in a single environment for each stage and substance for efficient and informed decision-making. This talk will provide an overview of a new software application (Luminata™) developed specifically to address these platform innovation needs.

AGRO 225

Mass spectrometry based structure elucidation of impurities in synthetic agrochemicals using modern instrumentation and software tools

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Identification of impurities in synthetic active ingredients is critical in two aspects: 1) registration of products with regulatory agencies; and 2) to understand mechanisms of side reactions in order to eliminate or reduce the presence of impurities in the final product. High resolution mass spectrometry (HRMS) coupled with either liquid chromatography (LC) or gas chromatography (GC) is an excellent tool for structure elucidation of impurities due to its combination of speed, sensitivity, and mass accuracy. The selection and application of appropriate software tools also become indispensable. Efforts to improve the confidence and throughput using commercially available software and hardware tools will be discussed.

AGRO 226

On-line measurements for process monitoring, development and manufacturing of Monsanto's crop protection products

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The traditional approach to quality within the agricultural industry is to perform testing post batch to ensure it has met the critical quality specifications. If the specifications are not met, then the batch would be a failure and discarded. Traditional analytical testing in a manufacturing quality control laboratory is generally very time-consuming and costly due to required manual sampling and sample preparation. What if we could reduce or eliminate failures and the time spent manually sampling and preparing samples? Process analytical technology (PAT) is a mechanism to design, analyze, and control manufacturing processes through the measurement and monitoring of process parameters preferably inline or online and thus being more time and cost efficient in testing while at the same time, enhancing consistency and minimizing failures. This presentation will discuss online or inline methods for process monitoring of Monsanto's active ingredient processes. This will include discussion of the specialized instrumentation, successful implementation and method development that went into establishing the PAT. Methods discussed will include the use of IR, Raman spectroscopy, and online gas chromatography.

AGRO 227

Challenges with site selection, monitoring well placement and sampling for groundwater monitoring of a pre-emergent herbicide in the upper Midwest

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To directly assess the possible flux of a pre-emergent herbicide into shallow aquifers through coarse textured soils in the upper Midwest, Bayer is conducting a five-year study that pairs well-defined chemical applications with periodic in-field and edge-of-field groundwater monitoring. Thirty-four monitoring wells with depths from 18 to 63 ft provide access to groundwater below 18 separate fields in Iowa, Minnesota, and Wisconsin. Site selection criteria included: 1) field sizes of 20 acres minimum, 2) corn grown treated with herbicide of interest in at least 3 of the 5 years, 3) groundwater depths of less than 25 ft for the fields with loam and between 25 to 50 ft for fields with sand or sandy loam, and 4) no restrictive layers present within the soil profile. Peristaltic and stainless steel submersible pumps and multi-meter-enabled flow cells are used in all wells to provide characterization of aquifer geochemical conditions and to produce groundwater samples quarterly. The samples and various trip and equipment blanks are frozen on site, shipped by next-day air, and analyzed using LC/MS/MS with ^{13}C-internal standards at Bayer laboratories in North Carolina. This presentation will review the challenges encountered at each step, and will report the current monitoring results.

AGRO 228

Atrazine Ecological Monitoring Program: Study design and conduct

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A high sampling frequency watershed monitoring program, Atrazine Ecological Monitoring Program (AEMP), which began in 2004, has collected over 28,000 water samples representing 284 site years from 75 watersheds across 13 states in the Midwest and the South. The AEMP consists of compliance-based, targeted monitoring in small watershed (< 40 mi^2) headwater streams, and it is designed to identify environmental conditions in corn and sorghum agricultural watersheds that are susceptible to high surface runoff potential. The AEMP sampling design captured atrazine runoff events following chemical applications to corn and sorghum agriculture when residue levels in the receiving stream are expected to be at their maximum. The breadth of atrazine concentration data from water samples accompanied by watershed characteristics, meteorological data and agronomic data provide a comprehensive understanding of atrazine transport mechanism. The AEMP monitoring data quantifies the upper 20^{th} centile of potential aquatic exposure to atrazine in corn and sorghum growing areas in the United States. The presentation will provide an overview of the site selection process, study design, sample and data collection process, and a summary of important findings.

AGRO 229

Relating sampling bias factors to surface water catchment characteristics for deriving confidence counds on available pesticide monitoring data

Rochelle F. Bohaty[1]*, rochellefhbohaty@gmail.com, Sarah C. Hafner*[2]*, Christine Hartless*[3]*, Charles Peck*[3]*, James Hook*[3]*, Dana S. Spatz*[3]*.* (1) Office of Pesticide Programs, Environmental Protection Agency, Alexandria, Virginia, United States (2) University of California - Davis, Davis, California, United States (3) U.S. Environmental Protection Agency, Washington, District of Columbia, United States

The Office of Pesticide Programs evaluates the potential for pesticides to occur in aquatic environments using screening level fate and transport models and an assessment of available monitoring data. A major limiting factor in the use and interpretation of pesticide surface water monitoring data is geographical area represented by the study design. The SEAsonalWAVEQ with EXtended capabilities (SEAWAVE-QEX) model developed by the U.S. Geological Survey can be used to impute missing or censored (*i.e.*, below the laboratory reporting limit) pesticide concentration data using streamflow as a covariate. SEAWAVE-QEX was used to generate chemographs of daily pesticide concentrations from less

frequent sampling intervals to assist in the development of sampling bias factors. Sampling bias factors are used to adjust concentrations of pesticides in infrequently sampled waterbodies to estimate a desired exposure concentration (*e.g.*, an upper 95th percentile exposure concentration). Sampling bias factors are compared to a number of catchment characteristics such as pesticide use, precipitation, and soil erodibility in order to extrapolate monitoring observations across the landscape.

AGRO 230

Evaluation of SEAWAVE-QEX as a tool to increase the utility of available pesticide surface water monitoring data

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The Office of Pesticide Programs evaluates the potential for pesticides to occur in aquatic environments using screening level fate and transport models and an assessment of available monitoring data. A major limiting factor in the use and interpretation of pesticide surface water monitoring data is infrequent sampling. The SEAsonalWAVEQ with EXtended capabilities (SEAWAVE-QEX) model was developed by the U.S. Geological Survey to impute missing or censored pesticide concentrations from surface water monitoring data using daily streamflow as a covariate. SEAWAVE-QEX was evaluated by masking daily or near-daily pesticide monitoring data to mimic potential monitoring program sampling designs. The results of SEAWAVE-QEX are compared to daily or near-daily pesticide monitoring data in order to evaluate the utility of the tool for use in pesticide exposure assessments.

AGRO 231

Interpreting water quality monitoring observations through modeling: PRZM/SWAT and SEAWAVE-Q

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Water quality monitoring data, specifically pesticides, can represent best-available exposure profiles related to ecological risk assessment; however, there are challenges in synthesizing these data toward making strong conclusions about the nature of the potential range of risk. Some of these challenges include: data collection frequency, monitoring period duration, interpreting exposure profiles from one location to another, and monitoring system scale. To address these challenges, statistical approaches may be applied to characterize empirical trends. Additionally, process-based numerical systems modeling approaches can offer a different perspective on synthesis and interpretation of monitoring data. A comparison of a statistical and process-based numerical model was conducted to evaluate strengths and weaknesses of representing, synthesizing, and conclusions from monitoring data. This comparison was developed from pesticide measurements of six intensively-monitored HUC12 headwater watersheds in the Midwest. SEAWAVE-Q is a regression model that incorporates a linear trend term, covariates accounting for seasonality, and a transformation of flow to represent a long-term pesticide trend at a specific monitoring location. PRZM/SWAT (pesticide root zone model/soil water assessment tool) is a spatially-distributed hydrologic and chemical transport numerical model that combines upland chemical and hydrologic processes from PRZM and stream flow and chemical transport processes from SWAT. The predictive quality and limitations of these two models was assessed against observed, daily concentration measurements as well as hypothetical data collection frequency and timing (derived from sub-sampling the same data sets). Results suggest that a process-based modeling approach, such as PRZM/SWAT, may be more advantageous when calibration data are available.

AGRO 232

Assessing the effectiveness of vegetative environmental buffers in mitigating ammonia and PM emissions from animal agriculture

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Atmospheric emissions from animal production, such as particulate matter (PM) and ammonia, have raised concerns due to potential negative effects on human health and the environment. Vegetative environmental buffers (VEBs), composed of tolerant trees, shrubs, and tall grasses, can be used to control and reduce the transport of these emissions. We conducted a series of experiments at poultry production facilities equipped with VEBs to quantify the effectiveness of this mitigation strategy. However, direct comparison to a control facility was not possible because many variables were different, especially meteorological conditions. Therefore, we conducted additional sampling campaigns to gather specific data for modeling efforts. A sampling campaign was carried out at a poultry production facility without a buffer where PM and ammonia emissions were measured to discern dispersion as a function of distance and the diurnal and nocturnal effects on emission plumes. Several simulated emission campaigns were also conducted. The first series used LiDAR and a known quantity of emitted PM to visualize plume changes as a function of meteorological conditions and time and to measure capture efficiency of the VEB. In the second simulation campaign, the interactions of ammonia with a VEB were quantified by comparing changes in ammonia concentrations to methane concentration changes. A modified small-scale Gaussian plume air dispersion model was developed to estimate changes in ammonia and PM concentrations due to dispersion. Results showed that the VEB enhances dispersion and that the VEB was responsible for capturing 25% of the ammonia and PM. In addition, the VEB was more efficient under low turbulence (low ambient wind speeds). These results will be useful for designing more effective VEBs and other mitigation strategies for atmospheric emissions.

AGRO 233

Estimating sulfuryl fluoride emissions during structural fumigation of residential houses

Jing Tao*, jing.tao@cdpr.ca.gov.* California Department of Pesticide Regulation, Sacramento, California, United States

California Department of Pesticide Regulation (CDPR) monitored sulfuryl fluoride (SO_2F_2) indoor concentrations of twenty-three structural fumigations of residential houses in

eight counties between September 2014 and March 2015. We placed Remote Data Acquisition (RDA) Fumiscopes® at the center of the fumigated houses, and RDA Fumiscopes® measured the SO_2F_2 indoor concentration from four locations of the houses once very hour. Assuming that the decreasing indoor SO_2F_2 was completely emitted to the outdoor environment, we used the monitoring data to estimate SO_2F_2 emissions during fumigation treatment and aeration periods. The results showed considerable variability of half loss time (11 – 60 hr) and mass loss (22 – 81%) during the treatment period. Due to the complexity and differences in residential structures, it is challenging to accurately estimate the fumigation volume prior to the application. Actual application rates for the same pest treatment also differed from house to house. Tarp and seal quality could be the most impactful factors for the mass loss of this period; however, they cannot be quantified. The decline of indoor concentrations followed the first-order kinetics. Therefore we were able to develop a model to calculate the hourly flux (g/m^2s) of a treatment period using the following variables: initial concentration, treatment period mass loss, and house height. Although California Aeration Plan requires a minimum 12-24 hours of aeration after a treatment, our monitoring data showed that 93±5% of the mass at the end of treatment period was emitted through the ducting system within the first 2 hours. The ratio of the loss amount in the first hour to the loss in the second was 6:1 on average.

AGRO 234

Investigation of atmospheric transport of the beneficial microorganism *Entomophaga maimaiga* using microspheres

***Harold Thistle**, hthistle@fs.fed.us.* USDA Forest Service, Morgantown, West Virginia, United States

A large field study to investigate the movement of spores produced by *Entomophaga maimaiga*, an entomopathogen that attacks the damaging forest defoliator, *Lymantria dispar* (Gypsy Moth), is being conducted. Understanding of the movement of this entomopathogen will allow optimization of limited resources to combat infestations of Gypsy Moth. As part of this study, fluorescent microspheres are used to simulate the movement of the spores. To release the microspheres, a novel mechanical system will be designed. In nature, spores are propelled from larval cadavers and thereby released in the atmosphere. After this initial event, the spores are airborne and may be entrained in the airflow and transported some distance or deposited on foliage or the ground surface close to the point of release. The percentage of these spores that are eventually transported regionally is the question of interest in this study. This presentation will focus on the use and analysis of the microspheres as a tracer and also the potential of this tracer technology to aid in various issues of atmospheric transport in pest control in the future.

AGRO 235

Estimating risk to non-target plants and animals from semi-volatile esticides

***Charles Peck**, peck.charles@epa.gov, Faruque Khan, Kris Garber.* EPA, Washington, District of Columbia, United States

The United States Office of Pesticide Programs assesses risks of pesticides to non-target animals and plants. Ecological risk assessments consider off-site transport of pesticides to non-target areas via several pathways, including drift, runoff, and volatilization. This talk will focus on risk assessments of semi-volatile pesticides and the volatilization and drift transport routes. Chemical-specific fate data are obtained from laboratory (*e.g.*, wind tunnel studies, humidome studies, etc.) and field studies (*e.g.*, spray drift field deposition studies, field volatility studies, etc.). These data are used to parameterize volatilization and deposition models that estimate exposure in non-target areas (*e.g.*, AERSCREEN/AERMOD, PERFUM, and AgDRIFT/AGDISP). Exposure estimates are compared to available toxicity data in order to evaluate risk to off-field, non-target plants, and animals from the application of semi-volatile pesticides to treated fields.

AGRO 236

Comparison of three regulatory methods for estimating volatile flux of pesticides from treated fields

***Jody Stryker**, jstryker@stone-env.com, Lauren Padilla, Jonnie Dunne, Brent Toth.* Stone Environmental, Montpelier, Vermont, United States

Evaluating pesticide volatilization under field conditions is a critical aspect of understanding the fate, transport, and exposure risk associated with agricultural applications of these compounds. Three methods are currently recommended by EPA for the purpose of estimating volatile flux of pesticides from treated fields: the indirect method, the aerodynamic (AD) method, and the integrated horizontal flux (IHF) method. The objective of this talk is to present the basis for choosing an appropriate method, including requirements for each method to be valid and necessary data inputs (such as those related to surface roughness and fetch). The indirect method is based on back calculating the flux that best simulates observed air concentrations off-field, and makes use of the EPA-supported air dispersion modeling system, AERMOD. The AD method uses the Thornthwaite-Holzman equations (developed to estimate evaporation) and is based on a logarithmic wind profile. The IHF method is based on mass balance principles and requires no atmospheric stability corrections. Practical guidance for parameterization of semi-volatile compounds is offered, and the theoretical as well as practical limitations of each method discussed. Uncertainty propagation from the flux estimation methods to subsequent modeling of downwind deposition using AERMOD is also evaluated. By examining methods currently used in regulatory assessments for flux estimation, the goal is to identify areas in which improvements can be made or new tools developed to better understand the mechanisms driving volatilization from plant and soil surfaces.

AGRO 237

Methodology to more realistically compute deposition rates for volatilized pesticides: Refining the deposition velocity term in dispersion models

***David A. Sullivan**, sull_env@ix.netcom.com, Ryan D. Sullivan, Dennis Hlinka.* Sullivan Environmental Consulting, Alexandria, Virginia, United States

Droplets of vapor drift can produce offsite deposition to crops and other surfaces. Such effects can be modeled for current cultural practice or for alternative application practices designed to provide mitigation options. Deposition of gas-phase pesticides has two major terms: (1) dry deposition of droplets and vapor, and (2) wet deposition of vapor, such as during a precipitation event. This paper focuses on the dry deposition term. The core term to link near-surface modeled concentrations to the dry deposition term is deposition velocity. Deposition velocity (m sec-1) times near-surface airborne concentration (µg m-3) produces the deposition rate to the surface (µg m-2 sec-1). In a dispersion model such as AERMOD, deposition velocity is automatically calculated based on the wind speed, atmospheric stability, and the aerodynamic roughness conditions at the source of the meteorological data. An important limitation is that the source of the meteorological data in most cases is an airport, where an ASOS or AWOS tower operates adjacent to a runway surrounded by aerodynamically smooth clipped grass. When modeling deposition to grass, bare ground, and other smooth surfaces, these standard treatments are reasonably accurate. Many agricultural areas have common crops grown during the

same season and in close or adjacent proximity. When modeling off-field deposition to a crop in this situation, especially a relatively tall crop or orchard, the deposition velocity term may be substantially miscalculated when based on surface conditions representative of an airport. This paper describes how meteorological analysis can be performed to more accurately account for the differences between wind inputs (mean wind speed and friction velocity) and atmospheric stability between the source of the long-term meteorological data and the modeling domain of interest. Through such meteorological analysis, domain-specific refinement of the AERMET output file can be done to refine the meteorological input data to AERMOD and tailor the analysis to more represent the modeling domain of interest.

AGRO 238

Novel aromatic surfactants

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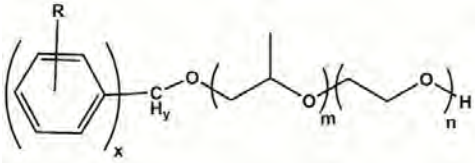
Aromatic surfactants such as alkylphenol ethoxylates and tristyrylphenol ethoxylates are commonly used in agrochemical formulations, but have many environmental and toxicological drawbacks. As such, there is a need in the industry to investigate other hydrophobic scaffolds on which to build new surfactants. The development of new aromatic surfactants will be presented, with a focus on the synthesis, basic properties, and applications of said surfactants in agrochemical formulations.



AGRO 239

Overcome common stability challenges in agricultural formulation development

***Jinglin Liu**, jliu21@dow.com, Mei Li, Ricado Acosta Amado, ke min, Paul Larsen, Derek Hopkins.* Dow AgroSciences LLC, Indianapolis, Indiana, United States

With increased customer demand for novel and more effective agricultural solutions, the crop protection industry has been continuously developing innovative products, not only by launching new active ingredients, but also by advancing novel formulation technology. A successful formulated product requires robust physical and chemical stability throughout the product's shelf life over a wide range of temperature and humidity storage conditions. Common stability challenges include crystallization, phase separation, chemical degradation, etc. Full understanding of intrinsic physical and chemical properties of active ingredients, strengths and limitations of each formulation type, and common causes for stability issues will benefit a scientifically sound formulation design approach. Case studies in this work clearly exemplify how essential formulation strategies can be identified and applied during the formulation development process to overcome common stability challenges.

AGRO 240

Compatibility agents for complex tank mix systems

***Julia Sheehan**, jsheehan@stepan.com, Jacob Bell.* Stepan Company, Chicago, Illinois, United States

For decades, it has been common practice among growers to combine pesticides and fertilizers into one tank to reduce time and money spent on spraying fields. While an efficient approach, this practice can lead to many issues of physical and chemical incompatibilities of the various tank mix partners, a problem often made worse with today's complex and sensitive active ingredients. To combat this issue, compatibility agents can be used to enhance stability of the fertilizer and pesticide combination. Among the various compatibility agents on the market, more than 75% involve the use of phosphate esters. However, most commercial phosphate esters are nonylphenol based and furthermore, their mechanisms as compatibilizers are poorly understood. This presentation will discuss a wide range of phosphate esters evaluated in fertilizer and pesticide systems, and examine trends regarding mono/di ratio, ethoxylation levels, and molecular weight.

AGRO 241

Effect of carbon chain length and degree of unsaturation on skin sensitization potential of fatty acids and their corresponding methylated esters

***Ricado Acosta Amado**, racostaamado@dow.com, Sean C. Gehen, Raja S. Settivari.* Dow AgroSciences, Indianapolis, Indiana, United States

Methylated seed oils (MSO) are gaining importance as co-solvents/adjuvants in agricultural formulations because they can enhance the biological efficacy of the increasingly complex lower soluble new active ingredients. The skin-sensitizing results reported in the literature for some unsaturated carbon-carbon double-bond lipids (MSO constituents) suggested positive results, more frequently in the murine local lymph node assay (LLNA) compared with those in the guinea pig maximization test (GPMT) and/or in human subjects. As the LLNA is one of the gold-standard methods for regulatory registration purposes, it is relevant to elucidate factors leading to positive skin sensitization results of MSO components. In the current study, chemical structure-skin sensitization relationships which can rationalize patterns of varying sensitization potential among the carbon chain length and degree of unsaturation were investigated for a series of individual fatty acids and their corresponding methylated esters using the *in-vitro* KeratinoSens™ assay. Results show that the degree of unsaturation increased the skin sensitization potential and suggested an inverse relationship with increasing carbon chain length.

AGRO 242

Urea-hydroxyapatite-polymer nanohybrids as seed coatings for enhanced germination of seasonal crops

Dasuni Pabodha[1,2], Dasuni N. Rathnaweera[1,2], Gayan Priyadarshana[2], Chanaka Sandaruwan[2], H. W. K. S. L. Kumara[2], Kosala Purasinhala[2], Surani Chathurika[2], Samitha Daraniyagala[3,1], Veranja Karunaratne[2], ***Nilwala Kottegoda**[3,2], nilwalaK@slintec.lk.* (1) Institute of Chemistry Ceylon, Rajagiriya, Western, Sri Lanka (2) Sri Lanka Institute of Nanotechnology , Homagama, Western , Sri Lanka (3) Chemistry, University of Sri Jayewardenepura, Horana, Sri Lanka

In developing countries, agriculture plays a vital role in the economy. To maintain a high-quality yield in agricultural crops, advanced technological applications should be practiced. Several attempts have been made to enhance the nutrient supplying process to the plants in different growth stages, but little effort has been made to enhance the nutrient status of the seeds at seedling stage. Nitrogen and phosphorous are the essential nutrients in plants that are needed for the root growth. We focus our work on the synthesis of urea coated hydroxyapatite nanoparticles encapsulated into a seed coating which supply nitrogen and phosphorus to the seedlings in a controlled manner throughout the early growth stage. Sodium alginate-carboxymethyl cellulose polymer mixture has been selected as the compatibilizer due to its biocompatibility. The nanohybrid suspension was synthesized using in situ sol-gel method and was mixed with the polymer mixture to obtain the coating mixture. Seed coating was realized using dip

coating method with calcium chloride as the cross-linking agent. Seed germination experiments were conducted at the laboratory under constant light conditions and controlled humidity and temperature. Scanning electron microscopic data were used to analyze the morphology of the seed coating. Fourier transform infrared spectrometric data revealed the compatibility of the coating with the seed surface. Hydroxyapatite: urea treated seeds revealed a higher germination percentage, germination rate, and a higher nutrient content compared to the control.

AGRO 243

Hydroxyapatite-citric acid nanohybrids for optimum release of phosphorus in fertilizer applications

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Each year world population is grown by 83 million while one in every nine people suffer from chronic hunger. Sustainable agricultural practices should be explored in order to feed the increasing global population. In this context, efficient practices in using agrochemicals, particularly fertilizer, play a major role. However, the socio-economic and environmental issues pertaining to current commercially available fertilizers demand for the need of next generation sustainable and smart fertilizer systems. Being one of the macro plant nutrients, the sufficient amounts of phosphorus (P) are required for plants to attain utmost crop yields. As soil is not capable of providing this requirement, P has to be supplied to plants externally via fertilizers. Globally, the main source for the production of P fertilizers is a non-renewable rock mineral which makes it an unsustainable process due to the absence of gaseous phase in the phosphorous biogeochemical cycle. Among the phosphorus fertilizers used by farmers, the solubility of ground rock phosphate is low, and whereas commercially available superphosphate fertilizers are highly soluble, thus resulting in lower phosphorous utilization efficiency by plants.

Identifying these facts, our study was aimed at a nanotechnology based solution for developing an efficient, smart P fertilizer with optimum solubility to maximize P plant uptake leading to higher crop yields. For this purpose, we introduced citric acid modified hydroxyapatite nanoparticles synthesised via a wet chemical precipitation approach, as a P source. Characterization of nanohybrids showed that the citric acid is bonded via hydrogen bonding to hydroxyapatite nanoparticles resulting in surface modification without altering its major mineral phase. Water release studies and bioavailability studies conducted with *Zea mays* as the model crop showed that the nanohybrids have elevated the availability of P in contrast to pure hydroxyapatite nanoparticles and rock phosphate.

AGRO 244

Encapsulation of biologics for agricultural applications

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There is increasing interest in using beneficial microorganisms as alternatives to synthetic chemical pesticides and fertilizers in agricultural production. Application of beneficial microorganisms to seeds efficiently places microbial inocula into soil where they will be well positioned to colonize seedling roots and protect against soil-borne diseases and pests. Despite the long history of inoculation of legume seeds with *Rhizobia spp.* and clear laboratory demonstration that a wide range of other beneficial microorganisms improve crop performance, there are still very few commercially available microbial seed inoculants. Further research is needed before the benefits of a wide range of environmentally sensitive potential seed inoculants can be captured for use in agriculture, ecosystem restoration, and bioremediation. There is no single solution to improving the ability of seed inoculants to establish and function consistently in the field. Development of novel formulations that maintain the viability of both inoculant and seed during storage will result from multidisciplinary research in microbial and seed physiology and adjuvant chemistry. In this study, alginate immobilization was used to encapsulate microbes for industrial seed treatment. The technology has been demonstrated to encapsulate and stabilize *Pseudomonas fluorescens*, *Penicilium bileae*, and *Bradyrhizobium japonicum* for biological seed treatment. All encapsulated microbes were stable, showing less than a 1 log decrease in viable cell counts, for at least 8 weeks at room temperature. The encapsulated *P. fluorescens* formulation was stable as a liquid dispersion on shelf for 6 months, and as a dried, on-seed coating for 4 months. The encapsulated *B. japonicum* showed no loss in viable cell counts after 8 weeks while the unencapsulated control *B. japonicum* showed a two-log loss in viable cells at the same time point: a dramatic improvment in stability for a microbe that is particularly sensitive to environmental stresses. The process developed in this work yielded microbe encapsulation efficiencies of approximately 60% for all three microbes. We are improving this efficiency by minimizing the number of cells lost to the process equipment, and reducing stresses on the cells to mitigate cell damage. Overall, the results show that this novel technology may be extended to an array of different microbial species for a variety of stabilized on-seed and on-shelf liquid applications.

AGRO 245

New emulsifier system with improved Clethodim stability for emulsifiable concentrate formulations

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Historically, Calcium Dodecylbenzene Sulfonates (CaDDBS) have been used as emulsifiers in Clethodim Emulsifiable Concentrate (EC) formulations. These emulsifiers have been shown to offer good emulsification properties but tend to promote the degradation of Clethodim, while also displaying unfavorable regulatory handling language. In many instances, these products are formulated above the target amount of Clethodim to account for this degradation. The practice of using increased amounts of Clethodim is costly and could lead to regulatory issues. As a result of the problems associated with using CaDDBS, Stepan Company created a new anionic/nonionic emulsifier system for Clethodim EC formulations that shows comparable emulsification performance with improved Clethodim stability and regulatory handling language over the traditional CaDDBS-based emulsifiers.

AGRO 246

Synthesis of radiolabeled standards of bicyclopyrone and sedaxane to support product development

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Carbon-14 isotope is one of the most useful tools in tracking the movement of a molecule in a living system. Due to its long half-life and low energy, carbon-14 continues to be the cornerstone in the development of pharmaceutical and crop protection chemicals. The presentation will highlight the ^{14}C-syntheses of bicyclopyrone, a HPPD class of herbicide and a metabolite of sedaxane, a SDHI fungicide. The key step in the

synthesis of ^{14}C-bicyclopyrone is the replacement of a low yielding thermolysis reaction with a phosphazene base-induced elimination of the benzenesulfonyl group to form the desired vinyl sulfone, which results in an improved and scalable synthesis. A bioinspired synthesis of a ^{14}C-labeled sedaxane metabolite will also be discussed. The radiolabeled standards of both bicyclopyrone and the sedaxane metabolite were used in environmental fate and metabolism studies required for product registration.

AGRO 247

Production of isotopically labelled natural products and metabolites by microbial fermentation and biotransformation

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The preferred strategy for the production of natural products and mammalian / environmental fate metabolites is chemical synthesis. However, total synthesis is not always a viable option due to the structural complexity of natural products and many metabolites. Microbial fermentation and biotransformation are established routes to access unlabelled natural products and metabolites as alternatives to chemical synthesis. Combining microbial fermentation and biotransformation processes with radiochemistry enables the development of microbial production methods which can then be transferred to radiochemistry facilities for optimum production using radiolabelled material. Using case studies, this presentation will describe strategies for the microbial production of [^{14}C] labelled complex natural products *via* labelled precursor feeding, as well as the production of metabolites of [^{14}C] labelled small molecule compounds.

AGRO 248

Case study: Natural product stability

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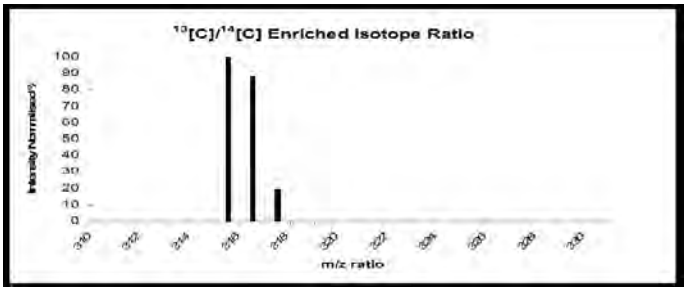
Selcia was approached by a customer who required storage stability / mass balance on their natural product for a registration dossier to be submitted for regulatory approval of the compound. As the compound was a mixture of components with one key active component, the customer was concerned that 40% of the mass balance was unaccountable for after 6 months stressed (40°C) storage. The target active component has no natural isotope ratio, and the majority of the anticipated degradation products were already present in the extract. Therefore, Selcia proposed a ^{14}C mass balance study to quantitatively follow the key active component and its breakdown on storage. Additional ^{13}C labelling would aid elucidation of the degradants. The presentation will take the form of a case study on how a mixture of ^{14}C, ^{13}C and unlabelled active compound, was used to generate an artificial isotope ratio for structural elucidation of the breakdown products resulting in a novel test system for following the natural product storage stability. The ^{14}C label component helps to target MS peaks of interest and provides mass balance details. The ^{13}C label provides a 'handle' for the structural elucidation.



AGRO 249

Carbon-14 labeling and synthetic strategies of imazamox and metabolites

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Imazamox is an imidazolinone herbicide labeled for the control of numerous terrestrial and aquatic weeds. Imazamox was first approved for use on soybeans in 1997 and has received the designation of reduced risk pesticide due to low human health impacts, low toxicity to non-target organisms, favorable environmental fate profile, and compatibility with integrated pest management techniques. Carbon-14 labeled imazamox and metabolites are used to evaluate the safety of imazamox in environmental fate and ADME studies. The chemical structure of imazamox affords the possibility to synthesize multiple metabolically stable carbon-14 ring labeled isotopomers, specifically in the imidazolinone and pyridine ring moieties. The carbon-14 custom syntheses of the isotopomeric imazamox, [imidazolinone-5-^{14}C] and imazamox, [pyridine-3-^{14}C], as well as the metabolites CL-354,825 [imidazolinone-5-^{14}C] and CL-312,622, [imidazolinone-5-^{14}C], were successfully completed. The synthesis of imazamox, [imidazolinone-5-^{14}C] is relatively straightforward with a good overall yield starting from potassium cyanide, [^{14}C], while the synthesis of imazamox, [pyridine-3-^{14}C] is more difficult requiring additional synthetic steps in low overall yield starting from sodium acetate, [2-^{14}C]. The synthesis of CL-312,622, [imidazolinone-5-^{14}C] was accomplished by further chemical elaboration of imazamox, [imidazolinone-5-^{14}C]. The synthesis of CL-354,825 [imidazolinone-5-^{14}C] required a re-design of the overall synthetic route and departure from known chemistry to accomplish this more challenging synthesis.

AGRO 250

Strategies for labelling test substances for regulatory studies

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Registration of crop protection chemicals requires a detailed risk assessment of the compound under consideration towards the environmental as well as mammalian exposure targets. In order to understand the risk associated with a product, not only the parent compound, but all significant degradation products need to be identified and their toxicological significance established. A meaningful risk assessment therefore, requires identification of the metabolites/degradation products in a variety of environmental and animal studies which are conducted for the registration purposes. These studies often require the use of isotopic labelling of the test substances in a strategic manner, so that potentially toxic molecules generated as a result of the product use may be identified. Some of the strategies employed in various registrations will be discussed.

AGRO 251

Strategies for isotopic labeling of agrochemical active ingredients to enable registration

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In order to register a new agrochemical product, the metabolic fate and degradation profile of an active ingredient must be determined. To enable this, a number of studies are conducted in different matrices, which include soil, water, crops, and mammals using a ^{14}C labeled radiotracer of the active ingredient. The ^{14}C radiolabel is positioned within the molecule so that all significant metabolites/degradants formed can be tracked and the metabolic pathway fully elucidated. To rapidly identify these metabolites/degradants, stable isotope labeled active ingredients (^{13}C, ^{15}N, and ^{2}H) in conjunction with high resolution mass spectrometry techniques are utilized. This talk will focus on the strategies and synthetic routes used for incorporation of isotopic labels into several commercial active ingredients for Corteva Agriscience™, Agriculture Division of DowDuPont™.

AGRO 252

Using radio-HPLC and radio-TLC in tandem for the quantification and confirmation of known metabolites in support of agrochemical product development

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The robust quantification and confirmation of known agrochemical metabolites from radiolabelled animal, plant, and e-fate studies is an ongoing challenge in new product development. The challenge is often exacerbated by the trend in lower application rates, coupled with complex matrices. Furthermore, concentration of sample extracts can lead to degradation of unstable metabolites, which in turn result in fundamental changes in the quantification of the final radio profile. In addition to techniques capable of positive structure identification (*e.g.,* MS and NMR), the OECD guidelines permit the application of co-chromatography of the metabolite(s) with known standards using "two dissimilar systems." Whilst the use of truly orthogonal radio-HPLC methods and/or LC-MS is often regarded as the primary tools in industry for these purposes, far less merit is afforded to the application of radio-TLC, which is often seen as an awkward, ageing or even obsolete technique. This presentation will highlight advantages of combining radio-HPLC methods and radio-TLC assays for the quantification and confirmation of known agrochemical metabolites. The application of these technologies will be underpinned by real world examples which will show that older techniques, such as TLC, can still have utility in the age of million dollar analytical instrumentation.

AGRO 253

Use of radiolabeled and stable labeled test substances in regulatory metabolism studies for agrochemicals

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Radiolabeled agrochemicals are commonly used for the regulatory studies such as metabolism and environmental fate in plants, animals, and soil. A radioactive ^{14}C-label is the most popular tracer for these studies in terms of a long half-life with low energy β-emitter. The technology for detection of ^{14}C in sample matrices is also mature. Typically, ^{14}C-radiolabeled agrochemical allows for quantification such as mass balance, and total radioactive residues in RACs, and identification of major components by co-chromatography of known metabolite standards.

An objective is to select a specific activity which provides limits of quantitation below 0.01 mg/kg. For example, 250 dpm determined by combustion of a 100 mg subsample with specific activity of 25,000 dpm/µg will result in limit of quantification of 0.01 mg/kg whereas 250,000 dpm/µg is 10 times lower. Another consideration of specific activity is the higher the value results in reduction of sample matrix interference by using a smaller size of aliquots for qualification of ^{14}C residues in extracts and post-extracted solid samples.

Radiosynthesis can incorporate ^{14}C at a specific site or incorporate ^{14}C into an aromatic ring system (uniform label). The advantage of ring incorporation is stability of aromatic moiety to xenobiotic transformations. Another advantage if one carbon is useful for MS analysis where detection is for a single ^{14}C addition of [M+2] peak. Incorporation of ^{14}C into an aromatic ring usually results in detection of several ions due to incorporation of one ^{14}C, two ^{14}C, three ^{14}C, etc. which reduces the usefulness as a marker detected by mass spectrometry.

Use of stable labeled analogs such as deuterium, ^{13}C or ^{15}N test compound to a mixture of ^{14}C and cold test compounds can facilitate identification of metabolites by mass spectrometry.

Examples will be presented to show how this selection of specific activity and position of incorporation of radiolabel into molecules are used to aid in determination of xenobiotic transformation pathways of agrochemical.

AGRO 254

CESI-MS for agrochemical analysis

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This work addresses the application of capillary electrophoresis with mass spectrometry (CESI-MS/MS) to a diverse variety of agricultural, ecological, and environmental scenarios. Commercialized by SCIEX, CESI-MS/MS has revolutionized the spectrometric analysis of highly polar agrochemicals, including protic organics, formerly prone to matrix suppression and chromatographic complications associated with reversed phase and ion exchange techniques. We detail the CESI-MS/MS analysis of a suite of polar pesticides and nutrients of great interest for a number of reasons, including: highly publicized concerns about health effects (i.e., glyphosate), MRL violations caused by biogenic and anthropogenic interferents (i.e., fosetyl-aluminum, and phosphonic / phosphoric acids), and the large amounts of

these compounds that are used each year to maximize the global food supply. With respect to ecological applications, we report amino acid nutrient dynamics associated with sucking and piercing plant pests, including glassy-wing sharpshooter and Asian citrus psyllid. Moreover, we characterize prominent semiochemical constituents contained in the oral secretions of key Tephritd fruit fly pests, such as those from the genus *Anastrepha*. Finally, CESI-MS/MS was applied to the identification of alkyl and aryl amines prominent in agriculture, personal care products, and disinfection technologies. Of particular interest was the CESI-MS/MS-quantification of amines that are subject to N-nitrosation, a structural modification that is not typically chromatographically distinct, at least with conventional approaches, and often is spectrometrically masked, such as during electron-impact ionization.

AGRO 255

Strategies for extraction and cleanup prior to LC-MS/MS determination of dicamba and other acidic herbicide residues in agricultural samples; consideration for bound and unbound compounds and metabolites

***Michael S. Young**, michael_s_young@waters.com, Kim Tran.* Waters Corporation, Milford, Massachusetts, United States

Herbicides are commonly used for agricultural weed control in production of many types of crops. To help ensure public health and safety, reliable analytical methods are necessary to determine residues of these herbicides in agricultural samples. Most unbound (free) acidic herbicides can be extracted from agricultural samples in the same manner as other pesticides using standard QuEChERS methods but the PSA based dSPE cleanup often employed for this analysis is not appropriate for acidic compounds. Although PSA is effective for removal of polar lipids and other anionic interferences, it also retains many acidic herbicides resulting in unacceptable low recoveries. For multi-residue screening, a simple pass-through SPE cleanup protocol was developed using a novel reversed-phase sorbent. This protocol is suitable for simultaneous determination of unbound acidic herbicides and base/neutral pesticides from a single aliquot of QuEChERS extract; recoveries of acidic and non-acidic compounds are comparable. However, some acidic herbicides, such as dicamba and 2,4-D, can exist in bound forms (esters or conjugates); determination of the total of bound and unbound residues requires hydrolysis prior to QuEChERS or other manner of extraction. A modified QuEChERS extraction protocol, including a hydrolysis step, was evaluated for determination of dicamba, dicamba metabolites, and 2,4-D. Cleanup options for the modified QuEChERS method will be discussed.

AGRO 256

Expansion of pesticide analysis screen by high resolution mass spectrometry in fresh produce in a regulatory environment

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The Florida Department of Agriculture has been monitoring pesticide residue in fresh produce for over 25 years. The main challenge over the years has been to find the right tool for the job for both GC and LC analyses. On the LC side, we recently moved from MSMS analysis by triple quadrupole instruments to single stage high resolution mass spectrometry to screen regulatory samples for pesticide residues. The advantages such as ability to expand the amount of screened analytes indefinitely, ease of instrumental method creation, improved selectivity, ability to retroactively question the data without reinjecting the extract, and excellent quantitation with almost no matrix interferences represent a giant leap forward. The challenges in implementation of HRMS such as cost of standards, establishment of validation criteria for a qualitative screen, and establishment of analytical workflows that can handle the huge amount of data generated will also be discussed. For the last 5 years, we have been monitoring our regulatory samples by HRMS. An increase in almost 50% of detected analytes was recorded.

AGRO 257

Coating-free, "quick-and-easy" scanning electron microscopy imaging of agricultural samples

***Nathaniel J. Carter**, ncarter21@gmail.com.* API Analytical Sciences, Monsanto Company, Saint Charles, Missouri, United States

Scanning electron microscopy (SEM) enables direct visualization of sample features of interest that are too fine to be resolved by light microscopy. However, many agricultural samples – *e.g.,* plant and insect tissues, crop protection product active and formulation ingredients, etc. – are sufficiently resistive to electrical current or sensitive to the low pressure under high vacuum conditions that they require special preparation techniques to be suitable for viewing in the SEM. Such preparation techniques are generally very time-consuming and complicated, require additional and costly equipment and lab space, need to be optimized for each different type of specimen, and can incur damage to the sample features of interest or otherwise prevent the desired information from being obtainable. These issues pose the potential of rendering SEM imaging of many agricultural samples impractical, especially in an industry research setting where throughput and turnaround time are often paramount, and personnel cannot be dedicated to extensive sample prep method development and laborious routine procedures. Moreover, while utilization of low vacuum conditions in environmental SEM (E-SEM) addresses some of these challenges, the higher incident electron beam energies typically required to produce a useful image increase the likelihood of damage to the specimen at high magnifications and fundamentally reduce the resolving power of the microscope, raising the lower limit on the size of features that can be analyzed. This presentation will discuss a simple methodology for sample cryo-preparation and imaging under high vacuum and low beam energy conditions that alleviates the need for meticulous preparation procedures requiring expensive equipment. This method is suitable for many common SEM configurations and readily applicable to varying specimen types; application to plant and insect tissues as well as crop protection active and formulation ingredients will be demonstrated, along with the impact of SEM imaging on research and commercial projects.

AGRO 258

Analytical methods to quantify off-target movement of dicamba

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Dicamba herbicide has been in use in the United States since the 1960's for selective control weeds in several crop and non-crop use patterns. Recently, a biotech product was developed to allow in-crop applications of dicamba for the control of broad-leaf and hard-to-control (herbicide resistant) weeds. As with all herbicides, it is important to understand the potential routes of off-target movement following application using a given use pattern. This presentation will focus on development and validation of two LC-MS/MS methods used to characterize off-target movement of dicamba. These methods determine the mass of dicamba on either filter paper or polyurethane foam (PUF) air samplers to characterize the mass loss off-target via spray drift or volatilization, respectively. Optimization of analytical method parameters will be presented along with results of inter-laboratory and intra-laboratory method validations that

assessed method performance characteristics including dynamic range, accuracy, precision, selectivity, and capability of dilution.

AGRO 259

Application of Raman microscopy in pesticide formulation analysis

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Raman microscopy is a nondestructive analysis technique that allows for the characterization of components of formulations with regard to chemistry, isomer and polymorphic form, particle size, and distribution. A review of Raman theory, instrumentation and analysis considerations will be followed by application examples using Raman microscopy. Raman can be used for the *in situ* characterization of active ingredients to determine their stereochemistry, such as whether an active is in the cis or trans form of a molecule; a determination not possible with techniques such as mass spectroscopy or liquid chromatography. And Raman can also be used to identify the polymorphic form (hydrate/anhydrous, crystalline form) of a compound which will have an effect on how the active will perform when formulated with carriers and when applied in the field. Raman is a very flexible technique and can be used to analyze active and excipient materials in different forms including powder formulations, emulsions, or as neat samples. The amount of material needed for a single analysis is less than 1 nanogram making it possible to use Raman in product development to verify molecular information of a compound. With the sensitivity of Raman microscopy and utilizing automated sample stages, spatially resolved Raman data can be collected of a formulated sample producing a map of the components in a formulation. The Raman map can be evaluated for the distribution and uniformity of a product with high spatial resolution. And with that distribution, the particle size of the active material in a formulation can be determined to the micron particle size level.

AGRO 260

Analytical method lifecycle through crop protection product phase advancement

***Wen Su**, wen.su@monsanto.com.* Monsanto, St. Louis, Missouri, United States

Analytical Chemistry plays a critical role in advancing crop protection products from discovery, through multiple development phases to growers' fields. Analytical method lifecycle refers to the combined activities of analytical method development, improvement, qualification, validation, transfer, and maintenance related to product development and commercialization. An integrated approach to analytical lifecycle ensures that analytical methods evolve from initial development to commercial production use in a manner that is best suited for their intended use at the various stages on the way to commercialization. The evolution of a HPLC method used for the development of NemaStrike™ seed treatment product is described to illustrate analytical method life cycle and key attributes that the method should possess at each stage.

AGRO 261

State of our world: An argument for watchful optimism

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When considering the state of our environment and our health, it seems that the only news getting attention is bad news. Television and internet are dominated by depressing stories and statistics: global warming, increased rates of autism, deforestation, viral epidemics. Regardless of the headlines, the actual news is surprisingly good. Despite public perception, many of the problems facing the health of our environment and our population have seen steady improvement. Air quality, renewable energy, agricultural production, food safety and security have all improved and are predicted to continue to improve. However, politicians and fear mongers would have us believe differently. And from the beginning of society, we have always been inclined to believe that we are heading quickly toward our own destruction. People want to believe the best, but it is in our nature to always fear the worst.

Yet, health and environmental scare-mongering can be harmful, resulting in misaligned priorities and detracting from constructive, science-based policy. We must guide our future attention not on the scariest stories or loudest crusaders, but on objective assessments of the real risks that challenge us.

AGRO 262

Chemophobia – Simply semantics or something deeper?: How to have a discussion with a non-scientist

***Del A. Koch**, dkoch@agrodiv.org.* Inovatia Laboratories, Columbia, Missouri, United States

The phrase chemical-free, which is virtually always a misnomer in its contemporary (mis)usage, contains within it the inherent (but unfortunate) corollary that chemicals are in general harmful. The resultant chemophobia, however, often goes beyond the obvious (to us!) error in semantics. As such, simply providing an accurate definition of the word chemical may not be a convincing argument. Everyone (scientists included) has filters, or lenses, through which information is evaluated. Understanding what filters are in place may be a necessity for having a meaningful conversation. While the scientific discipline is somewhat immune given its dependence upon observable facts, it is hard to argue against the proposition that we live in a post-modern culture, the cornerstone of which is that absolute truth does not exist. Thus, facts not fitting one's preconceptions, if not outright rejected as industry propaganda, at best may be acknowledged by a begrudging "Well, that may be true for you, but…" As an example, this statement from Wikipedia regarding the term "Chemical-free": "**From a chemist's perspective**, the term is a misnomer…" In other words, not factually inaccurate, just a matter of opinion. Granted, what the layperson may mean by chemical-free is **harmful,** or **man-made,** chemical-free, but the use of this term inevitably tends to increase chemophobia, as harmful and non-harmful substances, without any context as to the nuances of dose and exposure, are conflated. Strategies for having meaningful discussions on this topic, such as asking questions to elucidate the underlying fears and concerns regarding chemicals, will be presented.

AGRO 263

Politics and the news cycle: How to cut through the noise

***Genevieve OSullivan**, gosullivan@croplifeamerica.org.* CropLife America, Washington, District of Columbia, United States

It seems that the ever-changing political climate has made it more difficult than ever to ensure your voice is heard. We'll discuss strategies to engage with communications and government relations professionals to ensure your message is not only reaching the media, but also decision makers. It is important to get the facts straight, but even more important to create a narrative that drives interest and engagement.

AGRO 264

Hogwash: Battling misinformation on the front lines of the public sphere

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Although scientists are typically more comfortable in the structured and peer-reviewed format of the scientific literature, increasingly public perceptions and debates are shaped in less rigorous free-range sources such as social media, activist publications, and even documentary films. In these formats, narrative tales take precedence over facts and evidence. Science-based perspectives can still have influence in this sphere, and non-traditional routes can be used by practitioners of science to influence the discussion. Here a case study of a response to an activist's book (*Whitewash: The Story of a Weed Killer, Cancer, and the Corruption of Science* by Carey Gillam) will provide a framework for discussion of effective strategies for combatting misinformation and dispersing accurate information through various channels and networks. Relying in part on the science communication strategy of "inoculation" to describe the strategic deception, followed by evidence-based debunking of claims and omissions, a social media post provides a base of scientific information that can be propagated far beyond a single blog to much wider reach. Examples of the additional outreach routes will be provided, with evidence of the ongoing impact. Other suggestions for unconventional but successful science-based outreach strategies will also be discussed.

AGRO 265

When analytical data deceive: Separating fact from fiction

***William Reeves**, william.r.reeves@monsanto.com.* Monsanto Company, St. Louis, Missouri, United States

Pesticide residue data, whether generated by government agencies or private labs, inform consumers about an important aspect of food safety. Typically the results demonstrate that dietary exposures are low and unlikely to pose health risks. It is possible, however, to contort these findings to produce narratives that stoke fear and prompt changes in food purchasing choices. Recent examples of claims regarding adverse pregnancy outcomes, dramatic increases in pesticide exposure over time, and the presence of pesticides in popular children's foods illustrate how consumers can be misled into believing their food is not safe. These examples also reveal the difficulties in communicating factual information before the news cycle moves on. This talk will review how inaccurate claims and exaggerated findings create technical and communication challenges and how they can be overcome.

AGRO 266

Moms, milk, and Monsanto: The precise conditions for a perfect storm

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In the fall of 2012, we received an e-mail from someone claiming to be a Welsh farmer asking if we knew of any research that had been done to investigate whether glyphosate (the active ingredient in Roundup) could be detected in human milk. Having conducted research on human and bovine milk composition for decades, this question represented a typical inquiry for us. Not able to locate any published papers addressing this person's question, we reached out to the scientists at Monsanto (the manufacturer of Roundup) to find out if they were aware of any research in this regard. The answer, to our surprise, was 'no.' Two years later, in the spring of 2014, Moms Across America – an anti-GMO, pro-organic group founded and championed by Zen Honeycutt – reported on the internet that they had detected "shockingly" high levels of glyphosate in several human milk samples. However, their sample collection and analysis methods were ill-defined and questionable. Circling back to our earlier discussions with Monsanto, we decided to collaborate in such a way as to more rigorously answer the question at hand: *Does human milk contain detectable levels of glyphosate?* We were, however, relatively naïve to the perfect storm into which we had just unwillingly and unwittingly stepped. This presentation will tell "the rest of the story," including a complex web of lies, *ad hominem* attacks, rampant science illiteracy, orchestrated social media blitzes, partial journalism, and an extensive US Freedom of Information request. We strongly urge foods-and-nutrition researchers, their employers, and their professional societies to take a stand and speak up against these types of attacks by "advocacy" organizations aimed at purposefully misleading the public and (ultimately) attempting to drive policy decisions using emotions, unsubstantiated claims, and junk science as their evidence.

AGRO 267

An agroecosystem approach for endangered species

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A lot has been written in public venues about the interaction of crop protection products used in agriculture and the Endangered Species Act (ESA), with most authors focusing on the potential for impact on listed species with the use of individual products. This individual crop protection product approach also dominates the regulatory story lines, since the approval and registration review processes led by EPA are oriented toward individual products; this individual product approach is carried forward in the EPA interaction with the Services that have the responsibility of implementing ESA. Frequently Services take an individual species approach when considering their listing decisions and designing their recovery programs. The premise of this presentation is that continuing to focus on individual products and species in public dialogue as well as in regulatory systems does little to benefit endangered species, as it deflects attention and resources away from what should be the primary goal: to manage the agroecosystem to the benefit of both agriculture and endangered species.

AGRO 268

Deficit model: Avoid it

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The "deficit model" of communication holds that a presenter need only bring an audience up to speed on the facts and then that audience will agree with the presenter's ideas. However, communications research indicates that audiences often filter presentations for the information that reinforces their personal beliefs. Thus audiences have been known to draw conclusions that are wholly opposite to presenters' arguments. Belief systems are informed by combinations of personal experience, authority figures, and feelings. But individuals may apply different weights to these factors as they choose their actions. Effective messages therefore need to include emotional hooks, along with facts, to strike up conversations that could lead to more informed decisions.

AGRO 269

Analysis of weather and environmental factors associated with off-target dicamba movement

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Introduction of dicamba-tolerant soybean and cotton to the market and the subsequent increased use of dicamba herbicide has renewed interest in off-target pesticide movement. Over 2,700 dicamba-related injury complaints were filed with state departments of agriculture in the U.S. in 2017. Extension scientists estimate that approximately 3.6 million acres of soybean were injured; however, complaints encompassed a variety of crops and ornamentals. Multiple factors contributed to the off-target dicamba movement observed in 2017. They include physical drift, spray tank contamination, temperature inversions, and volatility. The work presented here will focus on our research efforts to study the movement of dicamba from both designed, research experiments as well as 'real world' occurrences of off-target movement. This will include studies on characterization of low-level surface temperature inversions; air sampling studies designed to detect dicamba during inversion-like conditions; examples of reported field incidences; and comparisons of environment and weather conditions surrounding successful dicamba applications and applications where dicamba moved off-target. Studying dicamba movement from different angles allows us to better understand how this specific chemical is moving but may also provide a more general understanding of pesticide movement.

AGRO 270

Dicamba emissions after application appear related to temperature, formulation, and adding glyphosate to the spray mixture

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Dicamba use in the United States greatly increased in recent years due to the need to control glyphosate-resistant weed species in dicamba-tolerant soybean and cotton cultivars. While weed control has been good, there have been significant incidents of off-target movement of dicamba onto non-target vegetation. This report examines factors that affect the possible movement of dicamba following an application. Dicamba emissions following application appear to be directly related to temperature, with more dicamba emissions detected as the temperature increases. There also appears to be a minimum temperature of ~15 C where dicamba emissions from all formulations essentially cease to occur. The addition of glyphosate to dicamba formulations decreased the spray mixture pH and increased the observed dicamba emissions when compared with each dicamba formulation alone. The BAPMA and DGA + potassium acetate formulations resulted in reduced emissions compared with DGA formulations. These data suggest volatility from all the dicamba formulations tested could contribute to dicamba drift. Dicamba emissions can be reduced by avoiding high temperatures and not adding glyphosate to spray mixtures.

AGRO 271

Evaluating spatial scale effects of dicamba applications on off-target vapor movement

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The active ingredient dicamba (3,6-dichloro-o-anisic acid, $C_8H_6Cl_2O_3$) is an herbicide registered in the United States for selective control of broadleaf weeds that has been used successfully since the 1960's. As with all herbicides, it is important to understand the potential routes of off-target movement following application. Off-target movement of dicamba can potentially occur via two routes. Physical spray drift can occur when droplets are blown off-target before reaching the target application area. The second route is vapor drift where pesticides are applied on target but later a small fraction of applied (<0.2%) changes state from a liquid to a gas, of which an even smaller portion has the potential to move horizontally off-site during certain meteorological conditions. To evaluate potential dicamba exposure outside of the application area due to volatility, standard air dispersion models were used along with field-measured flux data (rate of volatility) and meteorological data to estimate air concentrations that could exist outside the spray application area. The results of this evaluation are helpful for understanding the fate of dicamba in the atmosphere and to better understand the conditions that influence the magnitude of dicamba air concentrations following applications of dicamba.

AGRO 272

Monte Carlo modeling methods for county-wide and regional analysis of pesticide airborne concentrations and drift for volatilized pesticides

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Pesticides used in high density growing regions for common crops can produce county-wide residential exposures in terms of airborne concentrations and deposition as well as crops and other environmental exposures. At the County level of analysis and larger, it is necessary to realistically account for the timing and range of key input values when considering the combined impact of many sources. While highly conservative modeling can simplify the analysis based on simplifying assumptions, realistic assessments need to simulate more accurately the range of inputs and the timing of applications occurring at up to hundreds of different agricultural fields within the modeling domain. While it is normal that certain crops are dominant in certain counties with pesticide applications occurring within common application windows. Pre-emergent and post-emergent applications will have commonality in general, but obviously will not occur on the same week or same day. Such variability needs to be replicate for realistic assessment. Similarly, a range of application rates may be allowed for certain products, and a range of timing for the start and stop times for applications within label restrictions.

In order to sample within these ranges, and replicate cultural practice as accurately as possible, Monte Carlo sampling methods based on sound replication of cultural practice is a sound way to produce an analysis that is realistic and provides decision makers in terms of registrants and regulatory agencies with a range of model outputs that describe acute, chronic, and subchronic human exposures and environmental exposures, including off-site drift to other crops and environmentally sensitive areas. This paper

describes how expert elicitation methods can be used in conjunction with regional modeling to replicate cultural practices in specific counties of interest, and then output airborne concentration and deposition rates at various percentiles of modeled distributions.

AGRO 273

SOFEA3 modeling of 1,3-dichloropropene concentrations in ambient air in high fumigant use areas of the United States

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The soil fumigant 1,3-Dichloropropene (1,3-D) is used to control nematodes in high valued fruit and vegetable crops in the USA and globally, and it is currently undergoing registration review at the USEPA. One of the conditions of the re-registration is to monitor 1,3-D concentrations in ambient air in high fumigant use areas for the purpose of estimating bystander exposure. Dow AgroScience has developed and validated a computer modeling framework (SOFEA3) to simulate 1,3-D concentrations in ambient air based on grower product use data. SOFEA3 predicts acute and chronic pesticide concentrations in air for large air sheds resulting from representative agronomic practices, and it has recently been updated to include EPAs regulatory air dispersion model AERMOD. SOFEA3 is being used to simulate 1,3-D concentrations in four high fumigant demand areas in the USA, the Southwest, Pacific Northwest, mid-Atlantic coastal states, and the Southeast coastal plain. The model is parameterized with product use data including field location, treated field area, mass of 1,3-D applied, and depth of application. Agronomic data are essential for characterizing the 1,3-D source strength in the model and were obtained from growers in the defined study regions. Weather data for each region were obtained from state or federal meteorological stations. Modeled 1,3-D concentrations in high use areas in WA, NC, and FL are consistent with the concentrations measured and modeled in ambient air in Merced, CA. The modeled short- and long-term 1,3-D concentrations in ambient air will be used to assess human exposure and risk.

AGRO 274

Drought-induced effects on buckwheat (*Fagopyrum esculentum*) floral traits and honey bee visitation

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Buckwheat is commonly grown as a honey plant because of its long-flowering period, generous, stable standing nectar crop, and attractiveness to honey bees. Abiotic stressors like drought can modify a variety of floral traits, including nectar chemistry and volume, and floral scent and density. Droughts are predicted to increase in severity and duration in the future and so a richer understanding of the effects of drought on plant floral traits, and subsequent effects on plant-pollinator interactions, is needed. To evaluate the effects of drought on buckwheat floral traits, plants were grown under controlled greenhouse conditions with three water regimens: well-watered, moderate drought stress, and extreme drought stress. Floral volatiles were identified and analyzed by gas chromatography mass spectrometry, while nectar volume and carbohydrate content were evaluated by liquid chromatography evaporative light scattering detection. To compare honey bee affinity between drought stressed treatments, plants from all three treatments were placed nearby an apiary, where honey bee visitations were observed. After the observation period, plants were excluded from further pollinator visitation and allowed to set seed to evaluate crop yield potential and plant fecundity. Results will be described.

AGRO 275

Comparative analysis of diamide formulations on pest and beneficial insects

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Honey bee (*Apis mellifera* L.) decline is a nationally-recognized problem that demands attention from both the scientific community and beekeeping industry. The decline of bee colony numbers in recent years presents an economic and ecological threat to agricultural systems and the pollination services provided by bees. One outstanding threat is the unintended exposure of these pollinators to agricultural pesticides. The ryanodine receptor (RyR) is a non-voltage gated calcium channel found in cardiac, neuronal, and muscle cells which mitigates calcium flow out of the sarcoplasmic reticulum for muscle contractions. The RyR is an exploitable insecticide target site with minimal incidence of cross-resistance to currently available modes of action. Emphasis on this underutilized target site led to the discovery of a novel insecticide class, the ryanodine receptor modulating diamides. Many studies regarding efficacy of diamide insecticides report their selectivity for lepidopteran or hemipteran pests over beneficial arthropods, but do not elucidate on the possible mechanisms for this increased efficacy over other insects. There are no available studies that provide an in-depth analysis of diamide effects on beneficial insect pollinators, such as honey bees. This study focuses on diamide insecticide exposures to honey bees, a crop pollinating insect, and the fall armyworm, *Spodoptera frugiperda*, a crop pest insect. The aims are to examine toxicological, physiological, and behavioral parameters for both insects following exposure to diamide formulations. The data gathered will provide the acute toxicity, metabolic detoxification, behavioral fatigue, and RyR modulator manipulation for honey bees and fall armyworms exposed to three current formulations of chlorantraniliprole (Coragen® 18.4% a.i., Altacor® 35% a.i., and Prevathon® 5% a.i.). The future directions of this study will be discussed with emphasis on RyR modulation on muscle contraction, actin-myosin filament motility, and calcium mobilization. This mechanistic study presents a top-down approach to examine diamide insecticide exposures to a beneficial species at the cellular, organ, and organismal levels and, in turn, the gathered data will serve to bridge knowledge gaps related to diamide insecticide exposures and pollinator health.

AGRO 276

Monoterpenoid and phenylpropanoid esters as long-lasting mosquito repellents

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Plant essential oils are composed of complex mixtures secondary plant metabolites, typically including a variety of monoterpenoid and/or phenylpropanoid alcohols. While these alcohols and their parent oils often possess substantial repellent activities against insects over a short time period, use as long-term repellents has been hindered by the high volatility and rapid dissipation of the repellent compounds.

Esterification of the alcohol moiety provides a simple method of producing new repellents with higher molecular weights with concomitant reductions in volatility and dissipation rates. Moreover, because the skeletal structure of the parent monoterpenoid or phenylpropanoid remains unmodified, the synthetic ester retains its intrinsic repellent characteristics. A series of 21 isovalerate esters were synthesized and tested against the northern house mosquito (*Culex pipiens*) for both short-term and long-term spatial repellency using a static-air chamber assay. A structure-activity relationship (SAR) was produced by classifying the parent alcohols into groups based on the skeletal structure. When the esters were categorized into phenylpropanoid or acyclic, monocyclic, or bicyclic monoterpenoid groups, substantial correlation in short- and long-term repellency behavior was noted. Amongst these groups, the bicyclic monoterpenoid esters consistently provided the highest levels of short-term repellency, while the acyclic esters tended to have higher long-term repellency. To differentiate between the intrinsic potency and the concentration-dependent repellency of these esters, the diffusion equation was used to develop a model that estimates concentration of the repellent throughout the tube.

AGRO 277

Analysis of activity of monoterpenoid plant compounds on a nicotinic acetylcholine receptor

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Monoterpenoids found in natural plant products have been shown to have biocidal activity against agricultural, human health and veterinary pests. Specific neurological activity has been found to be highly variable between different monoterpenoids acting on different organisms and receptors. This research follows findings that carvacrol can act as an antagonist against the ACR-16 nicotinic acetylcholine receptor from *Ascaris suum*. We tested multiple plant derived monoterpenoids with the ACR-16 receptor. Receptors were expressed in oocytes from *Xenopus lavis*. We used two electrode voltage clamps to test the receptor response when exposed to monoterpenoids alongside acetylcholine, the natural agonist. Concentration response characterizations were performed at 100 µM and 10 µM concentrations of the monoterpenoid compounds. No monoterpenoids showed agonist activity on the ACR-16 receptor. Most compounds tested showed a significant inhibition of the acetylcholine response. Concentration response curves showed that the monoterpenoids tested had no significant effect on the EC_{50} values of the acetylcholine response. The maximal current was significantly lower than the acetylcholine only control for several compounds at the 10 µM rate. This research confirms previous findings that monoterpenoids as a class of compounds have varied but noticeable neurotoxic effects at a nicotinic acetylcholine receptor. We plan to characterize those compounds most active at our chosen target site.

AGRO 278

Natural compound spororium A protects tomato plants against *Botrytis cinerea* by priming the jasmonic acid pathways

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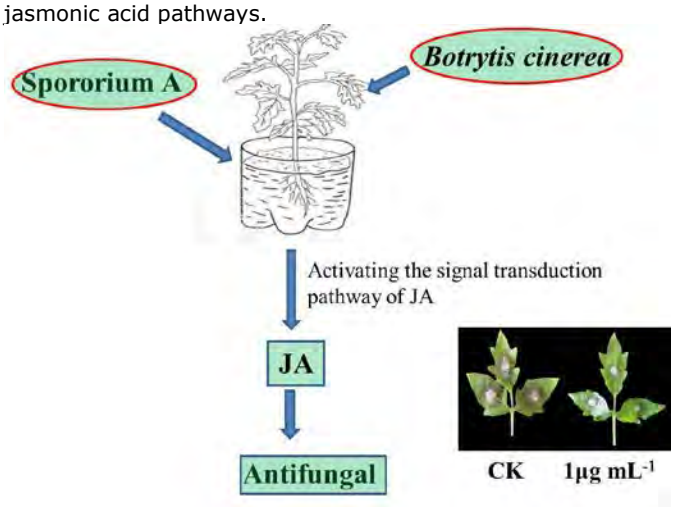
Plant induced resistance was considered as an efficient strategy helping plants resist disease to enable the reduction of agricultural chemicals. After treatment with biotic or abiotic inducing agents, the plant's level of resistance was enhanced by directly activating defence responses or priming the plant for a faster and stronger defence response after pathogen challenge. Tomato is a main horticulture crop throughout the world, and the gray mold is the main disease during the process of growth. The effective control of this necrotrophic fungus *B. cinerea* requires several chemical treatments and often leads to unsatisfactory results. In this study, we found a new resisitance inducer that can protect tomato plants against *B. cinerea*. A new natural compound spororium A was isolated from the fermention of endophytic fungus *Nodulisporium* sp. The compound had no inhibited activities against the growth of mycelium at 50 µg $mL^{-1}$and the germination of spore at 5 µg mL^{-1} of *B. cinerea*. But we found that the compound can help tomato leaf resist gray mold caused by *B. cinerea* using the hydroponic culture. When the tomato plant grew four leaf stage on soil, the plant was taken out carefully and put into Hogland nutrient solution with 1µg mL^{-1} of spororium A 7 days. Then the leaf was dropped spore suspension of 1×10^6 spore/mL and took samples every day until the seventh day. Protein activity of defense-related proteins polyphenol oxidase, guaiacol peroxidase, β-1,3-glucanase and chitinase was measured and the level of treatments had no significant difference with control. The expression of marker genes of jasmonic acid pathways were also measured and the expression of *PI2*, *PIN2* and *JEFR3* were induced. All the results tell us that spororium A can protect tomato plants against *B. cinerea* by priming the jasmonic acid pathways.



AGRO 279

Using biosolarization with almond byproduct amendments to disinfest almond orchard soil during pre-plant processing and improve soil quality

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Soil biosolarization (SBS) is a soil disinfestation technology that combines solar heating and the accumulation of fermentative organic acids (OAs) from organic amendments to remediate infested soils. Soil microorganisms under anaerobic conditions consume available carbon from organic amendments, such as almond byproducts, accumulating OAs

under tarped soil. Additionally, SBS offsets the environmental, health, and safety risks of conventional soil fumigant application. The objectives of the study were: 1) to assess the ability of almond processing byproducts—almond hulls (ALH) and a mixture of hulls and shells (ALMix)—to induce microbial fermentation and OA accumulation; 2) to monitor soil temperature during SBS; 3) to assess the nematicidal effect of the treatment; and 4) to assess the impact on soil fertility. SBS was applied during the preplant phase of a commercial almond orchard field infested with root lesion nematodes (*Pratylenchus neglectus*). OAs produced in the soil and nematode mortality were quantified across different treatments (control, solarized non-amended, solarized with ALH, and solarized with ALMix) and soil depths throughout the duration of the field trial. Soil macro-phytonutrients (N, P, K) were also monitored. ALH-biosolarized soil, with higher OA levels, outperformed ALMix-amended and non-amended solarized plots, resulting in complete nematode inactivation after ten days of treatment. Nematode inactivation in the non-amended plots indicated that cumulative heating likely played a significant role in nematode deactivation. Following the treatment period, mineralization of N, P, K was observed in solarized plots. In the months following treatment, the conversion of ammonium to nitrate was observed in ALH- and ALMix-biosolarized soil. These results demonstrated that biosolarization using almond industry byproducts could effectively deactivate orchard soil pests on a commercial scale. Further studies are needed to monitor soil nematode reinfestation and the additional benefits of the organic amendment on the orchard performance.

AGRO 280

Residue analysis of thiametoxam and its metabolite clothianidin during cultivation of strawberry and tomato

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In order to elucidate residual characteristics of Thiametoxam and its metabolite Clothianidin by application to strawberry and tomato, and to generate the data for the maximum residue limit (MRL) establishment in an importing country for Thiametoxam, Thiametoxam WG (10% a.i) was applied onto tomato and strawberry according to its safe use guideline. Strawberry was drenched 2 times at 10 day intervals, and tomato was foliar sprayed 3 times at 7 day intervals. Strawberries and tomatoes were harvested after final application from pesticide-applied plots, respectively. The residue of Thiametoxam and its metabolite Clothianidin were analyzed using HPLC-UVD. The average recoveries of Thiametoxam and its metabolite Clothianidin at three different fortification levels ranged from 82.1 to 98.1% and the coefficient of variations (CV) was less than 10%. The residue range of Thiametoxam in strawberry was 0.04 ~ 0.16 mg/kg, and the residue was decreased to below MRL (1.0 mg/kg, KFDA) in 0 day harvest samples after final application. The residue range of Thiametoxam in tomato was 0.04 ~ 0.13 mg/kg, and the residue was decreased to below MRL (0.2 mg/kg, KFDA) in 3 day harvest samples after final application. The biological half-life of Thiametoxam was 24 days.

AGRO 281

Import tolerances in Taiwan procedure, challenges & progress

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Due to the limitation of land, TW food self-sufficiency ratio is only 31%, and we import plenty of crops for food; therefore, the Import Tolerance is quite important for us. Companies submit plenty of Import Tolerances cases every year, since the public pressue, authority pend/withdraw our cases. Croplife Taiwan, some associations, and companies are dedicated to communicating with authorities. Let's see the Requirements, Process, Issues, and Progress in TW during the years...

AGRO 282

Comparison of pesticide residues in Korea cabbage and shallot by morphological characteristics of plant

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It is important to understand the residual patterns of pesticides for ensuring the safety of agriculture products. In this study, residual characteristics of fungicide trifloxystrobin were investigated in leafy vegetables such as Korean cabbage and shallot which have different surface area and growth characteristics. Trifloxystrobin (50% WG) was diluted 4,000 times with water and sprayed 3 times at an interval of 7 days before harvesting. The pressure of spray was 18 psi; the quantity of spray was 1,300 ml/min and foliar spraying. Trifloxystrobin in Korean cabbage and shallot was analyzed using analytical methods established by Ministry of Food and Drug Safety in Korea. The residual amounts of trifloxystrobin and its metabolites were analyzed by LC-MS/MS. The recovery rates for residual analysis of trifloxystrobin spiked with 0.02 and 0.1 mg/kg in Korean cabbage and shallot were ranged from 82.2-101.3% and 90.4-101.9%, respectively, and less than 20% of the coefficient variation. The residual amounts of trifloxystrobin in Korean cabbage and shallot ranged from 3.347 to 19.306 mg/kg and 1.547 to 7.901 mg/kg respectively. Shallot grew up vertically and had a smooth surface, while Korean cabbage grew up with making a head and had a rough surface. Furthermore, for measuring the surface area, the harvested leaf of Korean cabbage and shallot was drawn in section paper and then calculated by the computer. The leaf area of Korean cabbage was 4 times higher than that of shallot with 95.665 pixels. Therefore, the morphological characteristics such as surface area seemed to play a key role in determining the trifloxystrobin residual characteristics in crops.

AGRO 283

Toxicity evaluation of combined contamination of herbicide and heavy metals on earthworms (*Eisenia fetida*) in urban soil

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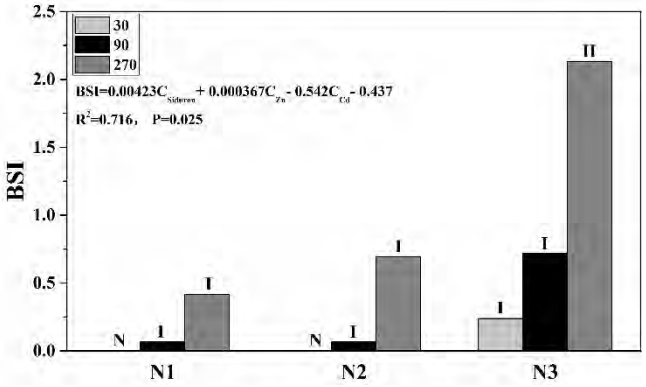
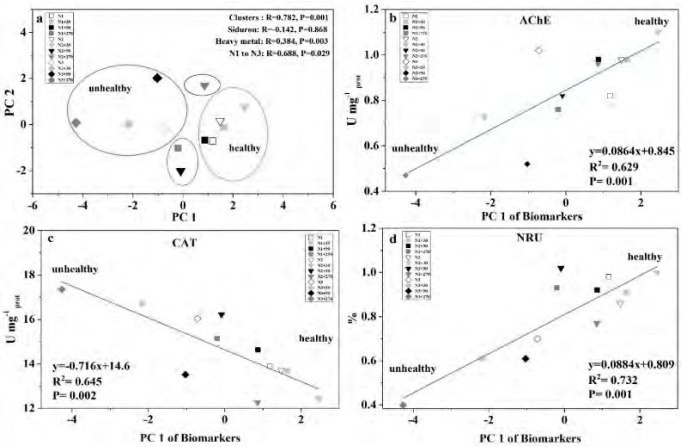
In terrestrial ecosystem, earthworms represent essential indicators for assessing the ecological risk of soil contamination and have gained increasing attention in the past few decades. In China, urban soil is heavily impacted by heavy metals and agrochemicals, such as herbicide (siduron), originating mainly from multiple sources including industrial and traffic emission as well as fertilizer application. To better understand the ecotoxicology of the combined stress between

siduron and heavy metals, especially in urban soils, in this study, earthworms (Eisenia fetida) were studied by measuring nine biochemical biomarkers after 14-day exposure to soils contaminated by herbicide and heavy metals. The results indicated that most earthworm biomarkers showed significant but irregular changes. Generally: (1) Synthetic stress (biomarker stress index) increased with the increasing concentrations of siduron and heavy metals; (2) Heavy metal contamination of soils contributed significantly more to the observed detrimental effects on earthworm health status than the stress of siduron; and (3) Activities of acetylcholine esterase (AChE) and catalase (CAT) and lysosomal membrane stability were identified as prognostic indicators for earthworm health status under combined pollution.



Integrated stress index of earthworm biomarkers (BSI) exposed to polymetallic soil



Multivariate analysis of earthworm biomarkers treated with polymetallic soil with siduron

AGRO 284

Field air SPME analysis of free-ranging giant pandas in Wolong nature reserve

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The elusive giant panda (*Ailuropoda melanoleuca*) conservation is a possible success story in the making. Habitat loss, population fragmentation, agriculture expansion, livestock grazing, road construction, and other distrubances have divided the wild population into isolate populations in six Chinese mountain ranges. Giant pandas use volatile chemical cues to determine identity, gender, and sexual receptivity of conspecifics across fragmented habitats. With isolated populations still at risk for extinction, novel methods for the detection of giant pandas would benefit conservation efforts. We hypothesized that free-ranging giant pandas have unique volatile chemical profiles detectable in air. Therefore, we aimed to collect volatile biogenic compounds produced by free-ranging giant pandas residing in the Wolong Nature Reserve (Sichuan, China) through field air analysis/solid phase microextraction (FAA/SPME). SPME fibers were secured approximately 1m above the ground to trees that appeared to have previous marking activity. Trail cameras were mounted across from the sampling location to confirm the presence of giant pandas. Volatile compounds were analyzed via gas chromatography mass spectrometry (GCMS). Trail cameras showed visits from masked palm civet, Asian badger, and giant panda. A single giant panda performed scent marking behavior at one of the sampling locations. The abundance of seven compounds were elevated in samples collected from the tree visited by the giant panda compared to the control. Three of these compounds (Ethane, 1,1-dinitro-; Octane, 4-ethyl-; 2(1H)-Pyridinethione, 3-ethoxy-6-methyl-) were only found in samples collected during the giant panda visit. Identification of the volatile compounds that free-ranging giant pandas use to communicate has the potential to provide valuable knowledge of population dynamics and communication across isolated populations.

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Ecological risk evaluation of combined pollution of herbicide siduron and heavy metals in soils

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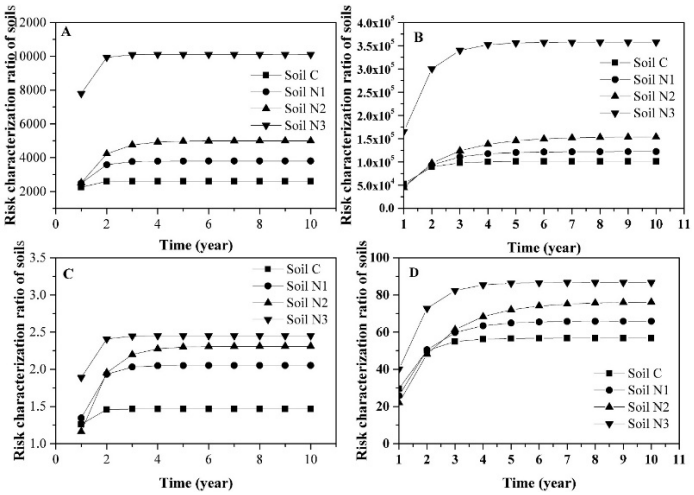
Combined pollution of agrichemicals and heavy metals were commonly observed worldwide, and growth concern about the issue led the administrations like EPA and European Union to require the consideration of mixture effects in environmental risk assessment. However, little has been reported on the ecological risk of combined pollution, especially in field due to lack of systematic methodology in assessing joint ecological effects and mechanistical data. In this study, mixture effects of herbicide siduron and heavy metals on mortality, DNA damage, gene expressions of metallothionein and heat shock protein70 in earthworm (*Eisenia fetida),* and cucumber root elongation were investigated and calculated based on single toxicology data of siduron and heavy metals using independent action and concentration addition model. Environmental behaviors of siduron in heavy metal contaminated soils were investigated with batch experiments in four soils (C, N1, N2, N3) from two public parks in Beijing, China, with similar properties but contrasting heavy metal contaminated level. Based on that the environmental exposure level of siduron was simulated with HYDRUS-1D model. Results suggested that soil organic matter rather than the co-existed heavy metals was the dominant factor affecting the fate and accumulation of siduron in soils. Then the predicted no effect concentration ($PNEC_{soil}$) of siduron was calculated with equilibrium partitioning method and extrapolation techniques. The $PNEC_{soil}$ of siduron derived from both root elongation and earthworm mortality was the lowest in the heaviest heavy metal contaminated soil N3. The risk characterization ratios (*RCR*) of siduron in four soils were all greater than 1. The highest RCR of siduron in soil N3 suggested that it was the joint toxicity of siduron and heavy metals to organisms determining the ecological risks of siduron in combined polluted soils.



Risk characterization ratioof siduron in four soils after 10 years' application under patterns of park & residential turf (A and C) and golf course greens (B and D).

AGRO 286

Food antibiotic residues in early life enantioselectively alter the murine gut microbiome and the immune response

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Antibiotics have been widely applied to the crops to prevent infectious diseases, yet the impacts of their residues from plant sources on gut microbiome have been rarely appreciated in the present mammalian-centric risk assessment. Because the crop's antibiotics that exhibit broad spectrum activity can be also effective as human antibiotics in clinical usage, given the fact that the crop's antibiotic residues exposure in humans is at daily basis, we proposed that such long-term administration alters the gut microbiota as well as its physiological parameters such as immune responses. To make an accurate assessment, we also included the charality of antibiotics in the study to address the possible enantioselective effects. We exposed the young female rats to the most widely used fungicide, metalaxyl that has one chiral center, at an environmentally relevant dosage and evaluated changes in the gut microbiota composition and the immune responses. Administration of environmentally relevant metalaxyl enantioselectively triggered the exaggerated basophil-associated TH2 cell responses that could implicate allergic inflammation. We observed enantioselectively profound composition changes in the gut microbiota that are associated with the changes in the cytokines profiles, serum IgE levels, hematology parameters. In this study, we found those immune response changes that may be deployed through collateral damage on the host inhabiting microbiota by the crop's antibiotic food residue, and so it is important to include the long-overlooked microbiome as a centerpiece in the next generation of risk assessment to make appropriate MRLs ensuring human health.

AGRO 287

Systemic stereoselectivity bioactivity study of chiral fungicide prothioconazole and its metabolite in agricultural management

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Chiral triazole fungicide has played an important role in pathogen therapy with the enantiomers often exhibiting different stereoselective environmental behavior, biological activity, and toxicity. Stereoselectivity degradation and bioactivity of prothioconazole and its chiral metabolite (prothioconazole-desthio) enantiomers in agricultural systems and theoretical evidence were investigated. The result of field experiment suggested that *R*-prothioconazole was metabolized to *R*-prothioconazole-desthio. There was remarkable stereoselectivity biological activity with *R*-enantiomer of prothioconazole and prothioconazole-desthio was 5.9-260 and 42-957 times more potent than the *S*-enantiomer, respectively. Furthermore, *R*-enantiomers inhibited biosynthesis of ergosterol and deoxynivalenol more effective than *S*-enantiomer for prothioconazole and its metabolite. Prothioconazole and its metabolite enantiomers exposure significantly changed the transcription levels of ergosterol (*CYP 51A, CYP51B, CYP 51C*) and deoxynivalenol (*Tri4, Tri5, Tri6, Tri12*) biosynthesis genes. Homology modeling and molecular docking studies suggested the distinct bioactivities of the enantiomers of prothioconazole and prothioconazole-desthio were probably due to their different binding modes and affinities to *CYP* 51b. The results showed that application of pure *R*-prothioconazole, with its high bioactivity and relatively low resistance risk, instead of the racemate in agricultural management would reduce the application dosage required to eliminate carcinogenic vomitoxin and any environmental risks associated with this fungicide, yielding benefits in food safety and environmental protection.

AGRO 288

Chiral amide herbicide metolachlor: Enantioseparation, stereoselective bioactivity and environmental behavior

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Metolachlor posses two chiral elements, an asymmetric carbon stom and a hindered rotation chiral axis, it can exist in four stereoisomeric forms (*αS,1′S- /αR,1′R-* and *αS,1′R-/αR,1′S*-metolachlor (*αSS-, αRR-, αSR-* and *αRS*-metolachlor for short). Metolachlor was introduced to the market in 1976 containing a mixture of all four metolachlor stereoisomers and in 1982, Moser et al. found that *S*-metolachlor (with respect to the C chiral center, including *αSS-* and *αRS*-metolachlor) showed the highest herbicidal activity. Nowadays, metolacholr and *S*-metolachlor are still widely used as the main amide herbicides in many countries and regions. Fist of all, we established a semi-preparative separation method of metolachlor by HPLC-PAD, *Rac*-metolachlor was first separated on a Ultimate Amy-S column to get baseline separated of *S*-metolachlor and other two stereoisomers and then the *S*-metolachlor was separated on a Chiralcel OD-H column. The four separated stereoisomers had purity of ≥99% and their absolute configuration were characterized by electronic circular dichroism. The enantioseparation of metolachlor by UPC2-MS/MS with a chiral AMY1 column was also established for fast and high sensitivity detection. The enantioselective bioactivity toward weed (*Echinochloa crusgalli*) was assessed by measuring the growth rates after the treatments with metolachlor stereoisomers, *S*-metolachlor and racmate. The order of the bioactivity to weed was found to be: *S>αSS>αRS>rac>αSR>αRR*. Investigations on the stereoselective degradation of metolachlor in rice field soil showed that highest-bioactive *αSS* stereoisomer display slower degradation rate than its enantiomers, the EF trended to increase little during 35 days of soil culture. Moreover, metolachlor was configurationally stable in the test soil matrices. This is the first time to study the differences of four stereoisomers, on the base of biological activity and environmrntal behavior, it is likely that the use of *S*-metolachlor instead of the racmate help to increase the bioactivity and will not increase the environmental pollution.

AGRO 289

Differences between C-chiral enantiomers and axial-chiral enantiomers on enantiomeric separation

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Chirality can be found in amide pesticides commonly. Hindered rotation about the phenyl-nitrogen bond and asymmetrically substituted C- atom in the alkyl moiety contribute to the chirality in these compounds. At present, all chiral amide pesticides are used in their racemic forms. However, stereoisomers usually differ in their pesticidal activity. Over the last few years, information to address the enantioseparation and enantioselective activity of these herbicides is limited, let alone the comparisons of them. This study applied metolachlor, acetochlor, and napropamide as the represents of chiral pesticides with different of chirality to make deeper understanding of the differences between canbon-chirality and axial-chirality on enantioseparation. It appeared that AY-H column and AS-H column were better for separation of metolachlor and acetochlor, which have axial-chirality in their molecules. Moreover, metolachlor and acetochlor could obtain satisfactory separation only on one column, while napropamide could achieve baseline separation on all columns expect OD-H column, indicating carbon-chirality only compounds are easier to separate. IPA provided higher resolution in the carbon-chirality herbicide napropamide, while EtOH in metolachlor and acetochlor. Generally speaking, our findings suggested that carbon-chiral herbicides and axial-chiral herbicides were different in their enantioseparation, which may be related to their distinct structures and their special interactions with CSPs on the chiral columns.

AGRO 290

Methods for improving chiral HPLC separation of agrochemicals that are present as multiple isomers in biological, soil and water/sediment matrices

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Many of the agrochemicals used in industry today have chiral properties, and oftentimes they are manufactured as a mixture of isomers; these isomers may present different stereoselectivities in environmental fate, toxicity, and even efficacy against the target species. A renewed emphasis has been placed by the regulators on characterizing the chiral components of a given agrochemical. When determining their biodegradation rate and pathway in environmental fate or metabolism studies, it can be challenging to characterize each isomer and quantitate their relative amounts, especially when unresolvable by reverse-phase HPLC. It may also be important to investigate potential isomerization during the course of a metabolism study, where the parent molecule can shift to a more stable configuration. Thus, normal-phase HPLC is typically used as a secondary chromatographic method to track each isomer individually. However, separation of all isomeric configurations by chiral HPLC may present challenges especially when analyzing soil, plant, and animal extracts. Several parameters, such as duration of column equilibration, sample diluent type, matrix interference, and injection volumes will be tested to determine their influence on separation of chiral components. A review of some sample preparation methods will be presented. In addition, an attempt at a 2-dimensional HPLC method will be presented, where reverse- and normal-phase HPLC will be used in tandem to increase efficiency.

AGRO 291

Modeling of nectar requirements for nectar foraging honey bees (*Apis mellifera*)

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The U.S. Environmental Protection Agency, Canadian Pest Management Regulatory Agency, and California Department of Pesticide Regulations developed a probabilistic model in 2012 to estimate nectar requirements of nectar foraging honey bees. The model is based on sugar requirements and nectar content, trip frequency, duration, and the fraction of trips spent flying. The median estimate from model simulations of 292 mg nectar/bee/day is endorsed in the Agencies' 2014 '*Guidance for Assessing Risks to Bees*.' The model inputs were based on limited data, and the structure allowed for some implausible results. To address these issues, recently, a literature review was conducted to collate quantitative information relating to nectar forager ingestion of nectar. These data were used in a simpler but comparable model, based on time spent outside the hive and recent radio-frequency tagging identification data from workers. This model, and its assumptions are presented. We compare results with a reproduction of the Agencies' model, with updated inputs based on data gathered. Sensitivity and correlation analyses are presented which call attention to the most important elements of the models. Suggestions are provided to enhance model inputs and improve exposure assessment for honey bees.

AGRO 292

Monitoring brood development in honeybee colonies: Which eggs to select and how many?

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It is often desirable to those who study honeybees to track the development of the brood; that is, the progress of development from egg to larvae to pupae and finally to adult emergence, as a sign of colony health. However, monitoring the developmental progression of worker brood in controlled experimental conditions in the field has proven to be extremely challenging, probably because the measurement techniques employed (so far) negatively and unpredictably affect that which is being measured. This presentation reviews untreated control and reference item data on brood development collected in recent studies conducted under the guidance of the Organization for Economic Cooperation and Development Document No. 75 (OECD 75) for consideration in pesticide registration actions. This guidance does not provide clear instructions nor a validated methodology for the unbiased, representative and statistically robust selection of eggs to be tracked in the monitoring of brood development, a remarkable omission given the ostensible purpose of the guidance document is the determination of effects on brood development. The data presented is derived from a study in which all of the eggs, young larvae, and old larvae present in all photographed honeycomb frames were tracked through development of two brood cycles with one cycle started during tunnel confinement and the other started just after confinement ended. The exhaustive tracking of all eggs and larvae was conducted to eliminate potential for bias in the OECD 75-recommended selection of a small subset of eggs (minimum 100) and to investigate the effect of frame location within the hive on brood developmental success. Recommendations for unbiased and statistically robust egg selection methods are proposed to improve the performance and reliability of the OECD 75 methodology.

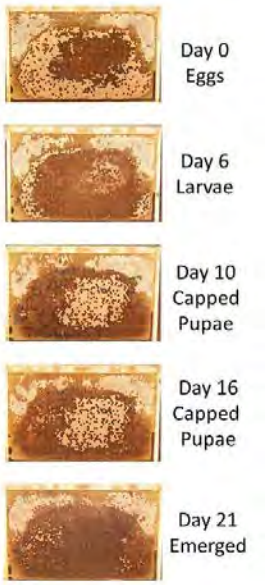

Figure 1 Brood development in honeybees tracked in 5 steps.

AGRO 293

LC-MS/MS method for estimating the exposure to neonicotinoid residues in pollinator attractive habitat adjacent to corn and soybean fields

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Recent research has suggested that neonicotinoid insecticides applied to crops can be detected in adjacent pollinator-attractive habitats and pollen collected by honey bees. Honey bees, native bees, and monarchs could be exposed to neonicotinoids through ingestion of contaminated pollen, nectar, and milkweed leaves. Although these studies indicate that neonicotinoids can be detected in pollinator-attractive habitats, the magnitude and extent of potential adverse effects to honey bees, native bees and monarch larvae is an active area of research. In this study, we developed and validated an innovative method to simultaneously evaluate concentrations of clothianidin, imidacloprid, and thiamethoxam, and two imidacloprid metabolites (5-hydroxy imidacloprid and imidacloprid olefin) in plant foliage. The results indicate acceptable ranges for the recovery, low and high quality control (between 75% and 110%), while the matrix effect indicates minimal ion suppression or ion enhancement for all analytes. The calibration curves were linear over the concentration ranges with r^2 at > 0.998. The data obtained established a validated, single extraction and LC-MS/MS analytical method for quantifying neonicotinoid concentrations to a method detection limit of 0.04 to 0.3 ng/g plant material. This method is comparable or in some cases better than existing methods that require separate extraction and/or LC-MS/MS methods. We are using the method to analyze leaf and pollen samples collected in 2017 from habitat patches adjacent to corn and soybean planted with neonicotinoid-treated seeds. This data will provide insights on spatio-temporal variability of neonicotinoid concentrations in Midwestern agroecosystems and help inform pollinator risk assessments.

AGRO 294

Gut symbiont viability of honey bees exposed to chemical stressors

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The honey bee gut microbiome is essential for the development of the immune system protecting this pollinator against gut parasites and pathogens. Dysbacteriosis of the gut is reported to cause immunodeficiencies and increased pathogen sensitivities, but little is known about their development. Few techniques are available to measure the development of a dysbiosis as it requires the rapid analysis of live and dead proportions of gut microbes. Previous studies require days or weeks for the digestion and excretion of dsDNA from non-viable microbes, by the host, to measure changes in relative abundance from DNA sequencing or qPCR[3-4]. Here, we will report a propidium monoazide (PMA)-based technique to characterize gut microbiome dysbiosis within 1 hr of treatment. After verifying this method using axenic *Drosophila melanogaster* inoculated with proportions of live/dead *Escherichia coli*, the antibiotic- and fungicide-mediated dysbiosis of the bee gut microbiome were measured. Gut microbes were significantly reduced in bees treated with oxytetracycline (55%), tylosin (74%), and chlorothalonil (40%) compared to the non-treated bees. The data reported here supports previous studies that demonstrated agrochemical effects on the bee gut microbiome. While this study focused on foraging bees, a dysbiosis in the initial stages of adult life (nurse bees) from exposure to agrochemicals or apicultural medications present in wax matrices, or from treatment, may be detrimental to the development of the host's immune system. Therefore, it is important to understand the agents that cause these depauperations, as well as effects on gut microbiome diversity, to help reduce or avoid dysbiosis, thereby improving the health of bees and their colonies. This PMA-based technique can be used to quickly identify chemistries that cause changes in gut bacteria viability as well as beneficial genera when coupled with DNA sequencing technologies, making it a powerful tool for studying microbes in insects.

AGRO 295

Fate and transport of brominated estradiols as surrogates for native 17ß-estradiol in an agricultural field

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Estrogens are steroid hormones eliminated from nearly all animals at reasonably high levels, and when released into the environment they can act as endocrine disrupting compounds,particularly to aquatic organisms. Tracing the movement of estrogens from animal waste to impacted waters is complicated by historical estrogen deposits in soils, as well as their uncontrolled introduction by wildlife. Tracer studies can be conveniently performed with radiochemicals; however, these are not suitable for field research. Stable brominated analogs of estrogens may prove a fruitful method to study the movement of these environmental contaminants in the field. Thus, the aim of this study was to synthesize, characterize, and introduce brominated 17ß-estradiol (Br-E2) analogs into a field setting and monitor the analog movement in soils. Two monobromo and one dibromo 17ß-estradiol analogs were successfully synthesized and introduced into the surface soil layer of agricultural plots (3 control, no Br-E2; 3 treated with Br-E2; 3 x 3 m) in North Dakota. Corn was

planted on the plots, and the plots were constructed with lysimeters 0.6 m below the surface to capture leachate water during the 2017 growing season. Due to drought conditions in the area, which lead to only a limited number of lysimeter samples, soil cores were also taken at the conclusion of the growing season. The cores were divided into 0.5 m sections and were extracted with water and acetone. Lysimeter water samples and soil extracts were analyzed by TTOF/LC-MS with standards. Downgradient movement of brominated estradiol analogs was quantified, and conversion to metabolites and degradates was determined. Results will be compared to those obtained previously with radiolabeled analogs in laboratory-scale batch studies in the same soil.

AGRO 296

Use of solid phase microextraction (SPME) in assessing volatility in agrochemical discovery lead optimization

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Pesticide drift, which is the off-target movement of pesticides, is recognized as a major cause of pesticide exposure affecting people as well as wildlife and the environment. During agrochemical discovery lead optimization, it is important to assess the vapor movement for analogs in lab-greenhouse and to better predict possible off-target movement for field candidates with potential volatility concern in field studies. Solid phase microextraction (SPME) is one of the most important solvent-free methods of sample preparation innovative techniques – simple, fast and efficient for organic compounds from gaseous, aqueous, and solid matrices. SPME method has been developed and applied in several Discovery insecticide and herbicide lead areas to assess volatilities for analogs and formulations along with bioassays. This poster will focus on applying SPME to a Discovery Herbicide lead area to assess the impact of different formulations on the volatility of sprayed moist and dry soils and on the volatility of sprayed glass plates on indicator plants. The results showed SPME is a simple and useful tool to identify potential volatility issues in discovery lead optimization.

AGRO 297

Spatial variability of DDT in aged contaminated soil and its bioavailability to indigenous earthworms

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Dichlorodiphenyltrichloroethane (DDT), an organochlorine insecticide with known detrimental environmental effects, was banned in the United States in 1972. However, the repeated applications of DDT and its persistence and hydrophobicity have given rise to highly-contaminated soils resulting in bioaccumulation in the local food chain and a concern for converted historical orchards. A primary exposure route of DDX (DDX = DDT and its degradation products) for indigenous fauna is ingestion of earthworms. Assessing the risk of DDX exposure requires calculation of the bioaccumulation factor (BAF) of the earthworms consuming the soil. Although BAF have been extensively investigated in the laboratory, field studies are lacking. Thus, the goals of this study are to investigate the spatial variability of DDX in soil and earthworms, to quantify the changes in BAF of DDX, to evaluate the factors influencing BAF, to establish a model that can predict DDX concentration in earthworm using soil concentrations, and to optimize a soil sampling strategy to assess ecological risk. A field study was conducted on a 1.2-ha field historical orchard where DDT was heavily applied. The soil was amended with compost to decrease DDX bioavailability. Control pots of soil (no compost) were placed in a nearby field. Soil and earthworms were collected at 53 sampling points on a by 15-m x 15-m grid before compost was applied (time = 0 days) and in the spring and the fall for several years following treatment. Soil properties and the DDX concentrations in soil and earthworms were determined. Geostatistical analyses were performed to characterize DDX variability and its spatial distribution. Preliminary results showed that the concentration of DDX ranged from 0.1 to 11 µg/g dry weight soil. A spherical model was used to fit the experimental variogram. Leave-one-out-cross-validation showed a mean error of -0.005 and mean square deviation ratio of 1.008. Results of this work will be useful to land managers developing remediation strategies for contaminated soils.

AGRO 298

Estimation of 1,3-dichloropropene flux by application method under California use conditions using HYDRUS 2-D

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In agricultural field fumigations, emission ratios (ERs) describe the proportion of applied material emitted from the soil surface in the days and weeks following application. ERs vary in response to factors including fumigant material, environmental conditions, and application mitigation measures. In California, ERs are used to inform regulatory decisions regarding buffer zone distances and are an important input in air dispersion models. Field estimates of ER are important but can only provide a limited snapshot of ER for a particular method under a specific set of environmental conditions, and field estimates of ER are limited or non-existent for a majority of methods. This study simulates ERs for 16 out of 18 of methods approved under California permit conditions for the fumigant 1,3-Dichloropropene. The vadose zone model HYDRUS 2-D is used to simulate flux from the soil surface for a period of up to 11 days following completion of application. Each application method is simulated across a soils dataset consisting of 15 agricultural soil types sampled from prepared fields prior to fumigation. Distributions of simulated ERs appear to correspond with literature-reported estimates where available. The results indicate nearly an order-of-magnitude difference in mean ER between the least- and most-emissive application methods with sometimes substantial within-method variation due to soil characteristics.

AGRO 299

Occurrence of antibiotics and antibiotic resistant genes in cow manure-fertilized *Zea mays*

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Zea mays (maize) seeds were planted under three different conditions: soil, manure-amended soil, and spiked manure-amended soil, where the manure had been fortified with antibiotics, in order to explore the occurrence of antibiotics and antibiotic resistant genes (ARGs). The *Zea mays* was harvested after three weeks to determine the uptake of antibiotics, and whether gene transfer had occurred. The study found that both tetracycline and sulfamerazine bioaccumulated in the *Zea mays*. Additionally, tetO and OXA-1 were detected in the soil, only after manure application, and no ARGs were detected in the plants. Maize grown using manure-amended soil were found to uptake a wider variety of sulfonamides and tetracyclines than those grown in soil only. Variable uptake factors were seen across the chosen

timepoints. Targeted resistant genes were found in the manure-amended soils but were not present in the soil control. ARGs were not detected in maize grown for three weeks in manure-amended soil. Future research is warranted to study the possible uptake of antimicrobials in maize grown under field scale conditions.

AGRO 300

Using geospatial techniques for effective product stewardship

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The goal of this project was to identify agricultural soils reflected in the acetochlor label use restriction, which overlap with shallow ground water. Acetochlor-based products are labeled for use within the United States to control annual grasses and certain broadleaf weeds and can be applied preplant, at-planting, preemergence, and/or postemergence to labeled crops. Acetochlor product labels restrict applications within 50 feet of any well where depth to ground water is ≤ 30 ft. The Acetochlor Registration Partnership (ARP - Dow AgroSciences and Monsanto Company) developed voluntary Best Management Practices (BMPs) for acetochlor to reduce the potential for the active substance and its major environmental metabolites in ground water and surface water, following application to agricultural fields. The ARP offers a website (www.arpinfo.com) with resources for end-users to help ensure the effective use and stewardship of products containing acetochlor. Depth to ground water information is often not readily available, which makes it challenging to depict the spatial distribution of areas where the use restriction should be observed. One can point to privacy concerns as a reason for the lack of ground water depth information available to the public. An assessment was conducted to identify crop lands with potential acetochlor use restrictions in Arizona, using geospatial techniques. Arizona's Department of Water Resources provides historical ground water depth data for point locations via a public monitoring database (http://www.azwater.gov/azdwr/GIS/). Groundwater depth information was extracted from the monitoring database for the period 1995 to 2015. As a data handling procedure, outliers were removed, then cluster and hotspot analyses were performed. The final dataset contained over 12,000-point observations of ground water depth for a 20-year period. Long-term average depths were calculated for each location. To generate a state-wide average groundwater depth map, a spatial interpolation technique was applied to the GIS vector or point dataset. The final ground water map was overlaid with National Agricultural Statistics Service (NASS) Cropland Data Layer (CDL) data to identify agricultural soils with potential concerns. For cotton, the assessment showed that just 3% of the agricultural areas overlap with shallow ground water.

AGRO 301

Degradation studies: Solvent systems including both polar and nonpolar solvents to extract residues from soil matrix

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As reflected in the EPA Environmental Fate and Effects Division (EFED) guidance for addressing unextracted residues in laboratory studies, residues that are weakly sorbed to solid matrix and thus available for degradation, should be adequately extracted in order to quantify the degradation and to avoid double-counting their sorption in exposure models. Additionally, transformation products may be less polar or more polar than the parent compound and may interact with the soil or sediment differently. Therefore, soil extraction regimes should include both polar and nonpolar solvents or solvent mixtures with a sufficiently wide range of chemical properties. At Innovative Environmental Services (IES) Ltd., Switzerland, an independent GLP-certified contract research organization, several GLP degradation studies have been conducted, for which soil extraction regimes included additional solvents in order to cover a wide solvent polarity range. The objective of this poster will be the presentation of soil extraction results for several degradation studies, possibly accompanied by correlation to the chemical characteristics of test items and transformation products.

AGRO 302

Improved extraction techniques for regulatory metabolism studies of agrochemicals

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For regulatory metabolism studies of agrochemicals in plants, animals and soil, characterization of residues in the sample from the test substance is of interest. Matrices are typically characterized using extraction techniques to solubilize the residues. Selection of extraction methods improving extraction recovery contributes a qualified study of the nature of residues for the agrochemical regulation. The solid samples for this test were pulverized wheat forage grown in soil applied with a radioactive ^{14}C-agrochemical. The samples were extracted with acetonitrile and water using three different instrumental extraction methods: Ultra-Turrax® homogenizer, Geno/Grinder® homogenizer, and conventional Wrist-action® Shaker. Data will be included that shows extractability of sample with Geno/Grinder is influenced by number and size of stainless steel balls used in the extraction process. EAG Laboratories-Hercules will present a comparison of these extraction techniques in terms of extractability, extraction time, laboratory cost, and high-throughput sample preparation approaches.

AGRO 303

Predicting environmental fate of agrochemicals in irradiated water-sediment systems

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The current regulatory requirements for environmental fate include aqueous photolysis and aerobic aquatic studies to evaluate degradation of agrochemicals in aquatic systems. For some test compounds, these studies can result in very different degradation rates, usually a short photolytic half-life in the irradiated water study and a long half-life in the dark water/sediment study. The current EU 2009/1107 guidance allows for the submission of irradiated water/sediment studies to address the issue of a long water/sediment half-life. As this study type is relatively new, no definitive study design has been written in guidance documents. This poster will describe one possible design of an irradiated water/sediment study as well as explore how multiple degradation processes, including photolysis, hydrolysis, microbial degradation, and adsorption, interact within controlled test systems/environments. Understanding how these processes interconnect allows for prediction of how a compound will behave in an aquatic environment to support the environmental safety assessment.

AGRO 304

Bioconcentration factor-based soil management guideline through uptake pattern of pesticide by radish

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Most of applied pesticides to crops often remain as residues in agricultural soils, a portion of which may be taken up by cultivated crops. However, the criteira such as MRL (maximum residue level) and safe use guidelines for pesticides, which are applied to ensure the safety of crops against pesticides, do not consider impacts of soil pesticide residues on crop contaminant concentrations. Thus, more detailed criteria were suggested through the plant uptake experiment of soil residue pesticide. The residues of Endosulfan(ED) which was used as an insecticide were analyzed, and soils treated with ED at concentrations of either 2 or 10 mg/kg in the radish were grown in such soils for 40-90 d. The residue amount of ED in soils and radish separated into shoot and root, and parts were analyzed using GC-MS. The sum of ED isomer and metabolites which taken up to radish shoot and root for 90 d from soil were 0.01-0.40 and 0.02-2.20 mg/kg, respectively. Also, distribution rates of ED-sulfate in radishes were the highest with 45.5%, followed by α- and β-ED. The results of uptake pattern were used to calculate the bioconcentraction factor (BCF), indicating the ratio of ED concentrations between radishes and soils. The BCF values to initial ED concentrations in soils were greater for root parts (0.0077 to 0.2345) than for shoot parts (0.0002 to 0.0429) and used to obtain regression equations by time. Long-term BCFs estimated by the obtained equations (R^2 of 0.86-1.00) were evaluated with the MRL (0.1 mg/kg) of ED for radish. Consequently, the safe management guidelines for ED in radish-cultivating soils can be suggested as 1.8 mg/kg considering the difference in crop species and soil properties, and the experimental error.

AGRO 305

Global aspects and demands on cooperation with a CRO

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Working with CROs has a lot of advantages. Outsourcing studies saves resources of the sponsor and allows companies to register their compounds even if they do not have their own labs. However, requirements for pesticide registration are increasing and consequently, the demands on studies increase as well. More efficient compounds with lower field rates challenge the analytical possibilities. In parallel more details have to be considered, new study types have to be performed, and opinions of authorities differ between (European) countries. All those aspects generate an increasing effort for registrants. But, this pressure is also perceived by the labs hired to perform high quality studies. By running studies in a routine way, a highly efficient study conduct is achieved. However, especially for efate and metabolism studies, this kind of study conduct is not suitable to deliver high quality studies. For those studies a lot of understanding and experience is required. Another demand is that the distance between authorities and the study conducting lab is big. Changing opinions and view of authorities are not directly visible to the CRO. That information is available only after publishing either at conferences or in journals and websites. A close cooperation between sponsor and CRO is essential to close those gaps, whereas the type of cooperation depends on the individual situation. Professional project management is important to warrant that all parties are at the same level of knowledge for a given compound. This level of knowledge is as better as earlier and as more extensive the CRO is involved in processes and projects. The poster presentation summarizes the demands but also the possibilities from the point of view of a CRO.

AGRO 306

Use of microtransplanted rat brain tissue in *Xenopus* oocytes to determine the toxicodynamic differences of pyrethroids on sodium channel isoforms in juvenile and adult mammalian brains

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Microtransplantation of mammalian neurolemma is a tool to examine the endogenous structure and function of transmitter receptors and ion channels associated with the central nervous system. Microtransplanted neurolemma can originate from a variety of sources, possess ion channels and receptors in their native configuration, and are applicable to examine diseases associated with different channelpathies. In these experiments, we examined the age-related differences in VSSC expression and concentration-dependent responses to pyrethroids in juvenile and adult rat brain tissue microtransplanted in *Xenopus* oocytes. Automated western blotting results indicate that adult neurolemma exhibited 2.5-fold higher level of expression of VSSCs compared with juvenile neurolemma when normalized to the housekeeping protein β-tubulin. The predominate isoform expressed in both tissues was $Na_v1.2$ with both showing a significant difference from zero. Adult neurolemma, however, expressed 2.8-fold more $Na_v1.2$ than juvenile and also express $Na_v1.6$ at a higher level (2.2-fold). In addition, neurolemma tissue microtransplanted into *Xenopus* oocytes showed reconstituted native ion currents in the plasma membrane of oocytes that was sensitive to TTX and abolished by choline ion replacement, functionally demonstrating the presence of VSSC. Increasing concentrations of permethrin and deltamethrin exhibited concentration-dependent increases in TTX-sensitive current from both adult and juvenile tissues. Concentration-dependent response curves were analyzed using the equivalence test and the slopes of the curves were different ($p < 0.05$). VSSCs associated with juvenile neurolemma was up to 2.5X more sensitive to deltamethrin than VSSCs in adult neurolemma. In contrast, VSSCs from juvenile neurolemma were less sensitive than adult VSSSs at lower concentrations (0.6-0.8X) and more sensitive at higher concentrations (up to 2.4X). However, because the expected brain concentrations in humans following realistic exposure levels are approximately 21- (deltamethrin) to 333- (permethrin) times below the threshold for response in rat neurolemma, age-related differences, if any, are not likely to be toxicologically relevant.

AGRO 307

Novel target for insecticide design: Mechanistic and structural analysis of arylalkylamine *N*-acetyltransferase from the red flour beetle

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The red flour beetle (*Tribolium castaneum*) is one of the most common secondary pests of all grain commodities in the

world. It has been held responsible for up to 20% of all grain storage contamination in the U.S., with this number rising to up to 50% in third world countries. This large-scale destruction results in substantial economic damage due to loss of the market price of product and decreased nutritional value. So far, against an arsenal of chemical species, this insect has avoided all attempts of control. One possible route to develop a novel type of insecticide for *T. castaneum* is based on the study of arylalkylamine *N*-acyltransferases (AANATs). These promiscuous enzymes (members of the GCN5-related *N*-acetyltransferase (GNAT) family) catalyze the *N*-acylation of biogenic amines to form *N*-acylarylalkylamines, and are vital to *T. castaneum* as they are involved in melanism, biogenic amine excretion, as well as hardening of the cuticle. In addition, the *N*-acylarylalkylamines have been suggested to act as potent neurotransmitters. For example, the acylation of serotonin is the rate limiting step in circadian rhythm. An AANAT-targeted insecticide would not only disrupt neural signaling, but would also inhibit cuticle development, decrease the structural stability, result in a non-conforming appearance (harming the insects' ability to mate), and diminish the cuticle-mediated protection against injury and infection. Presented here is a mechanistic analysis, which was determined to catalyze the formation of short-chain *N*-acylarylalkylamines, with acetyl CoA functioning in the role of acyl-donor via an ordered sequential mechanism. Also presented is the first crystal structure of AANAT from *T. castaneum*, bound to acetyl CoA. These combined data not only give new insight into the chemical mechanism behind this family of enzymes, but also confirms the hypothesis that despite high structural homology between AANATs from different organisms, a low sequence homology means insecticides could potentially be tailor-made to attack a particular insect, while quelling damage on other organisms.

AGRO 308

Sulfoximine derivative, sulfoxaflor, activates imidacloprid-sensitive nicotinic acetylcholine receptors on insect neurosecretory cells

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Sulfoxaflor is a recently developed sulfoximine drivative which was effective against sap-feeding insect and interacts with insect nicotinic acetylcholine receptors. We have demonstrated that sulfoxaflor acts as an agonist of cockroach nicotinic acetylcholine subtypes expressed on dorsal unpaired median neurons. Specifically, sulfoxaflor is able to activate the imidacloprid-sensitive nAChR1 and is ineffective on nAChR2 subtype. Pretreatment of DUM neurons with 1 µM imidacloprid displaces the current-voltage curve. Interestingly, mecamylamine a nAChR antagonist completely inhibited sulfoxaflor-induced currents whereas a-bungarotoxin partially reduced sulfoxaflor evoked currents. Our data are consistent with the finding that sulfoxaflor activates additional currents not evoked by imidacloprid.

AGRO 309

Photochromic imidacloprid for optical control of insecticide performance

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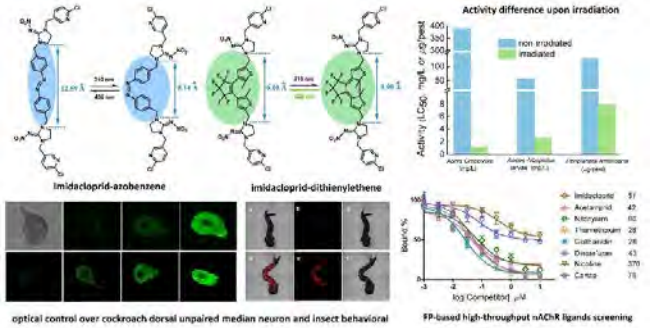
Photopharmacological ligands provide a powerful way for optical control of the activity of a molecule, which will facilitate better understanding of toxicological mechanism. By the blending of photoswitchable azobenzene or dithienylethene with a nAChR agonist imidacloprid, we prepared a series of photochromic ligands (PCLs) acting on insect nAChRs. Some PCLs showed different insecticidal activity before and after light irradiation. These PCLs were then used to optically control over the insect nAChRs, cockroach dorsal unpaired median neuron and insect behavioral responses of mosquito larvae (*Aedes albopictus*) and cockroach (*Periplaneta americana*). Unprecedentedly, photoswitchable imidacloprid-dithienylethene can also be used as an indicator for fluorescent polarization (FP) based high-throughput ligands screening.



Photoswitchable imidacloprid analogues

AGRO 310

Design of selective anti-juvenile hormone agents based on the structural analysis of apo, ligand-, and inhibitor-bound type II FPPS of the spruce budworm

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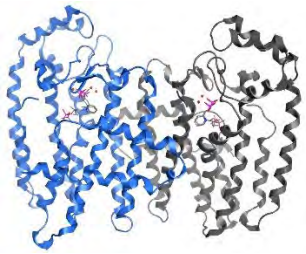
Farnesyl diphosphate synthase (FPPS) is a short chain (*E*)-prenyltransferase that catalyzes the condensation of two molecules of isopentenyl diphosphate (IPP) with dimethylallyl diphosphate to generate the C15 product FPP. In insects, FPPS plays a key role in the biosynthesis of juvenile hormone (JH), and in lepidopteran insects, a type-II FPPS (FPPS2) is responsible for the biosynthesis of homologous JH structures. Here, we present crystal structures of apo, ligand-, and inhibitor-bound structures of FPPS2 from the spruce budworm, *Choristoneura fumiferana* (Order: Lepidoptera). *Ortho*-substituted pyridinium bisphosphonates, previously shown to inhibit *Cf*FPPS2, bound to the allylic site as predicted; however, their alkyl groups were oriented towards the homoallylic binding site, with the bulkier propyl-substituted inhibitor, a selective inhibitor of *Cf*FPPS2, penetrating deeply into the IPP binding pocket. These results suggest a new approach for the design of selective anti-JH agents, as inhibition of enzymes involved in JH biosynthesis (such as FPPS) can have a profound impact on insect maturation and viability. Active site analysis of *Cf*FPPS2 was conducted along with a comparison to other inhibitor-bound FPPSs, from which we propose several approaches to improve FPPS2 inhibitor selectivity and potency. The results of these studies will be presented.



AGRO 311

Phytochemical synergists: enhancing pyrethroids with natural plant compounds

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Mosquito-borne disease accounts for the death of approximately 700,000 people annually throughout the world, with many more succumbing to the debilitating side effects associated with these diseases. Moreover, this is exacerbated in many countries where the lack of mosquito control resources and the lack of methods to treat and prevent human and veterinary disease coincide. As wild mosquito populations grow more resistant to currently utilized control chemistries, the need for cheap and effective novel chemical means for vector control are more important than ever. Previous work in our laboratory has demonstrated that plant essential oils and terpenoids are capable of enhancing the toxicity of permethrin against multiple mosquito species that are of particular importance as public health vectors. Our hypothesis is that plant essential oils are capable of enhancing and synergizing diverse insecticides utilized for the control of insect vectors. To test this, our group has screened numerous plant essential oils in combination with various pyrethroids against both pyrethroid-susceptible and pyrethroid-resistant strains of mosquitoes. A number of plant essential oils were shown to significantly synergize pyrethroids to a greater extent than piperonyl butoxide in these studies. To explore the mechanism of this enhancement, we assessed the degree to which plant essential oils inhibited various detoxification enzyme processes. We demonstrated that select plant essential oils significantly inhibited both glutathione s-trasnferase and cytochromes P450 enzyme activity in this study. Our work highlights the potential of plant essential oils as efficacious additives in future insecticidal formulations and illustrates their potential to synergize currently available insecticides on the market.

AGRO 312

The effects of coal tar as source material on the desorption kinetics of benzo(a)pyrene from contaminated soils

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The release of carcinogenic PAHs, such as benzo(a)pyrene (BaP), from soils contaminated with coal tar is crucial to bioavailability/bioaccessibility-based risk assessment of former manufactured gas plants (MGP). Due to the carbonaceous nature of these materials, they may act as both sink and source of PAHs. This study focuses on the release kinetics of BaP from two Australian soils with different textures and organic carbon contents (OC) as well as a silica sand artificially contaminated with BaP solution, coal tar pitch, and liquid coal tar, at BaP concentration of 50 mg/kg. The desorption kinetics of BaP were determined by sequential Tenax extraction and a three-compartment (rapid, slow, and very slow) desorption model was characterised after 1, 24, and 48 days of ageing with dry-wet cycles. The results show different desorption patterns of BaP from soils containing coal tar pitch and liquid coal tar compared to soils with BaP in solution. The desorption of BaP from silica sand and the clayey soil with higher OC (MTA) was inhibited by coal tar pitch and liquid coal tar compared to parallel treatments of BaP solution after 1, 24 and 48 days of ageing. For the sandy soil with low OC (GIA), a similar trend was identified at 1 day, but desorption of BaP was largely enhanced in GIA with liquid coal tar after 24 days of ageing. Overall, MTA yielded the lowest rapidly desorbing fractions (F_{rap}) for BaP (< 2.2%), followed by GIA (< 28%), and silica sand (< 33%). The strongest ageing effects were identified in GIA treated with BaP solution and coal tar pitch (27% and 2% decreased F_{rap} after 48 days, respectively). The ageing effects were less prominent in soils treated with pitched and liquid coal tar compared to BaP solution spiked soils. For MTA, declines in F_{rap} in these two treatments over 48 days were less than 0.1%. In GIA and sand with liquid coal tar F_{rap} increased after 24 days and decreased after 48 days of ageing. These findings show that coal tar as a source material will affect the release of the PAH in different ways, based on soil properties and its status (solid or liquid), during ageing process. This is important in the illustration of the re-mobilisation mechanisms of non-extractable PAH residues in MGP sites. The results of this study also help in the understanding of the changes in PAH bioaccessibility in aged MGP soils as the correlations between rapidly desorbing PAHs and their bioaccessibility have been reported in previous studies.

AGRO 313

Non-extractable residues of agrochemicals in soil in the regulatory context

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Knowledge on the biotic and abiotic degradation of chemicals is essential to assess their environmental fate and is a key factor in risk assessment and their registration. While the main focus lies on screening tests to assess biodegradation for industrial chemicals, extensive degradation tests are needed to investigate the route and rate of degradation of agrochemicals.

Generally, the degradation of chemicals in soil results in the formation of degradation products (i.e. metabolites), non-extractable residues (NER), microbial biomass and finally in their mineralization. Whereas degradation products and mineralization are relatively easy to assess, identify and evaluate in laboratory tests nature, identity and composition of NER is often unknown. Therefore, the question arises when NER should be considered a risk and when not. Major concerns of NER are the potential bioavailability as well as the release from soil over the long term. Therefore, NER are an important parameter not only in risk assessment (i.e. PEC/PNEC) but also in hazard/persistence assessment (PBT) of chemicals. With regard to EU regulatory aspects of NER there are generally two essentially different views. Firstly, a precautionary approach in the regulation of industrial chemicals considering residues as non-degraded parent which generally overestimates the exposure concentration. Secondly, a more conservative approach within the regulation of agrochemicals considering NER as degraded residues of the active substance which are of no further concern. Herein only a general cut-off criterion is laid down regarding the maximum amount of NER formed in combination with a low mineralization rate.

On basis of the nature and definitions of NER in soil the evaluation of NER in environmental risk assessment and PBT assessment within the regulatory context will be discussed. The focus will be laid on agrochemicals considering new findings of the recent past (e.g., biogenic NER) and their possible effects for regulation.

AGRO 314

Spray drift and pest control from aerial applications on soybeans

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Pesticide drift is an issue in modern farming, mainly for crops under constant spraying such as soybeans. This study aimed at assessing drift and pest control for aerial applications in soybean crops. Hydraulic nozzles and rotary atomizers, regulated to a wide spectrum of droplet sizes, sprayed thiamethoxam plus lambda-cyhalothrin using an agricultural aircraft Ipanema 202A at volume rate of 20 L ha^{-1}. Treatments consisted of testing two devices: a rotary cage atomizer (Micronair AU 5000) with blade angles of 65° for larger droplets, and with angle of 55° for smaller ones; and a set of adjustable nozzles (Stol model) with deflector angle of 90° for smaller droplets, and with angle of 30° for larger ones. Drift was evaluated through quantification of active ingredient, by means of liquid chromatography, on nylon strings set 20, 40, 80, 160 and 320 m downwind from the applied area. Control efficiency was measured by counting caterpillars and stinkbugs found five days after spray. Rotary atomizers produced lesser drift compared to adjustable nozzles at the designed setting. Furthermore, drift can be reduced through a suitable regulation of the devices, keeping an effective pest control.

AGRO 315

Initial measurement and evaluation of spray drift from an unmanned aerial vehicle

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Unmanned aerial vehicles (UAVs) are now being used to perform pesticide applications in commercial settings, but little data is available regarding pesticide drift from this mode of application. Several characteristics of UAVs fall outside of the validated range of existing spray dispersion models, and there is a lack of UAV spray drift data reported in the literature for use in model validation. The resulting uncertainty creates a regulatory challenge as it is unclear whether existing regulations are adequate for UAVs under all of their expected use conditions. A series of drift deposition studies is currently underway to measure downwind ground deposition resulting from a Yamaha R-MAX II UAV across a variety of agricultural settings. Those settings include a vineyard, an almond orchard, and a bareground plot. A drift deposition curve is fitted to measured data and the estimated proportion of material lost as drift under each application scenario is reported. Deposition results and supporting meteorological and crop canopy data are intended to provide sufficient data for assistance in validation of spray dispersion models for UAV spray drift which would ultimately allow estimation of drift across a range of environmental and crop conditions.

AGRO 316

Effect of evaporation rate and recent deposition dataset on AGDISP spray drift modeling for herbicide tank mix parners

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The aim of this study is to evaluate the modeling performance of AGDISP and REGDISP for spray drift deposition prediction of herbicide tank mix partners regarding the evaporation rate and droplet size distribution. The AGDISP model currently uses a single evaporation rate for active, additive and carrier components. This single evaporation constant has been previously seen to result in unrealistic estimation of spray drift deposition for multiple tank mix partners. In addition, the spray drift empirical data within the AGDISP model were generated 25 years ago, which provides an estimate of drift that often has a bias to over-predict. To test the implications of evaporation rate on tank mix partners, this study calculated evaporation rates using the theoretical equations parameterizing temperature, relative humidity, and nature of the formulation mixes. To assess the effect including newer deposition data, REGDISP modeling was conducted that incorporates the recent data (2000, 2004, and 2011) from Agriculture and Agri-Food Canada (AAFC). The results demonstrate that the use of tank mix-specific evaporation rate considering the latest experimental deposition data has significant impacts on AGDISP modeling for herbicide tank mix partners. This approach reasonably describes the REGDISP deposition modeling coupled with realistic prediction of evaporation rate is preferred over AGDISP for conducting herbicide tank mix risk assessments.

AGRO 317

Three dimensional plant modelling with open source software for use in spray particle deposition simulations

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Current approaches for modelling off-target drift of spray particles during pesticide applications do not account for the impact of different plant canopy geometries on localized airflow and the entrained particle trajectories that ultimately determine canopy interception and whether deposition reaches non-target plants. Computational fluid dynamics (CFD) presents the opportunity to improve understanding of these fine scale interactions, however efficient, low-cost methods for developing detailed digital representations of plant canopies are needed. In this poster, we demonstrate the development of high resolution 3D plant models via digital photogrammetry for use in CFD wind tunnel simulations. The source data for digital photogrammetry is a series of overlapping photos captured from video taken with a hand-held camera typically found in personal electronic devices. The video consists of several circles around the model plant at varying angles and distances in diffuse lighting. Capturing video in this way optimizes the number of matching points that can be identified between overlapping images and assigned 3D coordinates using photogrammetry software such as VisualSFM. The resulting point cloud of 3D coordinates was edited, and a surface model was generated using Meshlab. The resulting model is imported into SpaceClaim, CFD preprocessing software that generates a model ready for use with the ANSYS CFX solver. This approach can be applied to a range of plant species and growth stages including vegetables, flowers, and non-plant benchmarks like metal

discs or rods. CFX simulations using plant geometries developed by this method are the topic of a companion presentation.

AGRO 318

Advancing pesticide management strategies for citrus greening disease

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Citrus greening, or Huanglongbing (HLB) disease, causes citrus trees to produce green-colored, sour oranges. Trees are infected when psyllids, small phloem-feeding insects, feed on young citrus leaves. Currently, there is no cure for citrus greening disease, and pesticides seem to have a limited effect on the psyllid population. Thus, our interdisciplinary team at Colorado State University is quantifying the spatial distribution of insecticides to determine psyllid exposure rates to evaluate if a) the pesticide concentration is too low, b) the psyllids are not being exposed, and c) if the psyllids are developing resistance to the insecticides. One main goal is to develop analytical methods to quantify spatial distribution of pesticides to citrus trees by both aerial and ground application. This is accomplished by distributing Empore C18 sorbents and Whatman filter disks throughout citrus trees, covering all sides, the top and bottom canopy, and front and backsides of leaves. Three different mounting techniques have been tested for holding the disks: hanging 3D-printed holders, rigid-wire mounted 3D-printed holders that mimic a leaf, and disks clipped to the frontside and underside of the leaves. To visualize spatial distribution, a fluorescent dye is mixed with the pesticides before application. Then, the Whatman filters are collected and inserted into an ultraviolet (UV) lightbox outfitted with a Raspberry Pi camera. The UV light excites the fluorescent dye, giving an image of the droplets on the filter paper. The resulting image is analyzed in bulk using ImageJ software, which calculates the droplet size and outputs the spatial distribution of the dye. The C18 sorbents are collected for quantification of pesticide concentrations using a gas chromatography – tandem mass spectrometry (GC-MS/MS) triple quadrupole in the selected reaction monitoring (SRM) mode. Overall, this novel approach allows for more comprehensive quantification of pesticide distribution than current widely-used methods involving water-sensitive paper cards stapled to tree leaves. Preliminary results from field campaigns indicate that the underside of leaves may be receiving lower doses of pesticides than the topsides of leaves, both from aerial and ground applications. This likely results in the persistence of psyllids in the grove after insecticide applications, and further may be contributing to the development of insecticide resistance.

AGRO 319

Bayer's Science Transparency Initiative: Enabling access to safety studies

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Established in 2017, cropscience-transparency.bayer.com is an initiative to pro-actively share safety-relevant regulatory information about Bayer's crop protection products with non-commercial interested parties. By sharing safety data, Bayer is taking a leading role in breaking down barriers to build public trust and foster a fair, science-based dialogue around crop protection products.

AGRO 320

Urinary excretion and tissue residues of zilpaterol HCl after trace-level exposures

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Zilpaterol HCl is a β-agonist feed additive approved in the United States to improve feed and growth efficiency of cattle, but its use is banned domestically and internationally in most food animal species and in human and animal competitive events. The objective of this study was to determine levels of zilpaterol exposure that would return positive results using qualitative and quantitative screening and analytical methods. Sheep were fed 1.75 kg of feed containing 0.0075, 0.075, or 0.75 mg/kg of zilpaterol for 12 consecutive days. Urine and tissues were analyzed for zilpaterol residues. Zilpaterol was consistently detected by rapid screening assays (LOD = 1.7 ng/mL) in urine during the dosing and withdrawal periods. Rapid screening assays most consistently detected zilpaterol in tissues of animals exposed to the highest dose. Urine of sheep exposed to 0.0075 mg/kg of dietary zilpaterol consistently tested positive by quantitative LC-MS/MS methods.

AGRO 321

FOCUS and NAFTA degradation kinetics are too conservative? — Aged sorption affects the kinetic modeling of pesticide degradation in soil

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Degradation behavior of pesticides in soil is investigated by means of metabolism experiments, and their degradation rates are commonly derived using FOCUS or NAFTA approaches, in order to produce reliable modeling endpoints (DT_{50}) for environmental exposure modeling. Single first order (SFO) models are exclusively used to describe pesticide degradation in regulatory exposure modeling software. Pragmatic and guidance based workarounds using biphasic models, therefore, may lead to disputable modeling endpoints. Aged or time-dependent sorption could be among the processes causing the SFO model to fail. As sorption increases over time, the substrate appears to become less bioavailable to microbes, leading to slower degradation. In such cases, modeling endpoints can only be derived using multi- or biphasic models, resulting in overestimated and, hence, too conservative DT_{50}. This study compared kinetic evaluations of soil residue data using FOCUS and NAFTA procedures as well as inverse modeling to demonstrate the effect of aged sorption on SFO fits for soil degradation behavior of a parent compound. Results showed that the modeling endpoints derived by means of FOCUS and NAFTA procedures were significantly overestimated compared to the outcome of inverse modeling where aged sorption was included. Thus, the importance of aged sorption should be considered when performing soil metabolism experiments and when evaluating degradation kinetics for pesticides.

AGRO 322

Photo-enhanced soil metabolism of atrazine

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The OECD 307 Guideline 'Aerobic and Anaerobic Transformation in Soil' and the EPA Guideline OCSPP 835.4100 'Aerobic Soil Metabolism' define the degradation rate of an organic chemical in soil. The tests are conducted in field fresh moist soils at a constant temperature of 20°C under dark conditions. These parameters allow for consistent

testing between studies; however, these tests do not assess the simultaneous degradation contributed by natural sunlight that can occur in the field for some pesticides that are on the soil surface, at least initially. A soil metabolism study design based on the above guidelines was modified in this experiment by setting up treated soil samples with [^{14}C]atrazine and exposing them to artificial sunlight for several days prior to introducing the illuminated samples to additional fertile soil. Comparisons of the rate of degradation in the illuminated samples will be compared to treated soils incubated solely in the dark. This study design provides an example of higher tiered testing to assess a chemical's persistence.

AGRO 323

Estrone in aquatic systems in the presence of poultry litter and cow manure: Determination of its fate, degree of mineralization, and changes in its endocrine disrupting potential

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Application of animal manure to agricultural land has been identified as the main source of estrogens in the environment. This is of growing concern as the number of concentrated animal feeding operations(CAFOs) increases particularly in the United States. Poultry and cow manure contains free and conjugated forms of the natural estrogens17β-estradiol(β-E2) and estrone(E1) which can leach into the aquatic environment during runoff. Although, E1 is less estrogenic thanβ-E2, environmentally relevant levels of E1 cause feminization of male fish due to the ability of fish to convert E1 to β-E2. Our study focuses on the comparison of the degree of mineralization, fate, and changes of the endocrine disrupting potential of E1 in a simulated poultry litter and cow manure runoff. A radioactive ^{14}C-labeled E1 was used to determine the degree of mineralization E1 in poultry litter matrix. ^{14}C - E1 was spiked in the matrix of poultry litter and water to mimic runoff from poultry litter applied in farms. A time-course study was conducted by aerating the matrix with humidified air for 7 days. Samples were collected throughout the time course and analyzed for radioactivity using liquid scintillation counter. Another set of exposures was also performed using poultry litter and cow manure matrix spiked with a non-radioactive E1 standard and samples are analyzed using LC-MS/MS. Results show that aerobic microorganisms found in poultry litter was able to mineralize the estrogen present in the simulated water runoff; however, this was not observed in the cow manure. In the cow manure exposure experiment, it was observed that around 15% of E1 was also found in the suspended solids after exposure, suggesting that estrogens were not only dissolved in water but are also sorbed in the sediment. This research is important to fully understand the fate and transport of estrogens in aquatic systems and in assessing its estrogenic effect on aquatic animals as demonstrated by feminization of male fish.

AGRO 324

Soybean response to dicamba and 2,4-D in simulated furrow irrigation

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While much research has focused on damage to various crops resulting from drift exposure to auxin herbicides, there are no reports documenting the risk posed by exposure to auxins dissolved in irrigation water, such as may occur in recycled tailwater. The hypothesis for this research was that soybean would experience increasing rates of injury and reduced height as dicamba and 2,4-D concentrations increase in irrigation water. A susceptible soybean variety was grown in pots in a growth chamber and exposed to dicamba or 2,4-D dissolved in irrigation water using simulated furrow irrigation. Care was taken to avoid foliar exposure, and irrigation water was poured directly into the soil. Five herbicide treatment concentrations, ranging from 0-5ppm, were applied to soybean plants at two different growth stages. Plant injury (0-100% scale), height, pod malformation, number of pods, and number of pod bearing nodes were documented 14 days after treatment. Results indicate dicamba dissolved in irrigation water can result in visual injury to soybean at concentrations 0.5 ppm and greater. In particular, damage caused by dicamba at concentration of 5 ppm was substantial, and such damage is significantly different from that of 1ppm or less. Growth stage does not significantly affect damage under the same concentration when exposure is via root uptake. Linear and non-linear models for assessing EC50 will be reported. Results indicate that care must be taken in managing tailwater recovery systems that recycle water between dicamba-resistant and dicamba-susceptible soybean fields to avoid cross-crop impacts of residual herbicides dissolved in irrigation water.

AGRO 325

Residual characteristics of triflumizole in water dropwort and shallot

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This study was carried out to investigate residual characteristics of triflumizole in water dropwort and shallot under greenhouse conditions. The 2,000 time-diluted solutions of the test commercial pesticide (30% WP) were sprayed three times with a 7-day interval with different spraying days before harvest onto the test crops at an application rate of about 200 L per 10 a, and then samples were collected on the day of harvest. Limits of quantitation of triflumizole residues in the test crops as analyzed with an HPLC/DAD were 0.03 mg/kg. Recoveries of triflumizole in water dropwort and shallot were 75.3-80.7 and 74.6-80.0%, respectively. No residue change was observed from samples during sample storage periods. Residual concentration of triflumizole in water dropwort and shallot were 0.76-5.32 and 0.27-2.76 mg/kg, respectively. Residue level of water dropwort was higher than that of shallot because of characteristics of the test crops such as specific surface area and morphological properties. The estimated daily intakes of the test pesticide in two test crops were less than 1.0% of its acceptable daily intake.

AGRO 326

Changes of pyraclostrobin and its metabolite BF 500-3 residues in spinach and Korean cabbage

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In order to investigate changes of pyraclostrobin and its metabolite BF 500-3 residues in spinach and Korean cabbage under greenhouse conditions, the test pesticide (11% SC) was diluted by 2,000 times with ground water and then sprayed three times with a 7-day interval. Application was done at different days (28-21-14, 21-14-7. 17-10-3, and 14-7-0 days) before harvest, and then samples were collected on the prearranged days of harvest. The residues of the test pesticide and its metabolite were analyzed with an LC-MS/MS. Limits of quantitations of pyraclostrobin and its metabloite BF 500-3 were 0.06 and 0.02 for spinach and 0.03 and 0.01 mg/kg for Korean cabbage, respectively. The recoveries of pyraclostrobin in spinach and Korean cabbage were 85.3-95.6 and 74.3-88.3, and those of BF 500-3 were 81.1-98.4 and 73.0-87.9%, respectively. Average total residues (sum of pyraclostrobin and BF 500-3 residues) as treated three times with a 7-day interval at 14, 7, 3 and 0 days before harvest were 0.29, 0.82, 1.90 and 2.08 mg/kg for spinach and 0.50, 0.77, 0.99 and 1.20 mg/kg for Korean cabbage, respectively. These results represented that the amounts of pyraclostrobin residues in the test crops were higher as spraying closer to the harvest day.

AGRO 327

Dissipation characteristics of cyflufenamide and fenvalerate residues in perilla leaves

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This study was carried out to investigate the dissipation characteristics of cyflufenamide and fenvalerate in perilla leaves. The test pesticides, cyflufenamid 3.5% SC and fenvalerate 5% EC were diluted by 2,000 and 1,000 times with ground water and then sprayed 3 and 2 times, respectively, with a 7-day interval onto the test crop. Samples were collected 6 times at 0, 1, 3, 5, 7, 10 days after the last application. Limits of detection (LODs) of cyflufenamide and fenvalerate in perilla leaves were 0.002 and 0.02 mg/kg, and recoveries at 10×- and 50×LOD levels were 97.3-97.8 and 92.4-99.0% for cyflufenamide and 88.5-98.6 and 85.1-88.6% for fenvalerate, respectively. Residues of cyflufenamid and fenvalerate in the test crop were decreased gradually with a regression equation of $y=2.873e^{-0.116x}$ ($R^2=0.989$) and $y=4.806e^{-0.059x}$ ($R^2=0.942$) to show 5.9 and 10.3 days of biological half-lives, respectively. Dissipation rates of cyflufenamid and fenvalerate in the test crop were 64.7 and 46.1%, respectively, for 10 days after the last spray.

AGRO 328

Residual characteristics of fosthiazate and imidacloprid in spinach

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In order to determine the residual characteristics of fosthiazate and imidacloprid in spinach, the test pesticides, fosthiazate 30% SL and imidacloprid 8% SC, were diluted by 4,000- and 2,000-times, respectively, and then sprayed twice with a 7-day interval. Samples were collected on the day of harvest. The pesticide residues in spinach were analyzed with a high performance liquid chromatograph equipped with variable wavelength detector for fosthiazate and with diode array detector for imidacloprid. Limits of detection (LODs) were 0.02 for fosthiazate and 0.01 mg/kg for imidacloprid. Recoveries at two fortification levels of their 10×- and 50×LOD levels were 70.9-85.4 and 73.3-85.2 for fosthiazate and 84.9-85.3 and 85.3-86.2% for imidacloprid, respectively. Residual concentrations of the test pesticides in spinach were decreased time-coursely, while their residues were higher as spraying closer to the harvest day. The estimated daily intakes of two test pesticides by intake of spinach were less than 2.1% of its acceptable daily intake in all samples, representing that fosthiazate and imidacloprid residues detected in the test crop do not pose the immediate health risks.

AGRO 329

Bioavailability of HBCD/TBB/TBPH from dust and oil vehicles in Sprague-Dawley rats

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Recent data have revealed that homes, offices and cars are often more polluted with environmental contaminants than the outdoors due to high use of textiles, electronics, and insulation that are treated with brominated flame retardants (BFRs). BFRs volatilize and/or weather from consumer products and become encumbered in household dust. Human ingestion or inhalation of household dust is a suspected exposure route for BFRs, particularly for infants whose hand-to-mouth activities would facilitate ingestion, and BFR dust levels have been correlated to human plasma levels. Almost all major classes of BFRs have been identified in household dust, including hexabromocyclododecane (HBCD), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), and bis(2-ethylhexyl)-3,4,5,6-tetrabromophtalate (TBPH), at concentrations exceeding 1 µg/g. The goal of the present study was to determine the mammalian bioavailability of HBCD/TBB/TBPH at environmentally-relevant levels when bound to household dust or an oil vehicle in male rats. Standard reference material (SRM) 2585, a well-characterized

household dust, was used as the source of HBCD/TBB/TBPH. An oil vehicle was prepared whose daily administration duplicated the natural HBCD/TBB/TBPH exposure from 390 mg of dust/day. Male Sprague-Dawley rats were randomly divided into a Control group (n = 3), an Oil-dosed group (n = 4), and a Dust-dosed group (n = 4). Feces and urine were collected daily throughout the study. Twenty-four hours after their last exposure, the rats were euthanized and adipose tissue, brain, GI tract, kidney, liver, lung, muscle, blood, and skin were collected. Tissue and feces levels of HBCD isomers were determined by LC-MS, while the levels of TBB and TBPH were determined by GC-MS. HBCD isomers in household dust were bioavailable when ingested, although stereoisomerization or isomer bioavailability differences were evident. Analyses of the bioavailability and individual tissue levels of TBB and TBPH are on-going.

AGRO 330

Interaction of glufosinate and *Colletotrichum truncatum* on ammonia levels and glutamine synthetase activity in hemp sesbania

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The use of microbes and microbial products as bioherbicides has been studied for several decades. Researchers have also examined combinations of bioherbicides and herbicides in attempts to discover synergistic interactions applicable to weed control. Bioassays were conducted to assess possible interactions of the herbicide glufosinate [2-amino-4-(hydroxymethylphosphinyl)butanoic acid] and *Colletotrichum truncatum* (CT)[fungal plant pathogen and bioherbicide for hemp sesbania (*Sesbania exaltata*) control]. Glufosinate acts as a glutamine synthetase (GS) inhibitor leading to elevated ammonia levels, but the mode of action of CT is unknown. GS has also been implicated in plant defense in certain plant-pathogen interactions. The effects of spray applications of glufosinate (1.0 mM) and bioherbicide (8.0×10^4 conidia ml^{-1}), (applied alone and in combination) on seedling growth, GS activity and ammonia levels in hypocotyl tissues were monitored (88-h time-course). Growth (elongation and fresh weight) and extractable GS activity were inhibited in tissues by glufosinate and glufosinate plus CT treatments as early as 16 h, but CT treatment did not cause substantial growth reduction or GS inhibition until after ~40 h. Generally, ammonia levels in hemp sesbania tissues under these various treatments were inversely correlated with GS activity. Localization of hemp sesbania GS activity on electrophoretic gels indicated a lack of activity after 30 h in glufosinate- and glufosinate plus CT-treated tissue. Untreated control tissues contained much lower ammonia levels at 24, 64, and 88 h after treatment than treatments with CT, glufosinate or their combination. CT alone caused elevated ammonia levels only after 64-88 h. Glufosinate (0.25 mM to 2.0 mM) caused a 10%-45% reduction of colony radial growth, compared to fungal growth on agar without glufosinate, and the herbicide also inhibited CT sporulation. Although no major synergistic interactions were found in combinations of CT and glufosinate, further insight on the biochemical action of this bioherbicide on this weed was attained.

AGRO 331

Estimation of concentration percentiles for pesticide surface water monitoring data

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Extensive historical surface water monitoring data exist for many pesticides from various water quality monitoring programs, each often having differing objectives. These data may be collected at different frequency and/or times of sampling or targeted at watersheds vulnerable to storm-induced runoff from agricultural fields. However, data from many such sites and many years should contain information from both the high end as well the low end of the likely concentration distributions embodied in the historical multi-year and multi-region monitoring.

We will describe a weighting method that uses data of both low and high frequency sampling to obtain conservative estimates of "typical peak concentrations" at site-years within spatial regions. By "typical" we mean the median of the distribution of the target annual percentile (e.g., 90^{th}, 95^{th}, 99^{th}) across site-years. The method is illustrated using data obtained from the Atrazine Ecological Monitoring Database (AEMD) which includes all atrazine measurements collected at extensive monitoring programs in the United States, both targeted and non-targeted. Percentile estimates obtained are shown to be conservative by empirical comparison to direct estimates obtained using sites where higher frequency sampling data is available.

AGRO 332

Evaluation of SEAWAVE-Q Model for providing daily predictions from non-daily sampled atrazine surface-water concentration monitoring data

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The SEAWAVE and SEAWAVE-Q models are designed to test long-term trends of pesticide surface-water concentrations at specific monitoring locations where data have been sampled on a non-daily basis. The models are regression models which, in addition to having a linear trend term, include covariates for seasonality (SEAWAVE) and optionally a transformation of stream flow (SEAWAVE-Q). Model predictions are at the day-level, and provide a complete time series of predictions of daily concentrations over the sampling time-period. U.S. EPA has recently proposed to use the daily predictions from the SEAWAVE-Q model as a basis for the determination of bias factors, which are protective adjustments designed to address the bias of predictors of target quantities such as percentiles and maximum m-day rolling averages of concentrations derived from non-daily samples. This raises the question of SEAWAVE-Q model performance in predicting these daily time series and also of performance relative to other available methods. In this poster we present an evaluation of the performance of the SEAWAVE-Q model, and a comparison with other methods.

AGRO 333

Spatial and temporal analysis approach to quantify pesticide concentrations in surface water

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The Office of Pesticide Programs evaluates the potential for pesticides to occur in aquatic environments using screening level fate and transport models and an assessment of available monitoring data. The interpretation of pesticide surface water monitoring data in risk assessments requires an understanding of the impact of numerous variables (*e.g.*, sample site location, sampling frequency, watershed characteristics such as pesticide use, precipitation, and soil erodibility) on the frequency and magnitude of pesticide occurrence. Major limiting factors in the use and interpretation of pesticide surface water monitoring data are infrequent sampling and the limited geographical representation that may result in under or over estimation of pesticide exposure. This presentation discusses a framework the Office of Pesticide Programs is considering for integrating available surface water monitoring data quantitatively into pesticide exposure assessments using recently developed methods which employ the SEAsonalWAVEQ with EXtended capabilities (SEAWAVE-QEX) model developed by the U.S. Geological Survey and sampling bias factors.

AGRO 334

Association between a SNP and cytochrome P450-mediated herbicide resistance in *Lolium spp.* populations

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Multiple resistance to ACCase- and AHAS/ALS-inhibiting herbicides in *Lolium spp.* populations increases weed management costs in winter cereal production systems from Argentina. In most cases, cytochrome P450-mediated herbicide metabolism seems to be involved. The objective of this work was to develop markers associated with herbicide metabolism by cytochrome P450.
Genomic DNA was isolated from herbicide-resistant and susceptible plants obtained from a *Lolium spp.* population with multiple resistance (pinoxaden, iodosulfuron-methyl/mesosulfuron-methyl and pyroxsulam) mediated by cytochrome P450 monooxygenases. Five primers were designed from consensus regions of multiple cytochrome P450 sequences. Different levels of DNA polymorphism were detected combining the primers into pairs. A SNP was presumably related to the multiple herbicide resistance, where G and A alleles would be related to herbicide resistance and susceptibility, respectively. Tetra primer ARMS PCR method was developed for genotyping accessions obtained from 20 *Lolium spp.* populations (13 populations with multiple herbicide resistance to AHAS/ALS and ACCase inhibitors and 7 susceptible populations) from Argentina. The G or A allele-specific amplicon (140 and 225 bp, respectively) was detected in 90% of the cases. G allele was significantly associated to multiple herbicide resistance ($p<0.01$), and it was detected in nine populations which showed resistance to pinoxaden, iodosulfuron-methyl/mesosulfuron-methyl, pyroxsulam and/or sulfometuron-methyl/chlorimuron-ethyl. In these populations, herbicide detoxification was corroborated with cytochrome P450 inhibitors (malathion, piperonyl butoxide and 1-aminobenzotriazole). The A allele in homozygous state was detected in all accessions from susceptible populations. Future work will widen the number of populations studied.

AGRO 335

Metabolic resistance to tribenuron-methyl in *Descurainia sophia* L. conferred by cytochrome P450 enzyme (CYP96A146)

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D. sophia is one of the most notorious broadleaf weeds in China, and it has evolved extremely high resistance to ALS-inhibiting herbicide tribenuron-methyl. The metabolic resistance mechanisms mediated by cytochrome P450 in *D. sophia* were confirmed by P450 inhibitor, RNA-sequencing, liquid chromatography-mass spectrometry (LC-MS) and RT-qPCR analysis. The P450 inhibitor malathion significantly reversed *D. sophia* resistance to tribenuron-methyl with the resistance index (RI) decreasing from 116 to 28. Up-regulation of four P450s (*CYP96A146*, *CYP96A147*, *CYP96A15-like*, *CYP71A1-like*), three GTs and one ABC transporter were identified and confirmed in resistant (R) *D. sophia* by RNA-Seq and qPCR. The LC-MS analysis demonstrated the R plants metabolized tribenuron-methyl significantly faster than did the susceptible (S) plants at 3, 5 and 7 days after treatment (DAT) of tribenuron-methyl. The malathion significantly reduced the tribenuron-methyl metabolism both in S and R plants. The tribenuron-methyl metabolism in S and R plants, which were firstly treated by malathion, displayed no significant difference at 1, 3 and 5 DAT. In addition, the completely new P450 gene *CYP96A146* constitutively overexpressed in R plants, and the expression level of *CYP96A146* in R plants was about 512-fold higher than in S plants comparing to S plants. The *CYP96A146* expression in R plants was significantly induced by tribenuron-methyl at 1, 3, 5 and 7 DAT (1.9 to 2.9-fold). While, tribenuron-methyl induced *CYP96A146* expression in S plants only at 5 DAT (16-fold). As P450 inhibitor, malathion can significantly weaken or reverse tribenuron-methyl-induction of *CYP96A146* expression in S and R plants. *CYP96A146* expression in R plants reduced about 30-, 2.6-, 1.4- and 1.5-fold at 1, 3, 5 and 7 DAT, respectively. By contrast, *CYP96A146* expression in S plants decreased only at 5 DAT (4-fold). These results indicated the *CYP96A146* conferred *D. sophia* metabolic resistance to tribenuron-methyl.

AGRO 336

Metabolic and multiple resistance in junglerice from Mississippi

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A junglerice (*Echinochloa colona*) population, MS1, collected from a rice field in Sunflower County, Mississippi, was confirmed to be resistant to acetolactate synthase (ALS)-inhibiting imazethapyr and cross-resistant to other ALS inhibitors such as imazamox (3.3-fold), penoxsulam (9.4-fold), and bispyribac-sodium (7.2-fold). Addition of malathion to penoxsulam and imazethapyr reduced shoot dry weight and/or increased mortality compared to the respective herbicides applied alone, indicating possible involvement of herbicide metabolism driven by cytochrome P450 monoxygenase enzymes (CYP) as a mechanism of resistance. ALS enzyme assays or ALS gene sequencing analysis did not indicate a modified target-site based resistance. Lower levels of translocation of ^{14}C-bispyribac and ^{14}C-imazamox in the MS1 population compared to a susceptible population were recorded, perhaps indicating a result of metabolism. RNA-seq

analysis of gene expression before and after herbicide treatment revealed that the MS1 population exhibited a stress response upon exposure to imazamox. Additional studies revealed that the MS1 population was multiple resistant to fenoxaprop-P-ethyl, an acetyl coenzyme A carboxylase (ACCase) inhibitor, (11-fold; but susceptible to sethoxydim and clethodim, also ACCase inhibitors), propanil, and quinclorac. Sequencing of ACCase of MS1 did not reveal the presence of any known resistance-conferring point mutations. An enzyme assay confirmed that the ACCase in the MSI population was herbicide sensitive. Further investigations with two CYP inhibitors, malathion and piperonyl butoxide, and a glutathione-*S*-transferase inhibitor, 4-chloro-7-nitrobenzofurazan, did not indicate involvement of any metabolic enzymes inhibited by these compounds. Treatment of MS1 plants with quinclorac in combination with malathion indicated increase in susceptibility to the herbicide, implicating a role for CYP.

AGRO 337

Environmental metabolism studies with carbon-14 labelled plant protection products

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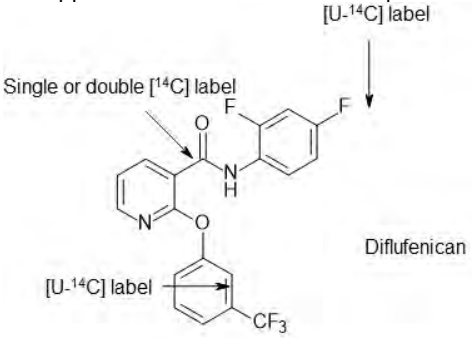
For the requirements of a metabolism study on a carbon-14 labelled plant protection product (PPP) test item, consideration should be made on the label position (OECD Guidelines for the Testing of Chemicals), specifications and stability. Using [^{14}C]diflufenican as an example, the factors taken into account when producing the test item for one or more studies over a period of time can be demonstrated. The molecule has three different rings each of which require a ^{14}C-label. Each label is manufactured by a separate route and the effect of the label position, specific activity and storage conditions on stability will be discussed. Furthermore, the different studies using radiolabelled compounds, such as metabolism studies in livestock and plants and environmental fate have additional requirements for the carbon-14 labelled compound. The purpose of those studies is to investigate the degradation pathway of a test item like diflufenican. Therefore, metabolites and degradation products observed in the course of a study have to be analysed and identified. Especially, for analysis and identification by high resolution mass spectrometry experiments, the labelling position and degree is important. Consequently, the discussion will also include aspects and requirements from the point of view of the application of radiolabelled compounds.



AGRO 338

Considerations for selection of ^{14}C radioactive tracers and ^{13}C stable label analogs to aid metabolite identification by mass spectrometry

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Regulatory studies for metabolism in plant and animal matrices commonly use agrochemicals with ^{14}C radiolabeled test substances. At the initiation of a study, isotopic dilution of the radiolabeled test material determines the radiochemical specific activity of the test substance applied to the test system, which affects the limits of quantification of radioisotope detection. In conjunction with the specific activity, the ^{14}C labeled position affects the ability to distinguish metabolites originating from the test material in mass spectral (MS) data. The location of the ^{14}C tracer can be exploited to aid identification of metabolites via liquid chromatography-mass spectrometry analysis (LC-MS) by adjusting the isotopic enrichment to give unique ^{12}C and ^{14}C isotope ratios. Uniform ring labeled compounds may be necessary to trace test substance metabolites, but generally do not give the advantage of a unique isotopic ratio with high intensity. Dilution of the isotopic enrichment is mainly due to the mixture of incorporated radiolabel ($^{14}C_1$, $^{14}C_2$, $^{14}C_3$, $^{14}C_4$, $^{14}C_5$, $^{14}C_6$) resulting in spectra that are not as easily differentiated from other endogenous materials without extensive purification. The presence of halogens in the tests substance and its products may also contribute to the observed MS isotopic ratio due to naturally occurring heavy isotopes, but during biotransformation any halogens present may be lost. Therefore, a strategy of incorporating stable labeled analogs such as ^{13}C should be considered. Examples where use of a single carbon incorporation compared to uniform ring labeled incorporation of the ^{14}C isotope will be presented. The molecular ion isotopic patterns observed by LC-MS analysis will be compared, along with examples of how halogen presence affects the isotopic ratios. The incorporation of ^{13}C stable labeled analogs to control the predicted unique isotopic ratios of test substances and metabolic products will be presented as a strategy to allow for easier metabolite identification in less purified matrices.

AGRO 339

Water quality influence on dilution properties of an oil-in-water emulsion agricultural formulation

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Emulsion, oil-in-water (EW) formulations are used in the crop protection industry to formulate non-water soluble active ingredients with minimum or no addition of solvent to improve handling and environmental profile. In addition to use rates and spray volumes, water quality (pH, water hardness, solids content, temperature) can influence the dilution properties of the tank mix. The quality of water used in Brazilian agriculture is not well characterized due to the diversity of soils and water resources available in the country. Farmers usually monitor and control only the pH, but usually not water hardness. In this study, a water quality mapping of key Brazilian agricultural regions was done in order to better understand pH and water hardness. In addition, a case study EW-type agricultural formulation will be presented to illustrate the influence of pH and water hardness on emulsion performance. An example of a tank-mix study to identify commercial water conditioners and adjuvants to mitigate the effect of water quality on dilution properties will also be discussed.

AGRO 340

Systematic approach to identify and solve tank mix incompatibility of crop protection products

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Tank-mixing crop protection products is a common practice to reduce application time and cost and increase the spectrum of pests controlled in a single application. However, mixing diverse crop protection products can cause various unexpected results. Physical or chemical interactions in the spray tank can have consequences in reduced pest control efficacy, carryover crop response or un-sprayable and difficult-to-clean deposits in the tank. Therefore, the assessment of tank mix compatibility during the early stages of crop protection product development becomes a critical criterion to define when a prototype advances to a commercial product. A case study presented here is an example of a systematic approach to mitigate physical or chemical tank mix incompatibility issues between crop protection products and its utility to leverage learnings into the development of robust crop protection formulation development.

AGRO 341

Stabilization of a suspension concentrate agricultural formulation with xanthan gum in high electrolyte environment

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Polysaccharides, including xanthan gum, guar gum and diutan gum, are used widely in agricultural formulations as viscosity modifiers to stabilize suspensions of particles and emulsion droplets in heterogeneous water based formulations including suspension concentrates (SC), oil in water emulsions (EW) and suspo-emulsions (SE). The suspending power of xanthan gum is dependent on a number of factors including xanthan gum concentration, molecular weight, trisaccharide side chain anionic structure, electrolyte concentration, pH and temperature. The suspension of solid active ingredients with xanthan gum in systems with high electrolyte concentrations due to either co-formulants or salts of carboxylic acid active ingredients is particularly problematic leading to physical destabilization, active ingredient sedimentation, shortening the product shelf life and application challenges. A case study suspension concentrate agricultural formulation containing a solid active ingredient at 0.76 wt % suspended in a carboxylic acid salt solution as second active ingredient at 8.95 wt % is used to study the impact of xanthan gum selection on the rheology of the formulation and suspension stability under accelerated storage conditions. A correlation between xanthan gum structure, rheology, and physical stability of the suspension is presented.

AGRO 342

Improving the chemical stability of emulsifiable concentrate agricultural formulations

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The need to deliver increasingly complex agricultural formulations containing multiple active ingredients with various properties creates innovation challenges to lend chemical stability over the product shelf life. Chemical instability could be the result of reactions between active ingredients and/or co-formulants, photo-degradations, oxidations, hydrolysis, etc. Therefore, technologies that can effectively isolate, hinder, or eliminate adverse reactions are critical for a successful stable agrochemical formulation. The methodology employed in each instance is usually specific to the type of degradation mechanisms expected in the particular formulation of concern. In this work, various stabilizing techniques (managing moisture content, hydroxyl groups, counter-ions etc.) used in formulation development will be discussed, and a formulation case study will be presented to exemplify an innovative approach to prevent ester active ingredient degradation caused by transesterification or hydrolysis.

AGRO 343

SLOPE PIT method to characterize surfactants

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Surfactants are amphiphilic molecules having both an hydrophilic and lipophilic interaction with molecules of a given system. Those interactions have been characterized by the use of HLB (Hydrophilic-Lipophilic Balance) since the mid-50s. Different calculation techniques have been promoted. One of the more popular methods is the Griffin's method which is based on the ratio of the hydrophilic portion of the molecular mass to the complete molecular mass and is expressed on a scale is between 0 and 20. Although this method gives accurate results for the characterization of pure molecules, it is less convenient in its use when working with mixtures of molecules which are abundant in the current chemical industry. Moreover, new biosurfactants that are manufactured thanks to biotechnology also lead to mixtures that are difficult to analyze due to the complex determination of the molecular weights. An empirical method has been developed by the University UCCS of Lille in order to measure the hydrophilic-lipophilic character of surfactants. This method is named SLOPE PIT (Phase Inversion Temperature) and is based on the principle of conductivity measurements of an emulsion before and after the phase inversion temperature. It has been first implemented using pure surfactants which enabled us to correlate the SLOPE PIT values to those predicted *via* HLB calculations. However, we didn't know if this technique is applicable for surfactant mixtures. The study evaluates the hydrophilic-lipophilic character of surfactants from Oleon's Crop Protection portfolio, which contains mostly mixtures, using the SLOPE PIT measurement. From a statistical point of view, we studied the correlation between surface properties and SLOPE PIT measurements with the goal of setting up a new scale using only SLOPE PIT.

AGRO 344

Challenging the accepted SAR of diaryl imidazole broad-spectrum fungicides

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Diaryl imidazoles have been reported to provide broad-spectrum fungicidal activity, and we describe here our independent work in this area. By challenging originally-accepted SAR requirements for diaryl imidazole substitution patterns, we discovered broad-spectrum fungicidal activity comparable to prior examples, and opportunities for optimization of other properties of the molecules.

AGRO 345

Mesoionic pyrido[1,2-α]pyrimidinones as insecticides

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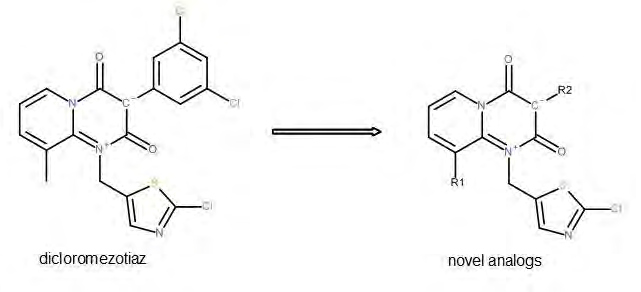
DuPont has discovered a novel class of mesoionic pyrido[1,2-α]pyrimidinone insecticides, such as triflumezopyrim and dicloromezotiaz. Here we will present the detailed optimization programs in support of the discovery of dicloromezotiaz. A series of mesoionic pyrido[1,2-α]pyrimidinones with various R1 and R2 have been synthesized for insecticidal screen. The chemical synthesis, biological activity and structure-activity relationships will be presented in detail.



AGRO 346

Pyraziflumid as a novel SDHI fungicide: SARs and synthetic methods

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Pyraziflumid was discovered as a novel SDHI fungicide chemically characterized by the 3-(trifluoromethyl)pyrazine-2-carboxamide group. This chemical series showed particularly high fungicidal activities against a broad spectrum of plant diseases in the case of *N*-(biphenyl-2-yl) as well as *N*-(1,1,3-trimethylindan-4-yl)carboxamides. Various *N*-(biphenyl-2-yl)pyrazine-2-carboxamides were synthesized, and their structure–activity relationships were studied. The optimization of the fungicidal performance of the series finally led to the identification of pyraziflumid, which could control a wide range of plant diseases. The details of structure-activity relationships from the view point of unique regiospecific substituent effect, and the synthetic methods of pyraziflumid will be discussed.

AGRO 347

Improved extraction and SPE cleanup protocols for LC-MS determination of ractopamine and other beta-agonist drugs in tissue samples

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Beta-agonist (beta-andronergic) drugs are used in animal husbandry as agents for growth enhancement and to promote leanness. Among these drugs, ractopamine and zilpaterol are approved for use in the USA and Canada for cattle, pigs, and turkeys. These and other beta-agonist drugs are not allowed for such use in the EU and in much of the rest of the world. Reliable analytical methods are necessary to determine residues of these compounds in samples obtained from animals raised for human consumption. These are challenging samples for residue analysis because they are high in fat and phospholipids, co-extracted substances that can lead to interference in the UPLC-MS analysis or contamination of sensitive instrumentation. The USA tolerance level for ractopamine is 30 µg/kg (ppb) in bovine muscle or 90 µg/kg in liver. Therefore, a suitable compliance method should show reliable performance for residue levels in the low µg/kg range. However, there is significant interest in monitoring for illegal use of beta-agonists wherein no measurable residue is allowed. In this study, after a simple and fast solvent extraction, a novel and rapid SPE cleanup protocol was employed for effective removal of phospholipids and other co-extracted substances prior to LC-MS analysis. The methodology was optimized for compliance monitoring (low µg/kg detection limits) and also for monitoring prohibited use (lowest possible detection limits) for ractopamine, zilpaterol, and eight other beta-agonist drugs.

AGRO 348

Fate of pharmaceuticals and other micropollutants during reverse osmosis of source-separated human urine for agricultural fertilizer application

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Human urine is responsible for the majority of nitrogen and phosphorous present in wastewater, but only constitutes less than 1% of the total wastewater volume. The source-separation of human urine is a viable way to reduce nutrient loading on wastewater treatment plants and thus mitigate eutrophication of the aquatic environment. Urine offers a natural, renewable source of nitrogen, phosphorous, and potassium nutrients that are vital for plant growth. Commercial fertilizers undergo energetically unfavorable processes to fix suitable amounts of nitrogen and mine enough phosphorous for an effective fertilizer. Urine contains pharmaceuticals and other micropollutants that are introduced into the wastewater, causing adverse effects on the organisms in the water. Using urine as a fertilizer feedstock could pose a risk to humans consuming food crops fertilized by human urine if the food crops uptake the micropollutants present in the urine. The goal of this project is to assess the risks of urine-derived fertilizers by analyzing the uptake of pharmaceuticals and other micropollutants in food crops. The urine-processing system in this study utilizes reverse osmosis and distillation to concentrate the nutrients in urine up to fourteen times prior to application as a fertilizer. Urine collected from the system is extracted by SPE, and pharmaceuticals are quantified by isotope dilution by LC-MS/MS. In addition to a targeted pharmaceutical analysis, utilization of the high-resolution capabilities of an Q-Exactive Orbitrap™ Mass Spectrometer allows for the untargeted analysis of other micropollutants found in human urine. Analytes of interest include pharmaceutical metabolites, natural and synthetic hormones and their metabolites, and opioids. The Orbitrap allows for a more complete risk assessment of urine-derived fertilizers because of the ability to do a suspect screening and detect compounds without the use of a standard. Fully concentrated urine from the system, as well as food crops fertilized by concentrated human urine are to be analyzed by Orbitrap MS to determine background levels of the analytes in the concentrated urine, and uptake of analytes by the food crops, respectively. The decreased discharge of nitrogen, phosphorous, and potassium nutrients from human urine into the water systems will improve the overall water quality of aquatic environments, while providing a natural source of key nutrients to be used as a fertilizer for food crops.

AGRO 349

Improving chromatographic performance of underivatized anionic polar pesticides in food to overcome renowned analytical challenges

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Various multi-residue LC-MS/MS methods are available to analyze food for pesticide residues, some pesticides are not amenable to such generic methods because they are highly polar and/or ionic in nature and so are not extracted and/or show poor retention under the generic C18 conditions typically employed. The chromatographic analysis of glyphosate, its metabolites and similar compounds are challenging due to the lack of retention by reverse phase techniques. Common alternatives include derivatization and ion chromatography. However, due to time-consuming sample preparation, MS incompatible solvents and the need for specialized equipment and/or reagents, the underivatized LC-MS/MS approach is still preferred. Due to the physicochemical characteristics of these compounds, repeatable peak shape and robust methodologies can be challenging to run routinely. A simple UPLC-MS/MS method will be presented for the direct analysis of highly polar pesticides, which provides analyte retention and excellent sensitivity, robustly, to exceed enforced MRLs. A panel of representative anionic polar pesticides, including aminomethylphosphonic acid (AMPA), glufosinate and glyphosate have been targeted in a selection of relevant foodstuffs prepared using Quick Polar Pesticides (QuPPe) extraction method. Chromatographic separation was achieved on a novel hydrophilic interaction liquid chromatography (HILIC) column, applying an ammonium formate mobile phase gradient. Method performance was evaluated by assessing chromatographic repeatability, linearity, accuracy and sensitivity. Satisfactory linearity was found for all pesticides over a range of 5 to 200 µg/Kg in food matrix (Corr > 0.995, residuals < 20%). The repeatability (%RSD) of the method was determined using spiked food matrix prepared in replicates (n=5).

AGRO 350

Comparison of cleanup efficiency for multiresidue analysis of pesticides in soybean by liquid chromatography tandem mass spectrometry

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In this study, we have evaluated the efficiency of different sorbents for the cleanup step in multiresidue analysis of pesticides in fatty vegetable matrices using modified QuEChERS method. The three different matrices tested (brown rice, soybean and soybean oil) were partitioned using acetonitrile and hexane prior to cleanup step. The extraction efficiency of solvents was compared to acetonitrile, acetonitrile+hexane. Afterward, the supernatant was purified using different sorbents; PSA, C18, C18+PSA, and Z-Sep. The different cleanup strategies were compared for a group of 321 representative pesticides in terms of recovery rates, matrix effects, extract cleanliness and precision using ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS). The best extraction efficiencies in soybean matrix were obtained using C18+PSA, while the results for matrices were pretty similar amongst the different sorbents with an overall lower performance in terms of matrix effects and recovery rates compared to soybean data, particularly in soybean oil due to the higher complexity and concentration of co-extracted species. On the other hand, the average reproducibility was clearly better when C18+PSA sorbent was employed in all selected matrices for most pesticides (RSD < 10% for 178, 115, and 152 pesticides in brown rice, soybean and soybean oil, respectively). The best results in terms of matrix effects were also obtained with C18+PSA; with signal suppression lower than 20% for 52%, 66% and 39% of pesticides tested in brown rice, soybean and soybean oil, respectively. Using C18+PSA as a cleanup sorbent, limits of quantitation using UHPLC–MS/MS, ranged from 0.01 to 0.5 mg/kg. As a conclusion, the combination of C18+PSA sorbent appears to be a more suitable method because it can be reduced the matrix effect rather than the other sorbents.

AGRO 351

Global reconnaissance of antimicrobial residues in wastewater and surface waters

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The occurrence of antimicrobials in the environment from discharges of wastewater treatment plants, and from the land-application of antibiotic-laden manure from animal agriculture is a critical global issue because these residues have been associated with the increased emergence of antibiotic resistance in the environment. Fluoroquinolones, macrolides, sulfonamides, and tetracyclines are approved for use in food-producing animals and are currently actively marketed. The problem is that these drugs are also considered to be important in human medicine, and their continued use for agricultural purposes may affect their efficacy in humans. Also, even though certain drugs such as ciprofloxacin, have already been banned for agricultural use in the U.S., they are still being used in other countries where there are no such regulations. In order to contribute to an improved understanding of antimicrobial resistance spread, a targeted analysis was performed to determine the levels of antibiotics in WWTPs and surface waters from four countries (Hong Kong, India, Philippines, and the United States) using LC-MS/MS. Results showed that the levels and composition of antimicrobials differ per country, with greater concentrations detected in Hong Kong, India and the Philippines, and lower levels in the United States. Ciprofloxacin was the most detected antibiotic and was found in all countries with concentrations from 23 to 2641 ng/L. Clarithromycin and Anhydro – Erythromycin were also commonly detected with concentrations from 4 to 877 ng/L, and 3 to 467 ng/L, respectively. High-resolution mass spectrometry was also used to do an untargeted analysis to find out what other environmentally significant compounds may be present in these samples.

AGRO 352

Automatic MS data analysis to reveal the metabolic pathway of flonicamid in oranges

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Automatic or semi-automatic methods for structure elucidation of metabolites for xenobiotics based on mass spectrometry (MS) data have been used in the recent years to a high degree of success. Typically, the biochemical reactions of xenobiotics are confined to a reduced set of chemical modifications that a compound can undergo. On the contrary, the analysis of samples from a pesticide like flonicamid in a complex matrix represents major challenge because the number of possible transformations is huge,

making it preferable to use a non-targeted detection approach like the "All Ion Fragmentation" to characterize the different potential metabolites. In this presentation we will show the use of a new procedure (MassChemSite) that can analyze this type of data, detecting the metabolites published and also assigning the structures to these metabolites with a high degree of accuracy and with no human intervention. In this way the known approaches for the structure elucidation of xenobiotics metabolites has been enlarged to a greater chemical and biochemical space. Methodology: Oranges treated with fonicamid (commercial formulation Teppeki®) at 1 mg/kg were evaluated. For that, 1.5 kg of fresh fruits (Navelate oranges) were crushed and homogenized, without neglecting any part thereof, skin or outer shell or bones as established by current regulations, storing it in the freezer at -21 C. 10 g of the homogenized sample was introduced in a 50 mL plastic centrifuge tube. Then, 10 mL of water and 10 mL of acidified acetonitrile (0.1% formic acid, v/v) were added and shaken for 1 min in a Polytron. After that, the sample was stirred for 15 minutes in a rotary shaker, and 1 g of NaCl and 4 g of $MgSO_4$ were added, and the mixture was shaken vigorously for 1 min in a vortex. Then, the samples were centrifuged for 10 min at 5000 rpm (4136g). 3 mL of the supernatant were collected and added in a 15 mL centrifuge tube with 50 mg of PSA. Subsequently, the samples were centrifuged for 5 min at 5000 rpm (4136g) and 1 mL of the supernatant was collected and injected into the UHPLC-MS-Orbitrap. The files from 1, 40 and 60 days after flonicamid application were directly analyzed by MassChemSite 2.0 and with a chemical book reaction developed for chemical degradation. Results: All the 3 previously reported metabolites were found and the structure was elucidated automatically by the software by comparing the fragmentation schema of the parent structures and the metabolites.

AGRO 353

Assessing the environmental risk of pesticides, biopesticides, and anthelmintics used in managing vector-borne diseases

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Insecticides, biopesticides, and parasitical agents are among the arsenal of tools used to control the transmission of vector-borne diseases. In the United States, these products are regulated by the U.S. Environmental Protection Agency under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) or by the Food and Drug Administration under the Federal Food, Drug, and Cosmetic Act (FD&C Act). Chemical and biological releases to the environment can occur from a variety of mechanisms including aerial or ground applications of pesticides to the landscape, wash-off of ectoparasiticides or excretion of anthelmintics from livestock or pets, and down-the-drain discharges from washing treated clothing. Approaches to evaluating the different delivery mechanisms and their potential adverse impacts to non-target organisms are presented through case studies.

AGRO 354

Comparison of the patterns of resistance and cross-resistance to insecticides conferred by the two major mechanisms of pyrethroid resistance in *Aedes aegypti*

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Mosquitoes are vectors of numerous human pathogens that kill or debilitate millions of humans each year. Because *A. aegypti* vectors four important human disease viruses, has a wide global distribution, and thrives in urban environments, it poses a serious risk to human health. Pyrethroid insecticides have been and are widely used for *A. aegypti* control, and their extensive use has resulted in resistance that is now found worldwide. Insecticide resistance is a major obstacle to the control of medically significant arthropod pests. To effectively manage the evolution of resistance it is important to know the levels of resistance conferred by specific mutations. To study the contributions of the different resistance mechanisms, we have created three pyrethroid resistant strains of *A. aegypti* that have the same genetic background as the susceptible ROCK strain: KDR:ROCK contains a pair of *Vssc* mutations S989P+V1016G (referred to as *kdr*), but no metabolic detoxification resistance; CYP:ROCK has cytochrome P450 (CYP)-mediated resistance but no *kdr*; and CYP+KDR:ROCK has both *kdr* and CYP-mediated resistance. Insecticide resistance of the individual and combined resistance mechanisms, *kdr* and CYP-mediated detoxification, were assessed by performing bioassays using structurally diverse pyrethroids on the three congenic strains and ROCK. Cross- and negative cross-resistance to organophosphates (OPs) was also assessed for the two strains containing CYP-mediated resistance. Our results indicate that there is a near multiplicative (more than additive) contribution to resistance to some pyrethroids when both mechanisms are combined. We found that resistance levels varied significantly, and that CYP-mediated resistance confers negative-cross resistance to some OPs. We also evaluated the contribution of *kdr* to resistance to two other classes of insecticides targeting VSSC (DDT and oxadiazines) and found that *kdr* has significant cross-resistance to DDT (> 2000-fold) and oxadiazines (13-38-fold). The implications of these results to the control of *A. aegypti* will be discussed.

AGRO 355

Chemical modulation of *Aedes aegypti* inward rectifier potassium ion channels prevents blood feeding and secretory activity of the salivary gland

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The mosquito salivary gland represents a critical tissue system that is essential for blood feeding by secreting hemostatic, anticoagulant, and digestive enzymes. This suggests that targeting the physiological mechanisms that enable proper gland function may represent a putative target site for the development of novel chemical agents to prevent mosquito feeding. Recent work from the Swale Laboratory has shown that inward rectifying potassium (Kir) channels are essential for fly salivary gland function, which has led us to hypothesize that Kir channels are a critical ion conductance pathway in the mosquito salivary gland that enables saliva secretion and pathogen transmission. To test this hypothesis, we employed structurally diverse chemical modulators of Kir channels to assess the influence to blood feeding of *Aedes aegypti*. Our data clearly show that multiple pharmacological modulators of Kir channels reduce the ability to ingest blood by 97.3%. To determine if the failure to feed was due to inhibition of salivary gland function, we quantified the fluid secretion from individual mosquitoes. Control mosquitoes were shown to secrete a mean of 1.2±0.3 nL whereas mosquitoes exposed to Kir channel modulators secreted a mean of 0.3±0.15 nL, which was a statistically significant ($P<0.05$) reduction in secretory activity. Next, we hypothesized that pathogen transmission would be reduced after exposure to chemical modulators of Kir channels. To test this hypothesis, we developed a model pathogen transmission assay with the use of the fluorescent probe, Rhodamine B. Indeed, inclusion of Kir channel inhibitors into the feeding solution reduced the transmission of the fluorescent dye by >99% for multiple Kir channel modulators. Our data suggest chemical modification of Kir channels represents a viable

mechanism to reduce mosquito blood feeding and transmission of pathogens.

AGRO 356

Chemical inhibition of inward rectifier potassium (Kir) ion channels prevents feeding and salivation of the cotton aphid, *Aphis gossypii*

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Aphids induce large economic losses to crops by feeding on sap and transmission of plant viruses. Importantly, resistance to currently deployed insecticidal classes is rapidly evolving and highlights the need for the development of novel insecticide targets for continued control of aphids. Aphids are phloem-feeding arthropods that rely on salivary gland secretions to digest plant cell tissue and ensure access of sap for feeding, suggesting the salivary gland may represent a target tissue for preventing aphid feeding and virus transmission. Unfortunately, an understanding of the physiological pathways required for proper salivary gland function of aphids is largely unknown. Proof-of-concept studies performed in our laboratory have shown that pharmacological inhibition and genetic ablation of salivary gland specific inward rectifier potassium (Kir) channels of *Drosophila melanogaster* dramatically reduces gland function and feeding success. Therefore, the objective of this study was to test the hypothesis that Kir channels represent a critical ion conductance pathway that is required for proper aphid salivary gland function and inhibition of these channels will prevent sap feeding. To test this hypothesis, we treated leaves of cotton plants with insect-specific Kir channel inhibitors to measure changes in feeding success rates. Our data provide clear evidence that contact exposure of *Aphis gossypii*, the cotton aphid, to Kir channel inhibitors prevents ingestion of plant products and nutrients. Analysis of feeding waveforms by the electrical penetration graph (EPG) technique showed a complete elimination of II-1 and II-3 potential drop (pd) waveforms after exposure to Kir channel inhibitors. Further, 0% of the aphids exposed to Kir inhibitors were capable of E1 (phloem salivation) and E2 (phloem ingestion). Importantly, inactive analogs to the Kir channel inhibitors did not alter feeding, suggesting the antifeedant properties are due to Kir channel inhibition. Our data suggests Kir channels represent putative target sites for mitigating aphid-mediated crop losses.

AGRO 357

Altering K^+ spatial buffering events through modulation of inward rectifier potassium (Kir) channels leads to nervous system failure and insect mortality

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Although greater than 90% of insecticides target the nervous system, a complete understanding of the physiological pathways critical for proper function of the insect nervous system is still lacking. In mammals, inward rectifier potassium (Kir) channels are known to maintain the resting membrane potential and regulate the firing threshold in neuronal cells, yet their role in the insect nervous system remains unexplored. Therefore, this work aimed to characterize the role of Kir channels in the insect nervous system and determine the potential for the development of insecticides targeting neuronal Kir channels. Exposure of *Aedes aegypti* and *Drosophila melanogaster* to VU041, an insect specific inhibitor of Kir channels, induced acute toxicity reminiscent of nervous system poisoning. We performed neurophysiological recordings of the excised *Drosophila* central nervous system (CNS) to test the hypothesis that Kir channels are critical for proper neural transmission in insects. Exposure to 300 nM VU041 increased the spike discharge frequency by 32 ± 6%, a statistically significant increase when compared to baseline spike discharge frequency ($P<0.05$). At increasing concentrations, VU041 was found to have a depressant effect on the *Drosophila* CNS activity with an IC_{50} of 23 µM. Immunohistochemical studies against the *Drosophila* CNS indicate that Kir channels are localized to the neural lamella, which is the extracellular matrix layer of the blood brain barrier and is important for maintaining the K^+ ion gradient of the CNS. Based on the data collected in this study, we hypothesize that the function of Kir channels in the insect nervous system is to regulate extracellular potassium concentrations $[K^+]_o$ during neural activity by distributing $[K^+]_o$ ions to areas of low internal K^+ concentrations, a process termed K^+ spatial buffering. Electrophysiological data supporting this hypothesis and the potential for neural Kir channels to be a target for insecticides will be discussed.

AGRO 358

Biorational products are effective spatial mosquito repellents against mosquitoes of multiple genera

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Diseases vectored by mosquitoes and other hematophagous arthropods are one of the largest medical issues impacting society today. Mosquitoes of several different genera are responsible for the transmission of the pathogens that ultimately lead to the development of malaria, West Nile Virus, Zika Virus, and a host of other life-threatening diseases. The common use of insecticides such as pyrethroids has given rise to the development of resistance to this class of insecticides in field populations of mosquitoes around the world. This development of pyrethroid resistance emphasizes the need for development of a new class of compounds to control mosquito populations. One major type of mosquito control strategy is simply to repel the insects from inhabited areas. Our lab has developed a series of biorational products, using plant-derived monoterpenoids as parent molecules, that have been shown to possess repellent properties. Using a bioassay developed in our lab we have been able to show varying degrees of repellency of these compounds against both *Culex pipiens* (northern house mosquito), the species closely linked to West Nile Transmission in the Midwestern United States and *Aedes aegypti* (the yellow fever mosquito), the species known to transmit the Zika and dengue viruses. The results provide evidence for the potential use of biorational products as part of a mosquito control strategy.

AGRO 359

Targeting ATP-sensitive inward rectifier potassium (K_{ATP}) channels to reduce the physiological burden of oxidative stress in European honey bees, *Apis mellifera*

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Decline of the economically important honey bee has become a globally recognized issue that has lead to a significant increase in the understanding of honey bee biology. Recent studies have shown that exposure of honey bees to pyrethroid insecticides during foraging elicits oxidative stress damage and, in turn, may affect the infectious disease tolerance of these individuals. There are several gaps in our knowledge with respect to the effects of oxidative stress in bees and further, little to no work has been performed to identify mechanisms that reduce the burden of reactive oxygen species (ROS) in managed honey bees. Yet in

mammals, ATP-sensitive inward rectifier potassium (K_{ATP}) channels are capable of reducing or eliminating the ROS-induced damage in cardiac, muscular, and neural tissues. Considering this, we aimed to test the hypothesis that honey bee K_{ATP} channels serve a protective role to the cell during times of metabolic stress by eliminating damaging free radicals. To test this hypothesis, we employed a pharmacological approach to assess the influence pharmacological activators and inhibitors have to the maintenance of oxidative free radicals. ROS was induced to individual bees by injecting known ROS inducers (i.e. H_2O_2, paraquat) that was shown to significantly ($P<0.05$) reduce total antioxidant activity by approximately 4-fold. Pharmacological activation of K_{ATP} channels was shown to increase the total antioxidant activity of bees exposed to ROS-inducers to levels that was not significantly different to control, suggesting a dramatic decrease in ROS. Conversely, inhibition of K_{ATP} channels reduced the levels of total antioxidant activity by approximately 2-fold when compared to H_2O_2 injected bees. Interpretations of these data will be discussed in an effort to advance our understanding of the mechanisms that protect bees against oxidative stress in an effort to improve the health and productivity of managed bee colonies.

AGRO 360

High-throughput screening apparatus for evaluating spatial repellency and vapor toxicity of commercially available and candidate repellent compounds

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Spatial repellents are essential for personal protection against mosquitoes that bite and transmit disease pathogens to humans. Current repellent screening methods, such as olfactometers and alternative choice tests, are complex systems that require a large quantity of compound (mg). This study validates a high-throughput spatial repellent screening method that occupies relatively little space and requires small amounts of compound (typically µg). Specifically, treated filter papers (5 cm^2) are held in caps on both ends of a 12.5 cm glass tube, and groups of mosquitoes (n = 16) are prevented from contacting the filter paper by netting. Spatial repellency is quantified by the proportion of mosquitoes on the treated side of the test tube. In this system, DEET had an EC_{50} (half effective repellent concentration on the filter paper) values of 31 (25-39) µg/cm^2 against *Ae. aegypti* wild-type (OR) susceptible strain. Screening of experimental fluorinated phenylamides identified several compounds with activity comparable to DEET (EC_{50} values, µg/cm^2, in parentheses): 1-4A (25), 4-2B (15), 4-3D (17), and 4-5A (17). Three anthranilate compounds also showed strong spatial repellency; ethyl anthranilate was most active, with an EC_{50} of 7 µg/cm^2 on OR, ca. 4-fold lower than DEET. A slightly modified version of the assay gave an EC_{50} for transfluthrin of 0.5 and 2.3 µg/cm^2 in susceptible OR and pyrethroid-resistant PR strains, respectively (4.6-fold resistance). The setup can also be used for assessing vapor toxicity. Transfluthrin-exposed OR strain had 1 hr KC_{50} value of 0.7 µg/cm^2 and 24 hr LC_{50} value of 0.15 µg/cm^2. The PR strain was 43-fold more resistant in 1 hr knockdown (KC_{50} = 30 µg/cm^2) and 53-fold more resistant in 24 hr mortality (LC_{50} = 7.9 µg/cm^2). To conclude, this high-throughput screening setup is useful for assessing fast-acting candidate spatial repellents and quantification of vapor toxicity against mosquitoes.

AGRO 361

Facilitating engagement on regulatory science in agriculture

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Higher Tier (non-guideline) studies are often conducted by industry and academics to address uncertainties associated with highly conservative Tier I guideline testing. Higher Tier studies are by nature more complex and more difficult to conduct and to interpret. Some classes of compounds have been the subject of numerous higher tier studies e.g., water monitoring, biomonitoring, multiple mesocosms, or spray drift studies. In addition, sometimes studies conducted by different investigators appear to give conflicting results. While scientists can often come to agreement on technical aspects related to a particular study, difficulties arise in the interpretation of multiple studies and use of these studies to make regulatory decisions. The 2017 Workshop on Innovation and Regulation in Agriculture was recently held at North Carolina State University with a topic of "Overcoming the Challenges of Incorporating Higher-Tier Data in Ecological Risk Assessments and Risk Management of Pesticides." Participants were experts from academic, government, including regulatory agency scientists, and industry. A set of recommendations were developed improve the process for designing and conducting, evaluating and utilizing higher tier studies for risk assessment and risk management of pesticides. These recommendations and potential actions will be discussed as part of this presentation.

AGRO 362

How higher-tier data can strengthen a pesticide risk assessment: examples with pyrethroids

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While a screening approach that focuses on worst-case scenarios using highly conservative assumptions can be a useful first step in assessing pesticide risk, lower-tier assessments are unlikely to provide definitive answers to many scientific and regulatory questions. Higher-tier data and analysis can support a more complete and accurate understanding of potential ecological effects and lead to more informed risk management decisions. However, incorporating higher-tier data into risk assessment and management requires a different paradigm than the screening-level approach of comparing simplistic risk quotients to pre-defined levels of concern. This presentation will use a comprehensive industry-funded aquatic assessment of pyrethroid insecticides to illustrate how different types of higher-tier data were applied to refine a screening-level regulatory assessment. The higher-tier approaches discussed will include species sensitivity distributions, refined exposure modeling and uncertainty analysis, joint probability curves, bioavailability calculations, landscape analysis, residue monitoring data, field experiments, mesocosms, and bioassessments. Each example will consider (a) the risk assessment/risk management question addressed, (b) technical aspects of the higher-tier approach to address the question, (c) methods for incorporating the higher-tier data into risk characterization, and (d) the usefulness of the higher-tier data for supporting risk management decisions. We conclude that the challenges to using higher-tier data are partly conceptual (i.e., the need

to refine risk questions in terms of magnitude and probability of exposure and effects) and partly institutional (i.e., the need for technical guidance and higher-tier regulatory standards).

AGRO 363

Probabilistic model for assessing risk to bird species potentially exposed to seed treatment pesticides

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Even though farmers use precise methods for sowing row crop seeds, small numbers may remain available on the soil surface. Thus, bird species foraging in agricultural fields could be exposed to unincorporated seeds treated with a pesticide. EPA's T-REX model for birds potentially consuming treated seeds does not account for seeds incorporated at planting, or for untreated waste grains present on untilled fields. Therefore, we developed the higher tier Seed Pesticide Avian Risk Assessment Model (SeedPARAM) for birds potentially exposed to treated seeds. The probabilistic model has an hourly time step and estimates acute and chronic risk to each of 20 birds per species on each of 1000 fields. Currently, the model includes eight bird species that forage for seeds in corn and soybean fields. Three routes of exposure are included in the model: consumption of seeds on the soil surface, consumption of below ground seeds obtained by uprooting young shoots, and consumption of young shoots. For each simulated bird, values are randomly selected for the major input variables (e.g., metabolic rate, proportion diet from treated fields) to estimate exposure. The simulation is repeated for 20 birds on each treated field. The model then moves to the next field. Fields differ with regard to pesticide dissipation rate, attractiveness to foraging birds, and waste grain availability. The number of waste grains on the soil surface affects the probability that a bird will consume a treated seed. The outer loop continues for 1000 fields. The model can either estimate probabilities of exceeding acute and chronic benchmarks or generate risk curves that define the relationship between cumulative probability and magnitude of effect. In this presentation, we illustrate SeedPARAM with a case study and discuss possible refinements for future versions of the model (e.g., considering foraging range, body size limitations for consuming large seeds).

AGRO 364

Evaluation of potential impacts of insecticides on aquatic invertebrates: Higher tier evaluations for risk management

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Many regulatory authorities, including U.S. EPA and Canadian PMRA, are required to perform ecological risk assessments in order to evaluate the likelihood that adverse ecological effects may occur as a result of exposure to a pesticide. The credibility of these assessments and their value for informing environmental decisions is based on the ability to accurately predict environmental exposures, understand ecological effects, and extrapolate predicted effects to population, community, and ecological function metrics. Screening level risk assessments, often the first step in evaluating pesticide risk, are based on worst-case exposure and effects predictions from baseline tools and studies. The uncertainty and assumptions of these assessments can be evaluated with higher tier effects, modeling, and monitoring data in order to revise the assessment to better predict real-world situations and allow for more informed risk management decisions. In this presentation, higher tier evaluations of neonicotinoid aquatic invertebrate risk assessments will be described as a case study.

AGRO 365

Opportunities and challenges of using NHDPlus connectivity data in refined modeling of aquatic exposure in flowing water bodies at the watershed scale

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Challenges in ecological risk assessment, including the evaluation of endangered species, require understanding exposure throughout networks of flowing water systems at the watershed scale. In these complex flowing water networks, the potential for exposure is largely driven by the connectivity and travel times of the upstream treated areas to the flowing water segments of interest. Current methods used in regulatory aquatic pesticide exposure modeling are based on a simplistic conceptual model of a single treated field adjacent to a static receiving water body. In flowing water bodies, however, upstream treated agricultural fields can be a significant distance to the receiving water body of interest, directly impacting time-dependent exposure profiles. This paper will focus on a methodology that takes advantage of nationally available NHDPlus watershed connectivity and hydrology data to reduce the uncertainty in potential runoff and drift contributions to aquatic exposure throughout flowing water networks. The methodology considers treated agricultural areas within NHDplus catchments independently to account for travel time of the source loadings to the flowing water segment of interest to realistically account for time delay and decay of upstream pesticide loadings. The approach represents a significant conceptual improvement in comparison to the existing screening level methodology and a simplification of process-based pesticide stream routing models with the advantage of using standard regulatory aquatic exposure models. Comparisons of simulated concentrations with monitoring data will demonstrate the more realistic results achieved. The approach developed can be combined with the nationwide available Cropland Data Layer to parameterize landscape-level PRZM model simulations and make refined, spatially explicit estimates of exposure in headwater and downstream catchments for use in ecological and endangered species risk assessments.

AGRO 366

Improving how we interpret results from the fish short-term reproduction assay (FSTRA) and the medaka extended one generation reproduction Test (MEOGRT)

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Higher tier endocrine tests like the FSTRA and MEOGRT are used to evaluate the potential for a chemical to cause endocrine disruption in tests. While variables such as fertility, fecundity, intersex and sex reversal have been identified as apical endpoints, other endpoints are used to evaluate mechanistic outcomes. Mechanistic endpoints are generally biomarkers that include secondary sex characteristics, vitellogenin, gonad pathology and plasma steroids. While guidance documents provide performance criteria and statistical methods for the analysis of these variables, there is little guidance on how to integrate all the variables into an overall assessment of whether a chemical is an endocrine disruptor. Not only is it important to evaluate statistically significant trends, but also whether the magnitude of the responses is statistically significant and biologically relevant

relative to controls. We argue that the pattern of the response is more important than the response of an individual variable. It is also important that variables are weighted in considering their importance in an overall pattern of responses. Jensen and Ankley (2014) provide guidance on breaking out results into overt toxicity, apical endpoints, and mechanistic endpoints for FSTRA studies, while Borgert et al (2011) have used weight of evidence methodology to evaluate endocrine activity for suites of studies. We have built on these methodologies to compare responses of known endocrine disruptors to the test chemical in order to identify clear signatures of responses of known ER-agonists, AR-agonists, or AR-antagonists for fish FSTRA and MEOGRT. Such an approach helps prevent using spurious results to draw erroneous conclusions in evaluating endocrine disruption in fish.

AGRO 367

Leveraging product specific residue data to refine dietary ecological assessments

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Glyphosate has low acute and long-term toxicity to birds and honey bees in standard regulatory studies. However, because of highly conservative assumptions in Tier 1 ecological risk assessments, there are challenges to pass a Tier 1 assessment for high use but low toxicity pesticides like glyphosate. Consequently, several exposure refinements have been introduced to these long-term glyphosate assessments to overcome the inherent conservatism of Tier 1 dietary assessments and achieve an acceptable ecological risk assessment for endangered and non-endangered species. Areas of exposure refinement that will be reviewed include residue studies with plants, insects, pollen and nectar. These studies were conducted to measure glyphosate-specific initial residues and rates of dissipation to calculate time-weighted-averages to refine exposure calculations for these long-term risk assessments. This presentation will describe how these data were developed and approaches to leverage these exposure refinements in higher-tier ecological risk assessments.

AGRO 368

Potential phototoxic response of Red Swamp Crayfish (*Procambarus clarkii*) to herbicides and fungicides

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In Louisiana, *Procambarus clarkii* are a staple in culture and cuisine, and they are raised in by unique farming method; farmers often alternate field usage between crawfish and rice. *P. clarkii* can inhabit a wide range of environments ranging from crop fields to bayous to roadside ditches. Often thriving in flooded rice fields, the crayfish are exposed to any and all pesticides that the crops were exposed to via water exposure, soil exposure, or ingestion of crops. Sunlight has the potential to enhance toxic effects of pesticides to organisms. The fungicide, dicloran, has shown to undergo photolysis and have phototoxic impacts to organisms such as inland silversides, fathead minnows, and eastern oysters; the herbicide, benzobicyclon, has also been shown to undergo photolysis and potentially accumulate in sediments. At concentrations of 0.50-mg/L dicloran, mortality can be observed for *P. clarkii*; 100% mortality was observed at 1.0-mg/L in the presence of light. *P. clarkii* that were exposed to benzobicyclon and light showed no mortality at concentrations ranging from 0.05-0.25 mg/L. Pesticides are not assessed for potential photo toxicity to non-target aquatic organisms as a part of the pesticide registration process and photo toxicity may be irrelevant in many situations for many compounds. However, this may be highly relevant for chemicals used in or near shallow water aquatic ecosystems housing economically important species.

AGRO 369

Biotechnology: RNAi, antisense oligonucleotides and CRISPR strategies to reduce psyllids and bacterial pathogens in citrus trees

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RNA-specific silencing like RNAi and antisense oligonucleotides, ASO, provide potent, sequence specific methods for animal and plant therapeutics. Topically applied RNAi, of double-stranded RNA, to tree foliage or soil continues to show promise for arthropod pest control. To target bacterial pathogens a next generation ASO called: FANA_ASO (2'-deoxy-2'-fluoro-D- arabinonucleic acid)_(antisense oligonucleotides) provides improved product stability and activity to reduce bacteria and other microbial pathogens in fruit crops and insects. Both strategies can target the RNA's in arthropod vectors: like hemipterans- Asian citrus psyllid, *Diaphorina citri*; the Glassy-winged sharpshooter leafhopper, *Homalodisca vitripennis*; Coleopterans (Curculionidae) and Lepidopterans. Results demonstrate that the improved stability of FANA_ASO, provides broader applications. Plant-delivered treatments were shown to reduce plant pathogens and insect pests. The first psyllid CRISPR-Cas9 knock-outs was able to result in psyllids with reduced fitness, and strategies are focused on altering psyllid's capacity to acquire and transmit the bacterial pathogen (C. Liberibacter asiaticus) linked to Citrus Greening Disease and tree death. Advances in gene suppression strategies now set a new foundation for the future of insect pest management. The suppression of pathogens, the pests, or modulation of the host plant defenses provides highly specific interdiction of agricultural problems.

AGRO 370

Genome editing: Technology for creating genetic variation in crop plants

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Plant breeders have been selecting upon naturally occurring genetic variation since the domestication of plants from wild populations. Genetic variation for traits like yield, flavor, nutrient composition, disease resistance, and drought tolerance has enabled improvements in plants that have resulted in the high performing crops that humans are familiar with today. Gene Editing techniques represent a new, diverse, and expanding set of tools that can be used to generate genetic variation, accelerate genetic gain, and further improve crops to accommodate the growing needs of humans as populations increase in the face of climate change. Some of these tools, particularly genome editing technologies, can generate variation in a highly specific manner that is similar or identical to naturally occurring genetic variation. In this talk we outline the need for these new technologies, the various techniques and potential applications, and regulatory considerations.

AGRO 371

Genetic improvement of potato by INNATE® and gene editing technologies

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Potato (*Solanum tuberosum* L.) is the fourth largest food crop after corn, rice, and wheat. Conventional potatoes are

typically vegetatively propagated due to inbreeding depression of heterozygous tetraploids. Simplot Plant Sciences (SPS) has used biotechnology to circumvent challenges associated with conventional breeding and to develop potato varieties with improved quality and disease resistance traits. Agrobacterium-mediated transformation was used to create multiple varieties possessing multiple traits, including lowered bruising and acrylamide potential, resistance to cold-induced sweeting, and late blight resistance. These commercial varieties were created using INNATE® technology, relying on the introduction of potato DNA to either overexpress a native gene or use of RNA interference to reduce expression of native potato genes, specifically in tubers. More recently, SPS has used precision gene editing (GE) technology to create new varieties by inactivating specific potato genes or through directed insertion of DNA at a given location in the genome. We will provide an update on our progress towards creation of compelling potato varieties using each of these technologies with a comparison of the merits and challenges associated with each.

AGRO 372

Low-cost and scalable production of RNA via cell-free bioprocessing

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GreenLight Biosciences has developed a scalable process for the production of low-cost RNA for agricultural applications. At the outset of development, a technoeconomic model was constructed and used to guide the R&D required to take GreenLight's proprietary cell-free RNA production pathways from nascent concepts to a cost-effective, scalable process. Key cost drivers were identified from model sensitivity analyses, and R&D tasks were established and organized to assess and improve on those cost drivers in order to meet economic targets. The efforts were organized into three modules. The first module comprised strain engineering and fermentation development and sought to maximize the production of enzymes and template plasmid used in GreenLight's proprietary RNA production reaction. The second module comprised recovery process development and sought to define recovery processes for the reaction additives, while minimizing losses. The third module comprised pathway engineering and biochemistry and sought to improve the yield and productivity of the RNA production reaction. This talk will describe how these R&D modules were defined using the model and how these modules were integrated to achieve a cost-effective, scalable RNA production process.

AGRO 373

***SmartStax®PRO*: The first commercial transgenic crop expressing insecticidal dsRNA to control corn rootworm**

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Corn Rootworm (CRW) *Bt* technologies have been in the market since 2003 and have provided significant value to growers. However, these various Bt proteins are represented by only several modes of action (MOA) thereby increasing the risk for resistance development. The use of dsRNA to control CRW represents a new MOA, that when combined with existing Bt technologies, should not only increase overall CRW control, but increase durability as well. This presentation will describe research that characterized the IRM potential of DvSnf7 dsRNA (including resistance selection) that will be stacked with Bt Cry3Bb1 and Cry34/35 to produce SmartStax PRO, the first commercial product controlling insects using dsRNA

AGRO 374

Midgut RNAi-based gene target for western corn rootworm control

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Western corn rootworm (WCR), one of the most devastating corn insect pests in the United States, has potential to cost U.S. farmers over $1 billion yield loss annually if not controlled. Transgenic traits based on proteins from Bt (*Bacillus thuringiensis*) have been important tools for controlling this pest over the last decade and efforts to prolong their durability have included refuge deployment, pyramiding of traits, and emphasis on integrated pest management. Nevertheless, reports of WCR resistance to Bt corn have heightened the urgency for developing new traits that will sustain the technology. Thus, DuPont Pioneer has worked to develop new corn rootworm actives including actives derived from non-Bt microbes, and RNA interference (RNAi) based gene targets that can be developed into effective traits. Based on RNAi technology, we recently discovered a midgut gene target DvSSJ1, which encodes membrane protein associated with smooth septate junctions (SSJ) and is required for gut barrier function. Insect bioassays using DvSSJ1 double-stranded RNA (dsRNA) demonstrated target mRNA suppression, growth inhibition and mortality of WCR larvae. Transgenic plants expressing DvSSJ1 dsRNA provided significant plant protection against WCR damage. DvSSJ1 represents a novel mode of action against WCR compared to current commercial traits and highlights the potential of non-Bt sources of future insect control traits.

AGRO 375

RNAi - Registration requirements for risk assessment inputs

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RNAi based biopesticides are a growing area of interest with respect to crop protection strategies. Regulatory authorities generally classify RNAi based biopesticides as either exogenously applied agents that control pests (dsRNA is evaluated like a biochemical pesticide) or as substances produced endogenously by plants (classified as plant-incorporated protectants or PIPs). In both cases an environmental risk assessment is needed for registration. Key components of any environmental risk assessment include toxicity information for various non-target species of concern and potential exposure levels. There is currently limited information available related to RNAi interference that addresses these components of the risk assessment. PIP based RNAi biopesticides can be evaluated like other GMO traits such as Bt. Exogenous dsRNA can be evaluated like biochemical pesticides. Exogenous dsRNA is generally regarded as safer than chemically produced conventional pesticides. Organisms in fact consume naturally occurring dsRNA as part of their normal plant diets. As such, the regulatory requirements for registration may be less stringent than for PIPs or for conventional pesticides. The recent advent of exogenous RNAi biopesticides has blurred the registration requirements. Registration of exogenous RNAi biopesticides may very well require a new paradigm. This presentation will focus on the testing needed to understand the metabolism and environmental fate of dsRNA and subsequent analytical methodologies to determine residues that may remain in the treated crops or the environment.

AGRO 376

EPA registration of dsRNAi Plant Incorporated Protectants: Implications for gene edited products

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After years of study and regulatory review, the U.S. EPA, in 2017, registered a dsRNAi plant incorporated protectants (PIP) construct for general commercial use as an insecticide to protect crop plants (SmartStax PRO Enlist™). Registration of the SmartStax PRO Enlist™ dsRNAi product may be a significant milestone in how EPA regulates this new generation of genetically engineered crop protection products. This presentation will address whether EPA's registration of a dsRNAi product for commercial use has implications for possible regulation by EPA of gene edited ag biotech constructs. In developing its safety assessment framework for RNAi products, EPA held two FIFRA Scientific Advisory Panel (SAP) meetings. The first meeting (January 28, 2014), considered questions related to the risk assessment framework that EPA's Office of Pesticide Programs (OPP) would apply to RNAi products. On September 27-28, 2016, EPA held a second SAP to address issues related to the SmartStax PRO Enlist™ RNAi pesticide product. The findings and recommendations of these two SAP panels informed EPA's regulatory decision on SmartStax PRO Enlist™. Generation of new biological constructs through the use of gene editing is occurring at a fast-accelerating pace. A cursory review of the literature reveals that recent genome editing advances relevant to crop protection include: generation of canker-resistant citrus varieties through use of CRISPR-Cas9 editing, development of powdery mildew resistant tomato using CRISPR-Cas9, enhancement of powdery mildew resistance in wheat using CRISPR-Cas9, and CRISPR-Cas9 mediated mutagenesis that resulted in enhanced fungal resistance in rice. The question is what type of regulatory oversight will EPA require of such pesticidal gene edited constructs. This presentation will provide insights as to what the regulation of dsRNAi constructs may portend for the regulatory framework that EPA will apply to gene edited products.

AGRO 377

Planning, performing, recording, reporting and archiving of analytical impurity profiling studies in compliance with principles of GLP

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Characterization of the parent compound is important and required prior to initiating any toxicological and analytical study. This information could be in the form of mass spectra, NMR data and chromatographic analysis. While characterizing the main component it is advisable to know details about related impurities, their abundance and their relevance. Depending on the nature of impurity, different approaches have been developed, published and followed to characterize them quantitatively or qualitatively. To prove the toxicological relevance of an impurity, profiling may include biological testing as well as analytical characterization. Analytical impurity profiling is conducted using a wide range of sophisticated equipment, data processing tools, and traceable reference standards. In toxicological studies, control of the biological test system is important, whereas analytical characterization has its own challenges in terms of operation, maintenance, calibration and monitoring of modern equipment. The main concern for regulators and GLP authorities all over the world is the integrity of data generated by automated equipment. Therefore, it is equally important to focus on the GLP aspects of the whole process, starting from receipt of impurity till archiving and retention of data. Overcoming the risk factors and the challenges associated with the generation of analytical data requires insight and a proactive attitude on behalf of the study director and the quality assurance auditor. In GLP studies, it is of prime importance to identify each critical phase and establish a detailed protocol and auditing scheme for various types of analytical studies. This presentation will emphasize best practices for planning, performing, recording, reporting, and archiving of analytical impurity profile studies in full adherence of GLP requirements.

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Roles of the Study Director, Management, Sponsor and the Quality Assurance Unit: GLP test control, reference substance preparation and characterization

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The requirements for characterization of test, control and reference substances are well established under EPA's 40 CFR Part 160 Good Laboratory Practices. Requirements for the production or synthesis are not addressed in the regulation in the same manner as is the characterization; however, the accuracy and retention of such records to support the preparation of test, control and reference substances are critical to support the reconstruction of the study. Sponsors, Managers, Study Directors and Quality Assurance personnel all play a distinct and important role in the full lifecycle of a test substance. Regulatory requirements can be successfully achieved by having an effective test facility operation that is aligned with EPA's expectations; this includes establishing well-articulated, management approved standard operating procedures, regularly documented training of GLP personnel, clear organizational structure and responsibilities, and a capable QAU. Using the recent draft OECD GLP Advisory No. 19, "On the Management, Chracterisation and Use of Test Items," the roles and responsibilities of the Sponsor, Study Director, Management and the Quality Assurance Unit will be discussed as it relates to test substance preparation and characterization through the lifecycle of a pesticide product.

AGRO 379

Use of quality metrics to drive the culture of continual improvements

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EAG Laboratories in Columbia, Missouri, implemented the tracking of Quality Metrics in 2006. The primary objectives of capturing these metrics are to track potential risks to compliance as well as issues regarding the review processes for raw data and reports. Since 2006, these metrics have evolved over time to continue to become more effective at looking for root causes and to drive the continual improvement processes in the laboratories. This evolution has occurred, and continues to occur, as Management utilizes the information provided by the Quality Assurance Unit to evaluate and implement necessary changes to procedures, documentation, and reporting at EAG Laboratories, Columbia to ensure that the highest quality product is provided to EAG's sponsors. This presentation will highlight the evolution of the Quality Metrics at EAG Laboratories, Columbia, provide examples of how Operations have used the metrics to make changes to positively affect these metrics, as well as demonstrate how metrics can be accepted into the culture of continual improvement at a testing facility.

AGRO 380

Best practices for obtaining samples of known quality

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Why should we care about sample collection and obtaining samples of known quality in GLP studies? Because analytical data is only as good as the quality of the sample from which the data are obtained; thus, decisions made from that data are highly dependent on the quality of the sample. Therefore, it is essential that we consider the importance of the sampling process during study planning and include procedural and documentation requirements for sample collection in the study protocol and/or standard operating procedures. In this presentation we will discuss guidelines for obtaining justifiable samples to improve analytical data which is the foundation for defensible decisions. What is the definition of a "Sample"? What is a "Representative Sample," and what constitutes a "Good Sample"? We will discuss the importance of how the GLP standards work to improve sampling processes and planning. We will cover an outline of best practices for obtaining samples of known quality. We will not discuss specific sampling protocols but instead, provide minimum best practices to support assurances that the sample is traceable from point of collection through analysis. This is accomplished by understanding management's role in the sampling process. They must support good sampling practices by ensuring the availability of adequate resources, hire and train qualified staff, provide the necessary tools, equipment and supplies for sample collection, and conduct ongoing and periodic training. A good quality sampling process involves clear and routine communication between project management, the sampling staff and the analytical laboratory for maintaining sample integrity through the careful documentation and handling of incoming samples. We will provide guidance as these all relate to compliance with the GLP standards.

AGRO 381

EPA good laboratory compliance

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Data summary reports are submitted to the EPA for studies relating to health effects, environmental effects, chemical fate testing, and to support applications for research or marketing permits. Questions may arise regarding the integrity of this data. EPA's GLP program is the link that assures data submitted under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) or the Toxic Substances Control Act (TSCA), can be relied upon.This presentation will be an overview of the EPA 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 FIFRA and TSCA. Mr. Myers will share what policies, practices and procedures laboratories should have in place to meet the EPA GLP regulations and how to avoid potential EPA enforcement actions.

AGRO 382

Industry: A look at the challenges facing CROs in the 21st century

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The agrochemical landscape and its regulatory ramifications have evolved quickly in the last 30 years. The last three decades have seen a surge on new chemistry and innovative approaches to pest management as well as increased awareness of environmental impact, resulting in ever-evolving regulatory guidelines. Contract research laboratories specializing in providing regulatory services for the industry face a myriad of scientific, technological, regulatory, economic, and even geographical challenges. We will explore some of these challenges facing our industry today and our approaches to contract research services as we move into the 21st century.

AGRO 383

Safety evaluation: Transport of dangerous goods – guideline requirement, challenges and solution

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Explodability data of the plant protection product are required by regulatory bodies. OPPTS and EC test methods suggest to perform thermal as well as mechanical (shock and friction) sensible. No single instrument or equipment is capable to cover both the aspects. There is an absence of certified equipment in the market for the determination of mechanical sensitivity. Therefore, labs generally customize it or perform only thermal sensitivity testing, leaving mechanical sensitivity untouched. Interpretation on the basis of thermal sensitivity testing is not completely justifiable; therefore, there is a need to develop an approach which at least defines thermal sensitivity on the basis of experimental outcome along with theoretical interpretation for mechanical sensitivity. We propose an approach based on the oxygen balance calculation and criteria for thermal decomposition energy value (< 500 J/g). Applicability of the representation of such data will be discussed in detail.

AGRO 384

View from ten thousand feet: How has agriculture been impacted by legal changes over the past 20 years?

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Review of past submissions and the impact of plant protection products on legal considerations. Related new topics on registration and regulation of agrochemicals. New considerations for related agrobusinesses such as the new tobacco regulations. What have we learned over the past 20 years? What do we need to consider?

AGRO 385

Introduction to the systems for agrochemical patent term extension across Europe

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The processes for obtaining an extension to the term of an agrochemical patent are currently handled at the national level by each sovereign state on the European continent. This can make it rather more complex for a third party to obtain a comprehensive overview of when an active ingredient is due to lose patent protection. This presentation reviews the legal mechanism to the granting (or denial) of term extension, the sources of public data from a sample of major countries in Europe, and the possible impact of the proposed unitary effect registration for European Patents.

AGRO 386

Patenting the unpatentable? Opportunities for protecting trade secret processes under the America Invents Act

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The 2011 America Invents Act (AIA) changed the U.S. patent system from a "first to invent" system to a "first inventor to file" system for prior art assessment purposes. Under the pre-

AIA law, the commercialization of a secret process (such as a trade secret process for making an agrochemical composition) foreclosed the possibility of obtaining a patent for that process after one year had passed from the initial offer for sale. As a result, companies were faced with the decision of either filing a patent application on inventive processes or keeping them as trade secrets. Originally, most commentators and the US Patent & Trademark Office interpreted the AIA as eliminating this "secret" prior art, opening the door to filing patent applications on long-held trade secrets. Recent developments in the case law, however, in particular the Federal Circuit's *Helsinn v. Teva* decision, have created great uncertainty on this issue. The presentation discusses the old "secret" prior art standard, the various views of how the AIA should be interpreted, and recent case law developments. It concludes with practical recommendations on how best to protect trade secret processes in view of the uncertain status of the law.

AGRO 387

Resistance and trait considerations in plant protection products

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The presentation will look at registration considerations for plant protection products in regard to seed traits, trait resistance, best yield, grower considerations and best practices. Some topics of interest will be plant trait and yield history considerations, variety and germ traits, EPA and USDA efforts with industry and crop consultant groups to address resistance, and extending the life span of crop protection products. Success stories such as the Bt traits that have had a 20+ year of effective use will be discussed. Considerations for new chemistries and traits that are highly specific, pros and cons.

AGRO 388

Continuing evolution of the coordinated framework: Implications for agricultural biotechnology

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Toward the end of President Obama's tenure, his Administration released two documents intended to update the Federal Government's Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework) to make it more relevant to the current and expected future state of biotechnology in the United States. Consistent with this updating of the Coordinated Framework, EPA, USDA, and FDA have in the past year taken actions to update regulations and regulatory guidance affecting agricultural biotechnology. This presentation will discuss the changes to the Coordinated Framework, examine the recent regulatory actions, and explore their implications for future continuing advances in agricultural biotechnology. Particular attention will be given to implications for new products created through gene editing. Gene editing technology is poised to revolutionize agricultural biotechnology. It remains to be seen whether U.S. Government regulatory policy will facilitate or hinder this revolution.

AGRO 389

Opportunities and challenges for obtaining and defending patents in genetically modified or altered agricultural products, in creating new life forms, and in improved in agrochemical processes

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Laws relating to patents on novel forms of animals, seeds, and crops have undergone significant changes in recent years. Such changes have increased the challenges for researchers and industries to obtain effective patents to protect such inventions and their investments. The talk will review the various pronouncements that have come out in recent years from the United States Patent & Trademark Office on what subject matters are eligible for patenting in the above areas, in particular, DNAs or new life forms. The talk will also review some of the recent opinions issued by the courts in lawsuits involving agrochemical patents, for example, patents relating to modified soybeans, cloned animals, and herbicide-resistant corn. The talk will highlight what the courts are looking for in patent challenges, for example, in regard to patent eligbility as well as other requirements such as how to make and use the invention, and the need to fully distinguish the invention from other products or processes on the market or those already patented.

AGRO 390

GMO patents in the courtroom

***Carlyn A. Burton**, burton@oshaliang.com.* OSHA Liang, Houston, Texas, United States

This talk will examine the history of GMO patents in the courtroom. It will involve a discussion of cases in which patent holders enforced their patent rights over farmers, declaration judgments sought by growers, seed selling business and agricultural organizations against GMO patent holders, as well as suits between competitive agribusinesses.