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Abstracts

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AGRO DIVISION

AGRO 1

Agrochemicals: A cornerstone of agriculture

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Global food production has increased approximately 3-fold over the past 50 years. Such growth is attributed largely to advances in agricultural science and technology since the mid-20th century. Agrochemicals are essential to pest control, crop production and human health. Recent development and applications of agrochemicals are highlights of multi-disciplinary studies. In food and environmental analyses, chelate reagents such as Na_4EDTA are an effective modifier to improve extraction efficiencies of polar analytes from matrices, while chelates in root exudates enhance bioaccumulation of organic pollutants such as perfluorooctanoic acid in lettuce. Single-domain antibody and pattern recognition technologies have revolutionized immunoassay diagnostic kits to be robust, portable, multi-targets and easy to run. In addition, a few stories will be used to illustrate recent advances in mechanistic understanding of microbial transformation of agrochemicals, structural characterization of N-glycosylated plant and fungal peroxidase, and signal transduction between hosts and pathogens. This talk will also describe the potentials of enzymatic technology in bioremediation as well as sensitized photolysis and catalytic ozonation for wastewater treatment, where microbes, flavonoids, sunlight, zero valent iron, and ozone are utilized. The pi-cation interaction was first found in an antibody recognizing polycyclic aromatic hydrocarbons and has recently been used to improve the specificity and potency of glycogen synthase kinase-3β inhibitors for the interest in Alzheimer's disease treatment. Our recent discovery of a novel monoterpenoid insecticide and its mode of action will be briefly discussed.

AGRO 2

Dihydromyricetin targets 78-kDa glucose regulated protein in 3T3-L1 cells for anti-obesity effects

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Obesity is one of the most serious public health problems worldwide. Accumulation of excessive adipose tissue results in the onset of obesity. Therefore, the identification of compounds that inhibit adipose tissue accretion has been a significant effort in developing therapies to treat obese patients. Several herbs used by the Chinese for hundreds of years are known for their weight-losing ability. One such herb plant is *Ampelopsis grossedentata*. We have isolated a compound from this herb, and this compound inhibited fat cell differentiation. Subsequently, we identified this compound is dihydromyricetin, a flavonoid. Cellular studies showed that dihydromyricetin and the positive control epigallocatechin gallate (EGCG) have significant effects on reducing intracellular oil droplet formation in 3T3-L1 adipocytes, with a median effective concentration (EC_{50}) of approximately 400 μM and 75 μM, respectively. Although dihydromyricetin is known to inhibit adipogenesis, the molecular target has not been identified. The identification of the target protein of dihydromyricetin is crucial in the elucidation of its physiological function and clinical values. Thus, the objective of this study was to identify the cellular target of dihydromyricetin. Mouse 3T3-L1 preadipocytes were used to establish the optimal dihydromyricetin concentration to be used in subsequent experiments. We used the drug affinity responsive target stability (DARTS) and surface plasmon resonance (SPR) experiments to demonstrate the direct interactions of dihydromyricetin and EGCG with GRP78. The 78-kDa glucose regulated protein (GRP78) is associated with many disorders and is essential for adipocyte differentiation and adipogenesis. The DARTS and SPR experiments showed that GRP78 directly interacts with dihydromyricetin and EGCG, having a dissociation constant (K_D) of approximately 22 μM and 6 μM, respectively. The results suggest a new understanding of dihydromyricetin and EGCG in the modulation of obesity and hold promise for anti-obesity applications. Structural modifications of dihydromyricetin may be worthwhile for anti-obesity drug discovery.

AGRO 3

Recycling use of organic waste: New approach for a developed city in China

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Globally, annual organic waste generation is huge, and the management of organic waste becomes one of the major challenges in the twenty-first century. It might cause pollution to the water bodies, soil, and atmosphere, and could cause a loss of a mass of nutrient resources if managed improperly. Various technologies, as anaerobic digestion, aerobic composting and vermicomposting were developed to convert organic waste into bio-energy, compost, etc. But the resource use of end-products was relatively low, *e.g.*, only 15% in Wuzhong district, Jiangsu, China. Here, the Organic Recycling Research Institute (ORRI) of China Agricultural University, which was established in 2018, proposed a demo-project on the organic recycling at Suzhou city and tried to remove organic waste pollution in the urban and rural areas. The key concept of the project was the process of "continuous biological drying + aerobic composting," and all raw organic waste could be treated well by using different closed-type of reactors within one week. Such organic waste treatment processes shall be applicable to those de-centralized locations, like restaurants, schools, agro-product markets, and villages or townships. The end products, like organic fertilizer and soil conditioner can be used locally to support the development of ecological agriculture. Further, ecological environmental and economic benefits were analysed based on the case study at Linhu village and surrounding areas of the Tai lake. This could be of benefit by linking organic waste

recycling and environmentally friendly agriculture together in those densely populated regions in China and the World.

AGRO 4

Efficacy of non-psychoactive phytocannabinoids in a novel phenotypic drug-screening platform for old age-associated neurodegeneration

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Finding an effective therapy for old age-associated neurodegenerative disorders such as Alzheimer's disease (AD) is extremely challenging for modern medicine. The chemical scaffolds of many drugs in the clinic today are historically based upon natural products from plants, yet *Cannabis* has not been extensively examined as a source of AD drug candidates. Clearly, psychoactive phytocannabinoids such as tetrahydrocannabinol (THC) are unlikely viable AD drug candidates. However, the non-psychoactive counterparts as treatment options for neurological disorders have not been fully explored. Here, we test a hypothesis that non-psychoactive phytocannabinoids are neuroprotective in a novel preclinical AD and neurodegeneration drug-screening platform that is based upon toxicities associated with the aging brain. Several major phytocannabinoids and their synthetic derivatives were investigated for neuroprotection in phenotypic assays that recapitulate proteotoxicity, loss of trophic support, oxidative stress, energy loss, inflammation, and mitochondrial dysfunction. Nine phytocannabinoids were able to protect cells in these screening assays. They were able to remove intraneuronal β-amyloid, reduce oxidative damage, and protect from the loss of energy or trophic support. Their modes of action are independent on interactions with cannabinoid receptors (CB1 and CB2) in neurons. Structure-activity relationship analysis showed that functional antioxidant groups such as aromatic hydroxyls on phytocannabinoids are necessary for neuroprotection. Among them, cannabinol (CBN) showed a sub-micromolar potency in four of five distinct phenotypic screens. In addition, CBN modulated mitochondrial OXPHOS system against cellular stresses. Pairwise combinations of CBN and THC led to a synergistic neuroprotection. Together, the results indicate that non-psychoactive CBN exerts novel neuroprotective mechanisms and is a promising drug lead. Future efforts on the lead optimization might yield novel drug candidates for AD and other neurodegenerative diseases.

AGRO 5
Photopharmacological ligands for receptor, cell, and insect behavior modulation

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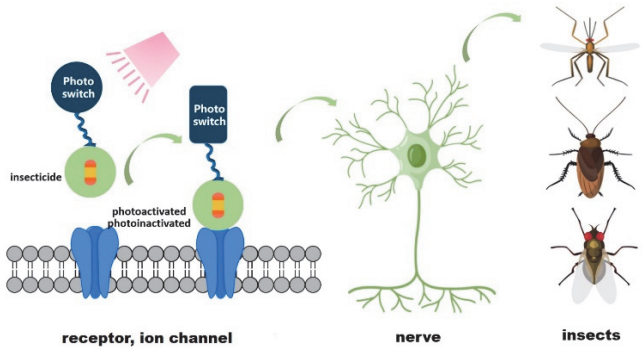
Photopharmacological ligands provide high spatiotemporal control over bioactivity and revolutionize our way for understanding toxicological mechanisms. However, applications of this technology in pesticide research remain exclusive. By modification of insecticides with photoswitches azobenzene or dithienylethene, we prepared a series of photopharmacological ligands for insect nAChR, GABAR, RyR, GluCl and voltage-gated sodium channel. These ligands enable reversible and spatiotemporal modulation of insecticidal activity, receptors, neurons and behavioral response of living organisms. For example, dithienylethene-imidacloprid can be activated by light and allows for optical control over insect nAChRs, cockroach DUM neuron and insect behavioral responses of mosquito larvae (*Aedes albopictus*) and cockroach (*Periplaneta americana*). These photoresponsive ligands provide novel toolkits for studying insecticide-receptor and insecticide-insect interactions.



Photopharmacological ligands for optical control of receptors, cells and insect behaviors

AGRO 6

Microcystins show high ecological and human health risks in vegetable fields

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Frequent cyanobacterial blooms in the eutrophic waters release massive monocyclic heptapeptide microcystins, greatly harming aquatic ecosystems and human health. However, little information of microcystins in agricultural fields is available. We conducted a large-scale investigation on three common microcystin variants (MC-LR, MC-RR, and MC-YR) in vegetables (n=161), soils (n=161) and irrigation water samples (n=23) sampled from southern China regions affected by cyanobacteria blooms. Microcystins are detected in almost all irrigation waters, soils, and vegetables, with concentrations up to hundreds of μg/L or μg/kg. Irrigation waters are the major source of microcystin accumulation in both vegetables and soils. Intracellular-MCs in the irrigation waters is found to play an important role in microcystins bioaccumulation in vegetables. Microcystins in more than half of soil samples pose high ecological risks. Microcystins in sixty percent of vegetables show moderate or high human health risk via diet based on the toxicity equivalents model, threatening food safety and human health. This study gives insight into the ecological and human health risks of microcystins in realistic vegetable fields, calling for both technical and management strategies to reduce the microcystins level and to ensure ecological and crop safety, particularly in the global-warming era.

AGRO 7

Quantitative detection of fipronil and fipronil-sulfone in sera of black-tailed prairie dogs and rats after oral exposure to fipronil by camel single-domain antibody-based immunoassays

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The insecticide fipronil can be metabolized to its sulfone in mammalian species. Two camel single-domain antibodies (VHHs) F1 and F6, selective to fipronil and fipronil-sulfone, respectively, were generated and used to develop enzyme linked immunosorbent assays (ELISAs) for the detection of the two compounds in the sera of black-tailed prairie dogs and rats. The limits of detection of fipronil and fipronil-sulfone in the rodent sera by the corresponding ELISAs were 10 and 30 ng mL^{-1}, and the linear ranges were 30–1000 and 75–2200 ng mL^{-1}. ELISAs showed a good recovery for fipronil and fipronil-sulfone co-spiked in the control sera of the black-tailed prairie dogs (90–109%) and rats (93–106%). The VHH-based ELISAs detected fipronil and fipronil-sulfone in the sera of the rodents that received a repeated oral administration of fipronil. The average concentration of fipronil-sulfone was approximately 3.2-fold higher than fipronil in the prairie dog sera (1.15 vs 0.36 μg mL^{-1}) and rat sera (1.77 vs 0.53 μg mL^{-1}). ELISAs agreed well with a liquid chromatography-mass spectrometry method for the quantification of both fipronil and fipronil-sulfone in real serum samples. Fipronil-sulfone was identified as the predominant metabolite of fipronil in the black-tailed prairie dog and rat sera.

AGRO 8

Establishment of an indirect competitive ELISA for flubendiamide and new insecticidal molecule discovery of phthalic diamide

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Flubendiamide (FBDA) as the first commercialised phthalic diamide insecticide that targets insect RyRs was launched in China in 2008-2009, and as an alternative of toxic organic phosphate insecticides methamidophos, acephate, etc., was used to control rice stem borer before 2016. With extensive application, it was found that the continued use of FBDA results in unreasonable adverse effects on the environment, particularly benthic invertebrates, and then FBDA registrations on rice in China were cancelled in 2016. Although its application in rice fields is limited, it is still applied extensively on other crops, vegetables, and fruits, and in particular it is still used worldwide against bollworm outbreak, and by value category it still ranked in the top ten of the global pesticide market. Thus, it is significant to establish a quick method to detect its presence in environment and foods.

In this study, a rapid and convenient method was established to detect FBDA. Upon keeping the integrity of FBDA structure, hapten was prepared with the following process, aliphatic section was obtained by coupling 2,2-dimethylacridine and ethyl 3-mercaptopropionate, and then cleaved 3-iodophthalic anhydride followed by condensation with substituted aniline, oxidation by hydrogen peroxide and hydrolysis. Hapten was conjugated with BSA and OVA via the active ester method as immunogen and coating antigen, respectively. Corresponding mAbs were also produced by immunizing mice and used in indirect competitive enzyme-linked immunosorbent assay (icELISA) development for FBDA detection. The established icELISA showed a half maximal inhibition concentration (IC_{50}) of 17.25 ng/mL, with a working range of 4.06-103.59 ng/mL for FBDA, and showed no cross-reactivity with chlorantraniliprole (CATP), cyantraniliprole. Average FD recoveries from spinach, tap water, and soil samples were 89.3-112.3%, 93.0-102.1%, and 86.9-97.6%, respectively. Meanwhile, FD detection results of icELISA aligned to those of ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The comparable results verified that icELISA was suitable for rapid detection of trace FBDA in environmental and agricultural samples.

AGRO 9

Development of plantGlycoMS, a set of bioinformatics tools, to interpret mass spectrometry-based glycoproteomics data

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Plants are sessile organisms programmed to adapt to their environment. Decoding this program is key to developing robust and high yielding crops. The plant *N*-glycoproteome rapidly responds to stress. Mass spectrometry-based glycoproteomics, which studies *N*-glycans and the attached proteins, can decipher pathways regulated by glycosylation. We designed a workflow and tools for analysis of plant glycopeptide mass spectrometry data that addresses interpretation issues arising from unique plant glycosylation patterns, glycosylation heterogeneity, and complex fragmentation. We used a highly glycosylated palm peroxidase (a robust enzyme for synthesis of fine chemicals and nanomaterials) as a test case. plantGlycoMS is a set of tools, implemented in R, that validates glycopeptide spectrum matches (gPSMs). Validity of gPSMs is based on characteristic fragmentation patterns (gPSMvalidator), adherence of the glycan moiety to the known *N*-glycosylation patterns in plants (pGlycoFilter), and elution of the glycopeptide within the observed retention time window of its alternate glycoforms, *i.e.*, other glycopeptides sharing the same peptide backbone (rt.Restrict). plantGlycoMS also quantifies the relative distribution of glycoforms using selected ion chromatograms (glycoRQ). Our workflow provided a detailed glycosylation profile at the 13 sites of *N*-linked glycosylation on windmill palm tree peroxidase. Glycan microheterogeneity was observed at each site. Site Asn211 was the most heterogeneous, containing 30 different glycans. Relative quantitation revealed 90 % of each glycosylation site was occupied by three or fewer glycans and two of the 13 sites were partially unoccupied. Although complex and hybrid glycans were identified, the majority of glycans were paucimannosidic, characteristic of plant vacuolar glycoproteins. Future work is aimed at adapting the workflow to analyze an entire glycoproteome instead of a single

glycoprotein. Glycoproteome studies will be conducted with the model grass, Brachypodium distachyon. Accompanying objectives to validate glycoproteome studies include development of glycosylation mutants via targeted mutagenesis and assessment of phenotypes related to environmental triggers.

AGRO 10

Smartphone-based rapid quantitative detection of pesticide multi-residues using colloidal gold immunochromatographic strip

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A Colloidal gold immunochromatographic strip (CGIS) immunoassay with a smartphone-based readout system for simultaneous detection of imidacloprid, carbofuran, and procymidone was developed. The system has a multi-channel CGIS bearing platform (Fig. 1). The platform has 6 bearing grooves arranged in a circumferential array, which can place 1-6 CGISs one time, and the number of grooves can be increased in the platform. By setting a barcode on the absorbent paper part of the CGIS, the system can automatically identify the information of the each CGIS as well as its corresponding standard curve. Pictures can be taken at any angle and height above the platform by smartphone as the developed software system can automatically conduct image correction. The specific working process of the developed system is shown in Fig.2. The CGIS was inserted into the sample well containing the sample extract. After color development reaction, place the CGIS in the bearing groove. Take photos with the built-in camera of smartphone, and uploaded the pictures to the cloud computing server through the self-developed WeChat applet. The image could be automatically identified and corrected, and then the color intensity values of testing line (*It*) and control line (*Ic*) of each CGIS could be accurately extracted and calculated. Calibration curves were constructed by matching the *It*/*Ic* value of each CGIS and the concentration of the analyte, thereby achieving simultaneously qualitative and quantitative detection of multiple pesticide residues. The test results will be presented on smartphone interface in real time. Accuracy of the developed image recognition system is consistent with the professional CGIS readers (R^2>0.99) in the laboratory standard environment. Besides, simple and effective sample preparation methods were developed to extract imidacloprid, carbofuran, and procymidone from spiked leek and tea samples. Water alone was used as the extraction solution, without using any organic solvent, and high extraction efficiency was achieved (Recovery>90%), which had been verified by liquid chromatography-tandem mass spectrometry analysis.

AGRO 11

Immunoassay perspective

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A perspective on enzyme immunoassays will illustrate a short history of the technology and its evolution to a mainstay analytical method with wide ranging applications for human health and the environment. Methods range from simple on-site analyses to determine the safety of worker entry into Superfund sites or agriculture fields to highly sophisticated methods to determine the effects of exogenous compounds on xenobiotic metabolism and cellular activity. Concerns regarding high analytical costs and uncertainties in assessing human exposures to environmental contaminants have been expressed by risk assessors. The public has also strongly expressed a desire to know the health of their communities and the relationship of public health and exposures to environmental contaminants. Immunoassays were first introduced for medical and clinical settings for the quantitative determination of proteins, hormones, and drugs with a molecular mass of several thousand Daltons. After a proven record for these types of compounds, methods such as enzyme immunoassays began to make inroads into the analyses of pesticides and other small molecules. This large range of analytes enables complex studies to determine environmental concentrations, human exposures, and *in vivo* effects of contaminants including biomarker discovery, proteome and metabolome alterations, and cellular activity. The integration of these types of data are needed to safeguard public health and the environment. Immunoassays for paraquat, polychlorinated biphenyls, chlorpyrifos, TCP (3,5,6-trichloro-2-pyridinol), 3-phenoxy benzoic acid, and isoprostane will illustrate the applicability of antibody-based methods for human exposure assessment and environmental monitoring.

AGRO 12

Evolution of mass spectrometry from the Hawaiian Islands to modern pesticide discovery

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Dr. Li's lab at the University of Hawaii has long provided crucial analytical information of pesticide residues supporting both local and national studies of pesticide distribution and fate. In the mid-1990s, the expertise of the Li lab was called upon to identify the source of a mysterious area of dead wildlife observed by a U.S. Department of Fish and Wildlife team on an uninhabited island of the Hawaiian Islands National Refuge. The toxin was identified as Carbofuran, and distribution was quantified across a delineated spill zone. Metabolites were also identified, including one previously unidentified product, which was confirmed by synthesis. The techniques used to make this identification more than 20 years ago can still be found in the modern analytical lab supporting pesticide research and development. These mass spectral techniques have improved in sensitivity and resolution over the previous 2 decades as GC-MS has largely been replaced by LC / HPLC / UPLC techniques, and large quadrupole systems have given way to benchtop sized high resolution mass analyzers. Though the instrumentation is different, the approach of tackling important analytical questions relating to pesticide distribution and metabolism by MS is still a critical part of new pesticide discovery and optimization through product development and registration.

AGRO 13

Reducing "identity crisis" in suspect screening of contaminants of emerging concern in the environment by high resolution LC/MS

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Suspect screening using liquid chromatography with high resolution mass spectrometry is a valuable approach in expanding the detection coverage of contaminants of emerging concern (CECs) in the environment. Current suspect screening workflows suffer from high frequency of false positives or insufficient confidence in the true identity of the compounds detected. A workflow was developed to determine the occurrence of CECs in wastewater and surface water samples from various countries. Results revealed the presence of 68 contaminants that include pharmaceuticals, pesticides, and industrial chemicals. The antimicrobials metronidazole, clindamycin, linezolid, and rifaximin, were detected in this suspect screening approach at concentrations of up to 650 ng/L. Notably, antifungal compounds were detected in samples from all six countries, with levels up to 1,380 ng/L. The presence of the amoxicillin transformation products, penilloic acid (285 ng/L – 8047 ng/L) and penicilloic acid (107 ng/L), were confirmed for the first time with reference standards in wastewater samples. The workflow reported in this study provides an efficient approach in the identification of CECs using open access databases for suspect screening and prioritization such as lists from the Network of reference laboratories, research centers, and related organizations for monitoring of emerging environmental substances (NORMAN) database system and the U.S. Environmental Protection Agency Chemistry Dashboard.

AGRO 14

***In situ* detection of atrazine contamination in natural waters**

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Wetlands are impacted by agrochemical contamination due to their extensive use by farmers. Atrazine, a common herbicide in the U.S., has been detected in natural waters and can be harmful even at low concentrations, with a maximum concentration level (MCL) in drinking water of 3 mg/L. The gold standard for atrazine analysis, chromatography-mass spectrophotometry, is precise but expensive, complex, and time-consuming. We developed an inexpensive label-free photonic sensor for rapid detection of atrazine in the field. The sensor is composed of a molecularly imprinted polymeric (MIP) porous film that provides selectively and optical active pore structure, similar to an inverse opal. Silica particle colloidal crystals were obtained by vertical self-assembly from 0.1% volume fraction suspensions in ethanol at 50°C. MIPs were fabricated with acrylic acid as the functional monomer, ethylene glycol dimethacrylate as crosslinker and UV-polymerization. The films, approximately 5 mm thin, were supported on polymethylmethacrylate slides. The silica particles were etched in a HF bath and the imprinted atrazine eluted with a solution of acetic acid in ethanol. Colloidal crystals were imaged by scanning electron microscopy to characterize morphology and number of particle layers, which appeared to be around 10-12 layers. MIPs were incubated in atrazine solutions for 20 minutes to reach equilibrium, as indicated by the kinetic experiments. Their reflectance spectra were recorded before and after exposure to atrazine, using a benchtop double-beam UV–visible spectrophotometer. Due to the swelling of the film, the peak wavelength of the reflectance spectra shifted with increasing atrazine rebinding when the polymer was incubated in a sample. This wavelength shift was correlated with concentration in the original sample. The sensors had a dynamic range of 0.14-12.3 ppb. Calibration curves were generated using LC-MS validated standard solutions. The reported sensor is a simple, fast, and efficient tool for atrazine analysis in natural waters.

AGRO 15

Innovation of agrichemicals for plant disease: The current situation and future

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The growth of global population poses a huge challenge to our food supply. Continuous application of crop protection products also raises many issues such as resistance, residue, ecological damage, *etc*. Nowadays, it is challenging to develop agrochemicals with high efficiency, selectivity, environmentally friendly, low use rate, and cost-effective. In the past decade, many great efforts have been made. Many fungicides with novel structures and mechanism have been discovered and launched. However, many of them were banned due to some faultiness in toxicity, environment and ecology. Recently, many active ingredients have been discovered, some of them can be used as potential candidates. In the future, more efficient and low-risk pesticides with more unique mechanisms, novel structures, ultrahigh-efficiencies and higher selectivity should be developed for solving the problems of plant disease. Hence, cutting-edge developments in natural product chemistry, asymmetric synthesis, rational drug design, and plant immune induction, have provided potential solutions for the discovery of crop production products with the aforementioned properties. The comprehensive use of these technologies will help to tackle current challenges and issues.

AGRO 16

Effects of endophytic bacteria *Enterobacter cloacae* strain TMX-6 inoculation on the degradation of thiamethoxam in rice

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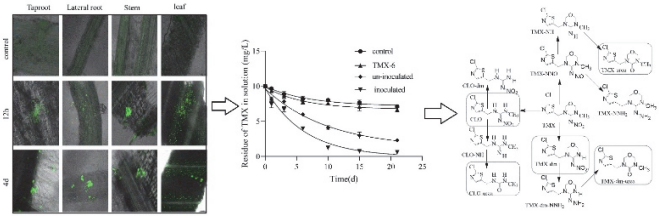
Thiamethoxam has become a widely used neonicotinoid pesticide over the last two decades. The residue of thiamethoxam in the environment has drawn great public attention. An endophyte bacterium TMX-6 capable of degrading thiamethoxam was isolated from wild *Ophiopogon japonicus*. TMX-6 was classified as *Enterobacter cloacae*. After being marked with green fluorescent protein (gfp) plasmid, TMX-6-*gfp* was successfully inoculated in rice (*Oryza sativa L.*). The colonization and distribution of TMX-6-*gfp* were observed directly in rice tissues with a confocal laser scanning

microscope. The inoculation of TMX-6-*gfp* enhanced the degradation of thiamethoxam inside the rice plants and culture medium. The half-lives of thiamethoxam in the nutrient solution, nutrient solution with TMX-6-*gfp*, nutrient solution planted with rice, and nutrient solution planted with inoculated rice were 46.2, 38.5, 9.9 and 4.7 days, respectively. Metabolomic analysis was used to investigate the effect of TMX-6-*gfp* inoculation on thiamethoxam metabolites and metabolic pathway in rice plant. No difference of the detected metabolites was observed between the treatments with or without the strain TMX-6-*gfp*; however, the abundance of the metabolites in different treatments was different.



Effects of TMX-6 inoculation on the degradation of thiamethoxam in rice

AGRO 17

Chiral enantiomers of the plant growth regulator paclobutrazol selectively affect community structure and diversity of soil microorganisms

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Paclobutrazol (PBZ) is a triazole plant growth regulator with a wide range of applications in crops and fruit trees. Paclobutrazol is often used as a racemic mixture in agriculture, although studies have shown that chiral paclobutrazol had only one enantiomer active against the target, the inactive enantiomer could migrate to the soil and adjacent environments and was toxic to soil microorganisms. However, the effects of paclobutrazol enantiomers on soil microbial community structure and diversity are unclear. Genetic markers (16S rRNA V3-V4 region and ITS) were used to study the effects of paclobutrazol enantiomers on soil microbial community structure and diversity. This study showed that the degradation rate of (+)-S-PBZ in soil was lower than (-)-R- PBZ. The microbial community structure and diversity in soil treated with (-)-R- PBZ were different from the microbial community structure and diversity in (+)-S-Paclobutrazol treated soil, and (-) -R- PBZ had a greater influence on soil microbial community. Furthermore, the study also observed that the microbial networks of (-)-R- PBZ-treated were less stable than (+)-S-PBZ-treated. These findings unveil a new role of chiral plant growth regulator in the development of soil microbial communities and provide scientific theoretical support for the application of high-persistence, highly active, eco-optical rotatory (-)-R- PBZ.

AGRO 18

Chemical fate processes in rice fields: Where have we been and need to go in the future?

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Rice culture offers unique environmental situations to investigate chemical processes not addressed in standard regulatory studies and not found in other cropping systems. The vast majority of rice fields are shallow water aquatic environments and sunlight plays a much greater role in fate processes then in deeper water bodies where the majority of sunlight is attenuated or scattered by suspended particulate matter. Secondary photodegradation processes including degradation by hydroxyl radicals can be highly significant as can degradation at sediment water interfaces. These processes have been shown to be highly significant to the degradation of the herbicide bentazon, bensulfuron-methyl as well as the newer rice pesticides like benzobicyclon that have recently entered the market. Mechanistic exposure models such as PFAM and EXAMS can provide estimates as to which processes will be important under various conditions. In addition, phototoxicity to aquatic organisms either residing in the rice field ecosystem or in aquatic environments receiving rice field tail waters is of potential concern and would not be addressed in the standard battery of tests conducted for pesticide registration. Recent work with the fungicide dicloran has shown that its presence at environmentally relevant concentrations can photochemically induce toxicity as well as sub-lethal impacts to crawfish, which is an economically important species in Louisiana and is harvested from rice fields. Measurements of a chemical's hydroxyl radical rate constant, fate in irradiated water-sediment systems, and phototoxicity to ecologically relevant organisms can allow better assessments of chemicals used in rice.

AGRO 19

Occurrence, variation and exposure risks of insecticides in air from metropolitan area of Vietnam

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Various pesticides are currently being used to maximize agricultural products and to control insects. In Vietnam, in 2017, pesticide active ingredient consumption was about 120,000 tons. Considerable pesticides are present in the atmosphere. Exposure to pesticides has been linked to significant adverse health effects, which are serious public health concern. To our knowledge, there are no previous reports in literature about using LC-QTOF-MS combined with automated identification and quantification system (AIQS) to simultaneously identify and quantify insecticides in the atmospheric particles. Nineteen of the 107 insecticides registered in the AIQS were identified and quantified in at least one sample of 47 samples collected in Hanoi for the dry season and the rainy season. Among the insecticides detected, 16 substances were found for the first time. Total insecticide concentrations averaged across all samples in the rainy season were lower than those in the dry season, and

concentrations of insecticides sampled in the daytime were lower than those collected in the night in both seasons. Their total value ranged from 0.47 to 27.0 ng m^{-3} (median, 3.6 ng m^{-3}), 3 compounds such as trichlorfon, propargite and carbofuran were detected in 100% of the samples, and the number of insecticides detected per sample was between 5-13 (median, 9). Four insecticides including trichlorfon, funobucarb, propargite, and carbofuran were the most dominant insecticides observed, accounting for 39.9%, 19.5%, 17.7%, and 6.6% of total level, respectively. The emission sources of some detected insecticides were related to agricultural usage and pest control in houses. We estimated the daily intake by inhalation, the Hazard Quotients, and Hazard Indices of insecticides, which altogether indicated that health risk by the measured exposure levels of those insecticides would be negligible; however, taking into account existence of highly sensitive populations to such insecticides, the cumulative exposure and the multiple exposure pathways should be elucidated.

AGRO 20

Trace level of pesticides in the lakes of Broknes Peninsula at Larsemann Hill area of east Antarctica

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More than 150 lakes at different islands and peninsulas are situated in Larsemann Hills. The Larsemann Hills are a series of islands and rocky peninsulas in Prydz Bay. It consists of two major peninsulas, Stornes and Broknes, four minor peninsulas and ~ 130 near shore islands. It is ~ 50 km^2 ice free polar oasis on the Ingrid Christensen coast, Princess Elizabeth land, located halfway between the southern boundary of the Vestfold Hills and the eastern extremity of the Amery ice shelf. Pesticides are toxic volatile organic compounds which persist in the environment for a long time. They are used globally to control the vector of malaria. Due to volatile nature, they are transported across the earth by the grasshopper effect. The present study was designed to evaluate the concentration of pollutants such as pesticides in the lake water samples, collected from five selected locations of Broknes peninsula during the 34th indian scientific expedition to Antarctica in austral summer of 2014 to 2015. A total of 15 lake water samples was collected from the different five lakes. In these samples, thirty-four compounds of different pesticides were estimated. Pesticides residue levels were found in lake water samples and varied from 10.33 pg/ml to 70.00 pg/ml. Presence of p,p'-DDT was detected in all different lakes, and the highest concentration was found in P4 lake. The presence of pesticides may be attributed to orographic effects, migratory birds, bio magnification, and anthropogenic sources. The presence of pesticides is an alarming situation and needs to be investigated further to maintain the pristine environment in Antarctica.

AGRO 21

Scientists observing, listening, and communicating to solve agricultural challenges

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Providing food, feed, fiber, and fuels to a growing global population while addressing food safety and environmental concerns will need bold, visionary, and highly collaborative research efforts. This collaborative research demands that scientists bring their curiosity and desire to learn new skills, but it also requires venues and opportunities to observe, listen, and communicate with other scientists, stakeholders, producers, and industry partners. This has been recognized by such established groups as the U.S. Department of Agriculture (USDA) multi-regional projects, the USDA IR-4 program, the Agriculture and Food Research Institute and Agricultural Research Service (ARS) National Programs, the Natural Resources Conservation Service's Conservation Effects Assessment Program, and the more-recently launched USDA-ARS Long-Term Agroecosystem Research (LTAR) Network. While engaged in these networks, scientists of different disciplines can explore new sciences and find new opportunities to solve agricultural challenges. Three examples will be considered. (1) Endosulfan isomerization provided evidence to discern the contribution of spray drift and volatilization after application. (2) Modifications to the Gaussian Plume Model allowed for evaluation of the efficiency of vegetative environmental buffers to capture ammonia, particulate matter from poultry house emission. (3) The chirality changes in degradation product of metolachlor (MESA) and its signature time stamp can assist in assessing transport of nitrate to streams in response to land management changes. The discoveries made in these large experiments have led to changes in guidelines and management practices to improve the environmental footprint of agriculture.

AGRO 22

Hemp and federal pesticide registration

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In accordance with the Federal Insecticide, Fungicide, and Rodenticide Act, EPA regulates the manufacture and use of all pesticides (insecticides, herbicides, rodenticides, disinfectants, sanitizers, and more) in the United States, including pesticides intended for use on hemp products. Under FIFRA, EPA ensures that, when used properly, pesticides provide significant benefits to society, such as controlling disease-causing organisms, protecting the environment from invasive species, and fostering an affordable, safe, and abundant food supply. FIFRA's safety standard requires EPA to weigh these types of benefits against harm to human health and the environment that might result from using a pesticide. In addition, EPA establishes maximum levels (tolerances), which refer to the

amount of pesticide residue allowed to remain in or on each treated food commodity in accordance with the Federal Food, Drug, and Cosmetic Act. Under FFDCA, EPA may establish a tolerance or a tolerance exemption for a pesticide residue in food or feed only if the agency finds that there is a "reasonable certainty of no harm" from consumption of the pesticide-treated food and from other non-occupational sources of exposure. The 2018 Agricultural Improvement Act (Farm Bill) provided for the cultivation, transport and sale of hemp. As defined in section 10113 of the Farm Bill, the term "hemp" refers to any part or derivatives of the plant *Cannabis sativa L*. with a concentration of tetrahydrocannabinol not more than 0.3% on a dry-weight basis. With this statute, EPA is able to work with pesticide registrants, who can submit FIFRA section 3 registration applications for use on hemp, and with states, who can register an additional use of a federally registered pesticide product under FIFRA Section 24(c) to meet special local needs. Tolerances may be set on raw and processed food and feed commodities that may move in interstate commerce. EPA is collaborating with Interregional Research Project #4 and other stakeholders to identify and generate data needed to register pesticides for use on hemp. EPA is also working to develop appropriate risk assessment parameters and define the hemp commodities that are used as food and/or feed and would require tolerances. This is a dynamic regulatory environment, contingent on questions related to cannabidiol oil, which is regulated by the FDA, the U.S. Department of Agriculture's approaches for marketing, and also the state programs and decisions.

AGRO 23

Regulatory challenges associated with pesticides use and hemp production

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Members of the Association of American Pesticide Control Officials (AAPCO) represent the officials charged by law with the execution of the state, territorial, provincial, and federal laws in the United States, including all its territories, and in Canada regulating the production, labeling, distribution, sale, use, and disposal of pesticides. AAPCO was formed in 1947, the same year that Congress enacted the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Pesticide related regulatory challenges associated with hemp production are varied and can be influenced by a number of factors, including: Why the hemp is being grown (CBD, fiber, or protein); processing methods, especially for CBD; pesticides used on surrounding crops; historic pesticide use on the land on which the hemp is being grown; and lack of legally available efficacious pesticide products. In addition, for states that have established legal medical and recreational marijuana programs, an infrastructure involving multiple agencies with varying degrees of pesticide related responsibilities could be involved. In some states, pesticide "action levels" have been developed. The regulation of hemp, including providing compliance assistance and enforcement, has resulted in an increased demand for staff resources. Some of these issues will be discussed.

AGRO 24

Update on the current federal regulatory scheme applicable to hemp agriculture

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The federal regulatory scheme applicable to hemp and hemp-derived products is currently in a state of flux. The Agriculture Improvement Act of 2018 (2018 Farm Bill) significantly changed federal law as applied to hemp agriculture. The U.S. Department of Agriculture (USDA) has taken a number of actions that are intended to implement the hemp mandates of the 2018 Farm Bill – some of which have proven to be problematic. In addition, the Environmental Protection Agency (EPA) has also acted under its authorities to make pest control products available to hemp growers. This presentation will discuss the current status of hemp regulation by USDA and EPA and discuss the outlook for hemp agriculture in the United States.

AGRO 25

Addressing U.S. growers' drive for hemp agricultural chemicals

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Gowan is a basic manufacturer and distributor of crop protection products. We develop, register, and market our products around the globe. As hemp acres increase, growers have increasing need for pesticides to control a wide range of pests – weeds, insects, and diseases. For most crops, the regulatory path is very clear. For hemp, there remain unanswered questions on what a registrant must do to add hemp to their labels. Registrants must consider many factors when adding any crop to their label but particularly one like hemp. This presentation will highlight and discuss the steps a registrant goes through before deciding to add hemp to a product label.

AGRO 26

IR-4 project's efforts to facilitate crop protection products for hemp

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The IR-4 Project (IR-4) is a national agriculture research program that is a partnership between USDA, the State Land-Grant Universities, the U.S. Environmental Protection Agency (EPA) and the crop protection companies. IR-4's primary objective is to develop data that is required to support registrations of chemical pesticides and biopesticides on fruits, vegetables, nuts, herbs and other specialty crops. IR-4 is needed because the crop protection companies focus their development resources on major crops/markets like corn, cotton, soybeans, etc., often leaving the specialty crop farmers with a limited arsenal of crop protection tools. Requests for Assistance in the registration of new crop protection products on hemp were submitted to IR-4 after the passage of the 2014 Farm Bill. With this law, it became legal

for IR-4 to perform research and develop data on hemp. However, EPA could not legally review submissions from IR-4 or registrants. With no clear path to registrations, IR-4 placed the hemp requests on hold. This regulatory barrier was changed with the 2018 Farm Bill. IR-4 was able to add several research studies on hemp to test the efficacy/crop safety with multiple fungicides and herbicides. IR-4 has been working closely with EPA to define the pesticide residue data requirements for hemp. Because of the significant variation in production systems, crop use and extraction methodology, developing data requirements for hemp is very complex. IR-4 has submitted a crop extrapolation proposal for the herbicide ethalfluralin on hemp. IR-4 has also submitted EPA Guideline 860 research protocols for the herbicide bromoxynil and the fungicide azoxystrobin to EPA. EPA has provided guidance, and IR-4 will be conducting residue studies in 2020. Details will be discussed.

AGRO 27

Crop protection in industrial hemp: The Canadian minor use experience

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Cultivation of industrial hemp has been permitted in Canada since 1998 and is regulated by Health Canada under the *Cannabis Act*, *Industrial Hemp Regulations*. Licenses are required for the production of industrial hemp, including for research purposes. Agriculture and Agri-Food Canada's (AAFC) Pest Management Centre (PMC) generates data to support regulatory submissions to Health Canada's Pest Management Regulatory Agency (PMRA) to obtain new minor uses of pesticides for Canadian grower identified priority pest management needs. The PMC conducts Magnitude of Residue studies under OECD Good Laboratory Practices (GLP), as well as efficacy and crop tolerance studies. Over 31,000 hectares of industrial hemp were produced in 2018, with the main production in the Prairie provinces. PMC conducts four residue trials in the Prairies and one trial in Ontario/Quebec to support registration. One trial is used to collect additional seed samples for determination of pesticide residues in cold-press seed oil, seed meal, de-hulled seed and flour processed fractions. Since 2007, PMC has generated data to support the registration of two herbicides for use in industrial hemp; quizalofop-p-ethyl and ethafluralin. An additional five projects are underway for weed, insect and disease control. Under the new industrial hemp regulations, growers can also harvest hemp flowers and leaves to sell to licensed cannabis processors for the production of CBD oil. Health Canada's PMRA is reviewing the regulatory requirements for the registration of new minor uses of pesticides related to the use of industrial hemp for CBD oil production.

AGRO 28

Pest management in *cannabis* crops: A review of insecticidal products currently in use and others on the horizon

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Cannabis sativa, whether grown in protected cultivation (greenhouse, indoor) for medicinal or recreational use, or outdoors for industrial use, suffers from insect and mite pests like all other crops. There is some overlap in pest species, especially those that are host specific to *Cannabis.* However, there are differences in the pest complex as well as in potential management tools between indoor and outdoor production systems. The legal use of insecticides in the U.S. has been confounded by differences in federal versus state legal status of non-hemp strains of *Cannabis*. As a result, the use of pesticides varies between states. In this presentation I will review insecticides currently in use in some of the major producing states (California, Washington, Colorado) and comment on their suitability for management of the major target pests. I will also review pesticides approved for use on *Cannabis* in Canada and Australia and compare regulatory approaches in those jurisdictions with the U.S. situation. Limitations on the use of pesticides in *Cannabis* crops will also be discussed.

AGRO 29

Challenges and initial assessment of products for suppressive control of hemp pests

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The cornerstone of pest control in modern agriculture is integrated pest management (IPM), an ecosystem-based pest management strategy that focuses on long-term prevention of pests through a combination of multiple methods based on ecological principles and socio-economic considerations. Each pest management system differs by crop, location, and production system, but most rely on insecticides as the primary insect pest control option. By default, IPM programs must be dynamic to address changing crop values, input costs, environmental conditions, and pest complexes. However, all IPM programs have five major components: (1) pest identification, (2) monitoring and pest population assessment, (3) action guidelines, (4) preventative strategies, and (5) suppressive strategies. Hemp, *Cannabis sativa* L., is unique in that there are currently no IPM programs because, until recently, it was illegal to grow. Both field and greenhouse producers will be clamoring for control strategies. To address these deficiencies, we conducted initial assessments of currently labeled products for suppressive control of key hemp pests. This talk will discuss the challenges we faced in assessment and evaluation and the results of our efficacy studies.

AGRO 30

Arthropod relationships with hemp and options for management in Virginia

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Current research and documented scientific information pertaining to hemp production and pest management is lacking due to cannabis prohibition that existed throughout most of the past century. The number of growers and interest in hemp cultivation in Virginia and other states is increasing at an extremely rapid rate, and accessible scientific information is vital to aid growers when pest issues arise. Our

observations have shown that although many arthropods are encountered in hemp, not all are pests. Corn earworm (*Helicoverpa zea*) has consistently been the most injurious arthropod of outdoor hemp. Other species of concern include hemp russet mite (*Aculops cannabicola*) and cannabis aphid (*Phorodon cannabis*) – both cannabis specialists with little known about their biology and life history. Beginning in 2019, trials were conducted to assess the efficacy of biological insecticides on the aforementioned pests since many states, including Virginia, allow biological pesticide use in hemp. Many products have shown promising results for management, but not all are allowed for use due to state restrictions or lack of language on the pesticide label to allow for use in hemp. For hemp russet mite, essential oils, sulfur, and soaps can reduce densities. For corn earworm, products containing *Bacillus thuringiensis* strain *aizawai* or the *Helicoverpa zea* nuclear polyhedrosis virus are the best options, yet efficacy is greater when young larvae are targeted. Spinosad has been the most efficacious product, but it is not allowed for use in hemp. In 2018 and 2019, studies were conducted to assess the effects of insect defoliation on yield and quality of grain (both years) and CBD (2019) variety hemp. Results suggest that defoliating insects are likely not a concern for physical yield (grain seed weight or CBD bud weight). However, questions remain as to whether phytochemical, or cannabinoid, production in hemp is altered by arthropod herbivory.

AGRO 31

Past, present, and future analytical techniques for pesticides in cannabis. Implications for export manufacturers

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Cannabis contains hundreds of components; many at percent concentration levels. This creates a complex matrix which makes it difficult to identify adulterants at ppb levels. Processes for detecting pesticides in cannabis have undergone dramatic evolutions in the last ten years. Since the first commercial cannabis samples were analyzed, techniques and equipment for testing have changed significantly. Action levels and target analytes vary widely by jurisdiction. Brand protection in emerging markets may require additional safeguards. This presentation describes a variety of techniques for broad coverage of pesticides to comply with regulatory requirements for a global market of manufacturing and exportation.

AGRO 32

Comprehensive pesticide residue screening in cannabis and cannabis products: A non-compliance laboratory perspective

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Beginning in late 2016, PAL began method development on a comprehensive pesticide residue screening procedure for cannabis and cannabis products. Without guidance at the federal level, states were implementing pre-sale compliance testing to address public safety concerns. This resulted in a multitude of prohibited pesticide lists and associated action levels. The U.S. EPA has not approved the use of synthetic pesticides for cannabis cultivation, so any residue found would indicate an off-label use of a commercial product. Different target lists and action levels can lead to a product that is "safe" in one state but not in another. With our history of serving the conventional agricultural industry, we developed our cannabis testing program using existing validated methods and GC-MS-MS and LC-MS/MS technologies. Using these two technologies in tandem, we can optimize each target compound for response and performance. Regulatory agencies can use the additional information from comprehensive screening to evaluate and address potential impact to public health.
Full scan data from both GC-MS/MS and LC-MS/MS was collected to evaluate the effectiveness of cleanup and dilution techniques. Reducing background matrix reduces negative matrix effects and decreases required maintenance on the MS/MS systems resulting in increased productivity. Currently validated for over 200 compounds, the scope can be expanded as new compounds of concern are identified.

AGRO 33

Comprehensive analysis of cannabis using one and two-dimensional gas chromatography with high performance time-of-flight mass spectrometry (GC & GCxGC-TOFMS)

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Cannabis characterization is not only important for determining its legality in the United States, but also for determining the effectiveness and safety of its many products. Regrettably, reported test results are often incomplete or inaccurate. In this study, Gas Chromatography – Mass Spectrometry (GC-MS) and comprehensive GCxGC-MS technology was used for untargeted analysis of cannabis botanicals. The methodology included pesticide analysis, potency determination, and terpene profiling. Data collection was comprehensive; however, both targeted and untargeted processing methods were utilized for the quantitative and qualitative analysis of samples. The analytical methodology provided data with superior chromatographic resolution and increased peak signal to noise values, which dramatically improved compound detection and identification. This is a direct result of improvements in S/N and production of rich, high-quality mass spectral data. Data processing was fast, reproducible and provided excellent results for database comparisons. Deconvoluted mass spectra were searched against large, well-established spectral libraries (*e.g.*, Wiley 11, NIST 17) and user TOFMS libraries. Pesticide, terpene, and cannabinoid standards were used to develop the internal libraries and for quantitative analysis. In addition, mass spectral delta values were used to support spectral similarity search results. The shift from GC- to GCxGC-TOFMS resulted in removal of background interferences (*e.g.*, column bleed), functional group clustering for easy data visualization, and over a two-fold increase in compounds identified.

AGRO 34

LC-MS/MS and GC-MS/MS to meet AOAC method performance requirements for multi-residue pesticide analysis in cannabis

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Although cannabis products are legal for medicinal or recreational use in many U.S. states and in Canada, there are currently no harmonized guidelines for pesticide residue tolerances. Consequently, each state or nation with legalized cannabis has its own pesticide residue list with limits that may be quite different in each region. The AOAC has issued a Standard Method Performance Requirements document (SMPR 2018.011) that describes minimum recommended performance characteristics to be used to evaluate methods for determination of pesticides in cannabis. AOAC has used the lowest tolerance level from any of the U.S. states or Canada as the target action level for any proposed method with a recommended LOQ at 50% of the action level. Although most of the target pesticides are amenable to LC-MS/MS analysis, many compounds have much lower detection limits using GC-MS/MS methods. Therefore, both LC-MS (ESI and APCI) and GC-MS (EI and API) methodologies designed to meet AOAC requirements will be discussed.

AGRO 35

EFSA guideline on chiral pesticides: From industry perspective

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In August 2019, EFSA published the guideline "Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers". The guideline had big impact on the development and the registration of Plant Protection Products (PPP). It requires industry to demonstrate the PPP chiral or stereoisomers purity, stability of these stereoisomer during the PPP metabolism by animals, live stocks, and plants, and provide chiral separation methods. If the stereoisomer is changed greater than 10% of the applied dose, it requires industry to treat the isomer as a different chemical component, and to produce the toxicology data of the stereoisomer and to conduct the risk assessment of the isomer. Apparently, this will add additional burdens to the PPP industry. In this presentation we will interpret the guideline from industry prospective, especially when the stereoisomers are not readily available or unstable. We will also discuss the strategies on how to deal with these difficulties.

AGRO 36

Chiral stationary phase comparison for the enantioseparations of 20 agrochemical compounds

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Approximately 30% of organic chemical pesticides are chiral molecules, and, since 2007, ~44% of new pesticidal molecules contain at least one asymmetric center. This is significant because, in some cases, only one stereoisomer is responsible for the targeted effects while the others are less active or entirely inactive. Differences in efficacy, toxicity, environmental effects, and degradation rates suggest the potential need for robust characterization and determination of enantiomeric ratios of pesticide products. Chiral chromatography provides both a way to determine these ratios and, if desired, a means to prepare enriched product with better specificity and/or lower toxicity. In this study, high-performance liquid chromatography (HPLC) chiral separations of 20 agrochemical compounds were performed by screening compounds on each of seven chiral stationary phases (CSPs) with each of two different mobile phases. "Hit" rates, determined by resolution values (R_S) ≥ 1, are reported for each column/mobile phase combination. The instances of highest resolution along with the number of unique hits are also reported for each column. Separations of poorly resolved compounds and compounds with two chiral centers (*i.e.,* four stereoisomers) are highlighted. This small data set may be helpful in guiding future chiral agrochemical screening protocols in terms of column and mobile phase prioritization.

AGRO 37

Update on the U.S. federal regulatory approach to genome edited agricultural products

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The U.S. federal regulatory approach to genome edited agricultural products is continually evolving. The Trump administration has generally put forth a science-based approach to regulating such products, but, as it currently stands, that regulatory scheme is incomplete. Of the Coordinated Framework agencies, the U.S. Department of Agriculture and the Food and Drug Administration have provided clear indications of their approaches to genome edited products. The Environmental Protection Agency has not - but it may soon. This presentation will provide a detailed description of the regulatory requirements and procedures that each agency applies to genome edited agricultural products, including the disparate legal underpinnings of each agency's approach.

AGRO 38

Nanomaterials for delivery of Cas9 plasmids and ribonucleoproteins to plants

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Introduction of CRISPR machinery to a host plant genome may be accomplished either through expression of

recombinant plasmids or direct delivery of the Cas9 ribonucleoprotein (RNP). However, introducing the biomolecular workhorses of CRISPR into model and crop species is hindered by the molecular delivery challenge across the plant cell wall. The cell wall presents a rigid barrier to biomolecule delivery which can be overcome by Agrobacterium or biolistic particle bombardment, the success of which is limited by host species, tissue type, and random transgene integration. Furthermore, the necessity of tissue culture introduces further limitations, as many crops are recalcitrant towards regeneration. RNP delivery simplifies the workflow of plant gene editing by circumventing the need for plasmid optimization and improves specificity by reduction of off-target cleavage. However, introducing Cas9 RNPs into plants is not easily addressed by current delivery technologies, as *Agro* is only amenable to DNA delivery, and biolistics rely on protein dehydration onto the carrier surface which can result in loss of Cas9 activity. Nanomaterials offer an addition to the workhorses of plant genetic engineering due to their ability to load diverse cargo, traverse the cell wall and plasma membrane, and selectively localize in tissues and organelles. We have developed a polyamine-carbon nanotube conjugate (PEI-CNT), which is loaded with DNA and administered by aqueous infiltration to the leaf abaxis. In *Nicotiana benthamiana*, we report indel generation in a GFP transgene via PEI-CNT plasmid delivery. We also present the development of Cas9-CNT conjugates for RNP delivery to mature plant tissue without biolistics – both through direct binding of an engineered Cas9 variant to the CNT surface and through the inclusion of a noncovalent peptoid intermediate for binding of WT Cas9 to the CNT surface. Peptoids, or poly-*N*-substituted glycines, possess remarkable biostability and peptide-like structural properties. We present the design and synthesis of modular peptoids with two domains, a CNT-binding domain and an RNP-binding domain, and demonstrate screening of a peptoid library based on these domains for non-covalent binding of Cas9 to peptoid-CNTs. We have identified charge and the protein:CNT ratio as key variables in the design of a stable conjugate, where a stable RNP-peptoid-conjugate can then enable cytosolic delivery of preassembled Cas9 RNP to mature plant cells.

AGRO 39

Destiny of palladium: Development of efficient palladium analysis techniques in enhancing palladium recovery

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The palladium-catalyzed cross-coupling reaction is a widely used protocol to build C-C or C-heteroatom bonds in organic synthesis both in academic research and industrial production. The Nobel Prize in Chemistry in 2010 was awarded to R.F. Heck, E.I. Negishi, and A. Suzuki for their contribution to the research area. For the past decade, the use of palladium catalysts in synthesis has become more frequently used in fine chemicals, pharmaceutical intermediates and active pharmaceutical ingredients. A serious problem results in residual palladium remaining in the final product, which leads to the rising need for cost-effective ways to remove palladium contaminants. Along with the scarce nature of this precious metal, the demand for palladium recovery is critical for a cost effective process. A recovery process using aqueous sodium bisulfite solution at elevated temperature offered 60-80% palladium recovery in the form of sodium palladium tetrasulfite dihydrate complex $Na_2Pd(SO_3)_6$ - $2H_2O$. Modified neutron activation analysis (NAA) technology was adapted to fully close the palladium mass balance. With this accurate palladium detection method, we were able to further optimize the palladium recovery process by a set of designed experiments.

AGRO 40

Application of 2D HPLC to address the need to investigate the chiral profile of active ingredients and their metabolites in environmental fate and metabolism studies

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The recently published guidance from EFSA[i] on risk assessments for active substances that contain stereoisomers provides recommended approaches to address and assess data requirements for active substances with the same molecular formula but different three-dimensional orientations. One approach is to study different isomers of an active ingredient and individually assess each isomer, but this approach is both costly and time consuming, requiring the synthesis of the individual isomers. Chromatography is a constantly evolving technique and while the concept and application of 2D HPLC has been around for a number of decades, recent improvements in equipment and column technology allows the routine use of 2D HPLC in a reliable and robust manner. The use of 2D HPLC to couple reverse phase profiling methods with reverse phase chiral methods along with tandem mass spectrometry – with or without radiolabelled 0analysis – enables the investigation of chiral profiles of agrochemicals and their metabolites without the need for separate isomer studies or extensive sample isolation, workup, and subsequent chiral chromatography. This presentation discusses the practical application of 2D reverse phase-chiral separations and their potential to become a routine approach to analysis in environmental fate and metabolism studies when considering the data requirements to fulfil the new EFSA guidance.
[i] EFSA Journal 2019; 17(8):5804

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Evaluation of unmanned aerial vehicles for application of chlorantraniliprole insecticide against navel orangeworm in almonds

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Adoption of unmanned aerial vehicles (UAV) for delivery of agrochemicals, initiated in Asia, is rapidly increasing worldwide. Advances in battery endurance, aircraft performance, payload capacity and navigation / autopilot systems are driving this expansion. Concurrently, the regulatory issues of biological efficacy, spray drift onto non-targets, operator certification and licensing, worker exposure, and label language are increasing. Registrants require performance data and field experience to guide usage instruction and label language. In markets and with products where conventional, manned aerial applications are common,

existing regulations and label language provide a base from which adaption to UAV's can proceed while other markets and products without a legacy of aerial application may require significant research. This study investigated using an electric hexacopter UAV to deliver insecticide to California almonds at hull-splitting stage to control navel orangeworm (*Amyelois transitella)*. Mature almond orchards present greater coverage challenges than field crops and smaller orchards or vineyards because of the tree height (6-8 m) and dense foliar canopies. Altacor® insecticide (chlorantraniliprole) was applied at two spray liquid volumes; deposition and efficacy were compared to a conventional air-blast ground sprayer using 10-20X greater liquid volume. The performance was compared by analyzing spray deposition from water sensitive papers, insecticide residues on filter papers and whole unhulled almonds at three canopy elevations. Biological control was evaluated by measuring pest damage at harvest. Overall residue levels on whole unhulled almonds across all pooled canopy strata are similar between treatments while significant interaction of canopy level and spray method showed distinct residue patterns between the unmanned aerial and ground air-blast applicators. Penetration and deposition at the lower canopy were remarkably high for the UAV application. Bio-efficacy results showed no significant difference in navel orangeworm damage among all treatments. The temporal and spatial variability of the pest development in this crop presents a potential use where UAV application may supplement conventional ground and aerial application to optimize efficacy and stewardship. This study presents insightful data that may guide the development of UAV labeling and usage recommendations.

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Quantitative fluorine nuclear magnetic resonance (^{19}F-NMR) method paired with liquid chromatography tandem mass spectrometry (LC/MS/MS) for a complete mass balance of per and poly-fluoroalkyl substances (PFAS) in biosolids

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Land application of treated sewage sludge, or biosolids, is a common disposal practice used to alleviate loading of bulk material into landfills. This process also improves soil characteristics and plant growth; however, soil contamination and plant uptake of chemicals of emerging concern have been observed as a result of wastewater irrigation and biosolid soil amendments. Per and poly-fluoroalkyl substances (PFAS) are chemicals commonly used worldwide since the 1950's. PFAS have been observed to elicit negative health effects such as developmental, immuno, and hormonal toxicity in animal and human models. Biosolids may be an important transfer route for PFAS into human consumers if edible crops take up the contaminants. Therefore, it is important to develop a method that can monitor PFAS in biosolids that are used as agricultural amendments. Other studies utilize targeted liquid chromatography tandem mass spectrometry (LC/MS/MS) for the analysis of common PFAS, which is limited to ionizable compounds. Due to the complexity of biosolids and PFAS, this approach faces a myriad of challenges including ionization and extraction efficiency. Because of these challenges, a method for the quantification of total PFAS was developed using fluorine nuclear magnetic resonance (19F-NMR). Because fluorine is not abundant in the environment and due to the minimal impact of matrix on 19F-NMR, this technique is ideal for PFAS analysis in complex matrices. Using the –CF3 shift that is characteristic of the majority of PFAS, the total concentration of PFAS can be calculated. A cumulative 19F-NMR approach with an LC/MS/MS analysis can elucidate a full understanding of PFAS in complex media. It is a valuable tool for risk assessment and mass balance to gain a broader understanding of the fate of PFAS as a result of biosolid reuse, especially when the contamination of food sources is a possible consequence.

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Use of multispectral sensors and unmanned aerial vehicles for agricultural applications

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Multispectral aerial imagery, particularly that with bands in the near-infrared portion of the spectrum, has a long history of use in agricultural applications. The rapidly-expanding use of unmanned aerial vehicles (UAVs), also known as drones, has opened several new avenues for the use of multispectral image analysis in agriculture. UAVs allow for much greater flexibility and higher spatial resolutions than are attainable with manned aircraft or satellite sensors. They are also considerably less expensive to deploy over small areas. This paper will review the use of multispectral image analysis in classifying and measuring the stress of crops and vegetation in general. The particular strengths and weaknesses of UAV-collected imagery will also be reviewed.

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Optimization of the multi-residue analysis method for 29 pesticides in meat using gas chromatography-mass spectrometry

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The optimization and validation of multi-residue analysis for 29 pesticides in various meat matrices were evaluated. The developed method was validated in five meat matrices (pork belly, sirloin, chicken drumstick, lard, and beef tallow according to the CODEX guideline in terms of recovery, precision, calibration curve linearity, the limit of quantitation (LOQ) and matrix effect. In this study, meat samples were extracted using a QuEChERS extraction followed by optimization of sample clean-up. The clean-up method was then compared and optimized based on the d-SPE(PSA, C18, Z-SEP) and SPE cartridges(aminopropyl, C18, florisil). For the optimized analysis method, it is suitable to use the SPE cartridge as a clean-up method rather than d-SPE in order to improve the sensitivity of pesticides and reduce the matrix effect. In the clean-up method using the SPE cartridge, the use of the C18 SPE cartridge met most of the pesticide recovery criteria. The results of the optimized method showed that above 85% of pesticides gained the LOQs results of below 0.01 mg/kg, and over 90% of pesticides achieved good calibration curve linearity results (R^2 > 0.99). As a result of the recovery test applied in an optimal method, more than 80% of the pesticides in the sample of pork belly, sirloin, and chicken drumstick satisfied recovery and precision criteria. In contrast, in lard and beef tallow, only 65% of pesticides met recovery and precision criteria. Therefore, it was confirmed that the recoveries measurement pesticide depends on the fat content of the samples. It can be seen that this fat content

also affects a matrix effect. The average matrix effect was 16.6 ~ 30.7% in the samples of pork belly, sirloin, and chicken drumstick, while the average matrix effect was over 50% in the pork and beef fat sample. Although the matrix effect was different for each pesticide, most of the pesticides were affected by ion enhancement. In conclusion, it is judged that it will be possible to use it as a multi-residue analysis method for 29 pesticides on the meat sample.

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Open-source QSAR models for pKa prediction using machine learning approaches

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The logarithmic acid dissociation constant (pKa) reflects the ionization of a chemical, which affects lipophilicity, solubility, protein binding, and ability to pass through the plasma membrane. Thus, pKa is a physicochemical property that affects absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) of chemicals. The goal of this work was to develop a free and open-source computational model to predict pKa values, which could then inform models that predict chemical ADME-Tox properties. Experimental acidic and basic pKa values for 7,912 chemicals were collected from the literature. Chemical structures were curated and standardized for quantitative structure-activity relationship (QSAR) modeling and then split into training (80%) and test (20%) sets. Continuous molecular descriptors, binary fingerprints, and fragment counts were generated using PaDEL then used to train predictive models using different machine learning methods, including support vector machines combined with k-nearest neighbors, extreme gradient boosting, and deep neural networks. The resulting computational models showed comparable predictivity on the training and test sets, with a root-mean-squared error around 1.5 and a coefficient of determination around 0.80. Performance of our three best models compared favorably with two commercial pKa predictors from ACD/Labs and ChemAxon that were used for benchmarking. The validated models were implemented in free and open-source packages and are available on GitHub.

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Assessment of potentially vulnerable use areas in western Africa

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The Acetochlor Registration Partnership (ARP; Dow AgroSciences and Monsanto Company) developed voluntary acetochlor Best Management Practices (BMPs) to reduce the potential for the active substance to reach ground water and surface water. As part of an ongoing global acetochlor stewardship support, a study has been conducted to determine the co-occurrence between acetochlor use on crops and potentially vulnerable soils in the Permanent Interstate Committee for Drought Control in the Sahel (French: Comité permanent inter-État de lutte contre la sécheresse au Sahel, aka CILSS) countries. The assessment was focused on high potential use areas of acetochlor, within the CILSS, for two representative crops (corn and cotton). The geospatial analysis performed identified approximately 462 million hectares (ha) of potentially vulnerable soils in the CILSS of which 65.7 million ha of soils are within agricultural areas. Acetochlor product labels approved in the U.S. by the U.S. Environmental Protection Agency, restrict applications on vulnerable soils within 50 feet of any well where depth to ground water is ≤ 9 m. Other agricultural BMPs for applying acetochlor products in the U.S. are designed to minimize run-off to surface water. Approximately 0.24% of agricultural fields (0.159 million ha of soils) in the CILSS are in areas of shallow groundwater. In addition, 0.02 % (0.0128 million ha) were determined to be adjacent to surface water bodies. The analysis provides evidence that only small portions of vulnerable agricultural soils in the CILSS may be at risk for acetochlor contamination by means of leaching to groundwater or surface runoff. The approach could be expanded to other regions to inform water quality management decisions for products containing acetochlor.

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Effect of biogas slurry on the remediation of petroleum contaminated soils through composting

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Soil pollution by petroleum hydrocarbons and their adverse effects create widespread environmental problems. Composting is one of the potentially cost-effective solutions for hydrocarbon contaminated soil by indigenous functional microbes. However, some petroleum hydrocarbons and their metabolites remained after composting due to low efficiency of organics transformation, leading to high phytotoxicity of composts. Biogas slurry is the by-product of biogas production with high content of organic nutrients for microbes, possibly conditioning the substrate and altering the biological activity. Herein, biogas slurry was proposed to be added into the composting of hydrocarbon contaminated soil as an activator for organics degradation and humification. The parallel factor (PARAFAC) of excitation-emission matrices spectroscopy with UV–Vis spectroscopy was applied to analyze humic acid for evaluating compost maturity and the germination index was assayed for phytotoxicity. The relationship among petroleum hydrocarbon, organic matter and humic acid as well as the phytotoxin level of composts was analyzed by redundancy analysis (RDA) and structural equation model (SEM). The results showed that the degradation rate of organic matter and petroleum hydrocarbons were increased by 7.8% and 4.6% with biogas slurry addition. Composts with adding biogas slurry had higher proportion of petroleum hydrocarbon compounds with C<16 than the control after composting. UV–Vis spectroscopy and excitation-emission matrices of the fluorescence spectra indicated the humification degree of humic acid in composts with biogas slurry addition were higher than that of the control. biogas slurry addition increased 18.0% of germination index than the control. Though ~0.3 mg/kg estrone was introduced as adding biogas slurry, the removal

of estrogens occurred during composting. Four components containing tyrosine-like, tryptophan-like, fulvic acid-like and humic-like substances (C1–C4) were successfully developed by PARAFAC. RDA indicated that the degradation of total petroleum hydrocarbons was significantly related to the C4 of humic acid and total nitrogen content derived from the biogas slurry. SEM demonstrated that petroleum hydrocarbon degradation was also a key factor in humification. These findings suggest that the added biogas slurry drives the degradation of petroleum hydrocarbons and the humification of humic acid, leading to the composts with lower phytotoxicity for soil application.

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Chinese classical composting in Southern Song Dynasty

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Ancient China was a country of agricultural cultivation civilization, which had a long history of using agricultural organic wastes. The earliest document on compost referred to *Chenfu Agricultural Book* in the Southern Song Dynasty (1127-1279 AD.) Here, we attempted to revive this ancient composting using rice straw ash and sesame meal at the ratio of 1:1(T1), 2:1(T2) and 4:1(T3) with triangular shape pile (0.6 m diameter and 0.5 m depth) for 37 days, respectively. Results showed that T-1 was more suitable than T-2 and T-3 for composting, and the composting method of Chenfu can be revived today. It is incredible that ancient Chinese people had such high wisdom in recycling of agricultural organic waste 900 years ago. This classical method of composting is an important reference for recycling agricultural organic wastes.

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Microcystin-LR accumulation in leaf-vegetables under various polluted-treatments

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Microcystin-LR (MC-LR) is prevalent in water, and it can be transferred into farmland-crop system via irrigation, overflow (a pollution accident) and algae fertilization. MC-LR in soil can be absorbed and transported by crops into the food chain, which is harmful to agricultural products and threatens human health. In the present study, the bioaccumulation, toxicity and health risk of MC-LR in various treatments were investigated using pot experiments, including irrigation of polluted water, cultivation of polluted soil, and application of algae fertilization. The total MC-LR amount used in the different treatments are the same. Three leaf-vegetables that are widely planted in southern China were used for the pot experiments, including *Ipomoea batatas leaves*, *Brassica juncea*, and *Brassica alboglabra.* All leaf-vegetables can accumulate MC-LR under the three treatments. MC-LR bioaccumulation decreased in the order of algae fertilization application > polluted-water irrigation > polluted-soil cultivation. Totally opposite order was found in MC-LR degradation in soils of the treatments, indicating the slower MC-LR degradation in soils led to its higher bioaccumulation in leaf-vegetables, and vice versa. MC-LR bioaccumulation remarkably reduce biomass of the three leaf-vegetables, and makes their leaves prematurely age. MC-LR bioaccumulation in the edible parts of the leaf-vegetables, especially those with applied algae fertilization, showed medium and higher health risks. The findings of this study highlighted the concern on the application of algae fertilization.

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Development of the analytical method for hexythiazox in a traditional herbal medicine, *Angelica koreana L.,* using LC-MS/MS

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Hexythiazox is an insectcide which has been used to control mite in melon, apple, strawberry and traditional herbal plants. Analytical method of hexythiazox in general food crops was well developed using HPLC or LC-MS/MS, however, such method was not established for the traditional herbal medicines so far. The aim of this study was to develop an analytical method for hexythiazox in the traditional herbal medicines *Angelica koreana L.* using a LC-MS/MS. Kinetex C18 analytical column (2.1 × 100 mm, 2.6 *μ*m) was used and the mobile phases were methanol and water. The instrumental limit of quantitation (ILOQ) was 0.02ng. Linearity of calibration curve (r^2) was ≥0.99 between 0.02 – 0.5 μg/mL range. For the sample preparation, 5.0 g of sample powder in 50 mL falcon tube was wetted with 10 mL of distilled for 10minutes. And then sample was extracted with 10 mL of acetonitrile containing 0.1% formic acid by shaking vigorously for 1 minute. After that, the extract was partitioned with 4 g of MgSO4 and 1 g of NaCl by shaking vigorously for 1 minute, then centrifuged at 3,500 rpm for 5 minutes. The aliquot (5mL) was purified with SPE cartridge (1g, NH_2-GCB) by eluting of dichloromethane/hexane mixture. The eluate (15mL) was concentrated, dissolved in LC-MS/MS mobile phase (0.2 mL) and an aliquot (10 μL) was analyzed. The method limit of quantitation (MLOQ) was 0.02 μg/mL. A recovery test was performed to validate the established method at two fortification levels (MLOQ, 10 MLOQ), following the method described above. The recovery rate was in the range 70~105% with <10 % of coefficient of variation. Therefore, the present method was proved to be reasonable for the quantitative determination of hexythoazox in the traditional herbal medicines *Angelica koreana L.*

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Rapid and simultaneous analysis of 113 pesticide residues in chicken and egg using GC-MS/MS

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Chicken meat is the most consumed meat in the world. This study aims to develop a rapid and simultaneous analytical method of 113 pesticide multiresidues in chickens and their eggs, using a modified QuEChERS procedure combined with gas chromatography−tandem mass spectrometer (GC-MS/MS; Shimadzu GCMS-TQ8050). The pesticide residues were separated with BPX-5 column (0.25 mm × 30 m, 0.25 μm). The method limit of quantitation (MLOQ) was <0.01 mg/kg, and the correlation coefficients (r^2) of matrix-matched

standards were ≥0.99 within the range of 0.003−0.05 mg/kg. Triphenylphosphate (TPP) was used as an internal standard for quantitation. In order to optimize the sample preparation procedures, composition of extraction solvents and clean-up methods were compared in different ways. Various extraction solvent systems were used such as acetonitrile containing 0.1% formic acid (ACNFA), which was modified with ethyl acetate by 0, 20, 30 and 50% for chicken meat. The best extraction efficiency from chicken meat was obtained with ACNFA with 50% ethyl acetate. For eggs, only ACNFA resulted in the best extraction efficiency. For sample clean-up, three types of dSPE, (C18, Z-SEP, Z-SEP +) and two types of SPE (Prime HLB, Phree) were tried. Z-SEP was good for purification of both the chicken and the egg. For the recovery test, samples were treated with standard solutions at MLOQ, 10MLOQ and 50MLOQ levels and processed with optimized extraction/clean-up methods. The recoveries ranged from 70 to 120% (RSD ≤ 20%) at each spiking levels (n=5) with 111 compounds for the chicken meat, while 99 compounds for the egg. The method established through this study is expected to be used to quantitatively analyze pesticide residues in chicken meat and eggs.

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Application of exogenous enzymes in aerobic composting of food waste

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Food waste accounts for 37-62% of urban domestic waste and continues to grow at 7% per year. The disposal of food waste is critical for urban sustainable development. Considering that food waste is mainly characterized by high water content (80%-95%), high oil (8-20%), high salt content (up to 0.8-1.5%), and easily producing foul odor gas, it is difficult to degrade quickly through aerobic composting and obtain high quality organic fertilizer, and the separation of oil and water before composting will also produce secondary pollution and other problems, so the search for more harmless treatment measures has become the food waste aerobic composting technology urgently needed to solve the problem. Enzyme pretreatment technology can degrade macromolecular organic matter such as starch, protein, fat, etc. in food waste, which is beneficial for the removal of food waste odor, promoting the process of food waste treatment, etc. The dry matter of food waste includes 16.73% crude protein, 28.82% crude fat, 2.52% crude fiber and other minerals on average. It has been studied that proteins in food are hydrolyzed with proteases up to 74.18% and lipase has a good hydrolysis effect on lard and frying waste oil in food with a maximum hydrolysis rate of about 85%. However, the effect of applying compound exogenous enzymes in food waste is unclear. In this study, we add protease, lipase and cellulase to the food waste composting process at different times and in different ratios to reduce the fat content, promote the degradation of protein and cellulose, reduce the odor of the composting process, set up a control test and record the relevant data of the subsequent composting process of the food waste, determine the degree of maturity and quality of the finished compost, so as to determine the effectiveness of the enzyme treatment technology, and select a more effective food waste pre-treatment solution. This study suggested that the use of exogenous enzymes can reduce the cost of subsequent treatment of food waste and environmental pollution, which is an effective way for recycling food waste resources.

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Effects of kitchen waste compost on rice growth, yield, and grain quality

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The aim of this study is to compare the effect of different fertilizers especially kitchen waste compost on rice growth and yield. Rice "Nanjing No. 46" was used as the test material and five fertilizer treatments were set up in Suzhou City, Jiangsu Province, China, including primary fermentation kitchen waste compost (C1), secondary fermentation kitchen waste compost (C2), chicken manure organic fertilizer (C3), pig manure organic fertilizer, chemical fertilizer (C4), and no fertilizer applied as control (CK). The rice growth, yield, grain protein content, grain nutrient elements and heavy metals content were studied. Results showed: different types of fertilization treatments all can promote the growth and enhance the yield. The order of plant height in the yellow ripening stage is CF> C1> C2> C4> C3> CK, and the order of the aboveground biomass is C1> CF> C2 > C4> C3> CK. The yield is increased significantly in C2 compared to CK, and the output is up to 9379.65 kg/hectare. The protein content of each treatment group is less than 7%, and the sorting from high to low is C1> CF> C2> C3> C4> CK. Compared to CK, all the fertilization treatment increased the total nitrogen and total potassium content of grains. The total nitrogen content in C1 was 25.86% higher than CK, and the total potassium of C2 also reached a significant difference level, 23.92% higher than CK. The content of heavy metal As, Cu, Pb, Zn, Cd, Ni, Hg, and Cr in grains are all within the safe concentration range of China's GB 2715-2005 Food Hygiene Standards. The comprehensive analysis shows that kitchen waste composting can ensure the quality and the yield of rice, which has the potential for large-scale promotion and application.

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Development of a thermophilic microbial inoculant for aerobic composting of food waste

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Compulsory garbage sorting is increasingly applied in cities of China, with a large amount of kitchen waste separated. This kitchen waste normally contains high content of water and organic waste, thus it easily gets rotten especially when the temperature is high. Accelerated aerobic composting is embraced for treating these organic wastes. Here, we aim at development of complex microbial inoculants for aerobic composting of kitchen waste. A total of 192 thermophilic bacteria were isolated from continuously enrichments supplement with kitchen waste. Among them, 13 bacterial

isolates were strong on degrading protein, cellulose and oil, which are commonly present in kitchen wastes. The 16S rRNA gene sequencing analysis revealed that majority of these strains were affiliated to *Thermophilic Bacillus, Geobacillus* and *Aneurinibacillus.* A microbial inoculant with six strains was formulated, and its effects were tested on a 50kg composting reactor. The results showed that the microbial inoculant can reduce the energy consumption of the equipment (20%) and greatly enhance the dehydration rate of kitchen waste (40%) compared with other bacterial agents. This study suggested that thermophilic bacterial inoculants are of potential in composting treatment of kitchen waste.

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Vermicomposting of kitchen waste mixed with cow manure using earthworm *Eisenia fetida*

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Vermicomposting refers to the introduction of earthworms into organic waste treatment technology, where earthworms process organic waste into homogeneous and stable humus. Vermicomposting is generally higher in nutrients than traditional compost and is widely used in the treatment of organic waste. Here, composts based on mixing cow manure (CM) with pre-composted kitchen waste (PKW) in different ratios as a medium were conducted, *i.e.*, 100% CM, 4% PKW+96% CM, 12% PKW+88% CM, 20% PKW+80% CM, 28% PKW+72% CM. Earthworms (*Eisenia fetida*) were used to treat pre-composted kitchen waste. The results showed that the growth of *E. fetida* was increased significantly in the treatments of 100% CM, 4% PKW+96% CM and 12% PKW+88% CM feed mixture, no significant change in 20% PKW+80% CM feed mixture, and reduced significantly in 28% PKW+72% CM feed mixture in vermicomposting. After 20 days of vermicomposting, pH and the nutrients including nitrogen, phosphorus, and sodium increased from the initial to the final products. The weight of earthworms gradually decreased with increasing salt concentration. At salt content of 0.3% – 0.4% (dry weight), weight loss was extremely significant in 7 days. These findings indicate that pre-composted kitchen waste should not exceed 20%, and the salinity of kitchen wastes should not exceed 0.3% in vermicomposting. Vermicomposting with *E. fetida* was beneficial for kitchen waste management.

AGRO 56

Effects of halotolerant bacteria inoculation on kitchen waste composting

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Kitchen waste features high salt and fat content, especially for Asian countries. Halotolerant bacteria is able to grow under higher concentration of salt and can degrade organic matter in the composting process. The aim of this study is to accelerate the degradation of organic macromolecules and shorten composting time by using such halotolerant bacteria. We screened halotolerant strains, being tolerant to 1-10% (NaCl) mass concentration, from the leachate and soil samples and further verified their degradation ability by inoculating the halotolerant bacteria in the kitchen waste composting. Six strains of halotolerant bacteria with four functions of degrading organic macromolecules (protein, oil, starch, cellulose) were screened and identified as *Bacillus* and *Cellulosimicrobium*, respectively. A mixture of those 6 strains was prepared as a bacterial compound agent with 10^8 cfu/ml. The result of composting indicated that the moisture content and electrical conductivity were gradually decreased during the composting process, and the inoculation treatment (T2) showed higher decrease compared to the CK (T1) and the addition of the kitchen waste inoculation agent (T3). The T2 reached above 60°C on the third day of composting, which was faster than other treatments and held a longer period of high temperature. The results of pH and seed germination index also showed that T2 had higher maturity index at the ninth day. The results of the study show that halotolerant bacterial inoculation could accelerate kitchen waste composting.

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Effect of assistant materials on the maturity of food waste compost

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The amount of food waste is growing, accounting for 30% to 40% of urban domestic waste, and the annual amount of food waste removed is over 60 million tons. Food waste mainly contains starch, protein, fat, salts, crude fiber, and organic acids, with organic matter accounting for about 93% of dry weight. Considering the nutrient richness and high recycling value of food waste, it can be recycled using aerobic composting technology. Due to the high water content and low C/N value of food waste, it is difficult to achieve individual composting, so a certain proportion of assistant materials need to be added for material mixing and blending. There are four kinds of assistant materials: corn straw, rice husk, bran, and sawdust, were added to compost for 15 days, each accounting for 15% of the total weight of the raw materials. The results showed that the germination index (GI) of the treatment with straw, rice husk, and bran reached more than 80%, indicating that the samples were fully matured; the GI of the treatment with sawdust > 60% also reached the matured standard. Compared to the blank control group, GI values increase, EC values decrease, and N and S element losses decrease in the compost with the addition of assistant materials. Studies have shown that adding assistant materials to adjust raw materials at the moisture content of about 55%, pH value of 6.8-7.2, C/N value of 25-30, can help in rapid composting. The addition of assistant materials to food waste for co-composting can greatly shorten the composting cycle and effectively increase the nutrient content of the compost product.

AGRO 58

Development of analytical methods for pymetrozine in livestock products

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This study was conducted to review the pesticide residue method for livestock products in Food Code and develop the analytical method for pymetrozine. Test samples were chicken, beef, and pork. Pymetrozine is a polar compound with logPow value of –0.18, and its vapor pressure is <0.004 mPa. Pymetrozine residues on livestock products were detected by LC-MS/MS. The Method limit of quantitation (MLOQ) was 0.01 mg/kg. Pymetrozine was extracted from livestock products using acetonitrile as a solvent with magenesium sulfate and sodium chloride. The purification step was established with d-SPE. Good analysis reproducibilities were obtained (coeffiecient of variation <5.0%), and the linearity of calibration curves were reasonable (r2>0.99) in range of 0.001 ~ 0.1 μg/mL in various matrices. Recovery tests were carried out at three levels of concentration (MLOQ, 10 MLOQ, 50 MLOQ) and resulted in good recoveries (<70~ 120%). The analytical method of pymetrozine established in this study confirmed the recovery and reproducibility. This method can be used as an official analysis method for the inspection and safety evaluation of residual pesticides in livestock products.

AGRO 59

Development of analytical methods for spinosad in oriental herbal medicines

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Development of analytical methods for detecting residual pesticides in oriental herbal medicines ensures the safe management of pesticides and provides a safe food supply chain for consumers. Scientific and technical improvements on current MFDS official methods for analysis of pesticide residues in oriental herbal medicines were undertaken. This study was conducted to review the pesticide residue method for oriental herbal medicines in Food Code and develop the analytical method for spinosad. Test samples were Broadleaf Liriope. Spinosad is a nonpolar compound with logPow value of 2.0, and its vapor pressure is <3 x 10-5 mPa. Spinosad residues on oriental herbal medicines was detected by LC-UVD. The Method limit of quantitation(MLOQ) was 0.05 mg/kg. Spinosad was extracted from oriental herbal medicines using L(+) Ascorbic acid 0.25 g in 80% acetonitrile. The purification step was established with SPE-silica(1 g, 6 cc). Good analysis reproducibilities were obtained (coeffiecient of variation <5.0%), and the linearity of calibration curves were reasonable (r2>0.99) in range of 0.2 ~ 5.0 μg/mL. Recovery tests were carried out at two levels of concentration (MLOQ, 10 MLOQ) and resulted in good recoveries (<70~ 120%). The analytical method of spinosad established in this study confirmed the recovery and reproducibility. This method can be used as an official analysis method for the inspection and safety evaluation of residual pesticides in oriental herbal medicines.

AGRO 60

Exploring permanganate oxidizable carbon (POxC) analysis as an indicator of soil labile carbon

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Organic carbon in soil is an important component of soil nutrient cycling and is used as an indicator of soil quality. Permanganate Oxidizable Carbon (POxC) reflects the labile or active soil carbon, which is a small fraction of the total organic carbon in soil. Labile carbon is related to soil microbial biomass, nutrient cycling and availability, soil aggregation, and soil C accumulation. It has been linked to other soil health parameters and yield prediction. Our project focuses on understanding the variables that increase method variability, thereby finding a way to improve the method and use it to get better correlations between POxC and different agricultural management practices across different soil types. We report preliminary results in method optimization by introducing a quality control and studying how oxidation time, particle size, light exposure, and shaking affect the analysis. We expect method optimization to get better correlation with other soil quality parameters, therefore becoming a useful tool to design field management strategies and an early indicator of soil carbon dynamics.

AGRO 61

Simultaneous screening method for 439 pesticide multiresidues in yoghurt Using LC-MS/MS and GC-MS/MS

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In the case of pesticide poisoning/death incidences, it is very important to identify rapidly the unknown toxicant and determine its concentration for detoxification treatment or verification of death cause. Actually, in Korea we had a case for attempted poisoning using yogurt with pesticides. Therefore, the establishment of an accurate, precise, rapid and simultaneous multiresidue pesticide analytical method is critically required for forensic and toxicological investigation. In this study, an effective and simultaneous multiresidue screening method for the determination of 439 pesticides in yoghurt was developed using liquid chromatography-tandem mass spectrometry (LC-MS/MS, SHIMADZU LCMS-8060) and gas chromatography-tandem mass spectrometry (GC-MS/MS, SHIMADZU LCMS-8040). The multiple reaction monitoring was optimized with positive/negative ionization mode on LC-MS/MS and GC-MS/MS. Method limit of quantitation (MLOQ) was 10 ng/mL for 99.3% of all target analytes. To optimize the sample preparation, all target compounds were spiked in 10 mL of yoghurt at 5MLOQ, extracted with acetonitrile (10 mL) with/without 0.1% formic acid, and partitioned by using EN QuEChERS, and Original QuEChERS salt to get better result with 0.1% formic acid/acetonitrile extraction and Original QuEChERS. In the recovery tests at MLOQ and

5MLOQ, yoghurt was extracted with 0.1% formic acid in acetonitrile (10 mL), treated with Original QuEChERS(4 g $MgSO_4$, 1 g NaCl), and the extracts were centrifuged. To remove interference compounds as a second step, yoghurt extract was purified using QuEChERS dSPE (25 mg PSA and 150 mg MgSO4). Then the extracts (4 µL) were analyzed using LC-MS/MS, and the extracts (2 µL) were analyzed using GC-MS/MS. The total average recoveries of all target compounds were from 93.4 to 94.8% (CV≤20%) at two levels. The correlation coefficients (r^2) of matrix matched calibration curves were ≥0.99 for most of target compounds. In conclusion, the established analytical methods in this study can be effectively applied to the monitoring of pesticide multiresidue in yoghurt samples at hospitals and forensic facilities.

AGRO 62

Enabling nitrogen fixation by signaling the soil microbiome

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Using atmospheric nitrogen to provide fertilizer for cereal crops is a longstanding goal of plant science. A scalable, biological route to nitrogen fixation would allow reduced use of synthetic fertilizer, improving the sustainability of agriculture. Here, we present an orthogonal approach leveraging the capacity of the endogenous soil microbiome. Symbiosis between the root and soil microbiome is initiated and maintained through small molecule signaling, providing an avenue for microbiome programming in the field. We show that biomimetic small molecule chemistry can be used to activate nitrogen fixation in free-living soil diazotrophs, providing nutrition to the plant and providing harvest yield boost in field trials. Our data demonstrates that specific small molecule chemistry can enhance nitrogenase activity in cultures of model diazotrophs as well as in soil samples from agricultural production fields. Field trials show that use of this chemistry results in 5 to 15% yield gains in maize, and the potential to reduce synthetic fertilizer applications by over 40 pounds per acre.

AGRO 63

Exploring arid environments for stress-tolerant plant-growth promoting bacteria

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Global climate change has intensified various stressors that negatively affect agricultural productivity worldwide. Plant-growth promoting bacteria (PGPB) living in hotter, drier soils are predicted to adapt to changing climates faster than the plants living in these environments. To this end, we isolated bacteria from under the canopies of native plants growing in arid soils, such as southern California and Israel. Various *Bacillus* species, actinomycetes, and nitrogen-fixing bacteria were isolated, characterized, and tested on legumes, but also on wheat and corn. *Bacillus* species, e.g., *B. subtilis,* and *B. simplex* were effective biocontrol agents, not only killing *F. oxysporum* pathovars but also promoting growth and yield of the treated plants. Because of the essentiality of fixed nitrogen for sustainable growth, we also tested a number of diazotrophs as well as symbiotic nitrogen fixers for their effectiveness in enhancing crop plant growth. Although many bacteria were isolated from dry and saline soils, *Dietzia cinnamea* 55, an actinomycete, was one of the most efficient in *in vitro* experiments. Treating plants with strain 55 significantly enhanced both corn and legume growth over the uninoculated controls in both greenhouse and outdoor microplot experiments as evidenced by significant increases in shoot and root length, plant biomass, and yield of bacteria-treated plants. Preliminary field studies on corn indicate a similar growth promotion effect. The genomes of the most promising PGPBs were sequenced, and genome analysis showed that genes with the potential to confer temperature and desiccation tolerance were present in the strain 55 genome, and that the *Bacillus* genomes have genes encoding hydrolytic enzymes as well as fengycin and other lipopeptide antibiotics. Virulence assays with *C. elegans* or *Galleria mellonella* did not demonstrate any inhibitory effect of the PGPB on the survival or motility of the nematodes or negatively affect wax worm viability in contrast to *P. aeruginosa* PA14, which did.

Corn ears from control plants compared to plants inoculated with strain 55.

AGRO 64

Unraveling the modes of action of alkaline *Ascophyllum nodosum* extract-based biostimulants: From molecular priming to plant–microbe symbiosis

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Alkaline extracts of Ascophyllum nodosum seaweed are complex mixtures of potent bioactive compounds that improve crop quality, stress tolerance, and yield. The work described here stems from an ongoing effort to gain deeper understanding of the molecular modes of action of these extracts through combined phenotypic, transcriptomic, and metabolomic investigations. Specifically, these studies demonstrate stimulation of specific physiological processes that prime plants for oncoming adverse environmental

conditions, including elicitation of stress signaling, biosynthesis of osmoprotectants, and facilitation of rapid bZIP transcription factor-mediated stress response regulation. In addition to direct stimulation of plants, indirect effects through stimulation of the soil microbiome and its interaction with plants present other potential modes of action of seaweed extract-based biostimulants. Of particular interest are the pervasive mycorrhizal fungi, which play a critical role in promoting soil and plant health. To this end, we demonstrate the positive influence of A. nodosum extracts on the symbiosis between the model legume *Medicago truncatula* and arbuscular mycorrhizal fungi, including effects on rhizosphere signaling, fungal establishment, and intracellular development. Overall, our work provides novel insight into both the direct and indirect modes of action of alkaline *A. nodosum* extracts, demonstrating how this class of biostimulants enhances natural plant processes and primes plants for tolerance of abiotic stresses.

AGRO 65

Field screening approaches for monitoring whole-plant response modulated by biostimulants

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Biostimulants are the most rapidly growing segment of the Agricultural Chemicals industry, nevertheless, considerable uncertainty exists with regard to application rates, timings, crop responses, and mode of action. Skepticism among consumers and regulators as to the role of these products in modern agriculture further hampers adoption. To address this issue there is a need to develop university managed, rapid screening protocols that are independent, statistically robust, and low cost. The UC Davis Biostimulant Field Screening Trial is an investigation of physiological parameters related to biomass accumulation and energy balance of tomato plants (*Lycopersicon esculentum*, Mill.) in order to characterize whole-plant response of biostimulant treated plants to multiple-stressor conditions in commercial fields. This trial utilized the latest in sensing technologies and ground-truth devices to characterize plant phenology and to identify critical periods of biostimulant activity.

AGRO 66

Novel biostimulants and bionutrients from biochemicals and microorganisms: Status and potential

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The global biostimulant market is growing at approximately 10-15% compounded annual growth rate per year and is estimated at about $2-3 billion. In the past few years, the segment is going through rapid change as large amounts of investment capital have supported startup companies. As such, there are many new entrants into the sector who bring a higher level of business sophistication, science, and data behind their products. This talk will review the status of the biostimulant market and the challenges for existing companies to show a competitive advantage and gain grower adoption. Also, what are the prospects for new companies wanting to enter this already crowded sector? What are some technologies that have the most promise and cost/benefit to growers?

AGRO 67

Production and formulation of methylotrophic microbial products for agriculture

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Pink-pigmented facultative methylotrophs in the genera *Methylobacterium* and *Methylorubrum* (M-trophs) are highly abundant members of the plant microbiome. NewLeaf Symbiotics is harnessing the power of these beneficial bacteria to improve yield as both biostimulant and biocontrol agents. By focusing on two closely related genera with many beneficial characteristics, we have developed a powerful comparative genomics approach to deliver products that are well adapted to sustainable agriculture. Every M-troph in our collection is sequenced, annotated, and stored in our computational bioinformatics platform, the Prescriptive Biologics Knowledgebase® (PBK). The PBK allows NewLeaf Symbiotics to nominate strains for field trials selected specifically based on their genetic potential resulting in higher probabilities of success. However, despite such deep knowledge and a powerful tool like the PBK, there are several challenges to successfully ferment and process M-trophs into stable products. For example, M-trophs are Gram-negative bacteria and have a thinner peptidoglycan layer and outer membrane which can be easier to disrupt through downstream processing and/or excipient use. As well, M-trophs do not sporulate, and therefore are unable to make spores that could significantly increase shelf stability. Despite these challenges NewLeaf Symbiotics has been able to successfully ferment, formulate and process M-trophs into commercial products. This talk will focus on some of the ways in which NewLeaf Symbiotics has utilized a deep expertise in M-trophs as well as tools like the PBK, to enable this success of M-trophs from fermenter to the field. Examples of this success will be given such as selection of media to optimize cell mass production and the effect of processing methods to improve shelf stability. Excipient screening and selection will be discussed with respect to their effect on biological stability and physical/chemical handling properties.

AGRO 68

Improving viability and fitness of microbial biostimulants for commercialization

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Microbial biostimulants are emerging as environmentally sustainable products for improving crop nutrition, yield, and tolerance towards abiotic and biotic stresses. In particular, climate change effects are expected to impose more extensive environmental stresses on crops worldwide impacting productivity; microbial biostimulants can assist plants in counter-balancing these stresses. When associated with plants, beneficial microbes produce a range of small molecules including organic acids, phytohormones, and other bioactive molecules that can help improve plant health and growth. In exchange, the plant feeds the microbes carbon

through root exudates allowing for the microbes to continue metabolizing and provide extended benefits to the plant throughout its growth. Wide adoption of microbial biostimulant products, however, has been largely limited due to inconsistent field performance. Many of these products consist of concentrated spore-forming bacteria that were selected to survive long-term storage and supply chain conditions. Decades of research has identified many non-spore forming bacteria that can provide enhanced benefits to crop health. Further, recent advancements in discovery screening techniques and synthetic biology are leading to promising new microbial candidates, many of which are non-spore forming. However, translating results from a research setting to consistent performance under variable field conditions remains challenging. Production, formulation, and delivery of a high concentration of highly viable, fit microbes is central to improving colonization of the plant and increasing reproducibility of the microbes' beneficial effects. Since these microbial biostimulants are dynamic living products, manufacturers must ensure that the microbial strain that is produced and formulated is the same as what is applied to the field after storage. Phenotypic variation of the microbe as a result of sporadic mutation has been observed during scale-up of production and storage, which may, in part, explain deviations in microbe fitness and beneficial function contributing to inconsistent field results. Innovation in production, formulation, packaging and delivery is needed to improve the quality and efficacy of these living microbial products being commercialized.

AGRO 69

State and federal regulatory activities impacting the plant biostimulant industry

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The emerging plant biostimulant industry shows tremendous promise. One of the potential barriers to growth for the industry, however, relates to the current regulatory uncertainty for plant biostimulant products. Several governments, including the United States and European Union (among others), have begun to evaluate the potential regulatory and legislative reforms necessary to ensure regulatory certainty for this product category. The term "biostimulant" is in broad use by industry globally, but no U.S. agency, at the state or federal level, includes a legal definition of plant biostimulants. The Agriculture Improvement Act of 2018 described a plant biostimulant, but no formal legal definition currently exists in the U.S. It is certain that an extensive list of related subcategory terms and definitions will need to be developed to provide all stakeholders greater understanding of this broad category of products. USDA issued a report to Congress in December of 2019 including two alternate definitions to the one above. Plant biostimulant manufacturers/developers are prohibited from calling their products "biostimulants" and limited in the benefit claims they can make. Companies must either register their product as a pesticide with EPA or as a soil amendment, plant amendment, beneficial substance or fertilizer in every state they wish to market. Neither path is truly appropriate for these products, and both can be unnecessarily burdensome, costly, complex, and confusing for developers, regulators, and consumers. We will address potential regulatory and legislative reforms that will help resolve some of the above issues for all relevant stakeholders. This includes an update on federal regulatory activity by EPA and USDA and state regulatory activity including at AAPFCO and AAPCO.

AGRO 70

Update on the US EPA guidance for plant regulator products and claims, including plant biostimulants

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The document provides guidance about plant regulator products and product claims, including claims for products known as plant biostimulants (PBS). PBS are a growing category of agricultural products containing naturally-occurring substances and microbes that are used to promote growth, reduce the effects of abiotic stress, and enhance water and nutrient use efficiency. PBS are not intended to provide any nutritionally relevant fertilizer benefit to plants, although they may contain fertilizer nutrients in their formulations. The goal of the guidance is to provide examples of products and product claims that are considered to be plant regulator, as defined in FIFRA Section 2(v), and examples of products and product claims that are excluded from the definition of a plant regulator as plant nutrients, plant inoculants and soil amendments. The first draft of the guidance was released for public comment in March 2019 with a comment period that ended in July 2019. The Agency is currently developing responses to the comments.

AGRO 71

Update on U.S. Department of Agriculture and Environmental Protection Agency regulatory actions regarding biostimulants

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The 2018 Farm Bill passed by Congress at the close of the 115th Congress mandated that the U.S. Department of Agriculture (USDA) prepare and submit to Congress a report on U.S. government regulation of biostimulants. In this report USDA was to analyse and assess the current regulatory process for biostimulants. USDA submitted the required report in December 2019. In addition, in 2019, the U.S. Environmental Protection Agency (EPA) released a draft guidance on the regulation of plant regulators under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). EPA's draft plant regulators guidance will have significant impacts on the distribution and use of biostimulants in the United States. This presentation will provide an update on the status and effects of the actions by USDA and EPA.

AGRO 72

Reflections on our AGRO division: Fifty years of engagement

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This talk will cover AGRO Division's fifty-year relationship with the broader aspects of agrochemical history. We will embark on a historical look back at AGRO's role as a crucible for discussions of emerging issues in the protection of agricultural productivity, public health, and the environment, with engagement across industry, academia, and key government agencies. We will highlight key aspects of our division's governance that have positioned us for meaningful AGRO awards, international participation, student engagement, and career development opportunities for all.

As part of the celebration plans, a 50th Timeline Team formed to capture submitted milestones from across our AGRO membership; the results of those efforts inform this talk.

AGRO 73

Innovation and evolution – Perspectives on crop protection discovery and the industry

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Crop protection discovery and the industry itself have evolved over the past 80 years. With the anticipated population growth in the coming decades, changing regulatory environment and continued emergence of resistance to commercial products, there is a constant need to discover new chemistries with novel modes of action and expand the approaches taken to control pests of agriculture and vectors of disease. An analysis of the crop protection industry highlighted that despite these challenges, the discovery of new crop protection compounds and associated tools continues. This presentation will focus on how the discovery of crop protection compounds and tools, especially related to insecticides, has evolved and will provide a window to future directions.

AGRO 74

Biopesticides and natural products for sustainable pest management and plant health

Pamela G. Marrone*, pmarrone@marronebio.com. Marrone Bio Innovations, Davis, California, United States*

The growth of biopesticides (bioprotection) is projected to continue at a 15-20% compounded annual growth rate, outpacing that of the flat $60 billion chemical pesticide market. What is driving that growth? When integrated into crop production and pest management programs, biopesticides offer the potential for higher crop yields and quality than chemical-only programs. Added benefits include faster speed to market, reduction or elimination of chemical residues, therefore easing export, enabling delay in the development of resistance by pests and pathogens to chemicals and shorter field re-entry, lower carbon footprint and low risk to non-target organisms, including pollinators. Challenges to the adoption of biopesticides continue to be perception of weaker efficacy and higher cost, lack of awareness and education in how to deploy their unique modes of action in integrated programs, and testing products alone versus in integrated programs. We are still in the early stages of discovering and developing biopesticides from microbial and plant-derived natural products, including the application of modern molecular tools, big data, and precision farming to enhance and enable more holistic integrated pest management systems.

AGRO 75

Role of the UC Davis Department of Environmental Toxicology in the founding of the division of Agrochemicals

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The ACS Division of Pesticide Chemistry, now the Division of Agrochemicals, was founded in 1970. Coinciding with the development of the Department of Environmental Toxicology at UC Davis, Professor Emeritus Donald G. Crosby was a founding member and first chair of both the division and department. In the United States during that same year, Earth Day was first celebrated and the Environmental Protection Agency was established. It was the dawning of an exciting era devoted to developing a better understanding of the fate and impacts of chemicals, including agrochemicals, in the environment. Having arrived in Davis a decade earlier with a doctorate from Cal Tech and research experience at Union Carbide, Professor Crosby established one of the first research programs focused on the environmental fate of pesticides. The division and department, firsts for both, were the natural results of his pioneering activities. The Division of Agrochemicals is now celebrating its 50th anniversary of serving as an international platform for the dissemination of cutting-edge information and a forum for the sharing of new ideas. Much has been learned over the years, from synthetic and fate processes to modes and mechanisms of toxic action in both target and non-target species. This has significantly advanced the management of agrochemicals to greatly enhance their benefits while minimizing their risks. A truly pivotal year, 1970 represents a turning point in both the focus on environmental quality and the role that deriving a better understanding of agrochemicals can play. Both the Division of Agrochemicals and the UC Davis Department of Environmental Toxicology have led the way ever since. Over the five decades that have passed, Professor Crosby had witnessed many changes and will share his reflections on both the past and future.

AGRO 76

Barriers to transformational science communication

Trity Pourbahrami*, trity.pourbahrami@gmail.com. Betty Moore Foundation, Palo Alto, California, United States*

We can all think of instances when effective science communication has been transformational for individuals, communities, organizations, and governments. Is there a secret? What is holding us back? In this interactive session Trity Pourbahrami, who is an experienced practitioner and

educator in science communication, will discuss some of the common barriers faced by scientists and engineers when trying to engage the general public. These include misalignment of the content with the audience and strategic purpose. She will use specific examples to illustrate how these barriers can impact the level of trust and credibility extended to the experts by the general public. In addition, she will share specific tactics and tools that have successful been used by scientist and engineers to overcome these barriers.

AGRO 77

Lessons learned from communicating plant science to the public

***Gloria Jaconelli**, gloria.jaconelli@croplife.org, Leighona Bernstein, Deb Carstoiu, Michael Stebbins. CropLife International, Brussels, Belgium*

Despite 25 years of science showing that GMOs do not have any adverse side effects, there are still many consumers today – especially mothers – that are apprehensive about buying food produced using this technology. The basis for science communication is to increase public understanding of science, but when it comes to plant science – biotechnology and crop protection – the scientific facts are not always given the credibility they deserve, leaving consumers confused as to which products they should buy and not fully understanding what scientific risk means. CropLife International – the voice of the plant science industry – has supported projects like GMO Answers and Pesticide Facts to address consumer questions and concerns around plant science, as well as to improve accurate science communication on crop protection and plant biotechnology. This talk will share some of the successful examples from these two platforms.

AGRO 78

Innovative conversations: Changing the way we talk about pesticides

***Genevieve OSullivan**, gosullivan@croplifeamerica.org. CropLife America, Washington, DC, United States*

How does the average consumer feel about pesticides? What are their questions? What are their concerns? We have an opportunity to listen and address these issues. Find out more about these questions and answers and learn the best way to meet consumers where they are and avoid the pitfalls that we all encounter when dealing with complex scientific topics.

AGRO 79

Engaging in new ways: Building trust by enhancing transparency

***Aimee Hood**, aimee.hood@bayer.com. Bayer, Ofallon, Missouri, United States*

To meet global food demands today and tomorrow, we need to work together to develop safe, sustainable tools and practices. We understand that meeting these challenges is not only dependent on innovation, but also on engaging society openly and transparently to bridge the differences that might separate us. Bayer Crop Science began releasing our safety related Crop Protection studies online in 2017 and have recently added submissions for our GM crop products. This year we will pilot an "OpenLabs" program inviting visitors to get a thorough overview of Good Laboratory Practice (GLP) and observe scientists performing a regulatory study. In this symposium, we will highlight all of the components of our transparency journey and explore the future activities that will continue to build trust with our stakeholders as well as a challenge to each of us to amplify these and other ways we can build trust.

AGRO 80

Communicating science on social media: Tips and recommendations for getting started

***Sarah Mojarad**, mojarad@usc.edu. University of Southern California, Los Angeles, California, United States*

Social media is a powerful tool for science communication. Interacting with the public and providing credible scientific information is increasingly important. Conducting outreach on social media is a fun way to share research and engage the public. This talk provides beginners with motivations and tips for effectively communicating via social media.

AGRO 81

How to grow understanding of formulating crop protection products

***Brittany M. Rauzan**, brittany.rauzan@corteva.com, Philip Ranly. Corteva Agriscience, Indianapolis, Indiana, United States*

At Corteva Agriscience, we are developing innovative, sustainable products that allow us to provide solutions to the increasing demand for food production as the global population expands. Traditional communication approaches regarding the formulation development process have been focused on the scientific community, regulators, and customers. In this presentation, we will present how we are changing the communication strategy to focus on our consumers. Highlights will include our work at our global research center to translate in a relatable way to visitors (stakeholders, customers, and consumers) how we formulate crop protection products. We also will preview our future initiatives to branch out beyond our company and partner with society to improve understanding of crop protection formulation product development.

AGRO 82

Effectively communicating regulations and mitigation strategies to professional pesticide applicators in urban areas in California

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The success of adopting a mitigation strategy related to pesticide use relies heavily on effective communication to professional pesticide applicators. To address concerns of pesticides found in surface waters, the California Department of Pesticide Regulation's (CDPR) Surface Water Protection Program (SWPP) has promulgated three source control mitigation strategies to reduce pesticide pollution in urban waterways over the past 9 years. In 2011, CDPR and the

manufacturers of bifenthrin (a pyrethroid insecticide) signed a memorandum of agreement (MOA) to restrict professional applications on impervious surfaces. In 2012, CDPR adopted regulations restricting professional structural and landscape applications of 17 pyrethroid insecticides. In 2018, CDPR established California specific mitigation for fipronil insecticide products through label-based use restrictions. Timed with availability of newly labeled products, SWPP initiated a comprehensive outreach program to communicate the details of all three mitigation strategies to professional applicators statewide. There are three main components of SWPP's outreach strategy: (1) direct outreach to professional pesticide applicators at continuing education events; (2) development of specialized workshops to provide hands-on or online training to pesticide applicators; and (3) increased coordination with the enforcement arm of CDPR, as well as the California County Agricultural Commissioners who directly enforce pesticide laws and regulations. Since 2018, SWPP scientists have reached over 4,200 applicators at 35 continuing education seminars and workshops. This presentation will share lessons learned and methods used to evaluate the outreach efforts.

AGRO 83

NewLeaf Symbiotics' prescriptive biologics knowledgebase: A microbials company data repository and analytics platform

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NewLeaf Symbiotics, an agricultural biotechnology microbials company, has created a proprietary web-based analytics platform and centralized repository of all company data, known as the Prescriptive Biologics Knowledgebase (PBK). Regardless of a user's technical expertise, NewLeaf's PBK enables deep interaction with company data in its entirety: fully sequenced and annotated genome sequences, secondary and tertiary genomic analyses, phenomic data generated from bench, controlled environment, and field experiments, fermentation and production measurements, and more. These data are exposed to the user in both raw formats and through a suite of interconnected analytics tools, whose output can be fed back into other tools as input. This input-output common language framework allows users to construct their own flexible, custom analytics procedures. Through the PBK, NewLeaf users track end-to-end strain provenance, collate all known information on every strain, identify new product candidates, and uncover microbial modes-of-action.

AGRO 84

DNA-encoded chemical libraries: Cheminformatics - heterogeneous reactions - compound identification

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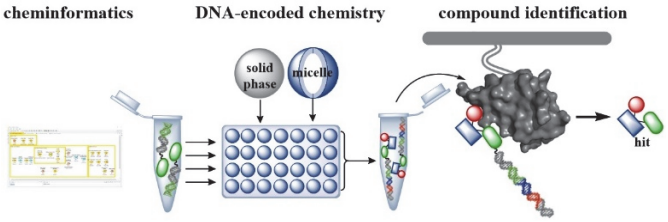
DNA-encoded libraries of chemically synthesized compounds (DELs) are a widely used small molecule screening technology. DELs are efficiently synthesized by a combinatorial workflow that encompasses alternated enzymatic DNA tagging and preparative organic synthesis steps. In contrast to discrete screening libraries, encoded library formats enable identification of bioactive compounds from highly complex compound pools by a selection-based assay and subsequent barcode sequencing. Successful compound selection depends on both chemical space coverage and functionality of the genetic tag. A prime challenge in the field is the current lack of organic preparative methods for encoded compound synthesis. These need to tolerate water and keep the genetic information intact. Reactions mediated by low pH, oxidants, many metal ions and harsh reaction conditions carry the risk of damaging the DNA barcode. We exploit heterogeneous reaction systems such as solid phase- and block copolymer micelle-based approaches to open access to a larger scope of reactions on DNA-tagged starting materials. For reaction selection from the vast amount of reaction data we are developing a cheminformatics tool. It filters reaction data by relevant reaction conditions and clusters reactions for efficient navigation of reaction space to prioritize translation of novel reactions to DEL and aid in DNA-encoded library design. Screening of a proof-of-concept DNA-encoded library and analysis of DNA barcode sequencing data with an in-house-programmed algorithm led to identification of an inhibitor of the TEAD4-YAP protein-protein interaction.



Research in DNA-encoded libraries: Computer-assisted reaction selection; heterogeneous approaches to reaction translation; and proof-of-concept DNA-encoded library synthesis and screening.

AGRO 85

Collaborative modeling project for predicting acute oral toxicity (CATMoS)

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Non-animal approaches are needed to assess an increasing number of chemicals for acute oral systemic toxicity potential (LD50). NICEATM and the ICCVAM Acute Toxicity Workgroup organized an international collaborative project to develop *in silico* models to predict LD50 and bridge data gaps. Participants from 35 groups submitted a total of 139 predictive models built using a dataset of 11,992 chemicals split into training (75%) and evaluation sets (25%). Crowdsourced models were developed for five endpoints: LD50 point estimates, EPA hazard categories, GHS hazard categories, very toxic (LD50 < 50 mg/kg), and non-toxic (LD50 > 2000 mg/kg). Predictions within the applicability domains of the submitted models were evaluated, then combined into consensus predictions based on a weight-of-evidence approach. The resulting consensus model, forming the Collaborative Acute Toxicity Modeling Suite (CATMoS), leverages the strengths and overcomes the limitations of

individual modeling approaches. The consensus predictions are fully reproduceable and performed at least as well as independent replicates of *in vivo* acute oral toxicity assays. The CATMoS consensus model is available via the free and open-source tool OPERA (Open Structure-activity/property Relationship App). OPERA also provides predictions for physicochemical and pharmacokinetic properties and other toxicological endpoints with applicability domain and accuracy assessment. CATMoS predictions for the ~850k chemical structures in DSSTox are being made publicly accessible via NTP's Integrated Chemical Environment and the EPA's CompTox Chemicals Dashboard.

AGRO 86

Modeling PROTAC-mediated protein degradation: Case studies and recent developments

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Targeted protein degradation using bivalent small molecules (such as PROTACs) is a new modality that provides a means to control protein levels *in vivo*. Despite many clear advantages, numerous challenges exist in PROTAC development, particularly concerning the rational design of efficacious molecules. In this presentation, multiple computational methods that enable the *a priori* evaluation of putative PROTAC molecules will be discussed. Numerous case studies will be offered, where the application of these computational tools can successfully recommend both potent PROTAC candidates and molecules to avoid. Results from scenarios across different target proteins, E3 ligases, and PROTAC architectures will be presented.

AGRO 87

Opportunities and challenges for deep learning in pesticide structure-activity analysis

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Deep learning is a powerful and easy-to-apply technology that is causing sweeping changes across science. Agrochemical discovery and development is no exception to this trend, with the most visible applications to date involving pest recognition. This talk will attempt to put deep neural networks into a broader context vis a vis other machine learning methods and highlight some of the intrinsic challenges posed to deep learning by the small data set sizes, inherent biases and uneven data quality characteristic of agrochemical research and development.

AGRO 88

Leveraging human-curated data to help solve India's pesticide formulation challenge

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The agrochemical market in India has strong growth potential, especially related to crop protection. From 2019 to 2024, the projected compound annual growth rate is 3.73%, with market drivers including the ever-increasing demand for food, growing emphasis on food safety, and the introduction of herbicide-tolerant seeds. Crop protection in India involves the control of a number of crop- and region-specific plants and insects. To develop new, effective and environmentally safe solutions, agrochemical formulators must consider previously used and existing active ingredients and formulations, as well as delivery systems and possible regulatory restrictions. Here, we discuss how agrochemical companies can leverage formulations data, combined with regional regulatory information, to deliver the most cost-effective solutions. This big-picture approach addresses market drivers, while ensuring a non-negative impact on consumers and the environment.

AGRO 89

"Spray and pray please go away!": Efforts to establish a cheminformatic strategy aimed at rationalizing and optimizing the agrokinetic parameters of pesticides

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Enormous strides have been made over the past three decades to understand the key determinants of pharmacokinetics as they apply to pharmaceutical drug design and the underlying causes of drug attrition. However, despite the knowledge gained from active research in pharmacokinetics and ADME (absorption, distribution, metabolism and elimination) of pharmaceuticals, minimal attention has been given to the optimizing and understanding "agrokinetics" as they apply to the same problems in agrochemical discovery. Although "rules of 3" and their counterpart ("rule of 5") exist in the literature, useful molecular design/delivery rules and tailored informatics approaches are underdeveloped for pest species targeted by agrochemicals such as plants, insects, and fungi. Here we describe retrospective analyses using public datasets assembled from the literature on chemical uptake through plant foliar systems, insect oral absorption, and fungal uptake. These data have been applied to develop both (i) biophysical and (ii) QSAR/QSPR-based cheminformatic and machine learning models in MOE (Molecular operating Environment) to shed some light on the agrokinetic parameters of biologically active pesticides. These exploratory models can help pave the way for new experimental and *in silico* methods of capturing and understanding the key agrokinetic descriptors for modern pesticides. Rather than "spraying and praying" for new agrochemical solutions, it is our hope that both the rules and relationships gleaned from these experimental models will hold promise in reducing toxicity, enhancing potency, reducing biological resistance and streamlining the value of ultra-large agrochemical library datasets. These simple informatics-driven approaches for estimating agrokinetic determinants can bring a much-needed "rational" dimension to agrochemical design, and focus discovery or optimization efforts down to a molecular level of resolution.

AGRO 90

Revisiting and updating chemical categorizations using chemical fingerprint and high-throughput screening data

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Chemical categorization, or grouping, is routinely employed to capture and report salient chemistry and toxicity correlations as well as to consider analogs for chemicals that have limited empirical information. One prominent application of chemical grouping used by chemical regulators is the Ecological Structure Activity Relationship (EcoSAR) model, which predicts the toxicity of classes of chemicals to various aquatic species. There is a continual need to update chemical categories and predictive models as new information on chemical hazards becomes available, especially given that a large proportion of industrial substances are not classifiable by EcoSAR and similar tools. This study employed hierarchical clustering approaches to evaluate whether potential refinements could be made to the current EcoSAR classes using chemical fingerprint and *in vitro* biological activity information. Refinements included building sub-categories for broad, existing EcoSAR classes (*e.g.*, neutral organics), as well as identifying new categories to address substances currently unclassifiable by EcoSAR. An ensemble tree-based binary classification model was developed to predict narcotic or specific-acting aquatic toxicity modes of action. The model was trained on chemical fingerprint (ToxPrints), *in vitro* biological activity (ToxCast and Tox21 high-throughput screening data), or both. It was then used to predict aquatic toxicity mode of action (narcosis vs specific-acting) for chemicals classified as neutral organics or unclassifiable by the current version of EcoSAR. Chemotype and activity enrichments for those chemicals predicted to be specific-acting identified features useful for refining EcoSAR classes, including several bond chemotypes (*e.g.*, sulfonyl, sulfide, sulfonate, alkyl-tri-halo, and benzopyran) and *in vitro* assay activity (*e.g.*, Novascreen ENZ assays). This approach identified data gaps in the biological activity inventory, potential analogs for chemicals that may fit the suggested new categories, and suggests specific high-throughput assays that may be most useful for informing reductions in animal testing.

AGRO 91

Boosting chemical libraries using information from vast chemical spaces of tangibles

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The recent advent of reaction-driven and purchasable chemical spaces of vast chemical sizes has matured into a highly useful resource across pharmaceutical and agrochemical research. Enamine, WuXi, and other compound makers nowadays offer on-demand molecules that can be picked from millions to billions of virtual "tangibles". Yet the question remains how best to exploit the latent information in the neighborhood relationships amongst the compounds — notably with respect to existing in-house libraries. Relevant questions in this context are: "Where are molecular gaps in my library?", "How can I fill chemical space voids with compounds from purchasable chemical reaction spaces?", "Do two spaces, the sizes of which are too big for enumeration, overlap?" These questions can be addressed using a workflow that shall be presented in this paper. Using very fast, tree-based algorithms that exploit the combinatorial nature of the spaces, we retrieve statistically relevant subsets across chemical spaces that exceed the multi-billion member barrier. Applying machine learning, local information can be exploited to enhance the quality and information content while navigating the chemical spaces.

AGRO 92

Impact of modern, macromolecular QM/MM crystallographic refinement on our understanding of protein:ligand structure and function

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Modern, structure-based (computational) agrochemical discovery is dependent upon accurate computational characterization methods which are in turn dependent upon accurate 3-dimensional (3D) macromolecular structure. Traditionally, the conventional crystallographic methods used to determine these macromolecular structures rely on highly approximate methods which lack explicit, rigorous terms for electrostatics, polarization, dispersion, hydrogen bonds, and other interactions. In order to address this deficiency and capture a more complete understanding of the structure, we have developed a fully automated approach for macromolecular refinement based on a two layer, QM/MM (ONIOM) scheme, which consists of one or more "region layer(s)" characterized using linear-scaling, semi-empirical quantum mechanics, coupled with a "system layer" encompassing the rest of the protein described with a molecular mechanics functional. Armed with a more accurate tool, we not only gain a better understanding of overall protein:ligand structure, but we can also use X-ray data to correctly determine active site tautomer/protomer states and water site locations. The impact of these improved structures on our ability to predict protein:ligand binding in the context of modern agrochemistry will be discussed.

AGRO 93

Computational startegies for understanding hormone perception in plants

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Plant hormones are a series of small molecules that are naturally produced in plants, which regulate plant growth, development, and responses to biotic and abiotic stresses. There are 9 major classes of plant hormones that have been identified, including auxin, abscisic acid, brassinosteroids, cytokinins, ethylene, gibberellins, jasmonates, salicylic acid, and strigolactones. Over the past decades, substantial research studies have been trying to answer a set of fundamental questions in plant biology: how do hormones work in plants? Recently, the crystal structures of receptor proteins for 7 classes of plant hormones have been identified, except for ethylene and salicylic acid. These structural studies have led to a predominant "molecular glue" hypothesis to explain the mode of actions of these hormones. According to this hypothesis, plant hormones act as "molecular glue" after binding to their receptors, thereby promoting protein-protein interactions between the receptors and the binding partners, which triggers the downstream signaling cascades. While this hypothesis has been supported by a variety of experimental data, the nanoscale details of how plant hormones bind to their receptors and lead to the assembly of protein-protein complexes remain largely unknown. In this work, we utilize large-scale all-atom molecular dynamics (MD) simulations and advanced free energy calculation methods to examine the mode of actions for 7 plant hormones, whose receptor structures are currently available. We have performed hundreds of microseconds MD simulations to capture the plant hormone binding processes. Coupled with Markov state models, we fully map out the protein-ligand binding pathways and the associated conformational changes of receptor proteins, along with thorough quantitative thermodynamic and kinetic characterization. Our results unravel the complete structural, dynamic, and energetic basis of the protein-ligand binding processes involved in plant signaling. In addition, we have investigated the solvation thermodynamics of these plant hormone receptors via MD simulations and inhomogeneous solvation theory. Our results highlight the essential role of water molecules in plant hormone perception and affinity, which can be exploited for receptor engineering and agrochemical design.

AGRO 94

Creating focused libraries for protein engineering

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Protein engineering plays a pivotal role in modulating the function, activity and physical properties of enzymes. Representative strategies employed in protein engineering include rational protein design and directed evolution. When the mutation space is too large, multiple experiments must be conducted to enable an efficient and complete search. Ideally, *a priori* knowledge can be used to reduce this space by mutating residues that have higher probabilities of yielding enzymes with desirable protein properties. When this knowledge is lacking, one can turn to computer-aided techniques to generate models and assess enzymatic properties. Here, we establish a method for predicting mutation probabilities, in order to reduce the number of variants in a given library.

AGRO 95

Residues of methyl bromide following postharvest fumigation of sweet cherries from western USA to control spotted wing Drosophila, *Drosophila suzukii*

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Methyl bromide (MB) chamber fumigations were evaluated for postharvest control of spotted wing drosophila (SWD), Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), in fresh sweet cherry exports from Western USA at pulp temperature ≥ 8.3° C. As reported in Walse, et al. (2016), a kinetic model of sorption was developed based on the measurement of MB and how calculated exposures varied across the fumigation trials. The model describes how to manipulate the applied MB dose, fumigation duration, and the load factor so that the resultant exposure is adequate for SWD control across various pulp temperatures when cherries are fumigated in wooden versus plastic bins. Following each fumigation scenario, after packing the fumigated fruit into export boxes and packaging materials, the methyl bromide residues were quantified after 24h of cold storage at 0.5 ± 0.05 °C per USEPA-approved protocols. Residues of < 2ppm, less than the newly proposed 5ppm USEPA tolerance, were formed from a ≤ 88 mg L-1 (6 lbs 1000 ft-3) dose applied for ≤ 3.5 h to sweet cherries in plastic bins at loads ≥ 10%. This finding suggests potential to revise the most recently approved MB label, which limits sweet cherry fumigations to ≤ 80 mg L-1 (5 lbs 1000 ft-3) doses applied for ≤ 2 h.

AGRO 96

Analytical methods for fumigants in air

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Fumigants are important pesticides for producing and preserving agricultural products. They include well known and sometimes controversial chemicals like methyl bromide, ethylene oxide, methyl isothiocyanate, MITC, dichloro propane, and many others. Because of their exceptional volatility and reactivity, sampling and analysis of fumigants can be a challenge. We will review and evaluate analytical approaches for fumigants in soil and in air from commodities such as tree nuts and spices that undergo fumigation often for shipments overseas.

AGRO 97

Improved analysis of propylene oxide, propylene chlorohydrin and propylene bromohydrin

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The importance of propylene oxide (PPO) treatments for stored product protection has only increased in recent years, especially as the implementation of FSMA in the U.S. puts pressure on tree nut producers to pasteurize their product. Unfortunately, analysis is complicated by the ease with which PPO will undergo nucleophilic reaction with water to form propylene glycol, or with chloride and bromide to form propylene chloro and bromo- hydrin, which can artificially lower the detected PPO residue. Avoiding the formation of these halohadrins (PXH) is of particular importance as they face regulatory scrutiny as carcinogens. The benefits and deficiencies of several methods of analysis for PPO and PXH, including the aqueous extraction used in ASTA method 23.1 and the MTBE extraction method previously reported by the authors, will be discussed. Novel methods utilizing dynamic headspace extraction and solid phase microextraction (SPME) will also be reported with particular emphasis on preventing artefactual effects. Preliminary experiments have demonstrated that while headspace sampling methods can significantly improve sensitivity for PPO, PCH and PBH (~3 orders of magnitude decrease in LOD for PBH), great care must be taken to avoid artefactually raising PCH and PBH levels. The use of autosamplers (either dynamic headspace or L-PAL3 with SPME attachment) can greatly reduce injection to injection variability and reduce the number of person-hours required for analysis, but to fit walnuts or almonds into headspace vials they must be chopped or ground, exposing further chloride or bromide to react with PPO. Experiments using manual SPME sampling will allow the use of glassware that can accommodate whole nuts. The use of iodide, or other nucleophiles, to compete with chloride and bromide for the reaction with PPO will also be examined.

AGRO 98

Using chemical-specific and non-chemical specific data for assessing human exposures to new fumigant products

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New fumigants require the completion of human health risk assessments before registration for use in California. During this process, potential human fumigant exposures are assessed for handlers, re-entry workers, occupational bystanders and residential bystanders. This presentation reviews the fumigant exposure assessment methodology currently used by the California Department of Pesticide Regulation. For assessing the human exposures, chemical specific data from the new fumigant is preferred. However, data for new chemicals is not always available. When data gaps are identified, information from other fumigants with similar physiochemical properties and use patterns may be used instead (*i.e.*, "new fumigant surrogates"). Currently, the most commonly used soil fumigants in California include 1,3-dichloropropene, chloropicrin, methyl bromide, metam-sodium and potassium N-methyldithiocarbamate. These fumigant surrogates, due to their long use history, have environmental fate and human exposure information available to bridge the data gaps of new fumigants. During the exposure assessment process, information gathered from chemical-specific and surrogate data are grouped based on the application methods (*e.g.*, broadcast shank) and tarp usages (*e.g.*, totally impermeable film) and then evaluated for the data completeness, quality, and usability. In-field monitoring data were used for assessing the exposures of handlers and re-entry workers. Exposures for occupational and residential bystanders were estimated by measuring fumigant soil emission rates over time and modeling the air dispersions around the treated field. This presentation will also discuss some identified data gaps which warrant future studies to reduce the uncertainties during the fumigant human exposure assessment.

AGRO 99

Sorption and off-gassing of ethyl formate following postharvest fumigation of bulk citrus with eFUME®

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The aim of the "Special Citrus Use" is to safely disinfest citrus of key pests, none more important than Asian citrus psyllid (ACP), before fruit leaves the grove. The results below provide evidence that pest control will result following the application of <250 mgL-1 eFUME (<43 mgL-1 ethyl formate AI) to field bins of "bulk citrus" under tarpaulin and that, importantly, there is a "total utilization" of ethyl formate, such that applicator/worker reentry will not occur until ethyl formate levels in the enclosure (tarpaulin) headspace are < 100ppmv (and thus immediately compliant with the Cal/OHSA 8-h TWA PEL for ethyl formate). Evidence is presented to show that ethyl formate off-gassing from recently fumigated fruit occurs at levels that do not pose a risk to applicators or workers, as ethyl formate is rapidly hydrolyzed in the fruit, and that resultant "free" and "combined" formic acid residues are collectively expected to be < 80 mgkg-1 as well as difficult to distinguish from natural levels. Based on laboratory-scale fumigations with mass-labeled ethyl formate having Ct exposures recommended in the "Special Citrus Use," "free" formic acid residues are expected to be < 14mgkg-1, the analytical limit of quantification (LOQ).

AGRO 100

Postharvest fumigation with ozone to control brown marmorated stinkbug (Hempitera: Pentatomidae)

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Brown marmorated stink bug [BMSB; *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae)] is an insect pest of concern worldwide. American grape producers are particularly concerned because the pest has affected their crop directly and their ability to export to certain markets across the world. Due to the phasing out of methyl bromide as a non-restricted fumigant, there is a need for developing alternative fumigants. Ozone (O_3) in combination with carbon dioxide (CO_2) is currently under study as an alternative to those

treatments. Fumigant testing was conducted at the UC Davis Contained Research Facility where adult insects are treated with 10,000 ppmv in chamber at 3 ± 1 ^{0}C for 1 hour. Prior to fumigation with ozone, adult bugs are exposed to a range of concentrations of CO_2 for ten minutes at 3 ± 1 ^{0}C. The initial results of this study are promising towards indicating that ozone fumigation (when combined with pre-fumigation CO_2 exposure) can achieve the desired control of brown marmorated stink bug. Results of this study will show how adequate toxicological efficacy toward adult BMSB can be achieved by an ozone fumigation at a temperature of 3 ± 1 ˚C in combination with a pre-fumigation treatment of CO_2 at the same temperature. Overall, these results may lead to a schedule for commercial implementation of ozone fumigations to better control BMSB across the world.

AGRO 101

Haber's rule and insect fumigant interactions: a case study of ethyl formate, sulfuryl fluoride, and *Halyomorpha halys*

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Fumigation science is a broad field that is concerned with a variety of scenarios from postharvest and soil fumigation to structural and consignment fumigation. Application of fumigants is similarly varied and includes: solid phosphine pellets, sulfur dioxide pads, and direct gas injection to name a few. While the application methods and uses of fumigants are diverse, the end goal is always the same: maintain a headspace concentration of fumigant for a set duration until a particular mortality of the target pest is achieved. Haber's rule, $C^zt = \omega$, is the basis for relating concentration (C) and time (t) to an empirical level of response (ω) in fumigation science. The parameter z is an empirically determined ratio of the relative importance of C and t toward ω and can vary for each fumigant-pest combination. Here we discuss the differences in z for exposure-response curves of *Halyomorpha halys* treated with either ethyl formate or sulfuryl fluoride as well as differences in diapausing and non-diapausing physiological states as a response to both fumigants.

AGRO 102

Unknown effects of plant protection products and their metabolites: The case of fluopyram and growth disorder in *Vitis vinifera*

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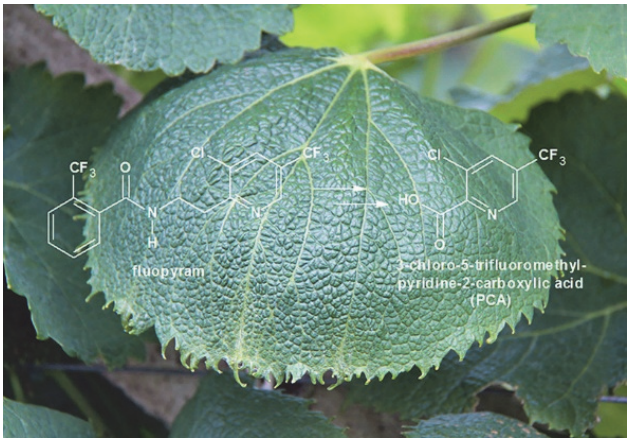
To feed an increasing world population, modern agriculture relies heavily on chemical plant protection products (PPPs). However, concerns over negative impacts on human health and the environment has led to the introduction of strict regulations in the Unites States, the European Union and beyond. Extensive experimental data are requested, leading to elevated costs and a reduced availability of synthetic PPPs in agriculture. Despite this significant registration effort, post-market surveillance is required to monitor efficacy and resistance and to discover unknown effects. We recently reported phytotoxic effects of 3-chloro-5-trifluoromethylpyridine-2-carboxylic acid (PCA), a metabolite of the fungicide fluopyram in *Vitis vinifera*. PCA was known from registration studies to be formed at ca. 1%, but phytotoxic effects have never been observed, including in trials performed at Laimburg Research Centre. Following the extraordinary rainy 2014 season, when fluopyram was applied repeatedly, unprecedented growth distortions were observed in 2015 in vineyards in South Tyrol, Italy, and throughout Europe. Using both field trials and greenhouse experiments, our study established a link between the application of fluopyram in 2014 and the emergence of phytotoxic effects in 2015. The results will be presented and discussed in the context of ever-rising regulatory and market requirements and the challenges for the development of future PPPs.



AGRO 103

High-resolution mass spectrometric approaches to evaluate the fate and effects of antibiotics in agricultural systems

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In response to increasing pressures on freshwater resources, the reuse of treated, and in some cases untreated, wastewater to meet irrigation demands has been adopted in many locations throughout the world. Wastewater is a known reservoir for numerous emerging contaminants including pharmaceuticals like antibiotics. The use of wastewater for irrigation represents a pathway for these bioactive compounds to enter the agro-ecosystem. Here we show how high resolution mass spectrometry (HRMS) can be used to 1) evaluate the impacts of environmentally relevant antibiotic mixtures on barley grain using a metabolomics approach, and 2) demonstrate how single cell mass spectrometry (SCMS) can be used to identify potential antibiotic degradants and provide insights into potential detoxification pathways used by plants exposed to antibiotics. Experiments with barley irrigated with a synthetic wastewater fortified with 11 antibiotics at their predicted concentrations in UK wastewaters showed potential toxic response in early stage growth. However, at crop maturity, there was little evidence of impacts on plant health or productivity. Metabolomic analysis of mature barley grain collected at harvest revealed no significant impacts on the extracted soluble metabolite profile between samples grown under increasing antibiotic exposure. Next, capillary extraction of individual plant cell vacuoles from a variety of common vegetable crops grown in antibiotic fortified hydroponic solutions was followed by direct

electrospray ionisation and HRMS analysis. Very high accuracy mass measurements allow the tentative identification of parent compound and metabolite elemental formulae. We show how SCMS can be used as a valuable tool in understanding the fate of xenobiotics once taken up by plants.

AGRO 104

Pesticide mixtures: Effects of combined application on the degradation of pesticides in soil (OECD 307) and aquatic sediment (OECD 308) test systems

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The environmental fate and behavior of pesticides is predominantly assessed by conducting laboratory studies according to OECD Guidelines, which are designed for testing of individual and single applied chemical compounds. Since most agronomic practice involves either sequential, or combined treatment of herbicides, fungicides, and otherwise insecticides in the production of agricultural crops, in one vegetation season different pesticides may co-exist in the soil environment, or in aquatic sediment in form of mixtures. Nonetheless, possible cumulative and/or synergistic effects and interactions between one and the other compound(s) in the environment, as well as indirect side-effects such as alteration of soil, water and/or sediment biomass have been very rarely investigated. As of 2019, in conjunction with RWTH Aachen University, Germany, Innovative Environmental Services (IES) Ltd, Switzerland, as an independent GLP-CRO has been investigating the environmental impact of tank mixtures applied to soil and aquatic sediment systems. Accordingly, the effects of broad spectrum fungicidal treatment of respective environmental compartments on the route and rate of degradation of a sulfonylurea herbicide have been studied under controlled conditions.

AGRO 105

New insights into an ignored issue of metabolite in biochar-amended soil: effect of biochars on dissipation of cyazofamid as an example

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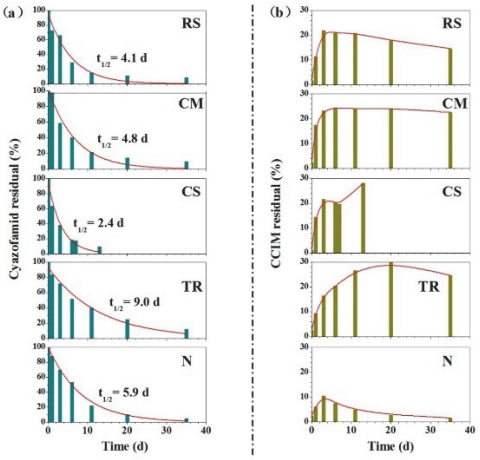
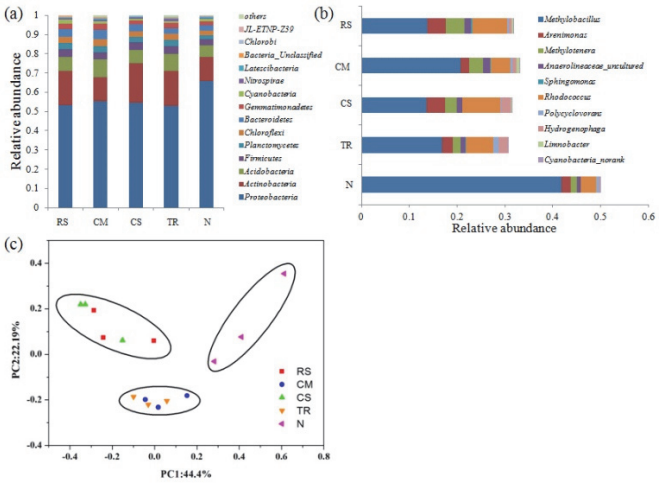
Biochars derived from rice straw (RS), corn straw (CS), chicken manure (CM) and tire rubber (TR) were applied to soil to investigate their effects on the dissipation of cyazofamid and its metabolite CCIM (4-chloro-5-p-tolylimidazole-2-carbonitrile), with high acute toxicity compared to cyazofamid. The enhancement of cyazofamid dissipation followed the order of CS > RS > CM, whereas TR depressed the cyazofamid dissipation. Adsorption, hydrolysis and microbial degradation were all involved in cyazofamid dissipation. CM and CS enhanced the contribution of biodegradation to cyazofamid dissipation, which might be related with the shifted microbial community. More importantly, CCIM residual was drastically increased by 8-15 times after biochar application, regardless of biochar type. In total, this study shed light on the issue of build-up of metabolites in biochar-amended soil, especially for metabolites having higher toxicities than parent compounds, providing new insights into potential risk of biochar application for soil remediation.





AGRO 106

Design, execution, and interpretation of analytical and impurity profiling studies in the support of the registration of an agrochemical

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It is prudent to say that information on 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 outcome. While characterising 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 characterise them quantitatively. To prove the toxicological relevance of impurity, impurity profiling may include biological testing as well as analytical characterisation. The analytical impurity profiling study is conducted using a wide range of sophisticated equipment, data processing tools, and traceable reference standards and in compliance with strict regulatory requirements. Different analytical studies applicable for characterization of technical pesticides include active ingredient content, identification and quantification of each expected impurity, spectra analysis / identity, vapor pressure, solubility in water, solvent solubility, water content, partition coefficient, and dissociation constant. Each of these

tests requires a careful selection of method to derive the results which meet the specific regulatory requirements. The risk factors in these studies could be the measurement and traceability of various equipment used and its periodic calibration, validation of a computerised system to ensure the integrity, reliability and traceability of data, traceability of reference standards, and its characterisation as well as various environmental factors affecting the stability of sensitive chemicals and standards. The challenges include the development of analytical method/s with sense to detect the impurities at trace level, validation of an analytical method/s to establish the method sensitivity to serve the intent and to meet regulatory requirements and the identification of impurities, and its quantitation using the precise and accurate analytical method. 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 to archiving and retention of data.

AGRO 107

Evaluation of DDT bioaccumulation in earthworms from a historically-contaminated orchard by Bayesian hierarchical modelling

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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. Thus, remediation of the contaminated soil is needed. Composting is a potential remediation technique, and the effectiveness of composting to mitigate bioaccumulation factor (BAF) has been confirmed by laboratory studies. A plot study was then conducted to examine the feasibility of composting under field conditions. However, it was found that field concentrations had great spatial variability and a traditional approach may not be feasible for data analysis. Therefore, it is necessary to find a more accurate data analysis approach. The goals of the current study include 1) to apply Bayesian hierarchical mode (BMH) to characterize soil and earthworm concentration, 2) to compare the performance of the proposed model with the traditional model, 3) to draw implications for remediation techniques and risk assessment. The data were obtained from an 18-month plot study, consisting of seven replicated plots. Each plot was divided into four subplots with three treatments (tillage, 2-year compost and 4-month compost) and a control. DDT and its metabolites (together referred to as DDx) in soil and earthworm were measured in each subplot. One-way analysis of variance (ANOVA) model and BHM were used for characterizing DDx concentrations. Cross-validation was applied for both models to evaluate out-of-sample predictive accuracy, quantified by normalized mean standard error (NMSE). Akaike information criterion (AIC) and Bayesian information criterion (BIC) were calculated to evaluate goodness of fit of the two models. The inferential results were used for evaluating effectiveness of composting. Results showed that the values of NMSE of BHM are smaller than values of NMSE of ANOVA model for soil concentrations. For earthworm, BHM and ANOVA model have almost identical NMSE. On the other hand, for both medium, AIC and BIC of BHM are consistently smaller. Therefore, BHM appeared to perform better than ANOVA model, and BHM would be a better data analysis approach than traditional approach. Future work will be focused on applying BHM to evaluate effect of composting to BAF and ecological risk.

AGRO 108

Comparison of EPA and ECHA guidance on characterization of non-extractable residues (NER) in degradation assessment

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Following the U.S. EPA publication of guidance for addressing non-extractable residues (NER) in laboratory soil and sediment studies in 2014, the European Chemicals Agency (ECHA) has recently published a discussion paper on improving the interpretation of NER in degradation assessment (June 2018). The EPA promulgated an approach aimed at removing weakly sorbed residues using solvents of different polarities. The ECHA recommendation offers a way of discriminating between the different NER fractions and identifying the relevant fraction for persistence assessment. This presentation will discuss results of several soil and sediment degradation studies performed based on each guidance and the challenges associated with their execution. These studies were mainly performed with plant protection products (PPP); but results of a few pharmaceutical API's and household products will also be presented. It was generally observed that the EPA recommended additional extractions with less polar and non-polar solvents collectively removed ≤5% of applied radioactivity following polar solvent extractions. More work is needed on the ECHA approach in order to better understand the NER and how to implement procedures in the routine work in most laboratories. Non-extracted residues were further characterized via kinetics modeling. The kinetics modeling approach provides an additional tool that was used to explain the formation of radioactive residues and CO_2 by fitting the data to NER and CO_2 as separate compartments.

AGRO 109

Unextracted or non-extractable: A Canadian regulatory perspective

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Pesticides undergo many transformation and transport processes in the environment. One process is the formation of non-extractable residues (NERs), a fraction of pesticides, including transformation products, that becomes tightly

bound to soil and sediment with low potential to remobilize. As such, NERs have negligible contribution to environmental exposure, and the Pest Management Regulatory Agency (PMRA) considers their formation a route of dissipation. In laboratory studies, pesticide residues can be difficult to remove from soil or sediment samples using conventional solvent extraction protocols. When unextracted residues from inadequate extractions are included with the NER fraction, degradation rate constants can be overestimated, and, consequently, persistence and estimated environmental concentrations are underestimated. From a Canadian regulatory perspective, the PMRA, therefore, takes a cautious approach by treating unextracted residues resulting from inadequate extractions as residues of concern and including them in half-life calculations. Historically, extraction protocols have often used solvents with similar polarities under ambient laboratory conditions. However, increasingly, advanced extraction technologies are being used to characterize bound residues in combination with multiple solvents of varying polarity. In 2014, the U.S. EPA published the *Guidance for Addressing Unextracted Pesticide Residues in Laboratory Studies*, recommending that multiple polar and nonpolar solvents are tested with each soil or sediment and across the duration of the study, as parent and transformation products vary in chemical properties and may interact with soil or sediment differently. The PMRA is developing a guidance for evaluating the quality of solvent extraction protocols that builds on the US EPA guidance and addresses unique Canadian policy issues. The PMRA guidance will describe current perspectives on NERs, steps for evaluating solvent choice and extraction methodologies, and, ultimately, the determination of unextracted versus non-extractable residues. The PMRA approach is elaborated with examples that illustrate the impact of suitable extraction protocols on regulatory conclusions for persistence in the environment and exposure to pesticides from drinking water sources and other ecosystems.

AGRO 110

Analysis of non-extractable residues (NER) for use in chemical persistency assessment – experimental results from a harmonised testing procedure

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In regulation of chemicals in Europe, NER in degradation testing is an issue. In European regulations it is treated incoherently with REACH considering NER as non-degraded substance if not proven otherwise while other, *e.g.* plant protection product regulation, consider NER as degraded up to now. As this might have significant impact on degradation half-lives determined, the German Environmental Agency is working on a harmonized approach. However, a standardized method for further characterization of NER is still lacking. In the last years, efforts have been made to differentiate NER into different categories of concern. A first approach was started on behalf of the German Environment Agency (UBA) in 2013. In September 2018 a follow up project was initiated by UBA order to harmonise those project results with the procedures described in the ECHA discussion paper on NER, published in June 2018 on experimental approaches to distinguish sequestered (type 1 NER), covalently bound (type 2 NER), and biogenic NER (type 3 NER). A parallel project was initiated by Concawe, which is closely related to the UBA project, but focusses on petroleum specific chemicals and includes additional bioavailability determinations to get a better understanding on relevant processes. In the UBA project we evaluate the applicability of the harmonised approach experimentally, supported by data from the Concawe project. We run these experiments using test substances labelled with ^{13}C and ^{14}C in parallel. In case of a positive correlation, data from literature using either label can be reconciled. Experiments are accompanied by the modelling tool *Microbial Turnover to Biomass (MTB)* that uses released CO_2 as indicator of microbial degradation and the theoretical biomass yield in order to estimate the biogenic NER formation. Experimental data determined in the current project shall be used to verify the outcome of this approach. We test a set of four substances in a standard OECD 307 approach. Different procedures for exhaustive extraction are applied in order to compare current NER definitions. NER are subject to a set of harsh extraction methods in order to identify and quantify the different NER types described in the ECHA discussion paper.

AGRO 111

Soil sequestration results in environmentally irrelevant residues of the herbicide MSMA

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Monosodium methylarsonate (MSMA) is a selective contact herbicide used for post-emergent control of a broad spectrum of weeds. In water, MSMA dissociates to ions of monomethyl arsenate (MMA) and sodium. MMA concentration in soil solution declines quickly by two processes: sequestration and metabolism by certain soil-dwelling microorganisms, which can methylate MMA to form dimethylarsinic acid (DMA), or demethylate it to inorganic arsenate. These two compounds are also sequestered in soil. The sequestration of MMA is a biphasic process: fast sorption followed by aging. The arsenic compounds sorb to minerals, especially to iron and aluminum hydroxides and also to clay minerals. Although substantial amount of arsenic compounds sorb to soil minerals rapidly via inner-sphere complexation, a portion of arsenic compounds initially bind to soil via outer-sphere complexes. Unlike most pesticides, MMA subsequently forms strong inner-sphere complexes over time due to chemical and physical aging processes. Therefore, essentially, the total arsenical residues ultimately become irreversibly bound in environmental conditions. In research labs, high concentrations of phosphate, ammonium hydroxide, or strong acids are used to extract arsenic compounds soils. These residues that can only

be removed in the lab with strong extractants should be regarded as environmentally irrelevant, and not available for leaching or runoff.

AGRO 112

Aged sorption and sequestration of pesticide in soil: Modeling and its implication for leaching risk assessment

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In degradation studies of pesticide in soil, conventional (organic solvent) and exhaustive (strong acid) extractions are often applied to ensure that soil residues are accounted for in leaching risk assessment. However, exhaustive extraction with strong acid such as HCl at elevated temperature can break down covalent bonds and release sequestered soil residues. As a result, exhaustively extracted soil residue DegT50 could be much longer than conventionally extracted DegT50. If the limited mobility of sequestered fraction is not considered, leaching risk could be well over-predicted. In this study, we report a unique behavior of a pesticide, which not only displays aged sorption but also sequestration. The exhaustive residue DegT50 is ~ 100 days longer than the conventional DegT50, suggesting leaching risk to ground water. However, both exhaustively or conventionally extracted soil residues barely leached beyond 30 cm soil in field dissipation studies. We estimated with a sequestration kinetics model that 10%-50% of soil residues was sequestered. The sequestration mostly occurred in the top layer of soil or exhaustively extracted residues show much bigger difference between top and lower soil horizons. This difference could not be simulated by assuming the release of sequestered fraction for leaching, as with conventionally extracted residues by aged sorption in inverse modeling with PEARL 4.4.4. This result demonstrates that the sequestered fraction is not available for leaching and is consistent with column leaching study with conventionally extracted soil samples. The study suggests that nature of exhaustively extracted soil residues should be clarified before included in leaching risk assessment.

AGRO 113

Speciation of non-extractable residues of pesticide in soil: Is it possible?

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The speciation of molecular entities contributing to non-extractable residues (NER) of pesticides in soil is critical in environmental risk assessment but has been a challenge because NER could not be experimentally extracted and identified. In this study, we explore a step-wise kinetic approach to determine the major contributors (either parent or individual metabolites) to NER, by analyzing laboratory degradation studies with multiple ^{14}C labels using a four-pool (parent compound, combined metabolites, CO_2, and NER) kinetics pathway model. SFO (the first-order) or DFOP (double-first-order-in parallel) is used as the best-fit kinetics model for degradation of parent compound in soil. DFOP is used as a model for the combined metabolite pool in which a major metabolite contributes to NER and a terminal metabolite degrades to CO_2. This four-pool kinetic analysis can definitively discern if parent compound or metabolites are responsible for NER. It can further be sequentially applied to degradation studies dosed with different stages of ^{14}C-labeled metabolites, to confirm major NER contributors or determine the speciation of molecular entities in non-extractable residues. Analyses with several molecules suggest that the molecular moieties such as pyridine with carboxylic acid or pyrimidine with multiple hydroxyls are responsible for NER. By combining with regular kinetic analyses, the approach has a potential to provide speciation of molecular entities in both extractable and non-extractable residues.

AGRO 114

Characterization of dispersion of particles from cotton gins and prediction of particle concentrations by AERMOD with dispersion correction factor

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The United States plays an important role in the global cotton market, where more than 20 million bales of cotton were produced in 2017, representing over 7 billion dollars in total value. In the United States, the cotton fiber must be separated from the seed at a cotton gin before the producer has a saleable product. Such process exhausts air to the environment through air pollution abatement devices to reduce the amount of particulate matter (PM) that a cotton gin emits to the atmosphere. The primary issue of the cotton industry across the country is lack of scientifically sound information about PM emissions. In addition, regulation-recommended air dispersion models have a potential to overestimate concentration for low-level sources by as much as a factor of ten. Therefore, a project was conducted to collect gin emission data to refute inaccurate data used by regulatory agencies. The current study is a part of the project, and the main goal of the study includes the following: 1) characterization of ambient PM concentrations; 2) evaluation of performance of EPA-recommended models for predicting PM concentrations; 3) development of dispersion correction factors for low-level sources. The sampling campaigns were conducted at a typical cotton gin. Each sampling period was about 10 hours when the gin was operating. The sampling arrays consist of samplers at 30° intervals, encompassing the gin at three radial distances at approximately 60-m interval from a predetermined center point located near the gin's main cyclone bank, and concentrations of $PM_{2.5}$, PM_{10} and TSP were measured. Results showed that the greatest observed concentrations occurred in the downwind direction from the source. In addition, the spread of the spatial distribution appears positively related to spread of wind direction. Correlation analysis shows that PM concentration was negatively correlated with height and distance from source. In addition, positive correlation was found between PM concentration and average ambient wind

speed while negative correlation was found between concentrations and deviation from wind direction for PM_{10} and TSP. Furthermore, negative correlation was found between standard deviation of wind direction and PM concentrations. These results enriched the knowledge of factors that influence pollutant concentrations, which will be useful for validation of air dispersion models and development of dispersion correction factor.

AGRO 115

Validation of field flux and AERMOD air dispersion model for estimating off-target pesticide exposure

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The EPA guideline for field volatility testing of a pesticide suggests calculating flux estimates from three methods: Aerodynamic (AD), Integrated Horizontal Flux (IHF), and Indirect (ID). The highest flux from these methods is used as an input to the AERMOD air dispersion model to estimate off-field exposure. This talk will first provide an overview of the field and modeling methods used by the U.S. EPA for assessing the risk of pesticide exposure from volatility. In addition, results will be provided from four model-validation field studies. Specifically, the AERMOD air dispersion modeling results based on estimated field fluxes and site-specific weather will be compared with off-target measured concentrations of a pesticide to evaluate the robustness of U.S. EPA approaches.

AGRO 116

VETPEC suite of models - modeling for regulatory evaluation of animal drug exposures

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The United States (U.S.) Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) conducts prospective environmental risk assessments to evaluate the potential for significant environmental impacts to occur from the approval of new animal drugs. To this date, U.S. FDA/CVM has not provided drug sponsors with methods for conducting environmental exposure assessments or calculating predicted environmental concentrations (PECs) of animal drugs. Therefore, U.S. FDA/CVM, in collaboration with the U.S. Environmental Protection Agency (EPA), developed the Veterinary Environmental Transport and Fate Models for Predicting Environmental Concentrations (VETPEC) suite. The VETPEC models calculate PEC values for animal drugs in manure (PEC_{manure}), soil (PEC_{soil}), and water (PEC_{water}). The modeling suite provides a singular framework to address the regulatory needs described in U.S. FDA/CVM Guidance for Industry (GFI) 89 and 166 (Phase I and Phase II Environmental Assessments, respectively), and will provide U.S. FDA/CVM and drug sponsors with an easy-to-use and consistent method to estimate exposure concentrations for use in exposure and risk assessments. In VETPEC v1.0, the exposure route evaluated is the application of drug residues in animal manure to cropland. For intensively-reared livestock, manure is often stored for a period of time (6-12 months) and subsequently applied to cropland as fertilizer. This exposure route is evaluated in both a farm-scale screening model and a farm-scale refined model. Both models use current animal characteristics and agricultural practices based on CVM animal scientists' expert knowledge and information reported in state and producer guidance. Animal drugs approved by CVM are not restricted to specific locations in the United States. Consequently, the VETPEC models account for differences in soil, weather, and industry practices, by leveraging crop scenarios originally developed by the U.S. EPA for model farms throughout the United States to predict daily and summarized PEC values for a 50-year simulation. The determination of whether impacts are significant will be based on comparison of these PEC values to predicted no effects concentrations and/or probability of occurrence (*i.e.*, only 1 of the 100 default scenarios may have impacts).

AGRO 117

Heuristic model of multispecies, sprayed, liquid sheet breakup in agriculture applications

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Atomization of agricultural compounds through nozzles is the primary mechanism for delivering many pesticides onto a target, but such mechanisms often generate negative externalities. For example, flat-fan (and other) nozzles used in agriculture produce a wide droplet diameter distribution. Droplets with small diameters, so called driftable fines, can entrain in wind gusts and spread great distances (drift). This phenomenon both reduces the effectiveness of the spray and potentially impacts surrounding non-target organisms. Driftable fine fractions can be controlled though the introduction of a secondary, immiscible phase to the agrochemical solution. When sprayed, the secondary species destabilizes the fluid sheet through the creation of holes, which expand and collide, forcing earlier breakup of the sheet into droplets with larger diameters. A heuristic model is proposed to approximate this multispecies breakup mechanism and predict diameters of the resulting droplets. Secondary phase objects are seeded into a fluid sheet, holes within the sheet are formed, and spontaneous pairwise interactions between holes are modeled. The decay of these interactions is then used to predict the diameters of the resulting droplets. This heuristic approach lends itself to a kind *of plausible physicality*, although it is not a full-fledged simulation of the true physics. These simplifications enable orders of magnitude speedup in prediction, but accuracy remains strongly dependent on choice of input parameters. Accordingly, these unknown parameters must be adjusted to minimize model predictive error when comparing against experimental data. There is no *a priori* means of determining these parameters from first principles, therefore an inversion learning technique is implemented. Predictive performance of this approach for flat fan nozzles is reported. This tool can enable the accurate prediction of driftable fine production, and drive rapid, multifactor engineering innovation in low-drift agricultural compounds.

AGRO 118

Terrestrial field dissipation studies: An assessment of spray application setup and sampling techniques to optimize zero-day recoveries

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Global regulatory guidance for the conduct of terrestrial field dissipation studies has evolved within the last 15 years. The need to generate fit-for-purpose studies has increased. This paper will evaluate the analytical data generated from numerous terrestrial field dissipation studies conducted in the last 10 years, and an assessment of data trends will be presented. Specifically, spray application setup considerations as well as application verification and soil sampling techniques to optimize zero-day recoveries will be covered.

AGRO 119

Field dissipation studies: A case study of applying a metabolite to bare-soil plots under exclusion of surface processes at four test sites located in France, Germany, and Spain

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Global regulatory guidance for the conduct of terrestrial field dissipation studies has evolved within the last 15 years. The need to generate fit-for-purpose studies has increased. This paper will evaluate the methodology and results generated from four terrestrial field dissipation trials where the principal soil degradation product was applied as a suspension concentrate formulation to bare-soil plots under exclusion of surface processes (sand cover) at four test sites located in France, Germany, and Spain. Specifically, test item application rationale, analytical results, and degradation kinetics will be evaluated.

AGRO 120

Pesticides in surface water: A California data story

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For decades, state and federal agencies as well as research institutes in California have been collecting water and sediment samples for the purpose of pesticide analysis. A wealth of monitoring data has been generated and stored in the California Department of Pesticide Regulation's (CDPR) Surface Water Database (SURF). This study aims to provide a unique comprehensive picture of the occurrence and distribution of pesticides in the surface waters of California. We use various statistical learning methods as well as GIS tools to characterize the spatial and temporal trends of pesticide occurrence in surface water. Important factors associated with the detections of pesticides are explored including pesticide use, dominant land use within the source area, and pesticide physico-chemical properties. Statistical models are used to characterize the relationships of these factors to pesticide detections. The acute and chronic aquatic life benchmarks developed by U.S. EPA are used as a screening tool to derive frequencies of exceedance among various pesticides to allow for a comparison of relative toxicity. The pesticides with the most frequent detections in California surface water are methoxyfenozide, chlorantraniliprole, imidacloprid, fipronil, diazinon, and bifenthrin, among which imidacloprid, fipronil, and bifenthrin had the highest exceedance rate of their respective lowest aquatic life benchmarks. Locations, where these pesticides were monitored and detected, are identified with respective sampling intensity and detection frequency. The results will help guide future monitoring efforts in California regarding where to collect water samples and for what pesticides.

AGRO 121

Evaluation of stream sensitivity to pesticide loading in the Willamette Basin, Oregon

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The Willamette River Basin, Oregon, a 30,000 km^2 HUC 4 watershed, is one of the most diverse agricultural regions on earth, producing more than 170 different crops. Over 500 pesticide active ingredients are registered to meet pest management needs. Pesticides are routinely detected in surface waters, raising concerns for additional resource protection. However, current surface water monitoring programs, limited both logistically and by scarce financial resources, provide limited understanding the true scope of the impact of pesticide use on water quality and give inadequate guidance on implementation of sustainable practices. However, landscape scale ecohydrologic modeling of pesticide fate can prospectively assess impacts on surface water quality across many scales, requiring significantly fewer resources compared to monitoring, as well as provide mechanistic understanding to guide implementation of best practices. To prioritize modeling resources across the Willamette Basin, presented here is a methodology for characterizing stream sensitivity using spatially explicit landscape, hydrologic, and climatic datasets in conjunction with cropping practices and associated pesticide use patterns. The Pudding River watershed, a well-studied HUC 8 subbasin, was chosen for initial method evaluation. Results indicate a correlation between the U.S. EPA Level IV Ecoregion classification and pesticide detections in surface water. Based on this finding, individual components of ecoregion characterization were evaluated to refine the methodology, identifying catchment scale characteristics that are best correlated to pesticide surface water loading. The applicability of this GIS-based stream sensitivity methodology to focus modeling efforts across the Willamette Basin will be discussed.

AGRO 122

Redesigning water monitoring programs to meet the needs of risk assessment: A proven and cost-effective approach

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Enormous data sets of water monitoring data for pesticides have been collected since the mid 1900's but their use in aquatic risk assessment (RA) for both human health and ecological risk is limited because the sampling programs were not designed to provide time weighted averages (TWA's). The USEPA and the PMRA are concerned that peak concentrations may be missed, and it has not been possible to directly obtain upper limit TWA's that match the durations of exposure in the various acute and chronic toxicity studies of interest (*e.g.,* 1, 4, 21 or 365 days) unless it can be shown the concentration does not change between samples. With monthly, weekly or even daily sampling, this is seldom the case. One approach is to arbitrarily use such data as TWA's, but there is no rationale that this represents a worst-case estimate. Alternately the EPA is developing statistical models to derive upper limit estimates of the required TWA's from monitoring data, but this has not proven to be easy. It would be limited to the few cases that required the highest Tier assessment and may not give more accurate estimates of exposure. The third option is to modify the sampling design to support RA. The criteria to be met are: No risk of missed peaks, direct quantitative measurement of TWA, adequate sensitivity to exclude concern for censored data, inclusion of all residues of concern, and cost-effectiveness. The design of water monitoring programs will be discussed, and two successful cases will be presented.

AGRO 123

Integrating a distributional approach to using percent crop area (PCA) and percent crop treated (PCT) into drinking water assessment

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The Office of Pesticide Programs (OPP) assesses human exposure to pesticides via all routes of potential exposure, including drinking water, based on the product use labels as part of the aggregate human health risk assessment. OPP utilizes a four-tiered assessment approach to conserve resources by only using time-intensive, refined risk assessment methods requiring more complex data input for pesticides with estimated concentration in the source water exceeding the drinking water level of concern at lower tiers. Lower tiered assessments (Tier 1 and Tier 2) assume that the modeled watershed supplying a community water system (CWS) is entirely planted with a crop of interest [*i.e.*, 100% Percent Cropped Area (PCA)] and that the entire cropped area is treated with a pesticide of interest [*i.e.*, 100% Percent Crop Treated (PCT)]. OPP recently proposed methods to integrate the entire distribution of CWS PCA values and state-level usage data to define the PCT in individual CWS watersheds in higher tiered (Tier 3 and Tier 4) drinking water assessments. These methods were evaluated by comparing estimated PCA and PCT values to usage data from more spatially resolved usage data sets and adjusted modeled pesticide concentrations to measured pesticide concentration data.

AGRO 124

Probabilistic co-occurrence analysis for suites of federally listed species

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Section 7 of the Endangered Species Act requires the U.S. Environmental Protection Agency (EPA) to consult with the U.S. Fish and Wildlife Service (FWS) about potential pesticide impacts to federally listed species. Consultation is challenged by the abundance of registered products and listed species, as well as by lack of consensus on best available species distribution data and co-occurrence prediction methods. Our previous work demonstrates that probabilistic estimates of species' ranges and pesticide use patterns improve these estimates. Here we demonstrate that such estimates can be made for suites of sympatric listed species. By focusing on two watersheds in Iowa and Mississippi, we obtained distribution data for 13 species of terrestrial and aquatic listed plants and animals occurring therein. We used maximum entropy methods (Maxent software) and bioclimatic, topographic, hydrographic, and other environmental variables to predict species' ranges. Using the principles of Bayes' Theorem, we constructed probabilistic spatial models of use areas for two pesticides by integrating information from the USDA Cropland Data Layer, USGS National Land Cover Dataset, pesticide usage data, satellite imagery, and other sources. We then combined species distribution and crop footprint models to derive overall probability of co-occurrence of listed species and pesticide use. For aquatic species, we also integrated an estimate of downstream residue transport. We report each separate species-by-use-area co-occurrence estimate, and also combine these modeled co-occurrence probabilities across species within focal areas to produce an overall metric of potential pesticide exposure risk for these listed species at the watershed level. These methods are robust to the challenges of risk estimation across diverse life histories and can be generalized to a variety of pesticide use patterns. We propose that the consultation process between EPA and FWS be based on such batched estimation of probabilistic co-occurrence for multiple listed species at a regional scale.

AGRO 125

Application of methomyl usage data in a probabilistic national endangered species exposure assessment

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Pesticide usage data has become increasingly important in improving pesticide exposure predictions in national endangered species risk assessments in the United States.

Although publicly available national-scale usage datasets have been available for several years now, it is only recently that usage data incorporation into the risk assessment process has been adopted by regulators. Outside of California, spatial allocation of usage data to appropriate crops at the sub-state scale has proven challenging. However, usage data are essential to deriving realistic exposure predictions (likelihood and magnitude) when evaluating potential risks to endangered species. These challenges include resolving usage by crop at the sub-state level, lack of consistency between spatial crop data layers and crop-specific usage estimates, and accounting for annual variability in usage amounts and application rates. Although California has superior usage information compared to the rest of the United States, applying the California Pesticide Use Record (PUR) data to generate usage footprints consistent with crop data layers poses data analysis challenges. We are developing a methodology for combining multiple sources of historical usage and crop location data to derive national methomyl usage footprints. The approach considers multiple combinations of crop-level usage, cropping patterns, and application rates to derive an ensemble of usage footprint realizations. These usage footprints provide probabilistic inputs to spatially-explicit aquatic exposure analyses that result in probability distributions of expected exposure concentrations (EECs) specific to each species assessed. Species EEC distributions that incorporate the usage analysis are compared to EEC distributions based on 100% crop treated assumptions to demonstrate the importance of accounting for actual usage data and its effectiveness in focusing refined analysis steps to listed species that are at highest risk.

AGRO 126

New perspective on the nanoplastics disrupting the reproduction of an endangered fern in artificial freshwater

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The potential risks of micro/nanoplastics on the ecological environment, particularly aquatic fauna, have been realized in recent years. However, information about their potential effects on aquatic plants is scarce. In this study, a four-week exposure experiment was conducted to investigate the effects of varying polystyrene nanoplastics concentration (PS-NPs, 0−100 μg/mL) on the early development of an endangered aquatic plant, Ceratopteris pteridoides. Fluorescent observations demonstrated that PS-NPs were adsorbed and accumulated on the spore surface of C. pteridoides rapidly and massively with increasing exposure concentration and time. The adsorption and accumulation of PS-NPs on the spore surface posed a negative effect on spore imbibition, causing 2.3−22.4% reduction in final spore size. Spore germination and gametophyte sex differentiation were both negatively affected by PS-NP exposure, resulting in 10.4−88.0% inhibition in germination ratio and 2.9−53.4% reduction in hermaphroditic gametophyte ratio. Additionally, PS-NPs were observed to penetrate into the roots of gametophytes. Higher concentration of PS-NPs (100 μg/mL) can even induce pathological changes on gametophytes, although with a low incidence (4.9%). The results above indicated that exposure to PS-NPs caused a series of disruptions from the spore imbibition to germination and gametophyte stages, and is likely to pose an eco-physiological risk on the reproductive success of endangered ferns.

AGRO 127

Flumethrin at sublethal concentrations induces stresses in adult honey bees (*Apis mellifera* L.)

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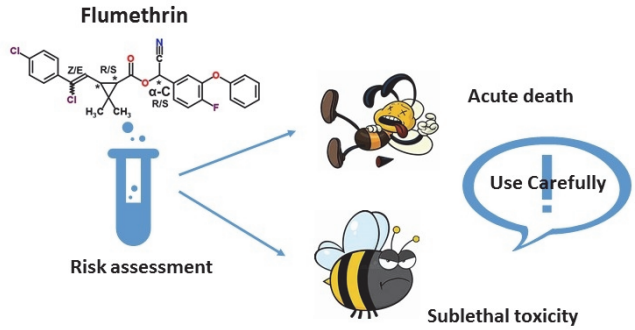
Flumethrin is a typical pyrethroid varroacide widely used for mite control in beekeeping, worldwide. Currently, information on the toxicological characteristics of flumethrin on bees at sublethal concentrations is still lacking. To fill this gap in information, we performed a 48-h acute oral and 14-day chronic toxicity testing of flumethrin in newly emerged adult honey bees under laboratory conditions. Results showed that flumethrin had high acute toxicity to honey bees with a 48-h LD_{50} of 0.47 μg/bee (95% CI, 0.39~0.57 μg/bee), which is higher than that of many other commercial pyrethroid insecticides, but lower than that of tau-fluvalinate. After 14 days of chronic exposure to flumethrin at 0.01, 0.10, and 1.0 mg/L, significant antioxidant response, detoxification, immune reaction, and apoptosis were observed in the midguts. These findings indicated that flumethrin had potential risks to bees, and it can disturb the homeostasis of bees at sublethal concentrations under longer exposure conditions. Flumethrin is highly lipophilic and easy to accumulate in beeswax; thus, careless practices might pose risks to colony development in commercial beekeeping and native populations. This laboratory study can serve as an early warning, and further studies are required to understand the real residual level of flumethrin in bees and the risks of flumethrin in field condition.



AGRO 128

Biodegradation and metabolic mechanism of strobilurin fungicides by the mixed populations of bacteria

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Strobilurin fungicides have become an integral part of disease management programmes on a wide range of crops in many countries of the world, including pyraclostrobin, azoxystrobin and picoxystrobin. The presence of fungicides in the natural environment, resulting from excessive use of pesticides, has become a serious threat to many ecosystems, including soil and open water. Biodegradation is the partial or complete hydrolysis of pesticide molecules by microbial activity through various physiological and biochemical metabolic pathways. However, the resource of strobilurin fungicides-degrading bacteria is still under development. Therefore, the study was conducted aimed at identifying soil bacteria of strobilurin degradation. Mixed populations of bacteria were obtained

from the soil samples of the Xiangshan Park and University of Hawaii that were simultaneously able to degrade azoxystrobin, picoxystrobin and pyrazoxystrobin 100 mg/L to 10 mg/L levels after 7 days. LC–MS/MS analysis revealed that new compounds accumulated in the growth medium as three strobilurins were degraded. We have identified the degradation pathways of multi-component that can demethylate strobilurins via hydrolysis of a methyl ester in the toxophoric region of the molecules. Additionally, the common bacteria in the mixed populations are probably *Pseudomonas* genus according to the microbial flora analysis by 16S amplicon sequencing. The Pseudomonas (69.79%), Sphingobacterium (27.17%), Delftia (6.31%), Achromobacter (1.64%), were highly associated with the removal of strobilurin in the system. Combining the experimental results of no degradation activity of a single strain, it is speculated that the synergistic effect of various strains may be the key link for the degradation of azoxystrobin, picoxystrobin, and pyrazoxystrobin. Based on the current work, the research will continue to explore and reveal the enzymes, genes and molecular metabolic mechanism. The research results are expected to provide scientific basis and method for the preparation of strobilurin fungicides contaminated sites, and have important scientific significance and relevant value for soil ecological health.

AGRO 129

Evaluating the environmental impact of food waste treatment model by life cycle assessment method

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Life cycle assessment (LCA) is an important tool to assess the potential environmental impact and resource consumption of solid waste in the life cycle. In order to understand the application of life cycle assessment method in the treatment of food waste, the relevant literatures since 2003 at home and abroad are collected and reviewed from the perspectives of evaluation object and scope, the function unit, the type of environmental impact involved, and its application in the technology optimization and decision support. The results show that the life cycle assessment method can effectively evaluate the environmental impact of food waste treatment, which has important reference value for the optimization of food waste treatment technology and decision-making. However, the relevant research and application in China is still in its infancy, and needs to be further improved in the unity of assessment methods, the establishment of a database, and the application of results.

AGRO 130

Multi-residue method for insecticides in livestock products by HPLC-MS/MS

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A multi-residue method of 56 pesticides in various livestock products (beef, pork, chicken, milk and egg) was developed by LC-MS/MS method after modifided QuEChERS procedure. These pesticides are insecticides with no specified MRL in livestock products in Korea. Modifided QuEChERS methods were evaluated several extraction and d-SPE clean-up (PSA, GCB, C18, Z-Sep) procedures. Limit of quantification, linearity, matrix effect and recoveries were performed in five matrices. The QuEChERS method with the PSA/C18 was exhibited a large number of pesticides with the limit of detection (<0.01 mg/kg), the good linearity (0.002, 0.005, 0.01, 0.02, 0.035 and 0.05 mg/kg, r2>0.98), and recoveries in the 70-120% range at three levels (0.01, 0.1 and 0.5 mg/kg). This study has validated the QuEChERS method for 45 pesticides, isomer and degradation product in livestock products by LC-MS/MS. The validated multi-residue method could be applicable to monitor the pesticides in livestock products for enforcement purpose in Korea.

AGRO 131

Get a (half) life! Improving the environmental relevance of laboratory studies

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Degradation of agrochemicals in aerobic soil laboratory studies often occurs more slowly than observed in field studies. Field soils are part of a non-confined, *in situ* spatial continuum and subject to multiple dynamic processes and exchanges. Laboratory soil systems are disturbed, physically isolated, and highly controlled to limit changes over time. In the guideline aerobic soil route and rate laboratory study, OPPTS 835.4100, bulk soil is collected, bagged, and shipped from agriculturally relevant fields to the laboratory. After receipt, the bulk soil is homogenized and sieved to remove plant debris, soil fauna and stones before use. This processing may result in direct loss of some specific microbial populations and/or exogenous enzymes and creates less favorable conditions for sustained microbial activity compared to intact soils. Current EPA agrochemical modeling guidance requires the use of laboratory half-lives as modeling inputs as opposed to field half-lives. The current research explores the aerobic soil degradation in intact soil cores incubated under controlled laboratory conditions as a higher tier bridge to more realistic modeling half-lives. Laboratory degradation results of processed soils and intact soil cores are compared with those generated under field conditions.

AGRO 132

Uptake and translocation of unpredictable soil residue of procymidone to rotational crop for positive list system in Korea

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This study has investigated the uptake and translocation of procymidone sprayed on a previous crop, "Korean cabbage" to rotational crop "spinach". The procymidone was applied at the recommended dose along with 2 and 5 times of that dose according to the safe use guidelines of chives belonging to the same group of leafy vegetables. For each treatment, procymidone was foliar sprayed twice at 21 and 14 days before harvest of Korean cabbage. Soil samples were collected at regular intervals (7, 14, 21, 30, 40, 50, 53, 56, 59, 62, and 65 days) after the first treatment of procymidone. Korean cabbage was harvested twice, first at the final treatment, and then on harvest day. After 4 days, spinach

was sown and grown for 40 days. The initial residual amount of proymidone after twice treatment on soil was 5.670-17.178 mg/kg and degraded by 3.098-9.956 mg/kg until the harvest of the previous crop. Procymidone residue remaining in soil was still 1.026-3.576 mg/kg till the final harvest of spinach. Moreover, the residual amount of uptake and translocation from the Korean cabbage cultivated soil to spinach edible parts were 0.020-0.048 mg/kg for recommended dose, 0.055-0.116 mg/kg for 2 times dose and 0.079-0.278 mg/kg for 5 times dose. Consequently, procymidone sprayed on previous crops can unpredictably uptake and translocate to rotational crops and may be detected above 0.01 mg/kg, resulting in violation of cause PLS standards.

AGRO 133

Modifying the SEAWAVE-QEX model for surface-water concentration monitoring data

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For pesticide surface-water monitoring data where the sampling frequencies are less than daily, the estimation of peaks or other target quantities such as upper percentiles and maximum m-day (m = 1, 7, 14, etc) rolling averages for regulatory assessment presents challenges because peaks are often missed in the sampled data. Various models have been developed that "fill in the gaps" of the time series so that better estimates can be obtained. In particular, the USGS developed the SEAWAVE-QEX model for this purpose. It is an extension of the SEAWAVE-Q model, which is a regression model with covariates for linear trend, a seasonal wave term to capture seasonality (*e.g.*, many pesticides have specific usage seasons), and a transformation of co-occurring streamflow data (*e.g.*, short-term flow anomaly or STFA). The purpose of SEAWAVE-Q is to estimate long-term trends of pesticide surface-water concentrations. SEAWAVE-QEX extends SEAWAVE-Q by modeling the serial correlation structure of the time series data, and the heterogeneity of the error variance as a function of seasonality. SEAWAVE-QEX is then used to generate multiple conditional simulations of the daily time series (called traces). An estimate of a desired target quantity is obtained from each trace, and the average of all the trace estimates is used as the final estimate of the target quantity in question. In practice, this approach tends to provide overestimates of upper target quantities. We present a time series model similar to SEAWAVE-QEX but using an alternative set of covariates for seasonality and flow. In the modified approach, seasonality is modeled using the deterministic Soil and Water Assessment Tool (SWAT) hydrological model, and modified versions of flow covariates that do not exhibit the right-skewness of STFA (which tends to cause overpredictions) will be examined. Other modifications include the exclusion of censored residuals in the determination of serial correlation.

AGRO 134

Upper percentiles of atrazine concentrations in SDWA and AMP water monitoring programs, as estimated using the pooled data method

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Extensive historical surface water monitoring data now exists for many pesticides, as collected by a variety of monitoring programs, each having differing objectives. Non-targeted programs may collect data infrequently, leading to sample sizes being too small to estimate upper percentile concentrations for any specific site-year. However, given many sites and many years of data, peak concentrations may occasionally be sampled. The question then arises of how to best use these data to characterize extremes within spatial regions. We apply a "pooled" method of estimating upper percentiles using the monitoring data from two drinking water monitoring programs: 1) the non-targeted SDWA (Safe Drinking Water Act) program, and 2) the targeted programs of AMP (Atrazine Monitoring Program), SMP (Simazine Monitoring Program), and SVMP (Stewardship Voluntary Monitoring Program). We provide pooled 90th, 95th, and 99th percentile estimates for finished water samples from flowing, mixed, and static water body types of the SDWA and AMP/SMP/SVMP programs, and also these same estimates for raw water samples from the AMP/SMP/SVMP programs. We extend earlier work by providing a direct derivation of the method effective sample size, the approximate sample size under simple random sampling. We also present further empirical evidence of upward bias of the pooled estimator, suggesting that estimates tend to be conservative in practice.

AGRO 135

Morphology-based transport of gold nanoparticles in mature plant leaves

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In recent years, nanomaterials have been utilized in various ways to address dominant challenges in agriculture, including as biosensors for pathogen, nutrient, and environmental stress monitoring, and as carriers for controlled agrichemical release and the delivery of DNA for genetic engineering. Accordingly, interest in studying the environmental impact of nanomaterial usage in plants and the soil microbiota has soared. The process of NP uptake, translocation, and accumulation in plants can be segregated into three scales: i) macroscopic – quantifying accumulation in plant tissues, ii) microscopic – studying NP transport throughout the plant, and iii) mechanistic – revealing the manner of association on a cellular level; most studies are macroscopic. While these studies are valuable for understanding how NPs and their surface chemistries might impact long-term accumulation and translocation throughout a plant, the uptake pathways and method of internalization into plant tissues and plant cells

remain relatively unstudied. Herein, we hypothesize that NP morphology, size, or surface chemistry can affect the performance of NP-based carriers regarding their microscopic and mechanistic transport into plant cells. To this end, we utilize DNA-coated gold nanoparticles (AuNPs) as model materials to elucidate the effect of NP morphology – diameter and aspect ratio – on internalization propensity, internalization timescale, and localization in mature *Nicotiana benthamiana* plant leaves. *Via* confocal and TEM microscopy, we find that by comparing NP fluorescence against cytosolic GFP fluorescence and quantifying co-localization values, smaller Au nanospheres interact with cell walls and possibly internalize to a greater extent and faster than larger Au nanospheres. Similarly, Au nanorods seem to experience peak association/ internalization faster and at a higher magnitude compared to nanospheres of a similar diameter. Furthermore, endocytosis studies suggest the mode of NP internalization is energy-independent for nanospheres, but energy-dependent for Au nanorods, suggesting a larger role of endocytosis to mediate cellular internalization in the latter. Our study suggests that NP morphology impacts NP internalization timescales, extent of internalization, and internalization pathways in plants. Taken together, our data can aid the rational design of NPs for bio-cargo delivery to plants towards efficient and targeted delivery or localization in plants.

AGRO 136

Adsorption of double-stranded ribonucleic acid (dsRNA) biopesticides to iron (oxyhydr-)oxides

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Genetically-modified crops expressing insecticidal dsRNA molecules, so-called RNAi crops, are an emerging crop protection technology. The use of RNAi crops results in the release of dsRNA to agricultural soils, demanding an assessment of dsRNA fate for proper ecological risk assessment. Adsorption of dsRNA to soil particle surfaces is a key fate process but remains poorly understood. This contribution reports on the adsorption of a model dsRNA molecule as well as of DNA to the three iron (oxyhydr-)oxides: goethite, hematite and lepidocrocite. Our working hypothesis was that structural similarities of dsRNA and DNA will result in comparable adsorption characteristics of these nucleic acids (NAs). We primarily used batch equilibration systems but complemented these with column transport studies to assess adsorption under dynamic flow conditions to iron oxide-coated quartz sand. We systematically varied both NA concentrations as well as solution chemistry (*i.e.*, pH, ionic strength, ionic composition) and assessed the effect of phosphate as co-sorbate. NA adsorption to the iron (oxyhydr-)oxides decreased with increasing pH, consistent with decreasing electrostatic attraction between the negatively charged NAs and the pH-dependent, positively charged iron (oxyhydr-)oxides. Adsorption increased when we increased ionic strength or added Mg^{2+} ions, suggesting that these conditions caused NAs to adopt a more compact conformation in adsorbed states -and hence to have a smaller footprint on the oxide surface- through intramolecular screening of negative backbone charges and cation bridging. NA adsorption decreased with increasing concentration of phosphate as co-solute, demonstrating that these molecules competed for iron oxide adsorption sites. Column experiments showed no NA adsorption to negatively charged pure quartz sand but increasing adsorption with increasing amounts of iron oxide coatings on the sand. These results support that iron oxides are important sorbents and that electrostatics govern overall NA adsorption. Comparable adsorption of all tested NAs supports our working hypothesis and thus implies that existing knowledge on DNA adsorption can inform dsRNA adsorption models.

AGRO 137

Mechanism of photodegradation in phenylurea herbicides

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Photodegradation is one of the primary abiotic mechanisms by which pesticides degrade. In the Central Valley of California, large quantities of herbicides are used every year to control unwanted plant growth and can ultimately affect agroecosystems, particularly through the water supply. Phenylurea herbicides such as diuron, and linuron are among those most widely used and also among the greatest concern for ground and surface water contamination. Here, we compute the most probable photodegradation pathways and products for a series of phenylurea herbicides through quantum chemical studies of the electronically excited states and their initial forces. The calculations were done using standard Density Functional methods as implemented in Q-Chem, coupled with a more recently developed algorithm for exploring potential energy surfaces. We found many similarities between these molecules but also some very interesting differences relating to their functional groups. Ultimately, the goal is to efficiently and accurately predict photodegradation products for a selected class of molecules, which can potentially lead to developing safer but effective alternatives for widely used pesticides.

AGRO 138

Can novel pesticide mitigation techniques impact agricultural water quality?

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Agricultural pesticides are utilized to keep up with the growing national and global need for food and fiber. Even with meticulous application methods, there is still a chance for pesticide leaching or detachment in runoff during certain rainfall events. Innovative mitigation techniques are required to reduce impacts of pesticides on aquatic receiving systems. Many of these practices focus on the use of aquatic vegetation (*e.g.*, constructed wetlands or drainage ditches) to physically slow the velocity of the runoff, allowing for sufficient contact time to either transfer (sorption) or transform (microbial degradation) pesticide components. Since there is no "silver bullet," or one-size-fits-all conservation practice that will completely mitigate all agricultural runoff, combinations of conservation practices offer the best attempt to decrease impacts of agricultural

runoff on downstream aquatic receiving systems. One such potential combination is the use of aquatic vegetation and woodchip bioreactors. A mesocosm-scale experiment examined the efficiency of three aquatic plant monocultures (*Sparganium americanum*, *Typha latifolia*, and *Leersia oryzoides*), each with a woodchip bioreactor at the outflow, at removal of the pesticides atrazine, metolachlor, malathion, and cyhalofop-butyl. Each mesocosm had an 8 h hydraulic retention time (HRT). Immediately before the experiment, 25% of the original water volume in each mesocosm was removed in order to simulate effects of a weir as an additional conservation practice for mitigation. Dosing of mesocosms was provided by calibrated piston pumps to deliver a constant concentration of each of the four pesticides over the 8 h HRT. Outflow water samples were collected at least hourly for 8 h. Samples were also collected during the non-flowing phase (10-48 h), and then again during the "clean" water flush from 49-56 h. Retention of dosed atrazine concentrations within the first 8 h (1 hydraulic retention time) ranged from 66±2% (controls) to 74±4% (*T. latifolia* and *S. americanum).* Only 7-8% of atrazine concentrations degraded during the non-flowing period. Between 25-28% of atrazine concentrations were retained during the clean flush in all mesocosms. Less retention among mesocosms was noted for metolachlor concentrations during the 8 h HRT, with control mesocosms only retaining 38±3%. Analysis of these novel methods will allow farmers and landowners to implement management practices to improve agricultural water quality.

AGRO 139

Microplastics in human environments: three pilot studies involving residential tap water and indoor air

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Despite fifty years of research, microplastic particles continue to turn up in new and surprising places. They have been identified in human food, commercial beverages, and municipal tap water. Atmospheric transport and deposition of microplastics are also now widely recognized as potential routes of contamination in indoor and ambient air. In light of these recent discoveries, the present investigation, which consists of three pilot studies, seeks to quantify microplastic abundance in tap water and indoor atmospheric fallout. The first study involved four separate homes, each with three treatments: tap water sealed, tap water exposed, and filtered (MilliQ) water exposed. MilliQ water was also used to run laboratory blanks. The average across three homes for the first treatment was 74.3 particles/400 mL (± 27.0) while the average across the second treatment was 85.3 particles/400 mL (± 37.4). This second study involved quantifying the synthetic particle load in air and tap water in a single home. It consisted of the same three treatments as the first study, but sampling took place in one particular home, in triplicate. Filtered water from a reverse osmosis (RO) system was used in study two for the third treatment and to run laboratory blanks. The average across the first treatment was 45.3 particles/400 mL (± 1.53), and the average across the second treatment was 55.7 particles/mL (± 15.9). The third study involved quantifying spatial and temporal variation within a single home. It included a single treatment, tap water exposed, in triplicate, over five consecutive days. For this final study, bottled HPLC water was used to run laboratory blanks. Particle concentrations ranged between 116 particles/400 mL (± 33.7) and 145 particles/400 mL (± 25.6). The sampling environments for studies two and three were similar in all ways but one, sampling jars in study three were positioned directly over an air-heating vent. This explains the higher concentrations found in study three compared to study two. Interestingly, in study three particle densities appear to rise and fall with changes in ambient temperature, likely due to an increase in activity of the air-heating vent when temperatures dipped. As research in microplastic contamination continues to advance, it is important to have a sense of variation between locations, within locations, and over time. It is also important to gauge realistic human exposures in an effort to assess risk.

AGRO 140

Long-term performance analytical methods for dicamba off-target movement studies

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Robust analytical methods are a key component to allow quantitative assessment of off-target movement of dicamba under field conditions. Two LC-MS/MS based analytical methods used to determine the amount of dicamba on filter paper or polyurethane foam (PUF) air samplers will be summarized. These methods have been applied to characterize the amount of dicamba loss off-target via spray drift or volatilization, respectively. These methods have been successfully transferred to multiple laboratories and utilized to collect data for off-target movement studies conducted by academic universities and industry for multiple years. This presentation will focus on the characterization of robustness of these methods by evaluating the analytical data of controls and fortified controls generated by two laboratories over multiple years.

AGRO 141

Wash-off potential of pyrethroids after use of total release fogger products

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Total release foggers (also known as "bug bombs") are widely used by the general public in an attempt to control indoor pests such as bed bugs, fleas, and cockroaches. These products often contain pyrethroids as active ingredients. Pyrethroids are frequently detected in wastewater effluents, and it was suspected that use of these total release fogger products might present a potential source of pyrethroids entering the wastewater. To understand potential transfer of pyrethroids via physical contact, a total release fogger (active ingredient: cypermethrin) was activated in an enclosed space, and pyrethroid transfer from various floor materials (wood, tile and vinyl) to an adsorptive material was quantified. Results suggested that the pyrethroids deposited on various surfaces can be subsequently transferred to other adsorptive materials via physical contact. We also found that the magnitude of transfer differed significantly between floor material types. Additionally, it was examined if cypermethrin can be extracted from the contaminated filter paper / fabric pieces using hexane, water, or water with detergent as a

solvent. The amount of extracted cypermethrin was significantly influenced by the solvent and material types (filter paper or cotton fabric) as well as an interaction between these factors. However, even with water only, part of the cypermethrin adsorbed on these materials was readily removed and dissolved into water. Overall findings support several possible routes through which pyrethroids from foggers can enter the wastewater, such as cleaning of exposed surfaces or washing of contaminated clothing.

AGRO 142

Pyrethroid residues in urban catch basins and their relationship with permethrin resistance in *Culex pipiens* mosquitoes

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Insecticide resistance, particularly to pyrethroids, in mosquitoes is a looming threat to effective pest control. Pyrethroids are frequently employed to manage mosquito populations, but excessive exposure may encourage resistance development and hamper long-term efforts. Urban settings also have multiple avenues, including concrete surfaces and catch basins, by which pesticides may be retained and released over extended periods of time. This study, conducted in collaboration with several California vector control agencies, measured pyrethroid concentrations in catch basins along with resistance in associated populations of mosquitoes to determine the presence of a relationship between the two. Samples of water and various solids were collected from several urban catch basins in Woodland, Elk Grove, and Ontario, California. Mosquitoes from the *Culex pipiens* species complex were caught near or in basins and used to raise colonies to a specific age for testing. Environmental samples were extracted for 8 pyrethroid analytes, and reared mosquitoes were subjected to resistance bottle bioassays using permethrin. Pyrethroids were detected in all catch basin samples, with the most prevalent analytes being bifenthrin, permethrin, and fenpropathrin. All field-caught mosquitoes exhibited permethrin resistance, and resistance ratios were determined by comparing their LT_{50} with that of positive lab colony CQ1. Correlations between pyrethroid concentrations and resistance ratios were not statistically significant, likely indicating that the number of basins sampled was not high enough. Subsequent work will focus on catch basin constituents' direct effects on mosquito development and resistance.

AGRO 143

Correlation between physicochemical properties and biological half-life of triazole fungicides in perilla leaf

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The biological half-life of pesticides applied to crops is the critical indicator for ensuring the safety of agricultural products. The biological half-life is affected by several factors like growing conditions of the crop, climate, application method, and physicochemical properties of pesticides. In this study, the biological half-life was calculated, and the degradation rates of six triazole fungicides sprayed on perilla leaves were evaluated. Moreover, the statistical analysis confirmed the correlation between the biological half-life and physicochemical properties of six triazole pesticides. The recoveries of the six pesticides were between 84.8 ~ 104.9%, which satisfies the residual pesticide analysis criteria. The biological half-life of six pesticides sprayed on perilla leaves, calculated using the first-order kinetics model, ranged between 6.4 ~ 15.1 days. When the biological half-life and the physicochemical properties were correlated using the principal component analysis: pKa and Log P, the biological half-life was found to be affected by PC1. The correlation coefficient between biological half-life and physicochemical properties (pKa), calculated by Spearman rank-order correlation, was R^2 = -0.928 (p <0.01). Biological half-life has been shown to correlate with pKa. In conclusion, it can be used as a database for the relationship between biological half-life and physicochemical properties and will contribute to ensuring a safe supply of agricultural products.

AGRO 144

Review of the availability of pesticide surface water monitoring data across the United States

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The Office of Pesticide Programs (OPP) has long desired to utilize available surface water monitoring data in pesticide exposure assessment; however, generally the data have not been sufficient for quantitative use in exposure assessments, and the tools available to assist in understanding the data were not robust. Recent model developments have made it possible to address the temporal limitations in available surface water monitoring data when the data meet specific data quantity and quality criteria. However, monitoring data available to evaluate a wide range of pesticides in varying locations is a limiting factor in the adoption of these tools in risk assessment. This work provides preliminary findings of a comprehensive review of the available pesticide surface water monitoring data to establish a baseline of the quantity and quality of data available and identify ways that future monitoring programs can be utilized such that currently available data becomes more useful for the evaluation of the newly developed tools or used in pesticides exposure assessments.

AGRO 145

Mitigation of surface runoff from agricultural fields by micro-dam technology and conservation tillage – results from maize field trials

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On sloped agricultural fields, water and sediment can be transported downhill as runoff 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 U.S. 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 or conservation tillage in maize on model input parameters. Innovative micro-dam and conservation tillage devices have been tested in the field. Application of micro-dams and conservation tillage alone, and micro-dams in combination with conservation tillage showed a consistent decrease of runoff, CN, and erosion (Table 1). The results support the approach to quantitatively consider in-field risk mitigation measures in the context of regulatory surface water exposure calculations, as proposed *e.g.* by the MAgPIE workshop. Based on these data, a robust case can be made to quantitatively consider innovative runoff mitigation for risk assessment purposes by *e.g.* lowering CN in the exposure scenarios or adapting parameters in the MUSS.

Table 1: Effects of micro-dams and conservation tillage in maize cultivation on runoff, curve numbers (CN; means), and erosion (percental means) in the two trials of 2018 and 2019

	Tillage			Conservation Tillage			Reduction conser. tillage vs. tillage	Reduction micro-dam + conser. tillage vs. tillage
	untr.	micro-dam	Reduction [%]	untr.	micro-dam	Reduction [%]	[%]	[%]
2018								
Runoff [L/ha]	72986	41528	43	12153	6597	46	83	91
CN	78	75	4	73	72	2	6	8
Erosion [kg/ha]	2371	1046	56	62	34	43	97	99
2019								
Runoff [L/ha]	96852	33148	66	30000	15370	49	69	84
CN	82	79	3	79	78	2	4	5
Erosion [kg/ha]	6655	1203	82	599	204	66	91	97

AGRO 146

Individual and joint toxicity of azoxystrobin and difenoconazole on zebrafish embryos

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Azoxystrobin and difenoconaozle are two major fungicides. In the present study, the acute toxicity of individual and co-exposure of the two fungicides was studied. Azoxystrobin, difenoconazole, and their mixture (former : latter, 1:2) caused apoptosis to zebrafish embryos at the stage of 96 hours post fertilization (hpf) observed by acridine orange (AO) staining and TUNEL assay. The 96 h LC_{50} of azoxystrobin and difenoconaozle were 0.618 mg/L and 1.478 mg/L, respectively. Both toxicological evaluation criteria - additive index (AI) and ratio of inhibition (RI) values - indicated that azoxystrobin and difenoconazole was antagonistic, but the biological effects induced by the mixture were severer than that of the individual one. The mixture induced a higher rate of tail abnormality and yolk sac edema, but significantly reduced the body length and hatching rates than azoxystrobin or difenoconazole alone. Exposure to azoxystrobin caused apoptosis in the tail area and the end of the abdomen, but difenoconazole in the head and heart area. Apoptosis bright spots induced by the mixture were all over the larvae body. The phenomenon of inconformity between toxicity and biological effects were rarely found in toxicology issues.

AGRO 147

Barriers to pathogenicity testing for microbial pesticides in adult and immature honey bees

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The interest in and use of biological materials in crop production is increasing globally at a rapid pace. Part of the interest is that these technologies are viewed as safer alternatives to conventional chemicals. While establishing the safety of these materials is as important as for conventional chemicals, there are important distinctions between them, such as an evaluation of pathogenic potential. Bayer, Smithers, and Eurofins Agroscience have explored the EPA honey bee pathogenicity test guideline (885.4380) and the OECD Guidance Document No. 239 on Honey Bee Larval Toxicity following Repeated Exposure for use with microbial pesticides. The EPA adult honey bee pathogenicity test guideline is technically challenging in a number of areas, including: 1.) High test concentrations test material as recommended often appear unpalatable, 2.) Test duration of 30 days is difficult to achieve regarding honey bee survival and average lifespan, and 3.) The particulate nature of the test material can result in clogging of the feeding syringe and difficulties maintaining homogeneity of the test material. Some options to consider that may reduce these challenges and improve the survival of control bees include, addition of pollen to the bee diet during the test, directly dosing the bees using a pollen-based diet, or feeding the bees a pre-determined quantity of treated liquid sucrose diet for a limited number of days and then supplementing with clean sucrose and pollen diet. We experimented with using a treated pollen diet for dosing the bees along with providing untreated liquid sucrose diet. OECD Guidance Document No. 239 is designed for chemical pesticides. An evaluation was conducted to determine if it could be adapted to microbial pesticides. Some pathogens may only affect the larvae, and/or are only infectious for a particular window (*e.g*. *Paenibacillus larvae*). We found that the larval bee diet (and royal jelly) inhibited growth of some of the bacteria and fungi, including a known bee larval pathogen. This finding may make the test unreliable for certain biopesticides. Additional research is needed to determine an optimal test method prior to the development of new guidelines by regulatory authorities.

AGRO 148

Synergistic and antagonistic effects of pesticides to the toxicity of organophosphate insecticides to *Apis mellifera*

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The worldwide decline in the population of pollinators is a worrying phenomenon, and a multidisciplinary effort has been launched to identify factors driving honey bee mortality. In North America, the extensive use of herbicides and fungicides may affect the health of non-target organisms, yet relatively little information exists regarding the toxicological impact these agrochemicals have to foraging pollinators. The herbicide atrazine and fungicide chlorothalonil are commonly used in agricultural systems and routinely detected within the bee, pollen, and wax samples of managed bee hives. Considering this, we aimed to determine the toxicological profile of atrazine and chlorothalonil alone and in binary combination with organophosphate insecticides. Atrazine or chlorothalonil alone at concentrations up to 1000 mg/bee did not result in acute toxicity to individual bees. However, atrazine and chlorothalonil (1 mg/bee) were found to enhance chlorpyrifos toxicity while reducing the toxicity of the O-substituted analog chlorpyrifos-oxon. Biochemical analyses of detoxification enzymes indicate atrazine and chlorothalonil significantly increase P450 activity in individual bees. The increased P450 activity indicates synergism of chlorpyrifos toxicity by the herbicide and fungicide is likely due to increased oxidative activation and enhanced detoxification of chlorpyrifos oxon. The changes to acetylcholinesterase activity after herbicide or fungicide exposure has been determined and will be presented. Our results suggest that concurrent exposure of organophosphates with select pesticides during foraging may pose increased risks to bees. These data fill significant gaps in knowledge regarding the toxicological profile of binary combinations of agrochemicals commonly contacted by foraging bees and can guide management decisions.

AGRO 149

Investigating lag time and microplastic dynamics in the Cannon River watershed

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Plastic debris is an environmentally persistent and complex contaminant that has permeated the aquatic environment. Microplastics are plastic debris that are less than 5 mm in size. Their small size increases the potential for these plastic particles to enter food webs, and plastics contain a plethora of chemical additives that can sorb toxic contaminants from the surrounding water such as PCBs, pesticides, and metals. This makes microplastics a potential pathway for contaminants to enter food webs, and not only harm aquatic life, but also humans who are dependent on freshwater ecosystems for drinking water and food resources. Microplastics are an emerging contaminant of concern given their ubiquitous presence and poorly understood interactions with human health and the environment. Although highly under-researched, agricultural practices including application of sewage sludge, plastic mulching, the use of tile drainage systems, polymer coated seed, and encapsulated pesticides has been suggested to generate high amounts of plastics in the environment. Additionally, agricultural practices generate other concerning contaminants including excess nitrogen and phosphorous, herbicides, fungicides, and pesticides that pose threats to the quality of our water resources. Management practices are commonly put in place to reduce contaminant losses, but it is difficult to determine the time it will take in order to see the effect of a given management practice at a watershed scale. Conveniently, the pre-emergent herbicide metolachlor was reformulated in 2000 from racemic to the more herbicidally active s-form, and this unique time marker can be exploited to measure residence time. Furthermore, metolachlor and nitrate are applied, infiltrated, degraded, and leached throughout the soil column simultaneously, and therefore metolachlor is a conservative tracer of nitrate in the water column. In this study, a novel method was utilized to quantify the lag time or residence time of surface waters using a watershed scale approach. Polar Organic Compound Integrative Samplers (POCIS) were deployed in 10 subwatersheds of the agriculturally dominated Cannon River in South Eastern Minnesota every 28 days (excluding the winter months) to capture the ratio of the racemic and s-forms of metolachlor for residence time analysis. Bulk 1 L water samples were collected every 28 days at the same locations for microplastic analysis. Discovered trends and results will be discussed.

AGRO 150

Geospatial model to estimate microplastics entering waterways from wastewater systems and land applied biosolids

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There is a need for exposure models to simulate the pathways and transport of such particles in waterways especially with increasing public awareness about the presence of microplastics in the environment. Microplastics may enter the environment from various sources and in many forms. One source includes personal care products containing plastic particles being washed down residential drains and entering municipal wastewater treatment plants (WWTPs). A large portion of these plastic particles are removed from the water phase during the treatment process, and generally end up in the solids (*i.e.*, sludge). Sludge disposal varies by country, region and locality, including landfill, incinerator, compost, or as land-applied biosolids. There is potential for particles in biosolid applications to reach aquatic systems depending on application location and subsequent environmental conditions. This poster will present a broad-scale model designed to estimate emissions and model the fate of plastic particles exiting WWTPs into the terrestrial and aquatic environments in Europe. The model uses geospatial information on WWTPs, river hydrology, and terrestrial transport potential. This regional/continental scale model is based on publicly available datasets and contained in a modular and transparent framework which is scalable and portable to multiple geographies. This presentation will demonstrate the utility of

the model and how the resulting information about ultimate mass disposition (*e.g.*, soil, freshwater, sediment, marine) and concentrations (surface water, sediment) can be used to help inform the discussion about prospectively assessing the presence and concentration of microplastics in the environment as emitted by WWTPs as effluent or transport from fields applied with biosolids.

AGRO 151

Effects of environmentally relevant concentrations of tire wear particles on estuarine indicator species

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Plastic debris, comprised of a wide range of polymers and synthetic materials, is a ubiquitous source of pollution in marine and estuarine ecosystems. Micro and nanoplastics are known to have adverse effects on the habitats, diets, and physiologies of aquatic organisms, but questions remain about the relative risk across different salinities, polymer types, concentrations, and sizes (*e.g*. micro vs nano). While studies confirm that high concentrations of microplastics can have deleterious effects on organisms, gaps remain in our knowledge of species response to environmentally relevant concentrations, particularly in estuaries where salinity can influence particle behavior and also potentially toxicity. Less is known about the effects of tire wear particles (TWP) as an organic aquatic contaminant. Generated from automobile traffic and composed of a complex mixtures of oil, rubber, plastic, steel, and additives, there is an estimated 1,121,000 t/a of TWP in the United States alone, frequently detected in the coastal environment near urban areas. In this study we used *Menidia beryllina* and *Americamysis bahia* as indicator species to study the sublethal effects of environmentally relevant concentrations of synthetic TWP in estuarine and bay environments. We exposed seven-day old *Mysid* shrimp (n = 9) to 4 concentrations of TWP (< 20µm) across salinities (15, 20, and 25 ppt) for seven days. Subsequently, we exposed *M. beryllina* to identical TWP concentrations from seven days post fertilization to 96 hours post hatch. Treatments of exposure included salinities of 5, 15, and 25 ppt, and TWP in two size ranges (1 – 20µm and < 1µm). A subset of fish and shrimp were used for behavioral analysis, while the rest were euthanized, preserved in fixative, and later evaluated for gut contents and growth measurements using microscopic morphometric analysis and bomb calorimetry. Preliminary results suggest that TWP size influenced swimming behavior in *M. beryllina* and that *A. bahia* shrimp were less sensitive. This could potentially have consequences for trophic transfer as less sensitive prey species may act as transport vectors, transferring TWP to more sensitive predator species. Though species exhibit varying sensitivities to low doses of TWP, the presence of adverse effects in *M. beryllina* indicates that even at current environmental levels, which are expected to continue to increase, aquatic ecosystems experience impacts dependent on the physical properties of the plastic.

AGRO 152

Municipal wastewater treatment plant effluent is our next water supply: Implications for pesticides monitoring, modeling, mitigation, and product design

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In response to growing populations and shrinking fresh water supplies, governments throughout the world have initiated moves toward "potable reuse" of municipal wastewater treatment plant (WWTP) effluent. Anticipating a future where people are drinking treated WWTP effluents, WWTP environmental managers have begun to pivot from their past focus on aquatic life protection to a broader approach that includes human health protection. Since WWTPs use biological processes not designed to remove chemical pollutants, even the most sophisticated WWTPs cannot fully remove toxic chemicals like pesticides, which pass through into effluents. Pesticides occur in WWTP effluent due to direct and indirect discharges after their diverse urban uses, such as pet flea control, cooling water system treatment, cleaning and disinfection, general indoor pest control, treated textiles, and sewer collection system root control. To support potable reuse of effluent, WWTPs typically provide additional treatment using one or more advanced treatment technologies like reverse osmosis (RO). These processes, which remove some but not all toxic chemicals, typically generate large volumes of a liquid waste ("RO concentrate") containing toxic chemicals at concentrations that can create difficulties or dramatically increase the cost for RO concentrate disposal. Since WWTPs do not control pesticide sales or use in the communities they serve, they focus on prevention through pesticide product regulation and product design to avoid adversely impacting WWTP operations, effluent, or biosolids quality. Human health protection and RO concentrate disposal challenges combine to make the upstream regulation and informed design of indoor pesticides critical for potable reuse to succeed. Additional monitoring and modeling studies that incorporate human health endpoints and RO concentrate management as a consideration are needed to inform pesticide product design and, as necessary, regulation and mitigation measures to allow society to obtain the full benefits of WWTP effluent. While WWTP effluent was once viewed only as a waste stream, today it is increasingly viewed as a valuable resource worthy of robust protections.

AGRO 153

New mechanism to mitigate slaking and dispersion

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In Australia much of the agricultural soils are clays, sodic and at times hydrophobic, which contribute significant complexity and compromise in the delivery of irrigation programs. Of all the functions that water serves in irrigation, none is more important than its mobility through these soils, and much effort has been expended at optimizing and conserving water usage. Our hypothesis that Polyether modified Siloxanes will enhance wetting tendency and assist in the movement of water through the soil profile, as well as the additional hypothesis that Silicone Quats will provide an avenue for ion exchange to displace sodium, are tested in field activity and reported in this poster. It is noted that when these

chemistries are used together, the effect of reduced surface tension and higher cationic charge provide a directional guide for water and electrolyte movement with significant implications of dispersion and slaking behaviour of the soil, as well as for environmental and cost issues arising out of irrigation processes. Laboratory evaluation of the slaking rate of reconstituted soil samples under immersion stresses demonstrate an interruption to dispersion tendency, increased coarse particle stability, and increase water stability for high slaking soils. Water stable clay soils are shown to increase their water uptake while retaining their water stability. The improvement in interrupting the slaking behaviour becomes evident in many sources of clay soils, and it is also clear from visual observation that dispersion is eliminated when the slaking behaviour is altered to such an extent. Data such as these suggest that the mechanism of non-metallic ion exchange introduced by the surface active polymer of Aqua-Sil is a real opportunity to counter the use of Gypsum and organic material in soil remediation strategies. A further observation that is also evident across many soil types is the reduction in variation of the slaking rate of the treated samples compared to the control. Field trial data from Werribee South (Broccoli), the Yarra Valley (vineyard), Shepparton (apples and stone fruit), and Armidale (potatoes) amongst others, support this concept and find that better friability and greater infiltration of soil can be achieved, resulting in greater water retention at depth and in the release and expulsion of sodium from soil, as well as achieving lower electrical conductivity in the growth zone.

AGRO 154

Overview of human exposure in relation to risks from glyphosate

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Since its discovery in 1970 by scientists working at Monsanto, glyphosate has become the most widely used herbicide in the world. Along with its increasing use, it has attracted attention from regulators and more lately the public. This public attention was largely driven by its use in genetically engineered glyphosate-tolerant crops, but, more recently, by the International Agency for Research on Cancer's (IARC) classification as '*probably carcinogenic to humans' (Group2A)*. IARC does not consider exposure in a formal risk assessment, but regulatory agencies have updated their reviews of glyphosate since 2015 and have set ADIs (and an RfD) for glyphosate that range from 3×10^{-1} to 1×10^{0} mg kg^{-1} b.m. d^{-1}. Converted to systemic dose, these values ranged from 6×10^{-1} to 2×10^{-1} mg kg^{-1} b.m. d^{-1}. A review of the exposure studies with glyphosate in the literature and in company reports was conducted. Based on data from published papers, exposure via air is very small with an estimated maximum systemic dose of 1×10^{-6} mg kg^{-1} b.m. d^{-1}. Exposures from consumption of contaminated untreated rainwater provided and maximum oral dose of 3.5×10^{-5} mg kg^{-1} b.m. d^{-1}. The 99th centile dose estimated from consumption of untreated (U.S.) surface waters was 4.3×10^{-3} mg kg^{-1} b.m. d^{-1}. Recent biomonitoring studies in the general public provided estimates of mean systemic doses of 8×10^{-7} to 0.20×10^{-2} mg kg^{-1} b.m. d^{-1}. Worst-case estimates of exposure via food indicated the greatest exposures in young children of 2.3×10^{-1} mg kg^{-1} b.m. d^{-1}. Dosimetry and biomonitoring studies in applicators provided normalized median estimates of systemic doses of 2.6×10^{-4} mg kg^{-1} b.m. d^{-1}. Maximum estimated systemic doses from dosimetry studies (3.4×10^{-2} mg kg^{-1} b.m. d^{-1}) were greater than the maximum measured in biomonitoring studies (4.6×10^{-3} mg kg^{-1} b.m. d^{-1}). None of estimated median or maximum exposures based on measured or modeled exposures exceeded even the most conservative ADI. Measures of exposures in domesticated animals gave similar results. Overall, it is concluded that use of glyphosate in production of food and fiber presents a *de minimis* risk to humans, domestic animals or pets.

AGRO 155

Glyphosate: A weight of evidence evaluation to assess potential interaction with the estrogen, androgen, and thyroid pathways

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Glyphosate acid was recently evaluated for its potential to interact with the estrogen, androgen and thyroid (EAT) pathways under the Endocrine Disruption Screening Program (EDSP). Eleven Tier 1 assays were run following the USEPA's validated 890-series test guidelines and included five *in vitro* and six *in vivo* assays. Results from estrogen and androgen receptor binding assays and an estrogen receptor transcriptional activation assay demonstrated that glyphosate does not interact with the estrogen or androgen receptors up to the maximum tested concentration of 1 mM. Steroidogenesis was assayed *in vitro* with the H295R cell line and with recombinant CYP19 (aromatase). Glyphosate was shown not to inhibit aromatase at the maximum concentration tested of 1 mM nor impact production of estrogen and testosterone at the maximum concentration 100 µM. Results from the *in vivo* uterotrophic assay demonstrated that glyphosate is not estrogenic at a limit concentration of 1000 mg/kg/bw day. The Hershberger assay demonstrated that glyphosate is not androgenic, anti-androgenic or inhibit 5α-reductase activity at the limit dose of 1000 mg/kg/bw day. The female pubertal assay demonstrated that glyphosate does not impact the estrogen and thyroid pathways at the limit dose of 1000 mg/kg/bw day. The male pubertal assay demonstrated that glyphosate does not impact the androgen and thyroid pathways at 300 mg/kg/bw day, with the limit dose of 1000 mg/kg/bw day resulting in overt and systemic toxicity, which confounded the interpretation of that dose level. Additionally, the amphibian metamorphosis assay demonstrated no interaction with the thyroid pathway up to the maximum tested concentration of 100 mg/L and the fish short term reproduction assay demonstrated no interaction with the estrogen and androgen pathways up to the highest concentration of 30 mg/L. A weight-of-evidence (WoE) assessment was conducted for each pathway that considered results from the Tier 1 battery as well as guideline regulatory and literature studies. A strength of this evaluation was that it included data across multiple levels of biological organization and mammalian and non-mammalian assays. There was strong agreement across the *in vitro* and *in vivo* Tier 1 battery, guideline studies and relevant literature studies, demonstrating no interaction with EAT pathways. The WoE demonstrates that glyphosate does not have endocrine disrupting properties through EAT modes-of-action.

AGRO 156

Systemic dose considerations clarify the interpretation of glyphosate epidemiology

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In 2015, the International Agency for Research on Cancer (IARC) classified glyphosate as a probable human carcinogen (2A). The classification was based largely on an interpretation of the toxicologic literature as showing sufficient evidence of animal carcinogenicity and sufficient evidence of mechanistic activity relevant to human carcinogenicity. The IARC epidemiology workgroup classified the evidence as limited – viz., *positive associations have been seen in the literature that were deemed credible, but chance, bias, and confounding could not be ruled out*. Most of the glyphosate epidemiology studies involve populations that reported only a few days of glyphosate use over an entire lifetime. With the exception of high dose radiation exposure or cytotoxic chemotherapy, it is just not plausible that such infrequent exposure could cause cancer (in this case non-Hodgkin's lymphoma). Accordingly, this review will look at the epidemiologic literature based on: the propitiousness of each study population, the likely cumulative systemic dose (not exposure) under study (with reference to comprehensive glyphosate applicator biomonitoring), and biologic plausibility based on systemic dose and toxicologic considerations. This approach discards as uninformative the studies where biological plausibility is obviously absent - where any positive findings have to be the result of bias or chance - and clarifies the interpretation of the epidemiologic literature in a way that is coherent with the broader scientific evidence for glyphosate. Recent methodologic research supports the view that the positive findings from the studies with very infrequent lifetime glyphosate use are the result of bias(es).

AGRO 157
Evaluating the genotoxicity and animal carcinogenicity of glyphosate and their relevance to humans exposed to pesticide residues

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In 2015, a working group convened by the WHO's International Agency for Research on Cancer (IARC) conducted a hazard assessment focusing on the carcinogenicity of glyphosate. It concluded that glyphosate, historically considered to be a very safe pesticide, was a probable human carcinogen. This prompted the WHO through its Joint Meeting on Pesticide Residues (JMPR) to conduct a re-assessment of the risks of glyphosate as a pesticide residue, which after reviewing the human, animal and mechanistic evidence, led to the conclusion that glyphosate residues in the diet were unlikely to pose a cancer risk. In this presentation, I will overview the JMPR's evaluation of the genotoxicity and animal carcinogenicity studies of glyphosate. Hundreds of genotoxicity studies on glyphosate and its formulation products were reviewed, and an unusual pattern of results has been observed. No increase in mutations was seen in the vast majority of bacterial test systems. However, DNA damage and chromosomal effects were frequently seen in cell culture models and in organisms that are phylogenetically distant from humans (*e.g.* plants, earthworms, frogs, fish, caiman, etc.). In contrast, in a large number of tests in mammalian models orally administered glyphosate, no increase in genotoxicity was seen. Similarly, glyphosate has been tested in a substantial number of animal cancer bioassays. While occasionally increases in tumors have been seen in various tissues, in most cases, these were not seen in other studies, often those in which higher doses were administered. The JMPR concluded that there was no consistent increase in the rat studies but that a clear conclusion could not be reached for some of the mouse studies. However, the doses at which the potentially positive effects were seen were extremely high and were considered not to be relevant to humans exposed to low levels in the diet.

AGRO 157

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***David Eastmond**, david.eastmond@ucr.edu. Molecular, Cell and Systems Biology, University of California, Riverside, Riverside, California, United States*

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AGRO 158

Overview of environmental fate of glyphosate and its effects on glyphosate-resistant crops

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Glyphosate is the most used herbicide worldwide, and it is a relatively high use rate herbicide. It is a non-selective herbicide with a unique mode of action that is ideal for vegetation management in both agricultural and non-agricultural settings. Its use was more than doubled by the introduction of transgenic, glyphosate-resistant (GR) crops. Glyphosate is tightly bound by most soils, so its movement to surface and ground water is relatively little compared to most other pesticides. It is readily degraded by many soil microbes, makings its environmental half-life relatively short. All of its phytotoxic effects are through inhibition of only 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), but inhibition of this single enzyme of the shikimate pathway results in multiple effects, both upstream and downstream from EPSPS, including loss of plant defenses against pathogens. This does not occur in GR crops. Most degradation of glyphosate in plants and microbes is predominantly by a glyphosate oxidoreductase or to a lesser extent by a C-P lyase. Its effects on non-target plant species are generally less than that of many other herbicides, as it has a low propensity to drift and is inactivated by soil. Glyphosate can benefit GR crops by its activity on plant pathogens that are sensitive to glyphosate. On the other hand, glyphosate can adversely affect some microbes that are beneficial to agriculture, such as *Rhizobium* species, although GR crop yield data indicate that such an effect has been minor.

AGRO 159

Glyphosate – a game changing technology

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The development of glyphosate for controlling weeds in production agriculture was a game-changer. Many of the earliest crop protection materials had unintended consequences for the environment and acute safety concerns for applicators. Many products were preventative in their mode of action and dependent upon timely rainfall to become effective. Others were only effective as burn-down compounds, requiring multiple applications for season long weed control. The development of glyphosate provided applicators with a herbicide option that was safe to use, effective on growing weeds, and provided long-lasting control. With the development of Roundup resistant crops, the need for preventative herbicides was reduced and in many crops eliminated. It also provided farmers the option to not spray for weeds at all, if the weeds were not present. With minimum and no-till farming becoming the norm, as farmers implement practices that reduce their carbon-emitting footprint, utilizing glyphosate for weed control is critical to reaching that goal. No single crop protection product has been developed that has had as great and positive an impact for modern agriculture.

AGRO 160

Update into the ecotoxicology of glyphosate, its formulants, and environmental degradation products

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New studies on the environmental exposure and effects of glyphosate and glyphosate-based herbicides are published at a rate which makes it hard to keep up to date. Despite this, the number of reviews publicly available in the peer-reviewed literature has not grown proportionally in the last two decades. In this presentation we aim to summarize the findings in the most recent literature. The conclusions drawn from this critical assessment are consistent with those presented in other reviews conducted in the early 2000's. The chemical and biological properties of glyphosate are key to understanding its fate in the environment and potential risks to non-target organisms. Glyphosate is polar and water soluble and does not bioaccumulate, biomagnify, or accumulate to high levels in the environment. It binds strongly to particles in soil and sediments and this reduces bioavailability so that exposures to non-target organisms in the environment are acute and decrease with half-lives in the order of hours to a few days. The mode of action of glyphosate is specific to plants which results in low toxicity and small risks to animals. Technical glyphosate (acid or salts) is of low to moderate toxicity; however, when mixed with some formulants such as polyoxyethylene amines (POEAs), toxicity to aquatic animals increases about 15-fold on average. However, glyphosate and the formulants have different fates in the environment, and they do not necessarily co-occur unless the organism or matrix is directly sprayed. Toxicity tests on formulated products in scenarios where they would not be used are unrealistic and of limited use for assessment of risk. Concentrations of glyphosate in surface-water are generally low with small risk to aquatic organisms, including plants. Toxicity and risks to non-target terrestrial organisms other than plants sprayed directly are low, and risks to terrestrial invertebrates and microbial processes in soil are very small. Formulations containing POEAs are not allowed for use over water but, because POEA rapidly partitions into sediment, aquatic organisms are protected from accidental over-sprays. We conclude that use of formulations of glyphosate under good agricultural practices presents a *de minimis* risk of direct adverse effects in nontarget organisms

AGRO 161

History and outlook for glyphosate-resistant crops

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Twenty-five years ago, glyphosate-resistant (GR) crops, commercially referred to as glyphosate-tolerant (GT), started the plant biotech crop revolution. Growers rapidly adopted GR crops whenever they became available. Adoption usually meant sole reliance on glyphosate for weed control. Not surprisingly, GR weeds eventually evolved resistance and

forced growers to change their weed management practices. Today, the widespread dissemination of GR weeds that are often resistant to other herbicide modes-of-action has reduced the value of the GR crop weed management systems. Still, growers continue to use the technology widely in six major crops throughout North and South America. Chemical companies promote sustaining glyphosate efficacy by combining glyphosate with other herbicides. Seed companies promote sustaining glyphosate traits by combining them with other traits that enable the use of glufosinate, dicamba, 2,4-D, HPPD, ACCase inhibitors, and other herbicides. Unfortunately, herbicide companies have not commercialized a new mode-of-action for over 30 years and have nearly exhausted the useful herbicide trait possibilities. Glyphosate-based crop systems are still the mainstays of weed management, but glyphosate and other existing chemical herbicides cannot keep up with the capacity of weeds to evolve resistance with existing technologies. Growers desperately need new technology, but no technology with the impact of glyphosate and GR crops is on the horizon. Expansion of GR crop traits is possible into new geographic areas and crops such as wheat and sugarcane. A non-transgenic metabolic trait that reduces glyphosate residues in a new cropping area could have a high value. However, the Roundup Ready® revolution is over. Its future is at a nexus and dependent on a range of issues.

AGRO 162

Evolved glyphosate resistance

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Glyphosate, a non-selective herbicide is extensively used worldwide for weed management. In sensitive plants, glyphosate binds to its target site, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an important enzyme in shikimate pathway and inhibits production of aromatic amino acids. With the introduction and wide acceptance of Roundup Ready crops in many countries, glyphosate has been used extensively for weed control, consequently, many weeds have developed resistance to glyphosate. Mechanisms conferring glyphosate résistance in weed species encompass both non-target site and target-site based. Non-target site resistance because of reduced translocation and increased metabolism of glyphosate have been reported. Target site resistance include, several types of mutations in the EPSPS gene, the molecular target of glyphosate, including amplification of EPSPS gene weeds. Our molecular cytogenetic analyses revealed fascinating information related to fundamental, evolutionary mechanisms of glyphosate resistance in *Amaranthus palmeri*. EPSPS gene amplification in glyphosate-resistant *A. palmeri* was driven by extra-chromosomal, circular DNA (eccDNA) molecules. Each eccDNA can multiply rapidly during the growth of the sporophyte and produce copy number variation in somatic cells. The somatic cells with amplified EPSPS survive the herbicide treatment, and this acquired trait is transmitted to the germ cells and the progeny. EPSPS gene may not have integrated into the chromosome but exist as extra-chromosomal (*i.e.*, eccDNA) elements. Based on our results, it is likely that in response to glyphosate stress, the extrachromosomal DNAs drive the amplification of EPSPS gene resulting in the rapid evolution of glyphosate resistance. This is a completely novel mechanism of evolution of glyphosate resistance and has vast implications for the development and management of glyphosate resistance.

AGRO 163

Glyphosate's role in supporting sustainable agriculture

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Sustainable agriculture has a legal definition under U.S. Code Title 7, Agriculture, Section 3103. The term refers to an integrated system of plant and animal production practices with five objectives. The system meets human food and fiber needs, enhances environmental quality, makes the most efficient use of nonrenewable resources, sustains the economic viability of farm operations, and enhances the quality of life for farmers and society. The research literature is replete with environmental and economic studies providing an overall weight of evidence that glyphosate use meets these five objectives. A quarter century of growing glyphosate resistant corn and soybean cultivars shows a steady continued increase of yields that suggests glyphosate has no adverse effects on productivity. Soil microbial ecology studies of landscapes where glyphosate has been used for multiple years show little effect on functional ecology. Soil erosion is arguably reduced when no-tillage practices have been facilitated in soybean and cotton by glyphosate use. Compared to other herbicides that glyphosate has replaced, its residues detected in aquatic systems do not exceed water quality criteria. Economic studies from different research institutions show enhanced profits to the growers. Greater profits mean more money is circulated through the communities where farmers live. The toxicological and environmental profile of glyphosate has been assessed globally by a diversity of regulatory agencies, and a mutual consensus has emerged that glyphosate is equivalent to a "reduced risk" pesticide, and thus significantly lessens health impacts. The argument that increased detection of glyphosate resistant weeds proves the herbicide is antagonistic to sustaining agricultural production is flawed because the problem is behavioral in nature owing to lack of appropriate management for resistance avoidance rather than due to the intrinsic properties of glyphosate. Nevertheless, continued contributions of glyphosate herbicide as a beneficial tool for agricultural production will require increased efforts to communicate to users the importance of product stewardship that includes attention to resistance avoidance and where possible an integration with other ecologically based pest management practices (for example, cover crops) that could reduce use of herbicides in general.

AGRO 164

Consideration of non-*Apis* bee species in pollinator risk assessment

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The pollinator risk assessment process for pesticides from regulatory agencies, including USEPA, PMRA, and CDPR, have historically relied on the Western honeybee (*Apis mellifera*) as a surrogate species to represent all *Apis* and non-*Apis* bees. However, the life-history characteristics of non-*Apis* bee species, including solitary, stingless, and bumble bees, indicate differences from the surrogate honeybee species. These differences introduce uncertainty in the exposure and

effects assumptions for the use of honeybees as a surrogate species, leading to the recent efforts of examining risk of pesticides to non-*Apis* species. The objective of this presentation is to provide a current state-of-the-science update on the specific life-history characteristics and specific risk assessment considerations for non-*Apis* species. Exposure assumptions will be examined, and comparative toxicological sensitivities will be presented with recommendations for appropriate use in the pollinator risk assessment framework. Challenges in the development of non-*Apis* laboratory testing methods will also be presented.

AGRO 165

Developing new methods to evaluate toxicity for different bee species in Brazil: Facing the challenges

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Brazil has the greatest diversity of bees in the world; however, several species are now listed as endangered or decreasing in abundance. Some of the agricultural pesticides currently used in Brazil may be harmful to bees. To ensure the protection of biodiversity within the context of sustainable agriculture, it is important for Brazil to have a system to evaluate whether compounds are safe for bees and effective in agriculture. Standard protocols for bee toxicity tests use species that are not native to Brazil, *e.g.* the honey bee, *Apis mellifera*. The distribution of native bee species in Brazil varies widely. Commercial sources of bees native to Brazil are not available, and few professionals are specialized in working with them. We have developed a proposed standard protocol using stingless bees from adaptations to OECD (1998a, b) protocol for *A. mellifera* using three different stingless bee species: *Melipona scutellaris; Scaptotrigona postica;* and, *Tetragonisca angustula*. The bee collection method, the experimental cage and anesthesia times were optimized. The proposed protocol was tested between October 2018 and August 2019 using dimethoate, a commonly used reference toxicant in bee toxicity studies. Currently, 14 laboratories in Brazil are involved in the standardization of the protocol, with more than 25 people trained to carry out the procedure. Partnerships with other countries with stingless bees are being established to apply this protocol there. The results will provide evidence to accept or reject *A. mellifera* as a surrogate for Brazilian native bees in toxicity studies of pesticides. In addition, we have already developed a protocol for testing larvae of meliponines and are working on the development of tests for native solitary bee species.

AGRO 166

Innovative approaches to evaluating the effects of insecticides on non-*Apis* bees

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Declines in some bee populations have been attributed to several environmental stressors including pesticides. Due to differences in life history and pesticide sensitivity, there is an ongoing international effort to develop toxicity testing methods that extend beyond honey bees (*Apis mellifera*) to include non-*Apis* bees. This presentation will discuss innovative research approaches and findings from laboratory studies performed using bumble bees (*Bombus impatiens*) and mason bees (*Osmia lignaria*). To evaluate the effects of chronic oral exposure to the insect growth regulator (IGR) diflubenzuron (0.1, 1, 10, 100 and 1000 µg/liter) on *B. impatiens* worker health and brood development, we used 6-week queenless microcolonies. Syrup and pollen consumption were reduced significantly for all diflubenzuron concentrations tested. Pupal cell production was decreased in the high-dose group. Drone production, a proxy for reproductive success, was inhibited in a concentration-dependent manner. To assess the toxicity of pesticides to *O. lignaria*, we developed and employed a novel modified spray tower design. Male and female *O. lignaria* adults were exposed by whole-body contact to low and middle label recommend rates of commercially blended insecticide mixtures (each with a different mode of action) for orchard crops. Mortality was monitored at 24, 48, 72 and 96-hours post treatment. Products containing neonicotinoid and pyrethroid insecticides induced rapid mortality, while diamide (chlorantraniliprole), molting hormone agonist (methoxyfenozide) and IGR (spinetoram) chemistries induced gradual mortality. High mortality was recorded for all insecticide treatments (LD_{50}<1 µg/bee) at 96-hours after application to adult mason bees. These studies provide insights into the effects of pesticides on under-studied species of non-*Apis* bees and provide a platform for continued methods development. Standardized methods are needed to support risk assessment activities and ultimately pollinator protection goals.

AGRO 167

Quantifying neonicotinoid insecticide residues in pollinator-attractive habitat adjacent to corn and soybean fields in Iowa

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Neonicotinoid insecticide residues can be transported from corn and soybean fields to adjacent pollinator habitat through dust-drift from planting treated seeds and/or through surface/sub-surface runoff following precipitation events. Bees could subsequently be exposed to neonicotinoids through consumption of contaminated pollen and nectar. Previous studies indicate neonicotinoids can be detected in pollinator-attractive habitats; however, the magnitude and extent of potential adverse effects to honey bees and native bees is an active area of investigation. In this study, the concentrations of clothianidin, imidacloprid, and thiamethoxam in pollen and nectar samples were quantified. Nectar and pollen samples were collected from six habitat patches immediately downslope of corn and soybean fields planted with neonicotinoid-treated seeds from May through August in 2018 and 2019 (~6 samples per site). Samples were extracted and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Results from 2018 indicate 80% of pollen samples and 10% of nectar samples had at least one neonicotinoid present above the method detection limit (MDLs ranged from 0.04 to 1.0 ng/g pollen and 0.05 to 0.3 ng/g nectar). For samples $\geq$ the MDL, neonicotinoid concentrations ranged from 0.14 to 5.12 ng/g pollen. These data are refining pollinator risk assessments by providing information on spatio-temporal variability of neonicotinoid exposures in Midwestern agroecosystems.

AGRO 168

Using bee foraging behavior to asses risk from pesticide exposure

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Pesticides have been shown to impair bee foraging and negatively affect bee health. These studies have been done mostly with honey bees and some with bumble bees, both social bees with colonies. But the great majority of bee species are solitary. We have approximately 40 native bumble bee species in the United States and 4,000 native solitary bee species. The honey bee is a non-native social bee. Pesticides affect all bees, and all bee species forage for pollen and nectar and exhibit specific behaviors when foraging. Here, we explore the different behaviors exhibited by different bee species while foraging. We contrast the foraging behavior of the European honey bee, *Apis mellifera*, the common eastern bumble bee, *Bombus impatiens*, and the alfalfa leafcutting bee, *Megachile rotundata*. We compare behavior at different scales. For honey bee and bumble bee, we contrast behaviors captured using Radio Frequency Identification (RFID) although such devices have not yet been adapted for use with solitary bees. We also compare all three species foraging within fields, using direct pollinator observations. While our research has focused on linking foraging behavior to gene flow risk for different bee species, we propose exploring the use of foraging behavior to assess risk from pesticide exposure. There has been a call for new techniques and protocols across the globe for making standard assessments on non-*Apis* bees. Bee species share foraging behaviors and such behaviors could be linked to pesticide exposure and ultimately to bee health. Foraging behavior could not only quantify the impact of a pesticide on the foraging of a bee species but also contrast the relative impact on different bee species. Foraging behavior as a potential assay for pesticide exposure would be applicable to all bees and could be standardized across bee species.

AGRO 169

Initial impurity screening for 5-batch analysis studies

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The most important part of 5-batch analysis is initial impurity screening to assess relative levels of impurities with respect to active ingredient (a.i.). While looking for impurities that are >0.1% relative to the a.i. content based on HPLC-UV, it is important to recognize that the response of some impurities may be less than that of the a.i. at a particular wavelength. This leads to underestimation of their levels at this stage as we depend on % area. To overcome this anomaly, it is important to screen impurities at varying wavelengths, covering the entire UV-Vis range, and selecting wavelength(s) that demonstrate the highest response for an impurity and the lowest response for the a.i. This may result in overestimation of the impurity levels compared to their actual content. However, this issue can easily be resolved/corrected when analysis is performed using the actual impurity standard.

The next level is to identify the impurities. For this, mass spectra of the impurities can be recorded on LC-MS or GC-MS. The obtained mass spectra help predicting the probable structures, though they sometimes lead to more than one structure for an impurity. A critical look at the synthetic process adopted for manufacturing the technical material and reaction mechanisms will lead us to the probable formation of the predicted impurity. When the impurity content is very low (around 0.1%) and if the ionization of that impurity on LC-MS is low, then the MS spectra do not give much information. In such cases, emphasis is to use preparative HPLC to isolate such impurities in order to get sufficient quantity for recording NMR. With mass and NMR spectra in place, it becomes easier to interpret and elucidate the impurity structure. After identification of the impurities, next step is to obtain the impurity standards that are essential for quantitative determination of impurity levels in batch samples. To generate required quantity of impurity standards, the best option is to chemically synthesize them in the laboratory. In cases where the synthesis is difficult, one can attempt to isolate the impurities from the technical material or corresponding crude material (before final purification) using preparative HPLC. The impurities thus obtained need to be sufficiently characterized and issued with a certificate of analysis for the purpose of using them as analytical standards. Once this process is done, a batch analysis study can be performed in compliance with regulatory requirements.

AGRO 170

Physical-chemistry testing guidelines: Complex challenges during simple tests

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Physical-chemistry testing of the technical and formulated pesticides is carried out to attain regulatory compliance. This may also be used for research and development, product development, and technical support. For registration and compliance with authority (such as the U.S. EPA, EU, Brazil, etc.) several physical-chemical tests are required, depending on the technical grade pesticides or type of the formulations. These tests are performed as per the various internationally accepted guidelines and methods. The OECD and CIPAC have published various guidelines and methods applicable to different types of formulations. All these (simple) tests, with different regulatory requirements, trigger a challenge which needs to be addressed for the successful registration of a product. As per the regulation of GLP, to avoid duplicate testing and a ready acceptance of data across the world, selection of the method or test guidelines, fulfilling the requirements of different regulatory agencies is of prime importance. Along with the challenges of selecting method and guidelines, these (simple) tests have a technical challenge of performance, selection/availability of equipment, variations in replicate testing, stringent acceptance criteria, interpretation of results, so on and so forth.
Different types of solid and liquid formulations are being used for plant protection. Each formulation requires a specific physical-chemistry test, over and above some of the common tests including purity, stability, and solubility tests applicable for technical pesticides. With a continuous improvisation of techniques, analytical methods, and modern equipment, the performance of these (simple) tests could be easy and faster; however, the challenges still exist concerning the selection of the applicable tests for different types of formulations, selection of test method/ guidelines, acceptance criteria, and interpretation of results. This presentation discusses the

challenges associated with different physical-chemistry tests, as per the various test guidelines while meeting the regulatory requirements.

AGRO 171

High performance film forming agent for seed coating applications

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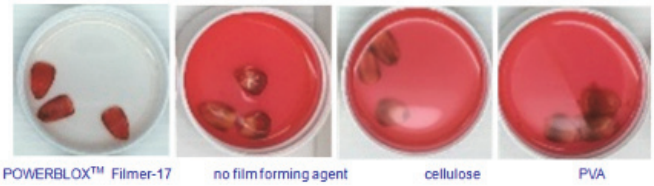
As more additives are applied to seeds, it drives innovation for improved Flowable concentrate (FS) formulations. Film forming agents (FFA) are an important component in FS as they improve adhesion and homogeneous distribution of the active ingredient (A.I.) on seeds and improve water resistance, without a negative influence on seed germination and growing. Commonly used FFA include Hydroxypropyl methylcellulose (HPMC), polyvinyl alcohol (PVA), polyacrylates, and other polymers with several adhesion mechanisms and film forming properties. However, FFA still have performance defects. We have developed a new polyacrylate that produces a uniform corn seed coating (Figure 1) when added at 10% w/w into a 30% w/v Thiamethoxam insecticide FS formulation. Determination of the seed coating expulsion rate, a key FFA adhesion performance metric, was done using 2 test methods: 1) shaking (280 rpm, 10 minutes), and 2) dropping (using a tube, throw-drop 50 times, height 2-3 meter). Subsequent, measurement of the residual A.I. concentration on the seed's surface or in the tube enabled determination of the expulsion rate, which for shaking was 2.7% w/w and for dropping was 5.5% w/w. These low values of expulsion exceeded HPMC and PVA performance. An additional test was a water soak test for 24 hours to measure loss of coating as determined by change in color of the solution vs. the control and other FFA (Figure 2). Finally, seeds were germinated at 25°C in the day and 20°C at night; seed germination rate (4 days) and seeding height (10 days) with and without polyacrylate were statistically the same. The polyacrylate developed here has good adhesion with decreased expulsion rate, good water resistance to avoid A.I. loss, and no negative effect on seed germination.



AGRO 172

Overcoming water hardness challenges in tank mix dilutions of high-load soluble concentrate agrochemical formulations

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High-load soluble concentrate agrochemical formulations have gained relevance in the market due to their associated sustainable attributes that optimize pest control through increased transportation efficiency, and minimum volume of containers to recycle or dispose, resulting in a reduced environmental impact. Development of these formulations, however, does not come without challenges such as low active ingredient solubility, high viscosity (particularly at cold temperatures), insufficient storage stability, and tank-mixing problems – particularly the formation of crystals, surface scum, gels, foam, or clumps of solid matter when formulations are diluted with hard water which may result in clogged spray nozzles or screens. Several active ingredients have a tendency to create insoluble salts when diluted with hard water, adding complexity to its use as water will need to be previously treated with conditioners. A high-load soluble concentrate case study is used to exemplify the use of an in-can crystal inhibitor system composed of a polymeric dispersing agent in conjunction with a chelating agent to assure good dilution properties over a wide range of water hardness and dilutions as well as excellent physical stability of the formulation when exposed to below-freezing temperatures over long periods of time.

AGRO 173

Predictive modeling: impact on agricultural formulation development and spray drift management

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The incorporation of drift reduction technology into a multi-active oil-in-water formulation is an attractive, yet challenging, approach to achieve formulation efficacy of each active ingredient and afford a sustainable agricultural formulation. Predictive modeling is a process that uses data mining and probability to forecast outcomes. Thus, a statistical model is used to gain an in-depth understanding of the interplay between spray solution physical/chemical properties, tank mix adjuvants, and application properties, which is critical to the optimization of formulation efficacy while ensuring spray drift reduction.

AGRO 174

Engineering the seed microenvironment

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Scientific discoveries in agriculture and sustainability are at the crossroads of material science, biochemistry, agriculture and biology. They underpin the innovative technological solutions that will impact water, energy, and food security (WEFS). These new technologies can then be implemented to address major societal problems that are linked to climate change, soil degradation and soil salinization. In particular, our objective is to augment agricultural outputs (*i.e.,* crop yield and production) while decreasing inputs (*e.g.,* water, energy, fertilizers, land, pesticides) by deploying plant-growth-promoting-bacteria (PGPBs) in the soil to alleviate plant stressors to be specific soil salinity. Synthetic fertilizer use has been instrumental in food production; however, usage tends to degrade soils (~1/3 of the world's soil is degraded) and salinize our soils while also contributing ~3% of world carbon emissions during fertilizer production. Using PGPBs as a substitute to fertilizer, our design approach engineers the seed microenvironment by coating the seeds with PGPB laden materials. PGPBs are well known to enhance crop production and protect plants from biotic and abiotic stresses, while decreasing the need for water and fertilizers[1]. However, the bacteria's delicate nature has hindered their use in current agricultural practices. We use a silk and trehalose mixture that is able to protect, preserve and deliver Rhizobium tropici to Phaseolus Vulgaris. The coated Phaseolus Vulgaris seed are shown to be able to significantly alleviate soil salinity stress in Moroccan soil when compared with uncoated (control) Phaseolus Vulagris seeds.

AGRO 175

Components of early product discovery and formulation in context of avoiding non-target exposure

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Development of a new active ingredient from discovery to registration can take well over 10 years and cost more than $250 million dollars. Thousands of compounds may be screened to eventually find one compound that will make it to market. The initial screening of new active ingredients will narrow the field through a series of many different tests. Many of these tests may be quite specific depending upon the targeted crop, pest, and geography of the new active ingredient, but they will typically focus on toxicology, metabolism, residues, ecotoxicology, physical-chemical properties, and environmental impact as well as efficacy of the new active ingredient. In this early stage of discovery, the new active ingredient will have many parameters defined including use rates, optimum formulation and also the registerability of the active ingredient in different parts of the world. All of these studies are performed to ensure that the new active ingredient will be safe to the workers, environment, crops, and the food produced by limiting the non-target exposure.

AGRO 176

Host plant effects on insecticide detoxification: Cardenolide and pyrethroid exposure affects gene expression in monarchs

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The monarch butterfly (*Danaus plexippus*) population has declined over the past two decades and is proposed for listing under the Endangered Species Act. Insecticide exposure has been cited as one of the multiple stressors contributing to the decline. Fall migrating adults may come into contact with late-season applications of pyrethroids in soybeans which has been shown to cause lethal effects if spray drift is deposited on the host plant, milkweed (*Asclepias sp.*), around field margins. However, milkweed itself also produces insecticidal defense compounds known as cardenolides. The concentration of these endogenous plant toxins varies based on milkweed species and may affect detoxification processes in monarch caterpillars. Here, we present findings from controlled laboratory experiments detailing the toxicology of bifenthrin within 5th instar monarch caterpillars with and without coexposure of cardenolides. Expression of Na^+/K^+-ATPase, esterase, P450, glutathione *S*-transferase and ABC transporter genes are reported across treatment combinations.

AGRO 177

Remotely-piloted aerial spray systems (RASS) as a tool to minimize plant protection products (PPP) impacts to listed species

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The agricultural industry is exploring strategies to decrease unintended exposure and thus minimize impact to listed species from Plant Protection Products (PPP). Unmanned Aerial Vehicles (UAV) have a well understood role in the mapping of habitats, identifying sensitive areas for risk mitigation. They are now authorized for the application of PPP. This activity is met with both excitement and trepidation. RASS could provide an excellent tool for minimizing impact as we utilize their ability to more precisely apply PPP. There is, however, a paucity of information of the spray distribution of these non-specialized machines, which could have a negative impact if used incorrectly. In recent years I have conducted numerous research studies that identify the role of RASS, highlighting their strengths and weaknesses. In collaboration with the Julius Kuehn Institute, Germany, China Agricultural University, and Bonds Consulting, in-swath variability and off target movement of the spray cloud was investigated

compared to traditional methods. Currently, Bonds Consulting, USDA Aerial Application Research Unit, and Harold Thistle, are working to characterize spray distribution and develop a surface deposition model for RASS. Within this presentation I will illustrate ways in which RASS could be used to improve precision in the application of PPP. I will compare their deposition profiles to those of traditional application techniques and assess the current state of knowledge to inform development of minimization of risk and resulting impact through RASS technology.

AGRO 178

Towards an efficient and improved approach for assessing risks of pesticides to endangered species in the United States: Methomyl case study

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Under the Endangered Species Act, the U.S. Environmental Protection Agency (EPA) and the Services (National Marine Fisheries Service and Fish & Wildlife Service) are required to assess potential risks to threatened and endangered (listed) species as part of the process to register pesticides. EPA issued interim guidance for developing biological evaluations (BE) for pesticides in 2015. Since then, the EPA, the Services, the regulated community, and others have continued to develop and refine national endangered species risk assessment (ESRA) methodologies, first with 3 organophosphates and recently with two carbamates, carbaryl and methomyl. In March 2020, EPA issued revised guidance for conducting BEs. Much, however, remains to be done to develop the most efficient, practical, and scientifically defensible approach. Therefore, we are developing an improved scientific approach with methods that more efficiently screen out listed species that are not at risk earlier in the tiered assessment process. The approach begins with simple methods such as off-ramping (*e.g.*, screening out listed species that have no potential for exposure); conservative, protective screening-level risk assessments to screen out entire receptor groups that are tolerant of exposure; and co-occurrence analyses to screen out species not present in pesticide use areas. We are developing spatially explicit, species-specific tools for each major receptor group to determine which listed species are potentially at risk using more advanced, but still protective, methods. Using methomyl as a case study, we demonstrate how these early tier methods can efficiently reduce the formidable scope of a national ESRA to focus on those listed species most at risk. The latter species would then proceed to probabilistic, more refined modeling and proper weight-of-evidence assessments.

AGRO 179

Evaluation of the potential effects of pesticide registrations on listed species: A case study with methomyl and the Dakota skipper (*Hesperia dacotae*)

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The U.S. Environmental Protection Agency (EPA) issued revised Method for National Level Listed Species Biological Evaluation (BE) of Conventional Pesticides (March 2020) and applied that revised guidance in the draft BE for two pesticides: methomyl and carbaryl. The scope of these national assessments has motivated ongoing development of efficient, practical and scientifically defensible approaches to address this issue. This talk showcases our efforts to develop an improved scientific approach for a terrestrial invertebrate, the Dakota skipper (*Hesperia dacotae*). Our case study initially focuses on accumulating the best available information on the Skipper life history and population dynamics, the species' range and distribution, and methomyl use as it relates to exposure of, and effects upon, individuals of the species and its critical habitat. The acquired information and data are incorporated into a tiered process to refine estimates of exposure and resulting risk to the Skipper. Refinements involve combining spatial and digital environmental data to redefine the pesticide drift zone and related estimates of exposure to individual Skippers and their critical habitat. This along with novel applications of surrogacy are used to arrive at close estimates of Skipper adult and larval body weights and daily food intake for calculating dietary exposure and drift effects distances of labeled application scenarios.

AGRO 180

Achieving effective ESA consultations in FIFRA registrations is the desired effect

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Registration of crop protection products and compliance with the Endangered Species Act continues to be complicated and ineffective. The ESA, and Federal Insecticide, Fungicide and Rodenticide Act, have different approaches to their species protection mandates. A history of narrowly framed approaches has resulted in obstacles to registration while not fully delivering on the intent of the laws. While refinements to EPA's environmental risk assessment and biological evaluation methods have occurred, the current process does not adequately link realistic individual toxicological endpoints to the species population impacts necessary for ESA decision-making. We will review and critique the March 2020 Carbaryl draft Biological Evaluation (DBE) and provide recommendations to inform and advance the consultation, including an equitable and transparent programmatic framework for all registrants, and the development of

methodologies for identifying best-fit conservation measures. The DBE evaluates 1,745 listed species identified as having ranges within the action area. EPA eliminates four species from further evaluation via "no-effect" determinations, another 199 received "not likely to adversely affect" determinations, with 1,542 species advancing for formal ESA consultation. New methods are needed for assessing, quantifying, and reaching agreement on conservation measures (CMs) for offsetting species impacts. Species impacts may be attenuated through CMs designed to avoid, minimize, or offset them. Avoidance and minimization examples include ESA-related labeling (restricted use areas, buffers, *etc*.). Offsets can take place through direct habitat conservation activities. As the timeline draws near for completion of the current round of registration review, it is in the interest of FIFRA registrants, as ESA applicants, to jointly coordinate their conservation measure approaches across both regulations. The desired effect is to better leverage the ESA and FIFRA authorization processes, resulting in improved endangered species viabilities (*e.g.*, fewer listings and more recoveries) and national scale pesticide consultations that are more practically linked to the landscape.

AGRO 181

Opportunities for listed species conservation during pesticide development and registration

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Currently, the focus of threatened and endangered (listed) species conservation is during generation and implementation of the Biological Opinion phase of section 7 consultation under the Endangered Species Act. However, there is great potential for listed species conservation to play a role in other stages of the pesticide development process, including pesticide research and development, risk assessment by registrants and EPA, and EPA's development of biological evaluations. Opportunities and benefits of early consideration of listed species conservation needs will be explored with the goal of starting a larger conversation on this topic with all stakeholders involved in pesticide use, registration and regulation. A brief review of how conservation measures fit into the consultation process will be covered.

AGRO 182

Evaluating the effects of pesticide use on endangered species: A tale of habitat management and fish recovery in the San Francisco Estuary

***Leanna Zweig**, leanna_zweig@fws.gov, Kim Squires, Jana Affonso. USFWS, Sacramento, California, United States*

Federal agencies that implement the Endangered Species Act (ESA) are tasked with evaluating the effects of pesticides on imperiled species and their habitat quickly, and often with little environmental data, or taxa-relevant toxicity threshold information. We review the USFWS ESA Section 7 consultation perspective for aquatic herbicide use in the critical habitat of the federally-threatened Delta Smelt (*Hypomesus transpacificus)* in the San Francisco Estuary and suggest opportunities for partnerships to ensure the use of best-available science for future consultations and improved risk assessments for listed species.

AGRO 183

Evaluating whether potential effects from a pesticide are 'reasonably certain to occur'

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On August 27, 2019, the Services (U.S. Fish and Wildlife and National Marine Fisheries Services, collectively) finalized regulations clarifying certain Endangered Species Act (ESA) terms. The clarifications are intended to make decisions related to the ESA (such as species listing, critical habitat designations, and agency consultations) more transparent and efficient. One of the terms addressed by this regulation is "reasonably certain to occur" which comes into play in ESA Section 7 consultations. Under Section 7 of the ESA, federal agencies are required to consult with the Services to ensure their actions are not likely to jeopardize the continued existence of listed species or result in destruction or adverse modification of critical habitat. An example of a federal action that may require Section 7 consultation with the Services is the registration of a pesticide under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). This presentation will explore the concept of "reasonably certain to occur" as it relates to FIFRA assessment and ESA consultation processes. Application of data and tools that can help to understand if an action is "reasonably certain to occur" will be discussed in the context of a pesticide registration, ESA consultation, and existing or potentially useful conservation measures. Some of these tools are aggregated and maintained by the FIFRA Endangered Species Task Force (FESTF).

AGRO 184

Population assessment and conservation (PAC) measures for pesticide consultations and meaningful stewardship outcomes

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The EPA pesticide environmental risk assessment process and resulting Biological Evaluation (BE) summarizes effects determinations for likely to be adversely affected (LAA) listed species. In this context, FIFRA-centric risk assessment methods are intended to characterize the likelihood that a threshold level of one individual of a listed species could result in LAA determination using highly conservative exposure and effects benchmarks. However, the methods used to flag an LAA determination are not adequate to characterize species population-level effects and viability, two factors which U.S. Fish and Wildlife Service and National Marine Fisheries Service use in developing their biological opinions. We propose a methodology for integrating output from the EPA's BE's toward application of a diagnostic, population assessment with conservation (PAC) measures. This new approach advances the consultation process by qualitatively and quantitatively developing conservation measures that are determined from an assessment of population-level biological change. The diagnostic PAC

provides flexibility to interpret, re-interpret, and synthesize information at the species population level. Population-level assessment is not currently conducted in EPA's BE framework, only risk assessment threshold analysis flags consultation. However, information and data in EPA's BE's can be used in the diagnostic PAC methodology, such as: potential chemical/product use and exposure footprint, habitat and species range and critical habitat (if available), and some species toxicological information. Additional data and information that is included in the diagnostic PAC methodology is population-level: species needs assessment (beyond habitat requirements), species life history information, and species population stressors, and stability against pressure from natural and anthropogenic sources. At the foundation, the diagnostic PAC is developed from currently-available data and information. At later stages or as the need arises during the PAC, there may be opportunities to develop meaningful conservation measures using analyses that incorporate quantitative extensions of currently available data and information, such as population models.

AGRO 185

Tale of two pests: examples from the battle against invasive species in Hawai'i

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Positioned in the middle of the northern Pacific, the Hawaiian archipelago is the most isolated island chain in the world. The arrival of colonizing species via wind, birds, and ocean currents over the course of millions of years, coupled with Hawaii's geographic isolation and diverse physical environments led to more than 18,000 native plant and animal species and the world's highest degree of endemism—native species found nowhere else on Earth. Like on many islands, the native biota is vulnerable to the effects of biological invasions. Isolation ensured that all but two species of terrestrial mammals—bats—could arrive and become established, and species like amphibians, land reptiles, mosquitoes, and even ants were also absent until brought by people. While not all introduced species become invasive, a small percentage do. Despite the use of predictive modeling tools, commodity import risk assessments, and the work of inspection agencies, new invasive species continue to arrive and many of them are not considered problematic elsewhere. The arrival of the coqui frog (*Eleutherodactylus coqui*) and little fire ant (*Wasmannia auropunctata*) to Hawai'i sometime in the late 1990s are examples. Because they were novel pests, there were no known or registered control products and application methods. Luckily, there were specialists that could focus their research efforts and resources to quickly look for control options, but the time it took to go from bench to market allowed these species to become established on Hawai'i island, where they are a constant source for invading the rest of the state and export market states.

AGRO 186

When the crop is an endangered species: Managing risk and efficacy in the use of pesticides on endangered species preserves in the western U.S.

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In non-agricultural contexts, such as stewardship of conservation lands, the definition and delineation of both crop and pest may be more challenging than in classic agricultural contexts. This situation may be represented in the extreme when managing endangered species *in situ*. Here, the "crop" is not only the endangered (focal) species, but the vegetation matrix, pollinators, prey and predator species—any biotic components that support its desirable habitat. Further, the "pest" must be considered in both its potential competitive and beneficial roles before being identified as a target for control. As such, the desirable (non-target) species are abundant and diverse and the risk threshold is very low. However, given that maintenance and improvement of conditions *in situ* for endangered species typically involve managing vegetation, essential conservation tools include tested, effective herbicides. Once a target (or targets) for control has been identified, the most appropriate control method (*e.g.*, mechanical, prescribed burn, chemical, etc.) must be determined. When the chemical approach has been selected as providing the best benefit:risk ratio, the pesticide's effective and safe use relies not only on strict adherence to label directions. Further risk management measures towards safeguarding the focal (endangered) species may include pre-application monitoring, conducting a pilot test, or combining two or more control methods. The Center for Natural Lands Management (CNLM) is one such organization that selectively uses herbicides on its preserves in California and Washington state, all of which are occupied by state- or federal-listed species or are sensitive wetlands. Although additional information on the effects of pesticides—particularly cumulative impacts and development of resistance—within ecosystem context is important, their safe and effective use will always require well-reasoned application because each situation is unique. The conservation applicator—like a farmer—is most effective when practicing in a long-term and adaptive management context.

AGRO 187

How growers make decisions: An economic perspective

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The quantity and quality of information available to growers is vast, particularly related to pest management. Once products are registered, growers must make informed decisions regarding when to apply based on localized pest pressure, what suite of products or methods of control to apply, how much to apply, and where to apply. While product labels provide guidelines, growers must adapt the label to their pest management needs, while also considering sensitive habitats and species protection. Many growers depend on professionals, such as crop consultants, to assist in making decisions; however, not all growers have access to professional services and must make complicated pest

management decisions themselves. Regardless of crop or location, poorly informed decisions can have significant economic consequences, from reduced yields to higher production costs. We will provide an overview of how growers make pest management decisions with focus on economic consequences. We will also explain how economic consequences are intertwined with the ecological outcome of grower activities and choices related to pesticide use.

AGRO 188

On-farm biodiversity: Alternative management practices to support multi-species habitat

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Not all farmland is made equal. In some cases, chronic underperforming acres exist, and rethinking how we characterize these lands can lead to new opportunities for alternative management practices such as establishment of biodiverse habitats that can benefit multiple species, including ground birds, pollinators, and other wildlife. Satellite imagery, vegetation maps, and yield analytics enhance decision making on farms and can be used to characterize and identify sub-field areas that may be better suited for activities other than cultivation. Ensuring the availability of functional habitats for species may appear to be at odds with land use objectives such as crop and livestock production, but The Climate Corporation and Pheasants Forever have found common ground to shift grower perspectives through partnerships, education, and science. Key elements of this partnership are to encourage cross-sector collaboration, decision support tools, and incentives to create beneficial outcomes for both the growers and the neighboring environment. By leveraging Climate's data science prowess and Pheasants Forever's vast knowledge of habitat practices, this partnership aims to convert non-profitable acres into conservation programs that can improve habitat and biodiversity while also improving the farmer's bottom line. With the goal of serving the needs of our farmers while creating and maintaining suitable wildlife habitats, this talk will describe the methodology used to gain insight into acres combined with pheasant habitat site suitability analysis.

AGRO 189

Modernizing inhalation risk assessment: Multi-scale mechanistic modeling, novel approach methodologies (NAMs) and modular workflows

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Many federal agencies have testing requirements or perform assessments of inhalation toxicity. These pose challenges as they entail consideration of both the physicochemical properties (PC) of the agent and its interaction with the complexity of the respiratory tract. Dosimetry models have proven effective to integrate PC properties with airflow parameters and airway architecture to translate dose across species. Model structures vary based on PC properties such as particles vs gases, and reactive gases vs volatile organic compounds. Recent scientific, economic, and ethical drivers have led to interest in developing human-relevant, mechanistically based approaches to testing, known as new approach methodologies (NAMs). The adverse outcome pathway (AOP) framework helps to organize data on adverse effects and identify key events (KE) of pathogenesis. Starting with a molecular initiating event, pathogenesis is characterized as a signature sequence of KE observed at various levels of biological organization. The aggregate exposure pathway (AEP) framework organizes exposure data, encompassing environmental transport and transformation as well as toxicokinetic interactions within organisms leading to the target site exposure (TSE). Coupling the AEP and AOP frameworks provides a source-to-outcome continuum to enable risk-based, hazard-based, or exposure-based decision making. Alignment of TSE resulting from various exposure platforms was emphasized by the NAS for evidence integration. Multi-scale dosimetry models allow quantitative description of the interactions among PC properties, key anatomical and physiological features, and major mechanisms of ADME (absorption, distribution, metabolism, and excretion); to characterize a TSE and aid *in vitro* to *in vivo* extrapolation, as well as advance quantitative AOP for evaluation of response-response relationship among the KE. Multi-scale dosimetry models coupled with AOP insights can guide strategic development of an integrated approach to testing and assessment, which is useful to informing testing requirements and interpretation and synthesis of results. Modular workflows and reporting standards based on the FAIR (findable, accessible, interoperable, reusable) principles will advance dosimetry models and scientific data management to ensure utility and impact.

AGRO 190

Cellular dose is the key driver for respiratory IVIVE

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There is need to identify non-animal alternatives to assess the acute toxicity of inhaled materials for hazard identification. In vitro exposure of 3D human airway cultures grown at the air liquid interface (ALI) may provide an immediate path to identify critical points of departure relevant for human risk assessment. We examined cytotoxic injury exposure-response profiles of large (Epithelix Mucilair HF; MAF) and small (Epithelix Smallair HF; SAF) human airway epithelial cultures to sodium dodecyl sulfate (SDS) applied directly to the apical epithelial surface via pipetting (50 µL) or as a liquid aerosol (VitroCell Cloud; n=3/SDS dose). Multiple functional endpoints including transepithelial electrical resistance (TEER; mucosal permeability), lactate dehydrogenase release (LDH; cytotoxicity), IL-6/IL-8 release (inflammation), resazurin assay (cell viability), and histology were used to assess the exposure-response profiles of MAF and SAF cultures. TEER was measured 3 days prior to exposure (D-3), on Day 0 test materials were applied for 24 hours and multiple endpoints were evaluated immediately (D1) and 7 days (D7) after exposure to assess acute and recovery/adaptation responses. The data show that 1) similar exposure-response profiles were observed in MAF and SAF cultures when the dose/unit cell surface area were equivalent between direct application and aerosol exposure and 2) that SAF cultures are more resistant to the direct acting toxicant SDS than MAF cultures. The results of this study underscore the need to measure multiple endpoints to evaluate the acute exposure-response profiles and the ability of the large and

small airway epithelial cultures to recover/adapt to injury. These data also confirm the need to use deposited/absorbed dose and not exposure atmosphere concentration when assessing the acute toxicity of inhalable test materials using alternative *in vitro* test systems. This *in vitro* model is a key component to develop an integrated approach for acute inhalation toxicity assessment that will integrate cheminformatics evaluation of the potential mode of action based on the molecular initiating event (MIE) and key events identified by the appropriate adverse outcome pathway (AOP). Future *in vitro* studies should include rat airway ALI cultures in order to compare regional dose in vitro with dose in vivo using known toxicants in rodents with extrapolation to human airways (IVIVE).

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High throughput toxicokinetic (HTTK) modeling of inhalation exposures

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The inhalation route is important for both occupational and general population chemical exposures. Unfortunately, *in vivo* data describing chemical toxicokinetics (that is, absorption, distribution, metabolism, and excretion) are typically unavailable for the chemicals in commerce and the environment. "High throughput toxicokinetic methods" (HTTK) combine relatively rapid *in vitro* measurements of toxicokinetics with generic mathematical models that make use of the *in vitro* data and physico-chemical properties. We present "httk," our HTTK software tool that includes and integrates both *in vitro* data and toxicokinetic models. We have recently added models for inhalation of both gases and aerosols. The structures of these inhalation models are refactored from previous models to allow for parameterization with *in vitro* toxicokinetic data. Because the models are generic, their expected performance for chemicals lacking *in vivo* data can be estimated based on chemicals that have *in vivo* data available. The inhalation models have been statistically evaluated using EPA's Concentration vs. Time toxicokinetics database (CvTdb). For the gas model, resulting blood and/or plasma concentrations of 41 volatile organic chemicals were modeled across 142 exposure scenarios and compared to published data. The slope of the regression line of best fit between log-transformed simulated and observed measured plasma and/or blood concentrations was 0.47 with an r^2= 0.45 and a log-scale Root Mean Square Error (RMSE) of 1.10. Additionally, log-transformed maximum concentration (C_{max}) and area under the curve (AUC) values were compared, resulting in direct comparison RMSEs of 0.47 and 0.49 respectively. HTTK inhalation models allow for *in vitro-in vivo* extrapolation of volatile compounds, enabling comparison of estimates of bioactive *in vivo* doses with estimates of chemical exposures. These approaches have the potential to integrate *in vitro* toxicity data for air pollutants into chemical risk evaluations.

AGRO 192

Link between delivered dose and cytotoxic responses: Exposing aerosolized materials to a lung cell co-culture system

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Traditional hazard testing models using animal inhalation chambers are time-intensive and financially expensive. Cell culture models combined with aerosol exposure systems have shown promise as surrogates in screening assays when multiple substances require preliminary information on hazard potential associated with aerosolized materials. This paper presents two different aerosol exposure apparati: a settling chamber and a gentle impactor. Both have been demonstrated as aerosol delivery mechanisms capable of exposing cells in culture at the air-liquid interface. The settling chamber was constructed with simple design specifications, but exhibited a low deposition efficiency. The gentle impactor required a more sophisticated design based on aerosol dynamics of target size for the droplet delivery, but ultimately produced 64.25% greater deposition efficiency than the settling chamber. This led to a shorter exposure duration for the same dosage. It also enabled consistent exposure to the same sized aerosols. From these results, the gentle impactor show promise as a high-throughput exposure tool that could be used in conjunction with cell-based co-culture models. Using a triple cell co-culture model of epithelial, dendritic, and macrophage cell-types, the induced inflammatory markers produced a more definitive response that aligns with known irritants versus sensitizers, *i.e* toluene-2,4-diisocyanate and trimellitic anhydride are known respiratory sensitizers and silica is a known respiratory irritant. On the other hand, when using single cell monolayers, no significant differential responses in inflammatory biomarkers was observed. Evaluation of the ability to deliver aerosolized substances consistently and measure cytotoxicity and inflammatory endpoints is possible and is illustrated as a potential high throughput screening tool.

AGRO 193

Applications of i*n vitro* to *in vivo* extrapolation in physiologically based kinetic models to refine inhalation risk assessments

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The USEPA Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry describes a generalized categorical approach for estimating inhalation exposures in the human that would produce internal doses in the airways or the systemic blood equivalent to those resulting from an animal toxicity study. These approaches are most suitable for inhaled chemicals and particulates that are directly toxic. However, when dealing with chemicals whose toxicity results from their metabolic activation in the respiratory tract, more complicated approaches may be necessary. In these cases, it is often necessary to incorporate *in vitro* data on metabolism into a

Physiologically Based Pharmacokinetic (PBPK) model. This presentation will illustrate the approach for incorporating in vitro metabolism data into human inhalation dosimetry predictions using three examples: (1) a hybrid PBPK/Computational Fluid Dynamic (CFD) model of the nasal dosimetry of naphthalene, (2) a PBPK model of the lung dosimetry of butadiene, and (3) a lung deposition/PBPK model of benzo-a-pyrene absorbed on particulate. In each of these cases, human inhalation dosimetry was predicted using in vivo data from the rat together with in vitro metabolism data from both rat and human. Finally, a similar approach will be described for performing in vitro to in vivo extrapolation (IVIVE) of the results of in vitro assays of effects in human cells or tissues. The use of PBPK modeling to support IVIVE will be a critical element as the field of toxicology moves from traditional animal studies to human cell-based assays.

AGRO 194

Deriving inhalation risk assessment endpoints from a new approach method (NAM)

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On September 10, 2019, the EPA administrator signed a directive to the Agency to reduce its requests for and funding of mammalian toxicity studies by 30% by 2025 and eliminate all such testing requests and funding by 2035. Prior to that, EPA held a Scientific Advisory Panel (SAP) meeting in December 2018 to evaluate a New Approach Method (NAM) to refine a point of contact toxicity derived endpoint for inhalation risk assessment for agricultural pesticide handlers. The case study used at the SAP meeting was an approach that Syngenta had proposed to the Agency for inhalation risk assessment. The approach used an *in vitro* system to determine the point of departure for adverse effects. The deposition of inhaled aerosol particles in different parts of the respiratory tract was estimated using a well-established computational fluid dynamics (CFD) airflow and material transport model for the human respiratory tract, developed by the Pacific Northwest National Laboratory. A respiratory clearance model published by the International Commission on Radiological Protection (ICRP Pub 130; Ann. ICRP 44(2)) was used to estimate the retained aerosol particles within the respective parts of the respiratory tract after deposition and clearance. These retained doses were compared to the *in vitro* concentrations to derive the Human Equivalent Concentration (HEC) for occupational risk assessment for pesticide handlers.

AGRO 195

Interpreting and translating nanoparticle cellular toxicity studies to human occupational exposures for better risk assessment

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Translating nanoparticle dose from conventional toxicity testing systems to relevant human exposures remains a major challenge for assessing occupation risk of nanoparticle exposures. As encouraged by *Toxicity Testing in the 21st Century*, researchers increasingly apply high-throughput *in vitro* approaches to identify and characterize nanoparticle hazards, including conventional aqueous cell culture systems for respiratory hazards. Efforts to identify hazards have primarily focused on quantifying adverse biological effects, usually with little consideration of nanoparticle dose or appropriate dose metric as evidenced by frequently published dose-response data using nominal nanoparticle concentration in cell culture media as the favored dose metric. In order to understand cellular dosimetry to semi-soluble silver nanoparticles, we developed a particokinetic model for predicting combined effects of particle sedimentation, diffusion, and dissolution on cellular dosimetry for *in vitro* systems (ISD3). We applied ISD3 to simulate particle and ion dosimetry for silver nanoparticle (20 and 110 nm) exposures to three different types of macrophages (RAW 264.7 macrophages and bone marrow derived macrophages from wild-type C57BL/6J mice and Scavenger Receptor A deficient (SR-A$^{(-/-)}$) mice). Dose response modeling with ISD3 predicted dose metrics suggest that the amount of ions in cells and area under the curve (AUC) of ion amount in cells best predict cell viability after silver nanoparticle or ion exposures. We simulated human occupational nanoparticle exposures using the Multiple-Path Particle Dosimetry Model (MPPD) and estimated alveolar ion amounts in cells using published silver nanoparticle dissolution levels in simulated pulmonary fluid. Using multiple models to align appropriate dose metrics allow us to translate results from *in vitro* studies to human pulmonary doses from occupational exposures. Translating *in vitro* studies to human exposures is a critical component for successful nanoparticle risk assessment and worker protection.

AGRO 196

Using dosimetry to improve exposure and risk assessments for firefighters

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Epidemiological studies have demonstrated that wildland firefighters are at increased risk of heart disease and cancer due in part to exposure to smoke and particles. While we have gained much of our understanding of the relationship between ambient particulate matter (PM) and cardiac morbidity and mortality with both short- and long-term exposure, wildfire fighters are exposed to conventional ambient PM and to PM from biomass combustion. The composition of wildfire smoke differs from that of conventional ambient PM, but still represents a significant risk factor for adverse cardiovascular health effects. The objective of this paper is to use dosimetric parameters to improve the assessment estimate of risk to firefighters of heart disease-related and cancer-related mortality. We had previously hypothesized that PM2.5 health effects data could be a useful surrogate to estimate health risks from wildfire smoke exposure and that, after correction for the mass contribution of coarse PM, PM2.5 and the PM3.5 (or PM4) one could use PM2.5-derived health outcome data to estimate wildfire fighter health risks. Data obtained from working firefighters doing various tasks and PM exposures measured while the tasks were being performed had been previously acquired, and risk estimates indicated that over a lifetime of exposures firefighters could be subject to increased health risks. Using ventilation measurements and a computational model of

respiratory tract deposition, we now have improved the assessment of dose and can better assess potential risks than had been done previously.

AGRO 197

Lung micro-dissection and *ex vivo* exposure to assess molecular binding

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Conducting an exposure and binding study with aerosols is a challenging safety design and engineering problem. *Ex vivo* dosing of fresh, metabolically active tissue is an alternative dosing procedure that mitigates safety and containment issues of an intentional aerosol. Nonciliated bronchiolar epithelial (Club) cells, as both the primary target for metabolically activated pulmonary cytotoxicants and the progenitor during repair after bronchiolar injury, are critical for distal airway epithelial function and regeneration. The role of Club cells in normal lung function for assessing sensitivity to cytotoxicants and expression of differentiation markers vary by airway level and species. Lungs of adult mice were inflated with 1% agarose and distal airways were micro-dissected using established procedures to isolate the metabolically active part of the lung for *ex vivo* exposure to ^{14}C-naphthalene (NA). The *ex vivo* exposure in culture with 250 µM NA produced a similar concentration of NA in the tissue by liquid diffusion as was produced by 10 ppm NA aerosol exposure for 1 h. Each mouse lung was split in half with one side serving as the exposure group and the other side serving as undosed control. Vials were sealed and incubated in a water bath for 1 hour at 37°C, followed by cooling and rinsing to remove all unbound NA. Tissue can then be processed to harvest protein or DNA to quantify and identify adducts. In this study, naphthalene-derived DNA adducts were quantified using accelerator mass spectrometry to measure excess ^{14}C in isolated DNA samples. Identification of specific NA metabolite adducts requires quantitative digestion of the DNA into individual nucleotides followed by a molecular separation and then quantitation.

AGRO 198

Modeling deposition and uptake of an inhaled puff emitted from an electronic nicotine delivery system (ENDS) in the human respiratory tract

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A puff from an electronic nicotine delivery system (ENDS) contains multi-constituent aerosols and vapors. The mixture is highly unstable and undergoes rapid thermodynamic changes. As a result, there will be significant changes in the characteristics of inhaled aerosols and vapors immediately after generation in the ENDS and while traveling through the respiratory tract. These changes need to be studied and accounted for when developing a dosimetry model to assess both vapor uptake and aerosol deposition in the respiratory tract. A coupled vapor uptake and aerosol deposition model was developed for a puff made up of water, glycerin, nicotine, propylene glycol, flavors, and byproducts. The deposition model included the coagulation of airborne materials at high aerosol number concentrations and vapor exchange between the liquid (aerosol) and vapor phases. The model accounted for the mixing of the puff with the dilution air at the end of mouth hold and reserve air when the puff reached the pulmonary space. The fate of all constituents in the puff was obtained at different sites of the respiratory tract. Overall, about 90% of the nicotine and propylene glycol were taken up by lung tissues whereas glycerin deposition was under 60%. Tissue dose from droplet deposition was higher than that for vapor uptake for all constituents of the puff. The fate of byproducts and flavors was highly dependent on the chemical characteristics of the constituents. The dosimetry model is a powerful tool to inform the exposure and health risk evaluation of ENDS use.

AGRO 199

Applying dosimetry models and software tools to facilitate risk evaluation of electronic nicotine delivery systems

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Electronic nicotine delivery systems (ENDS) heat liquids containing nicotine, water, propylene glycol, glycerin, and flavorings to deliver an aerosol mixture to the user. Potential health risks to ENDS users depend on the dosimetry of the ENDS aerosol in the respiratory tract, which is affected by factors such as the constituent concentrations, device characteristics, and user behavior. Dosimetry models were developed to describe ENDS aerosol dosimetry in the oral and lung airways of the respiratory tract. Aerosol dynamics integrated into the dosimetry models included droplet coagulation at high concentrations and constituent phase change from droplets to vapors. Additionally, a physiologically-based pharmacokinetic (PBPK) model was implemented to simulate nicotine disposition following lung deposition. The predictive models were integrated into a Java-based software platform that accepts inputs for user topography, ENDS profile, and constituent properties to simulate daily ENDS usage and compute the respiratory deposition of the ENDS aerosol and nicotine kinetics. Available toxicological data on individual ENDS constituents, including nicotine, humectants, flavors, and byproducts, were included in the software platform to compare with predicted internal lung dose following ENDS use. The software platform packages advanced aerosol dosimetry models and toxicity data into a user-friendly interface and demonstrates the utility of dosimetry modeling results to inform exposure and health risk evaluation of ENDS constituents.

AGRO 200

Monoterpenoids as nematicides

Joel R. Coats[1]*, jcoats@iastate.edu, Colin Wong*[2]*, James S. Klimavicz*[1]*, Edmund J. Norris*[1]*, Greg L. Tylka*[3]*, Richard J. Martin*[4]*. (1) Department of Entomology, Iowa State University, Ames, Iowa, United States (2) Entomology, Iowa State University, Ames, Iowa, United States (3) Plant Pathology, Iowa State University, Ames, Iowa, United States (4) Biomedical Sciences, Iowa State University, Ames, Iowa, United States*

Monoterpenoids and many of the plant essential oils from which they originate have been shown to have activity as insecticides, insect repellents, and synergists for conventional insecticides. One logical extension of our work in this area is the screening of a suite of monoterpenoids as nematicides. In this project we have evaluated a broad series of naturally-occurring monoterpenoids, from plant essential oils, against three very different types of nematodes: one was a plant-parasitic species, one was an animal-parasitic species, and one was a free-living species of nematode. The plant-parasitic species was the soybean cyst nematode (SCN); the animal-parasitic nematode was the swine roundworm (SRW), and the free-living species was *C. elegans*. This exploration involved testing the SCN in an egg-hatch bioassay in water in the laboratory, studying a nicotinic acetylcholine receptor from SRW cloned into *Xenopus* oocytes, and testing survival of *C. elegans* in a 96-well plate bioassay that quantifies metabolism of an exogenous substrate. The studies are valuable to identify lead molecules for directed synthesis programs to design, synthesize, and screen biorational analogs and derivatives for possible utility against SCN or SRW. It also provides some interesting comparisons in susceptibility of the three types of nematodes to similar series of monoterpenoids.

AGRO 200

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AGRO 201

TLC method for determination of kairomones for male Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), from tea tree oil

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The Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is an economically important invasive pest globally, attacking a wide variety of fruits and vegetables. In response to this threat, researchers are developing kairomone-based trapping systems to enhance medfly detection and control. In this study, we focused on the separation and determination of attractive kairomones from tea tree [*Melaleuca alternifolia* (Maiden & Betche) Cheel] essential oil using a combination of thin-layer chromatography (TLC) and laboratory bioassays with sterile male *C. capitata*. After TLC separation, the plate was exposed to the flies where they aggregated around kairomones directly on the TLC surface. To confirm the activity, the developed plate was cut into five zones which were then subjected to short-range two-choice bioassays. In addition, zones were separated using preparative-TLC and fractions were evaluated using electroantennography (EAG) to quantify medfly olfactory responses. EAG analyses and short-range bioassays provided similar results, indicating that the TLC-based bioassay system can be an effective, rapid screening method for identification of insect kairomones from plant extracts or essential oils.

AGRO 202

Novel mosquito-specific toxin from a marine strain of *streptomyces* for insecticide development

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Mosquito-borne diseases such as West Nile Virus, Zika, and chikungunya are on the rise in the United States and, with malaria, mosquitoes continue to contribute to the death of over a million people each year worldwide. New approaches for mosquito vector control are imperative as currently used insecticides show issues of non-selectivity amongst target and beneficial insects, and there is increasing rise of resistance amongst target organisms. With major challenges to developing new control efforts being insuring human safety, limiting off-target effects in the ecosystem, and confronting resistance, a novel mosquito cell-based screening platform was used to identify lead compounds with the potential to

safely and specifically eliminate this disease vector. Our library of marine microbial-derived natural products was screened to systematically search for compounds that were lethal to cultured mosquito cells while showing negligible activity against other insect cell lines, *Drosophila melanogaster* (Kc and S2R$^+$) and *Spodoptera frugiperda* (SF9). One compound, a novel boron-containing macrolide, NP-34, derived from a strain of *Streptomyces*, was determined to be a selective mosquito toxin after cellular screening. Exposure to NP-34 led to approximately 80% cell death at a concentration of 50 nM whereas toxicity to *D. melanogaster* and *S. frugiperda* cell lines was negligible with less than 10% death at the same concentration; this selective phenotype was further observed when tested against larval and adult stage mosquitoes at concentrations similar to that of permethrin treatment. Based on the above studies, along with initial mouse toxicity studies, we believe this unique boron-containing macrolide is a promising compound for the development of a novel, human-safe, and eco-friendly insecticide. Due to the selectivity profile of NP-34, we are taking a two-pronged approach to understand the mechanism of action against mosquitoes. 1) An affinity purification approach with NP-34 tagged for target pull down. 2) The development of resistant mutants combined with RNAseq and genomics. Preliminary results with permethrin-resistant mutant lines of *A. aegypti* have revealed susceptibility to NP-34. Additionally, we are in the process of isolating analogs similar to NP-34 produced by the same bacterial strain and creating semi-synthetic analogs to find the most potent and selective version of NP-34 for insecticidal development.

AGRO 203

Pesticidal activities of sesquiterpenolids from yacon leaf trichomes

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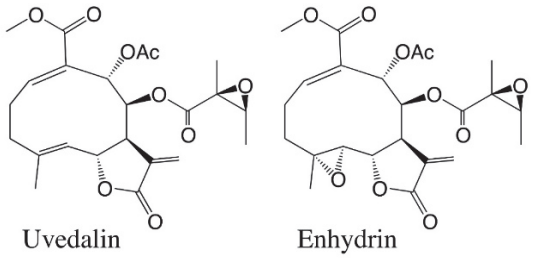
Yacon (*Smallanthus sonchifolius*) is an Asteraceae crop, and its enlarged roots are used as a foodstuff. Since this plant exhibits high tolerance to pests and inhibits growth of neighbor plants, it is easy to cultivate using small amounts of pesticides. A large number of glandular trichomes are present on the leaves of this plant and have been suggested to function as these resistance factors. In order to collect the constituents of glandular trichomes, leaves were rinsed using dichloromethane (DCM) to obtain the rinsate, and plant residues were subsequently extracted by DCM to obtain the DCM extract. Biological evaluations revealed that insect antifeedant activity against the common cutworm (*Spodoptera litura*) and phytotoxicity tests were performed using lettuce (*Lactuca sativa* cv great lakes) and Italian ryegrass (*Lolium multiflorum* cv wasefudo) seedlings. The main constituents of their glandular trichomes were isolated by silica gel flash chromatography using a hexane and ethyl acetate solvent system, and were identified as the sesquiterpenolids (SLs), uvedalin, its oxidative congener, enhydrin and uvedalinaldehyde, known as melampolides based on spectroscopic data. These SLs exhibited a strong insect antifeedant activity similar to the positive control azadirachtin A, and thus, may function as the chemical defense system in this plant toward phytophagous insects. Additionally, the two congeners, parthenolide from *T. parthenum* and erioflorin from *H. strumosus* showed moderate insect antifeedant activity against common cutworms. And almost test SLs also showed phytotoxicity, and uvedalin was the strongest inhibitor against lettuce seedlings, and uvedainaldehyde was the strongest inhibitor against Italian ryegrass seedlings. As the results, the substituent patterns of SLs maybe concerned with appearance of these pesticidal activities.



Uvedalin Enhydrin

AGRO 204

In pursuit of Inatreq™ active's unstable metabolite: M14

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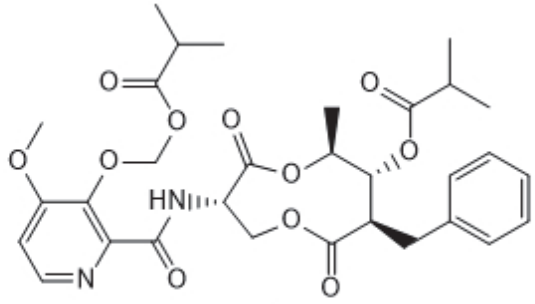
Inatreq™ active is Corteva Agriscience's newest commercial antifungal compound for the control of *Septoria tritici* on wheat. Inatreq is a semi-synthetic active ingredient derived from the natural product UK-2A. Multiple metabolite and degradation studies were performed to support Inatreq registration. Over 45 metabolites and degradants were identified. One particular metabolite, M14, was the last to be confirmed structurally due to its size (MW632), the number of possible candidate structures and, ultimately, its relative instability. The total syntheses of three compounds proposed as M14, along with the analytical methods used for structural confirmation of this unstable degradant, will be presented.



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AGRO 205

Fungicidal constituents from phytopathogens

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Phytopathogenic fungi use the host plant to survive by absorbing nutrients from the host plant. Certain fungi produce fungitoxic compounds to compete with other fungi that can

infect the host plant. Thus, we speculate that plant pathogenic fungi may be good sources from which to isolate and identify fungicidal compounds that can be used in crop protection. We have isolated and identified fungi that cause disease symptoms on crops and other ornamental plants. These fungi were grown in potato dextrose agar and were identified by molecular techniques. The liquid culture broths of various fungi were extracted with ethyl acetate followed by butanol. These extracts were tested on TLC bioautograpy to detect the presence of antifungal constituents. The fungi belonged to numerous genera such as Diaporthe, Phoma, Curvularia, and Pyricularia. In this paper, extraction, isolation, structure elucidation, and bioassay of antifungal constituents from various genera of phytopathogenic fungi will be discussed.

AGRO 206

Determination of ferulated sugars with activity against insect and fungal pests of maize

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Mechanisms of leaf and kernel resistance in sorghum and maize have been extensively investigated, but less information is known about stalk resistance mechanisms. Pith from low lignin mutant *bmr6* of sorghum caused much higher mortality of corn earworms and fall armyworms compared to pith from wild type plants. Freeze dried pith was extracted with methanol and fractionated. Multiple fractions had activity against insects. LC-ESI-MS characterization indicated that glucose and sucrose derivatives of ferulate, which were sometimes acetylated, were the principle components, but positive identity could not be determined. Large scale fractionation and purification followed by NMR was used to further characterize the components in the active fractions. Further activity against insects and plant pathogens was determined. Representative maize lines with varying stalk resistance to insects and plant pathogens were also examined for these compounds.

AGRO 207

Discovery of photostable abscisic acid analogs and novel formulations

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Abscisic acid (ABA) is an important plant hormone that regulates plant growth, signal transduction, and plant immune defense. Exogenous application of ABA has a variety of effects such as promoting the rooting and improving the drought resistance of crops, prolonging the flowering period, and increasing the fruit coloring rate. However, the half-life of ABA is only 24 min, and it is easy to isomerize and lose its biological activity under ultraviolet (UV) radiation, which increases the cost of field application of ABA. Under sunlight, nearly 50% of ABA can be converted to *trans*-ABA. Photodegradation products include 4-*trans*-3 methylene ABA and 4-*cis*-3-methylene. Potent and photostable ABA analogues were designed and synthesized. Further bioassays showed that under 150 mM salt stress, 10 nM a substituted benzoylaminocyclopropionic acid B2 could increase wheat biomass and salt resistance, and its mechanism of action was revealed at the biochemical and molecular level. In addition, the new plant growth regulator candidate showed improved photostability and showed better activity to inhibit wheat seed germination. To increase the photostability and biological activity of ABA, eco-friendly photoprotective agents were introduced for formulation preparation. Soluble 2-hydroxy-4-methoxybenzophenone-5-sodium sulfonate (BP-4) and lignosulfonates were found to be effective agents to maintain ABA activity under UV radiation. Approximately 26% and 52% ABA were kept when 280 mg/L ABA aqueous solution was irradiated by UV light for 2 h in the presence of 200 mg/L BP-4 and 2000 mg/L Ufoxane 3A, respectively. Recently, we encapsulated ABA into cetyltrimethylammonium bromide (CTAB) modified alkali lignin (AL) to induce the formation of self-assembly ABA@AL-CTAB nanoparticles. The controlled-release properties, anti-photolytic properties and thermal stability of ABA microcapsules have all proven to be excellent. In summary, it is very important to discover photostable ABA analogues and develop photoprotective ABA preparation. The latter is most likely to be used in agricultural production in the near future.

AGRO 208

Linking dicamba volatility from amine salts to principles of solid phase organic chemistry

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In recent years, the introduction of dicamba tolerant soybeans and cotton to the market has increased dicamba use rates, contributing to reports of damage to surrounding crops and vegetation resulting from off-target dicamba drift. The drift phenomenon occurs from two pathways: primary drift, when droplets containing the herbicide travel off-target during application, or secondary drift, when the herbicide volatilizes after deposition on soil or leaf surfaces. Secondary drift is controlled by the addition of amine additives such as dimethylamine (DMA), diglycolamine (DGA), and *N*,*N*-bis-(3-aminopropyl)methylamine (BAPMA) to the dicamba formulation. We hypothesized that the ability of amine additives to prevent dicamba volatility stems from the formation of a supramolecular assembly upon drying to a salt residue. When dicamba volatility is measured from amine salts, a nonlinear trend is observed for amines with multiple hydrogen bonding sites (*i.e.*, DGA, BAPMA). We hypothesize that dicamba volatility is therefore controlled by multiple intermolecular interactions involving the amine additive. Furthermore, when additional formulation components (*e.g.*, the herbicide glyphosate) are added, BAPMA is able to control dicamba volatility to a greater extent than DMA or DGA, indicating that inclusion of multiple hydrogen bonding sites in amine structures can substantially reduce volatility levels even from multicomponent mixtures. To further test the hypothesis that the number of hydrogen bonding sites determines the ability of amines to reduce herbicide volatility, we compared dicamba volatility from formulations prepared with eight different amine molecules. We determined the presence of multiple hydrogen bonding sites was a superior predictor of dicamba to control dicamba volatility than pK_a, molecular weight, or amine vapor pressure. Our results indicate that the number of hydrogen bonding sites on an amine, which likely promote the formation of a supramolecular assembly in the residue, is a key factor that

should be considered when designing low volatility herbicide formulations.

AGRO 209

Minimizing agrochemical spray drift via introduction of rheology modifiers

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Agrochemical spray drift is both a crucial challenge in sustainable agriculture and broadly impactful due to the manifold applications of such sprays. Therefore, there is a strong motivation to develop effective, low-drift formulations of these agrochemicals. These next generation tools should regulate the overall distribution of droplets to reduce the production of smaller droplets, while tightening the overall distribution of droplet diameters into an envelope of effectiveness. Existing research in antifoaming agents can be leveraged in this application. Antifoams alter the rheology of the thin films which constitute foams, driving their degradation and rupture. Atomization of sprayed fluid sheets can likewise be controlled via the introduction of rheology modifiers. This multispecies fluid sheet decomposes more rapidly and produces a droplet diameter distribution with a greater Mean Volume Diameter (MVD) than a single species sheet sprayed under the same conditions. Experimental observations have shown that the introduction, at a low volume fraction, of liquid rheology modifiers such as Methylated Soybean Oil (MSO) and Silicon Oil-in-water (SiO), triggers the formation of holes in the sprayed sheet which destabilizes the sheet altering the resulting droplet distribution. Further control of this distribution can be exercised by introducing solid silica particles to create a multiphase, multispecies mixture. In this presentation, we generate several statistical Design Of Experiments (DOEs) which employ commercially available nozzles to explore the parameter space of novel multiphase/multispecies agrochemical spray formulations and report their corresponding drift reduction potential. We find multiple formulations which reduce small droplet (driftable fine) production as well or better than current state-of-the-art (Interlock™, Synergen OS) low drift formulations, while keeping the larger drops of similar size.

AGRO 210

UAV spray testing in a wind tunnel

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Use of unmanned aerial vehicles for pesticide spraying on crops has expanded rapidly over the past 3 years, particularly in East Asia but also in many other regions including North America. Regulatory agencies, crop protection companies, and others have become increasingly aware of this expansion and the many open questions related to precision of application, air transport of spray material, and risk assessment. Multi-rotor UAV sprayers are unlike conventional aerial or tractor boom sprayers in many ways: payload and power constraints necessitating the use of ultra-low volume tank mixes, complex vortex effects, unusual nozzle configurations, different release heights, etc. There is an emerging body of literature describing field testing of UAV sprayers in cropped plots with a focus on foliar coverage and product performance but far fewer studies that address questions of environmental and human safety. In particular, greater understanding is needed in the areas of in-swath uniformity and off-target spray drift under a variety of spraying conditions. Thus, a series of studies were initiated examining the effects of a range of spray settings, such as height, speed, spray quality, and nozzle selection with the goal of understanding the factors that influence on-target and off-target deposition. A sufficiently robust body of data could help inform the development of a predictive modeling tool that can address the range of equipment and application scenarios that are foreseen with automated machinery. This presentation describes the phase that was conducted in a wind tunnel in 2019. The wind tunnel was selected for certain variables that were believed to be too sensitive and/or difficult to control in a field setting, specifically the effect of payload and wind speed on spray pattern and downwind drift. Three different nozzle types were affixed in the under-rotor configuration to a DJI Agras MG-1P octacopter and sprayed with a fluorescent tracer in a wind tunnel, and the deposition collected from the near-upwind to a distance of 20 meters downwind from the center target. A key feature of this study design was a framework that allowed the drone to hover with minimal constraints so that it would adopt its attitude (pitch, roll, yaw) autonomously in response to the height requirement and wind perturbation, thus simulating the effects of a realistic field spray in the crosswind direction.

AGRO 211

Modelling off-target plant canopy interception and surface retention of pesticide spray droplets

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Currently, plant canopy capture efficiency is not factored into the regulatory off-target drift risk assessments. For on-target applications, the pesticide application is carefully managed to optimize crop coverage by applying formulations with built-in adjuvants or tank-mixing with adjuvants to aid retention of spray droplets. These efforts take into consideration that plant species/canopy types have diverse morphology and surface characteristics, and as such will retain varying levels of applied pesticide loading. This concept is extendable to deposition of drifted spray droplets and the unique capture efficiency potential of off-target plants species, thus providing an opportunity to refine the current regulatory risk assessment practice where 100% drift interception and retention is assumed. This work presents a methodology to quantify plant canopy capture efficiency using combined plant surface wettability potential, spray droplet characteristics and plant morphology data. These data coupled with modelling has the potential to improve scoping and design of non-target spray drift studies and to inform the risk assessment process. We use wind tunnel experiments to demonstrate variability in spray drift capture efficiency between seven common field crops. We then use regression analysis of drift deposition rates to show the explanatory power of plant shape as the

ratio of plant surface area to volume (derived from photogrammetry), and plant surface roughness (derived from Scanning Electron Microscope imagery). An application of ANSYS CFX (a computational fluid dynamics (CFD) modelling tool) to simulate the wind tunnel experiments is also presented, with model-predicted plant canopy efficiencies compared to measured canopy interception.

AGRO 212

Assessing the risk of chemical mixtures in agricultural landscapes

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Chemicals used in crop protection are regulated and undergo an environmental risk assessment prior to authorization. Environmental risk assessments are always conducted on single active ingredients and may also be conducted for formulated products, which can include more than one active substance. In addition, some countries may request the assessment of pesticide tank mixes containing more than one formulated product. Beyond these intentional mixtures, applied concurrently in time and space, there is the potential for combined exposure of aquatic environments to multiple chemicals resulting from the combination of land uses, crop types and management practices within catchments. The detection of multiple chemicals in the environment has raised concern that current regulatory processes may be insufficient to assess the environmental risks of mixtures resulting from the use of different chemicals within agricultural landscapes. A scenario-based approach can be used to determine whether mixtures of chemicals pose risks over and above any identified using existing approaches for single chemicals. This presentation will cover approaches to assess chemical mixtures in agroecosystems though the lens of risk assessment. Terrestrial and aquatic examples will be presented to show how environmental mixtures can be evaluated with a risk-based approach.

AGRO 213

Application of SWAT+ in a high intense agricultural watershed in Belgium

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The Soil and Water Assessment Tool (SWAT) has been an internationally leading model for predicting the fate and transport of non-point source agrochemicals at the watershed scale for nearly two decades. SWAT is a semi-distributed model based on a hydrologic response unit (HRU), a conceptual component that aggregates areas with similar landscape characteristics within a watershed, including land use and soils, to simplify the calculation of water fluxes, as well as sediment and chemical loading to a channel system. Strengths of SWAT for the simulation of agrochemicals include the comprehensive hydrologic model, channel routing and in-stream chemical transport processes, and extensive customization of agronomic management practices. A recently released enhanced version of SWAT, SWAT+, provides two main advantages over the currently used SWAT model for landscape level watershed modeling for pesticide risks assessments: (1) flexible spatial representation of landscape features and their interactions and (2) advanced agricultural management practices, including rule-based probabilistic pesticide applications. Here, we provide an overview of the new features of SWAT+ and present the application of SWAT+ in a high agricultural intensity watershed in Belgium. We focus on assessing whether implementing the SWAT+ probabilistic pesticide application approach results in similar pesticide concentrations compared to what is achieved using detailed field-level application data available from a farmer survey.

AGRO 214

Novel approaches for assessing management of tile-drain agricultural chemical transport

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The study of conservation best management practices and their effectiveness at mitigating the off-site transport of agricultural chemicals is an established research field. While runoff and erosion have received much study focus, there is a growing need for new and novel approaches to monitor nutrients in tile-drained systems. A sophisticated water monitoring system was developed that utilizes technology to create an automated pass-through sampling site, employing state of the art nutrient sensor technology to monitor nitrate concentrations in thirty-seven discrete subsurface agricultural drainage tile laterals at a sampling rate of between two and four hours per sample at each tile. This design has proven highly efficient and provides a data resolution that would be impractical to consider under conventional sampling methods. It also allows for the replicated study of several treatments (different mitigation practice approaches) and control plots, with little additional cost per added plot. Additional benefits include the ability to view data in real-time, allowing researchers to observe real-time rainfall event-based plot responses without the analytical results delay from a laboratory. The developed site data provides the needed resolution for tile drain model validation or developments.

AGRO 215

Global parameterization of the APEX model based on simulation of edge-of-field phosphorus losses at monitored sites in Vermont and New York

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Edge-of-field losses represent an important pathway by which nutrients and pesticides can move off-field and be transported to adjacent waterways. USDA's APEX model was used to simulate field-specific land/soil conditions and agronomic practices, and to predict off-field surface and sub-surface (tile drainage) transport of phosphorus at twelve monitored sites in Vermont and New York. Calibration and validation of APEX models for seven sites identified a set of "global" parameters

for the model based primarily on minimizing model prediction bias and magnitude of model error in annual total phosphorus loss predictions. Performance metrics were then applied to an independent set of five validation sites to assess the robustness of the global parameterization. Monte Carlo analysis was conducted to assess parameter uncertainty in soils. The results of the global parameterization calibration using representative (SSURGO-derived) soils properties demonstrated that APEX-predicted average annual total P losses were less than 37% above or below observed values, with predictions for two thirds of sites deviating by less than 25% above or below observed values. Model simulations also showed no systematic bias. The Monte Carlo analysis demonstrated that uncertainty in SSURGO-derived soils datasets can account for the observed bias in APEX simulation results. The calibrated global parameter set has been incorporated into Farm-PREP, a Vermont-based tool designed to optimize land and nutrient management options at the farm scale to achieve P load reductions established in the Lake Champlain phosphorus TMDL. The calibrated and validated APEX model in Farm-PREP is being considered by the state of Vermont for use in quantifying water quality improvements as part of a Payment for Ecosystem Services program that would compensate growers who adopt conservation practices on their farms.

AGRO 216

Early process route to fungicide Adavelt™ active

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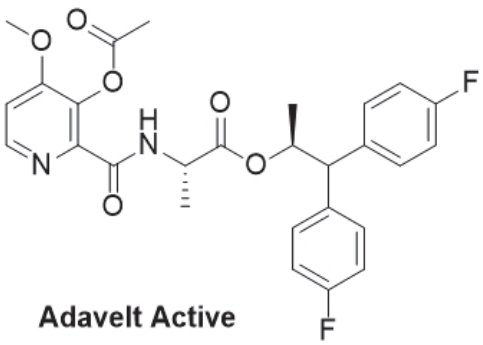
Adavelt is an experimental fungicide inspired by Corteva Agriscience's new commercial fungicide, Inatreq™ active. An early stage process route was identified and developed for the synthesis of Adavelt. These route development efforts led to a safer and atom-economical synthetic route for this structurally complex molecule. This presentation will highlight the overview of current process routes towards the chiral side chain and the pyridine moiety of new active ingredient, Adavelt.



Adavelt Active

AGRO 217

Systematic, risk-based approach to process hazards mitigation in agricultural product active ingredient manufacturing

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Ease of manufacturability is a key factor when estimating the probability of success and sustainability of a new agricultural product candidate. Due to the nature of the chemical reactions and processes central to chemical manufacturing, distinct hazards normally present themselves. These include the potential for rapid heat evolution due to intended and unintended chemical reactions, as well as rapid pressure increase due to gas formation, either of which can lead to over-pressurization of process equipment resulting in rupture, explosion, and/or deflagration. The destructive effects from these hazards can be exacerbated when highly energetic or unstable functional groups are present. The mitigation of the hazards associated with chemical manufacturing operations is essential to the safe production of active ingredients and intermediates. A systematic, risk-based approach to the identification, classification, and mitigation of hazards posed by the manufacturing process is advantageous in that hazards are assigned to specific classes, and each hazard is considered individually for its severity and potential for effective mitigation. This approach offers certain advantages over an ad hoc approach, in which the identification of hazards and mitigation strategies are developed based upon the awareness, experience, and knowledge of individual researchers or project teams. While the ad hoc approach may draw useful information from a wealth of different sources, and can lead to a reasonable set of testing and hazards evaluation, it can also result in an incomplete depiction of all the associated hazards. The systematic, risk-based management approach considers chemical reactivity in terms of heat generation, rates of desired and undesired reactions, rate of gas evolution, potential for destructive effects due to energetic functionality, and scale of manufacture to determine the required levels of evaluation and testing. Using a structured approach, a chemical process is thoroughly vetted to accommodate the wide array of manufacturing equipment and operating disciplines that are found across the globe. Details of this systematic approach to chemical process hazards mitigation will be presented.

AGRO 218

Data-driven development of new solutions to combat herbicide-resistance

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Increased emphasis on food and applicator safety in the agrochemical industry has led to the adoption of less toxic and more environmentally friendly crop protection solutions. However, many of today's most effective herbicides are under the microscope again as certain noxious weeds, such as *Amaranthus palmeri* (Palmer amaranth), develop resistance to the active ingredients. Identifying possible new herbicide ingredients and formulations that overcome resistance requires considerable time and effort to assess effectiveness and regulatory requirements. Having access to

information on existing ingredients, manufacturing processes and experimental test results can help increase confidence in formulation development and decrease research time. Learn how a new comprehensive formulations database and workflow solution is being used by agrochemical companies to streamline the development of crop protection formulations.

AGRO 219

Capillary electrophoresis coupled with electrospray ionization mass spectrometry for the analysis of phosphorus species residues in tree nuts

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Phosphorus oxyanions are important agriculturally as they are used as both fertilizer and pesticide. One of these chemicals, aluminum tris (o-ethylphosphonate) - also referred to as fosetyl-Al, is a registered fungicide. Several years ago, a shipment of tree nuts to the European Union (EU) was found to contain fosetyl residue even though fosetyl-Al is not used on tree nuts in California. The detection was based on H-phosphonate, a hydrolysate of fosetyl that is included in the EU residue definition of fosetyl-Al. H-phosphonate has many sources and sinks in agricultural settings, and this work aims to increase such understanding. A suite of commercially available agrochemical products was obtained, and capillary electrophoresis coupled with electrospray ionization mass spectrometry (CESI-MS) was used to characterize phosphorus oxyanion components, including H-phosphonate. With this setup we can achieve consistent retention times and sufficient resolution to differentiate between phosphorus oxyanion species. The developed method allows for a more accurate identification of phosphorus oxyanion species present and allows for the greater study of how these agricultural products affect the residues of the phosphorus oxyanion species present in tree nuts.

AGRO 220

Practical application and implementation of low pressure gas chromatography-tandem mass spectrometry (LPGC-MSMS) in a high throughput food testing laboratory: Lessons learned

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The premise of Low Pressure Gas Chromatography (LPGC) is not a new one. The first publications about the concept and application date back to the 1960's, but progress had been limited. More recently, this work has been championed by the USDA Agricultural Research Service, who have produced many papers on LPGC and its applications. In this work we present an adaptation and practical implementation of this promising technique in a high throughput food testing laboratory for routine analysis. We introduce a novel column configuration developed by industry for routine laboratory use. We discuss the lessons learned and pitfalls we experienced as well as advantages and disadvantages of the technique. Further, we evaluate the robustness of the technique with respect to different matrices using a QuEChERS extraction.

AGRO 221

Directly screen for pesticide residue in beer without sample preparation

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Barley, hops, and yeast are three of the main raw materials grown for the production and brewing of beer. A variety of pesticides are routinely applied to these crops to help protect them from fungus, disease, and pest attacks during the growing process. However, some pesticides that are applied to these materials can persist in the crops throughout the brewing process and are carried over to the finished beer products. We developed a method for the screening of pesticide residues directly in finished beer using DART-MS (Direct Analysis in Real-Time Mass Spectrometry). Because DART is an ambient ionization technique that can analyze samples in their native form, no sample or preparation of the beer is necessary, and the beer can be analyzed directly. Additionally, the novel pulsed gas ionization technique now associated with DART provides greater sensitivity for trace contamination detection as it eliminates ambient background noise much more effectively. It also provides greater throughput by permitting faster presentation of samples with our automated DART methods. Our high-throughput set up allows for 96 beer samples to be directly analyzed in 6 minutes amounting to only 3 seconds per sample. The DART analysis is subsequently paired with AnalyzerPro XD software for post analysis processing of the data to identify the target contaminants in a heat map visual of relative abundances. This workflow allows for both rapid and accurate high-throughput screening of many food and beverage products for contaminants and pesticides that may be present.

AGRO 222

Modifying methods for metabolite analysis: Method development for the determination of ethylenethiourea residues

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Analytical methods were successfully validated for mancozeb and its metabolite, ethylenethiourea (ETU); each method for the respective analytes was developed and validated in agricultural commodities, animal matrices (bovine), soil, and water. Developing analytical methods to analyze metabolites can require troubleshooting and problem solving, especially if the analytes and/or matrices are difficult to work with and extract. The structures of mancozeb and ETU differ significantly, and it was not possible to extract both analytes simultaneously using the same extraction method. Mancozeb is incredibly unstable after addition of solvent, and must be derivitized first before extraction, but after derivitzation, the analyte was easily extracted using the QuEChERS method. In contrast, ETU is very small and polar and has a stable ring structure, but the QuECHeRS method resulted in low recoveries. Instead, a simple extraction with formic acid in water followed by clean-up with C18 sorbent was used for ETU and resulted in acceptable recoveries. Column selection is a critical component of successful method development – due to the significant difference between the two analytes,

two different columns were also necessary. A HSS (high strength silica) column was used with mancozeb to provide additional stability and a PFPP (pentafluorophenylpropyl) column was chosen for ETU, which has been shown to retain small, polar molecules well. One radiovalidation for ETU was also performed and produced acceptable results. The methods developed and validated showed acceptable recoveries and relative standard deviations (RSD) based on EPA, OECD, and SANCO guidelines. These methods were also successfully transferred to outside laboratories for Independent Laboratory Validations to confirm the methods efficacy and were also used in field and ecotoxicology studies.

AGRO 223

Nitrite residue analysis in feral swine tissues

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Feral swine are a growing concern for farmers and ranchers in the United States. Estimated damage from feral swine exceeds $1.5B U.S. per year. The U.S. Department of Agriculture has established the National Feral Swine Damage Management Program to respond to this threat. This program is exploring multiple methods to mitigate this damage, and significant research into the development of a safe toxicant has been undertaken. Use of sodium nitrite baits has shown great promise in reducing populations of feral swine. Before use of this bait can be authorized on a larger scale, it is necessary to assess potential secondary hazards by analyzing tissues of exposed swine. Many techniques exist for analysis of nitrite in water and food products, but the ability to detect very low residues of nitrite in tissues which contain endogenous levels has proven challenging. Several analytical approaches were explored. Ultimately, a heated extraction of muscle, GI tract, and liver tissues with a potassium hydroxide solution followed by filtration and solid phase extraction was found to produce the best results. Sample quantification was achieved by ion chromatography paired with ultraviolet detection. The resulting method is reliable and sensitive with good recoveries for nitrite residues ranging from 10 to 1,000 µg/g. The developed method was used to evaluate residue levels of nitrite from feral swine fed a toxic bait.

AGRO 224

Novel approaches to detecting trace amount of alphacypermethrin in water using dispersive SPE

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The global regulatory environments for agro chemicals are continuously changing. Consequently, development of the analytical methods for residue analysis is becoming more and more challenging, especially the lowering of detection limits for pesticides in drinking and surface water has been a mandate due to public health concerns. Following this concept, a method with a low detection limit of 0.75 ng/L (0.75 ppt) for the determination of residues of alphacypermethrin in drinking water was developed using dispersive SPE for concentration followed by determination using LC-MS/MS. This low detection limit was required for this analyte in water for environmental risk concern. Due to the poor sensitivity of alphacypermethrin in LC-MS/MS, the method required significant concentration of water sample using a SPE column. Drinking water was used as a model matrix to mimic the situation of how this method would work for rice paddy water from the field. This presentation will discuss the detail of the method development work used for the detection of alphacypermethrin.

AGRO 225

Ambient ionization mass spectrometry for rapid analysis of a variety of analytes including opioids, active pharmaceutical ingredients, mitragynine, and pesticides

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Direct analysis in real time (DART) ambient ionization allows rapid analysis of many compounds by greatly reducing sample preparation time and by eliminating chromatographic separations. Here we describe both a DART-HR-MS (high resolution mass spectrometer) platform that has been utilized to screen for 240 pesticides from many different classes including amides, benzimidazoles, benzoylphenylureas, carbamates, conazoles, organophosphorus compounds, phenylureas, and triazines, as well as a system that couples the DART to a rugged single quadrupole nominal mass-mass spectrometer for rapid screening in the field (DART-MS). This platform is currently being implemented at an International Mail Facility (IMF) and to date this method has been used to analyze 851 Food and Drug Administration (FDA) regulated products in the past 4 months since the launch of the lab. Of them, 107 tested (12.6%) contained an active pharmaceutical ingredient (API) that violated FDA regulations, and 81 (9.5%) were determined to contain Drug Enforcement Administration (DEA) scheduled substances. Additionally, 96 samples of kratom products were screened for the presence of its primary alkaloid (mitragynine) using this platform, and DART-HR-MS results were compared to results obtained by LC-MS and/or GC-MS experiments. This method had a 100% true positive rate (88 samples contained mitragynine) and a 100% true negative rate (8 samples did not contain mitragynine) for this application. Sample preparation of commodities such as fresh produce, pharmaceutical products, kratom, and dietary supplements will be described for each system with an emphasis on rapid screening for a multitude of chemical classes. The sample introduction for each system will also be described. For instance, a transmission mode module works well for swabbing pesticides from the surface of produce, while a thermal desorption unit is imperative for safe analysis of acutely toxic compounds, such as fentanyl and fentanyl analogs, that might be encountered in the form of powders, liquids, or counterfeit pharmaceuticals in the field. The relative strengths and limitations of the DART-HR-MS and the DART-MS will be compared and contrasted, particularly in terms of sensitivity, ruggedness, speed of analysis, data processing, and ease of use.

AGRO 226

Elucidation of molecular mechanisms of insect olfaction in the pea aphid, *Acyrthosiphon pisum*

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Aphids (Homoptera; Aphididae) are major pests on arable and horticultural crops. Aphids communicate using olfaction, employing semiochemicals such as the alarm pheromone, (*E*)-β-farnesene, and the sex pheromone components, (4*aS*,7*S*,7*aR*)-nepetalactone and (1*R*,4*aS*,7*S*,7a*R*)-nepetalactol. An understanding of olfaction in aphids, specifically molecular recognition and discrimination of odorants, can help when designing novel protection tools. However, little is understood about the aphid olfactory system, specifically, the role of odorant-binding proteins (OBPs), small, soluble proteins that are found in high concentrations in sensory organs (antennae). This project will test current hypotheses relating to the molecular basis of olfaction in insects, specifically the role that OBPs play in the process. Initially, aphid sex pheromone components, (4*aS*,7*S*,7*aR*)-nepetalactone and (1*R*,4*aS*,7*S*,7a*R*)-nepetalactol, and their respective non-naturally occurring enantiomers, (4*aR*,7*R*,7*aS*)-nepetalactone and (1*S*,4*aR*,7*R*,7a*S*)-nepetalactol were synthesised and *Acyrthosiphon pisum* (pea aphid) OBPs were assessed for binding activity using *in silico* methods. The binding activity was then tested *in vitro* using fluorescence competitive-binding techniques. One OBP, OBP6, was shown to bind the aphid sex pheromone components and their respective enantiomers. Furthermore, potential strong-binding analogues of nepetalactol and nepetalactone were predicted through *in silico* methods. These analogues have the potential to act as olfactory disrupters and may be a valuable pest control method in future. Other methods, including mass spectrometry, nuclear magnetic resonance (NMR) and x-ray crystallography techniques, are currently being employed to fully assess protein-ligand interactions of the aphid OBPs and the structure of OBP6. In addition to OBPs, another key olfactory protein, odorant-receptors (ORs), of aphids have also been studied. Initial screenings are being undertaken using *in silico* methods, including the identification of conserved key binding sites. In future work, ORs will be cloned into a *Xenopus* oocyte expression system and electrophysiological recordings measured.

AGRO 227

Synthesis and biological testing of pheromone analogues for carposinidae moths

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Carposinidae moths are a family of fruit worm moths found predominantly in Asia and the South Pacific regions. There are thought to be about 280 species in the family, yet only three species have had pheromone components identified. All pheromone blends identified to date have at least one compound containing an alkene at C-7 and a ketone at C-11. Our aim was to use this structural commonality to create an analogue which could be used to disrupt the behaviour of multiple species of these pest moths. A variety of analogues were synthesised and initially tested via electroantennogram (EAG) on two carposinidae moth species; *Heterocrossa rubophaga* and *Coscinotycha improbana*. Compounds showing EAG activity were then tested on these two species using single sensillum recording (SSR) to ensure that the analogue was indeed stimulating the pheromone receptor neurons of the male moths. Two of the analogues elicited strong EAG responses and were taken forward for the SSR testing. Both analogues stimulated the same olfactory receptor neurons as the insect-produced sex pheromone. Field trapping trials were then conducted with these two analogues both individually and in combination with the pheromone of the two moth species. No attraction was shown to either of the analogues alone by either moth species. However, one of the analogues produced a strong inhibitory effect in *Heterocrossa rubophaga* reducing male moth trap catch to the sex pheromone by over 95%. No inhibitory effect on male moth trap catch was observed in *Coscinotycha improbana*. These results suggest the inhibitory analogue could be an effective mating disruption tool for *Heterocrossa rubophaga* and further testing at the orchard level is required.

AGRO 228

Environmental decomposition of cuticular hydrocarbons generates a volatile pheromone that guides insect behavior

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Abiotic environmental conditions may degrade semiochemicals and thus disrupt chemical communication. However, because habitat conditions may be unavoidable, some species may have adapted to detect environmentally mediated breakdown products as semiochemicals. Cuticular hydrocarbons (CHCs) are excellent targets for environmental degradation because they are species-specific blends that often include one or more double bonds that are susceptible to atmospheric oxidation. We demonstrate that CHCs of the American cockroach, *Periplaneta americana*, react with environmental agents to produce volatiles that impede cockroach aggregation. In behavioral assays, nymphs strongly avoided aggregating under shelters conditioned with breakdown products from cuticular alkenes using air, humidity or UV light as degrading agents. Chemical analyses of the headspace of CHCs exposed to these agents confirmed that volatile organic compounds (VOCs) were formed. Most of the VOCs were shared among the three conditions, although their ratios differed. Behavioral responses were then tested with mixtures of synthetic compounds that reconstituted the original amounts of VOCs. When short-chain fatty acids and other compounds were tested separately, no preference was observed between control and treated shelters. However, the avoidance behavior of treated shelters was restored when short-chain fatty acids and other compounds were tested together. These results demonstrate that environmental agents degrade non-volatile unsaturated CHCs into semiochemicals that function as volatile semiochemicals. This chemical communication mechanism is probably used by other insect species, including agricultural pests, providing a

new pool of semiochemicals that can be implemented in pest monitoring, surveillance, and management.

AGRO 229

Using semiochemicals to optimize biological control of invasive saltcedar

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The northern tamarisk beetle, *Diorhabda carinulata* (Desbrochers), is an established classical biological control agent for saltcedars (*Tamarix* spp.). Adequate control of *Tamarix* has not yet been achieved in certain areas where *D. carinulata* has been released. Retaining beetle populations on sites where it has been released is problematic, and accurately monitoring *D. carinulata* populations is difficult. Negative, indirect impacts have also resulted from the agent's establishment outside targeted treatment areas in the southwestern United States. Manipulation of *D. carinulata* spatial distribution with semiochemicals could potentially resolve or ameliorate these and other operational issues.
Lures utilizing a specialized wax-based matrix for the controlled release of semiochemicals were impregnated with a previously identified pheromone known to stimulate aggregations in *D. carinulata*. Observed release rates confirm that semiochemicals lures formulated with this matrix are a viable option for facilitating aggregation of *D. carinulata* under field conditions. The results of field-based assays indicate saltcedars treated the pheromone attracted and retained higher densities of *D. carinulata* than *Tamarix* that received a control lure. Treated *Tamarix* plants also exhibited more damage, resulting in a greater decrease in canopy volume than control trees. The attraction and retention of *D. carinulata* to pheromone treated *Tamarix* plants also arrested the dispersal of newly released individuals, resulting in greater population growth. Repellent semiochemicals were also investigated for their potential to manipulate spatial distributions of *D. carinulata* in the field. Volatile collections and Behavioral assays conducted with reproductive adults identified a repellent plant produced compound.
These results indicate that semiochemical-impregnated media could be useful for detecting, retaining, and directing populations of *D. carinulata*. The use of semiochemicals could be used to potentiate low density populations, increase monitoring efficacy, retain adults on release sites, and repel *D. carinulata* from sensitive habitat.

AGRO 230

Plants induce defense chemicals based on identity of parasitoid attacking an herbivore

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When plants experience herbivory they induce defense chemicals to increase concentrations. These compounds can have negative consequences for herbivores and their parasitoids. Parasitoids are insects which develop inside another insect host, ultimately killing it. Most parasitoids are thought of as an indirect defense for plants and are frequently used for biological control. However, some parasitoids cause their hosts to feed more, increasing plant damage. This could lead to increased expression of plant chemical defenses. I investigated whether (1) plants respond differentially to parasitoids which cause different amounts of feeding by their host, and (2) whether any plant response is based on feeding amount or parasitoid identity. I used two parasitoid wasps of the imported cabbageworm, which feeds on plants that produce inducible glucosinolates. The gregarious *Cotesia glomerata* has multiple larvae develop per host and increases herbivore feeding. The solitary *C. rubecula* has only one larva develop per host and causes the host to feed much less. I compared induction responses of *Brassica rapa* plants fed on by *C. glomerata*-parasitized, *C. rubecula*-parasitized, or unparasitized caterpillars at both equal and different development stages to determine the effects of parasitoid identity versus feeding damage on induction. Leaf samples taken before and after feeding were extracted through anion exchange, and glucosinolate content was analyzed using UHPLC-PDA-MS/MS. I demonstrated that parasitoid identity, more than the amount of feeding damage, influences induction responses of *B. rapa* plants. All caterpillars of the same instar fed approximately the same amount, but induction responses of glucosinolates were elicited only by *C. glomerata*-parasitized caterpillars independent of caterpillar size or damage. Plants may induce defenses against earlier stages of the gregarious *C. glomerata* in anticipation of increased damage later in development. In a follow-up experiment, brood sizes of *C. glomerata* were reduced when parasitized hosts fed on *B. rapa* compared to less toxic *B. oleracea* plants while *C. rubecula* survival was unaffected, indicating a negative effect of glucosinolates on *C. glomerata* fitness. This research indicates the role of parasitoids in influencing expression of plant traits. Further knowledge of plant responses to parasitoids is necessary to understand and predict the impacts of parasitoids used in biological control for agricultural systems.

AGRO 231

Monarch butterfly (*Danaus plexippus*) movement ecology and perceptual range facilitates effective habitat restoration

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Eastern North American monarch butterflies (*Danaus plexippus*) are a vagile species known for their multigenerational, annual migration. The overwintering population has declined significantly, and the loss of milkweed (*Asclepias sp*.), the monarch's obligate host plant in the Midwest United States, is considered to be a major cause of the decline. Monarch habitat restoration efforts are focused on increasing milkweed and native prairie plants. When considering habitat restoration for a highly mobile species, like the monarch butterfly, it is critical to include movement biology in the decision-making process. The spatial arrangement of milkweed in the landscape influences

movement patterns, habitat utilization, and reproductive output. New habitats should be established to facilitate movement within the adult's detection range to increase overall landscape connectivity. Handheld very high frequency (VHF) radio telemetry techniques were employed with monarch butterflies to track habitat utilization, flight patterns, flight-length, and perception distance. Thirteen radio-tagged monarchs were released in a 4-ha restored prairie, and locations were estimated every minute for up to 39 min by simultaneous triangulation from four operators. Assuming straight flights between locations, the majority of flights within the prairie were below 50m. While moving in the prairie, monarchs occupied 37.5% of the 4-ha. Flights associated with exiting the prairie exceeded 50m with high directionality; monarchs that left the prairie were tracked and relocated at distances up to 250m. To explore the perceptual range, radio-tagged monarchs were released known distances downwind from a single 'patch' of potted milkweed and nectar plants placed in a 16ha sod farm. Monarchs took flights ranging from one to 1,100m and often avoided the potted resources. Monarchs inhabiting surrounding corn/soybean landscapes provided an opportunity to empirically assess perceptual range. Monarchs downwind of potted nectar plants exhibited directed flights to these resources from distances of 50 to $\geq$ 75m. Current studies are taking advantage of more complex habitat scenarios to determine perception distance, as well as flight distances, behavior at habitat edges, and occupancy time in various landcover types. Results will inform conservation planning by assessing how different spatial arrangements of habitat patches in the landscape can optimize efficient resource utilization by monarch butterflies.

AGRO 232

Discovery and development of agricultural biologicals at Lavie Bio and their role in integrated pest management practices

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Biologicals play a critical role in agriculture with growing recognition of their role as standalone solutions and part of an integrated pest management program. Development and registration of these agents require less time and money compared to either traits or traditional chemistries. Lavie Bio is leveraging the Evogene computational biology platform to focus and refine discovery of biologicals. Based on tailored experimentation, a multidimensional database, and machine learning, Lavie Bio is able to dramatically improve the hit rate among nominated strains and discover entirely new taxa with targeted activities. Additionally, Lavie Bio is now using this same computational power to drive improvements in development of novel taxa including improved fermentation and formulations. This talk will highlight some of our successes in the development of novel bacterial biostimulants, biofungicides, and bioinsecticides.

AGRO 233

Application of dsRNA as a sustainable bioinsecticide: From laboratory to field

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Foliar-applied dsRNA is a new mode of action, environmentally safe biopesticide for crop protection. Orally delivered dsRNA can be designed to specifically direct RNA interference (RNAi) to downregulate mRNA for essential genes only in target insect pests, thereby avoiding negative impact on non-target organisms. GreenLight Biosciences' cell free RNA synthesis platform, GreenWorX™, provides a breakthrough solution to the previously unsolved problem of producing large scale quantities of dsRNA at a competitive cost for commercial field application. GreenLight Biosciences is using GreenWorX™ to produce the first sprayable dsRNA bioinsecticide product for control of Colorado potato beetle (CPB), *Leptinotarsa decemilineata*. dsRNA targeting a gene sequence unique to CPB was first characterized for biological efficacy and target gene knock-down in laboratory and greenhouse experiments. Extensive field studies in potato-growing regions across the United States have demonstrated the ability of GreenLight's CPB product to provide protection against CPB feeding damage equivalent to traditional chemical products. Low persistence under field settings and evidence for lack of non-target effects support the long-term sustainability profile of a dsRNA product for CPB control as well as other dsRNA-based products in GreenLight's pipeline.

AGRO 234

Design principles for biorational pesticides

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Green chemistry principles can be very successfully applied to agrochemicals. The initial step for creating green pesticides is the design of the compounds. Synthetic compounds that are designed off nature's chemistry are termed "biorational" compounds. Once a need for improved "green" pesticides has been identified, the process of design begins, with many green principles in mind. The major steps of that process are:
1. Screen natural products to identify the best lead molecule or molecules.
2. Consider need for new product in the targeted market.
3. Identify the limitations of the natural lead molecule: perhaps needs greater potency, longer residual time, safer toxicology profile, lower cost, different soil-binding properties, photostability, different volatility profile, etc.
4. Develop improvement strategies: to increase efficacy, lengthen residual time, enhance safety to non-targets, etc.
5. Consider synthesizing derivatives that incorporate the lead molecule into the biorational molecule. Maintain green principles.
6. Consider synthesizing analogues that substitute certain functionalities or groups for moieties extant in the lead molecule. Maintain green principles.
7. Test series of closely-related biorational compounds for obvious trends in efficacy or other important criteria.
8. Develop structure-activity relationships (SAR) or

quantitative structure-activity relationships (QSAR) to establish vectors for further exploration (SAR) or equations that can be used to predict efficacy of hypothetical biorational molecules, before actually making them.
9. "Toxicology considerations," *i.e.*, potential hazards that could be encountered in mammalian testing for LD50 or other tox endpoints.
10. Find out if patent protection is feasible for the most desirable of the biorational molecules?
11. Determine if there would be any likely special challenges in formulation of the best molecules.
12. Investigate costs of starting materials, number of steps in synthetic route, etc.
13. Assess commercial opportunity *viz.* available space in the market versus competitors' products. Superior properties of new biorationals.
The push for greener pesticides of all types has become more urgent. In certain situations, potency and persistence are still the dominant desirable characteristics for a pesticide. However, greener structures, environmental degradability, favorable ecotoxicology, and general safety have become very important principles for many pest-management scenarios.

AGRO 235

Impact of climate change on the relevance of TFD studies and OECD crosswalks

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Climate change studies confirm that the earth is warming up and that shifts in rainfall patterns are occurring. Various computer models predict that Earth's average temperature will rise between 1.8° and 4.0° Celsius (3.2° and 7.2° F). Consequently, these warmer temperatures will cause a higher rate of evaporation, resulting in a predicted increase of average global rainfall by 3-5%. This climate change, therefore, will impact the relevance of historical and current terrestrial field dissipation (TFD) studies. Under OCED guidance, TFD studies conducted in one country can be applicable to other countries if the characteristics of overlapping ecoregions are the same. These assessments are conducted using the OECD ENASGIPS tool. If these characteristics change, so will the similarity assessment and therefore the potential relevance of TFD sites. With increased rainfall, TFD sites in the Southeastern U.S., which typically have few similar areas, may become more relevant because other areas are receiving more rainfall. Likewise, TFD sites in Canada, characterized by cold winters, may become more applicable to larger areas in Europe with increasing temperature. In this presentation we will review some of the climate change scenarios, place existing TFD sites in context of new climates, and determine the impact on OECD crosswalks.

AGRO 236

Corteva Agriscience: Identifying next generation targeted crop protection solutions

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Farmers and ranchers often face difficult challenges when producing the food, feed, and fuel we all rely on for our daily lives. One such challenge is the societal demand to produce more using fewer resources to improve the overall sustainability of farming operations in the wake of critical global challenges such as climate change. Corteva Agriscience is actively exploring next generation targeted crop protection solutions which integrate digital technology and personal consultation with crop protection tools to address this growing global demand for greater sustainability. Targeted crop protection solutions can be broadly positioned in one of two ways, either improved spatial applications to target a pest exactly where it occurs in specific geo-spatial zones or improved temporal applications to address an emerging pest at the best possible moment in time using the most appropriate technology. One excellent example from Corteva of a recently launched targeted crop protection solution is LANDVisor. LANDVisor is a new U.S. rangeland management technology offered through certified consultants. It maps soil types, mesquite density, and other information to help plan mesquite spraying. Then it monitors foliage to help determine when it's most appropriate to spray ensuring maximum effectiveness of the crop protection tool. This targeted crop protection solution nicely demonstrates both spatial and temporal targeted crop protection practices and the ability to effectively and sustainably address a critical grower need when combining best available digital and crop protection technologies. In closing, Corteva is committed to delivering sustainable, differentiated, and intentionally crafted targeted crop protection solutions to help support the growing demand for improved agricultural sustainability.

AGRO 237

Landscape positions with more favorable growing conditions produce weed seeds with greater emergence rates in subsequent years

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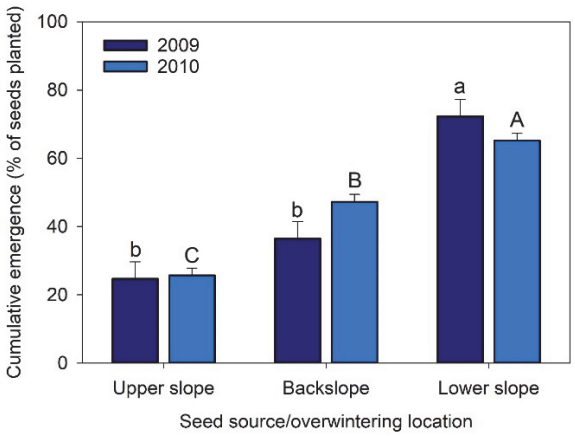
In a two-year field study, we evaluated the emergence and early growth of common lambsquarters seedlings as affected by the landscape position in which the seeds (a) developed, (b) overwintered, and (c) were planted. Results indicated that a higher proportion of seeds originating from lower slope positions emerged compared with seeds originating from the backslope or upper slope. The timing of emergence was the same for all seed source locations. There was no influence of overwintering location on weed emergence. Regardless of the seed source, we observed faster emergence and growth of lambsquarters planted in the lower slope, where soil conditions were more conducive to growth. These experiments will support the development of new strategies and decision aids to improve weed management.



AGRO 238

Sustainable approaches to formulation development at Corteva™ agriscience

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One of the greatest challenges facing today's world is to feed and support the growing population in a sustainable and environmentally responsible way. The demand for higher crop yields and agricultural productivity is ever increasing and so are concerns around the negative impacts on the environment caused by agricultural activities and products. At Corteva™ Agriscience, we discover, develop, and bring innovative products and solutions to enhance the overall sustainable profile of the agriculture industry and reduce the environmental impact of food production. Arylex™ active and Rinskor™ active are two new herbicides that allow farmers to manage herbicide-resistance and hard-to-control weeds with very low use rates (5 to 20 g ai per ha). Both products have favorable environmental and toxicological profiles. During formulation development, we develop and apply advanced technology to minimize the loss and maximize the delivery of active ingredients to the target site for enhanced biological performance. Co-formulants are evaluated and ranked based on ecotoxicology, environmental fate, and mammalian toxicology in a co-formulant toolbox, which guides formulators to select more sustainable co-formulants. Furthermore, predictive toxicology test methods are integrated in new formulation/product development to allow for earlier assessments and reduced animal testing. Drift reduction technology (DRT) represents a paradigm shift, where formulation technology is proven to be a substantial aid to application practices to minimize both vapor drift and unwanted spray drift. Corteva™ Agriscience has made significant investments in developing advanced formulation technologies and uses those tools and test methods to reduce the environmental impact of our products as a part of our strategy to incorporate sustainability into our formulation and product development decisions.

AGRO 239

Safety evaluation of the copper-mediated cross-coupling of 2-bromopyridines with ethyl bromodifluoroacetate

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The potential safety hazards associated with the copper-mediated cross-coupling of 2-bromopyridines with ethyl bromodifluoroacetate were evaluated. Thermal stability evaluation of the postreaction mixture of 50.6 mmol of 2-bromopyridine with 1.3 equiv of ethyl bromodifluoroacetate in the presence of 2.1 equiv of copper in 40 mL of dimethyl sulfoxide (DMSO) indicated a significant decomposition event with an onset temperature of 115.5 °C by accelerating rate calorimetry, which was significantly lower than that of neat DMSO. In contrast, the reaction mixture in *N*,*N*-dimethylformamide did not show any exothermic decomposition up to 400 °C by differential scanning calorimetry. Reaction calorimetry evaluation of this reaction in DMSO revealed a heat output (ΔH) of −13.5 kJ and an adiabatic temperature rise (ΔT_{ad}) of 129.5 °C, resulting in a maximum temperature of a synthesis reaction (MTSR) of 189.5 °C. The predicted heat of reaction using density functional theory with the BLYP functional was in good agreement with the experimental data. The scope studies with a variety of substituted 2-bromopyridines revealed similar magnitudes of ΔH and ΔT_{ad} compared to 2-bromopyridine when reacted at the same concentration. In all of the studied cases, the MTSR was significantly higher than the onset temperature of reaction mixture decomposition, indicating that in the absence of active cooling the system could quickly trigger the decomposition of the reaction mixture, resulting in a runaway reaction.

AGRO 240

Evaluation of biological insecticides to aid arthropod pest management in hemp

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The novel status of hemp as an agricultural commodity in the U.S. coupled with little to no published information about appropriate pest management tactics has led to many challenges. Since 2017, our research has focused on addressing arthropod management concerns in this crop. Corn earworm (*Helicoverpa zea*) is the most injurious pest in Virginia and many other states. There are also specialist arthropods found on hemp, both outside and indoors, including cannabis aphid (*Phorodon cannabis*) and hemp russet mite (*Aculops cannabicola*), but injury potential from these species remains unclear. Our research has shown that defoliation of hemp by beetles, caterpillars, and other chewing arthropods will likely not negatively affect grain hemp yield. In addition, stink bugs, although injurious to other crops, do not appear to be a concern for yield or quality loss in grain hemp at this time. Other research has focused on evaluation of several biological insecticides to manage insect and mite pests of hemp. We have shown that several natural products such as essential oils, sulfur, and soaps can reduce

hemp russet mite densities. Corn earworm has been the most difficult pest to manage, and many labeled biological insecticides have not provided adequate control. Spinosad provided the best control in all of our trials, but this product is not registered for use on hemp. The best options for suppression of this pest include *Bacillus thuringiensis* strain *aizawai*, as well as the *Helicoverpa zea* nuclear polyhedrosis virus product Gemstar. Most products evaluated in our studies are currently allowed to be applied to hemp in many states and will likely soon have hemp permanently added to the label. At the current time and looking ahead, successful arthropod pest management in hemp will continue to be a challenge due to legal restrictions surrounding pesticide use in the crop.

AGRO 241

Management of phytophagous mites in hemp using organic acaricides, and natural enemies

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Cannabis sp. has several key Acari pest species in different families: eriophyid (hemp russet mite), tarsonemid (broad mites) and tetranychid (spider mites) mites. This presentation will provide information on mite damages and discuss results of studies conducted in Kentucky to manage these pests using pesticides and natural enemies. Also, the hemp russet mite grown in open fields has shown differences on their abundances, speciation, and damages caused in hemp due to the geographical and environmental conditions. A hypothesis about the presence of the hemp russet mite across different states of the U.S. will be discussed.

AGRO 242

Terpenoids from plant essential oils can upregulate detoxification genes in *Aedes aegypti*

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A number of plant essential oils have been shown to enhance the toxicity of various insecticides. Cedarwood Moroccan, Cedarwood Texas, and Thymol have demonstrated enhancement of toxicity for pro-insecticides, compounds that must first be metabolically activated. This study is determining how these essential oils are acting on *Aedes aegypti*, at a genomic level, to enhance the effectiveness of pro-insecticides. An $LD_{05}$of the three essential oils were applied topically. After 24 hours, RNA sequencing of the surviving mosquitoes was conducted and analyzed by the respective treatments. A validation of the results using qPCR allowed confirmation of the sequencing results. Gene ontology and available *Aedes aegypti* gene mapping reported a number of detoxification genes being upregulated as a result of the selected plant essential oil and terpenoid treatments. Some variability was noted between types of oil or terpenoid, as well as between batch and year of essential oil. Cedarwood Moroccan, Cedarwood Texas, and Thymol have been demonstrated to increase detoxification enzymes in *Aedes aegypti*, resulting in a proportional increase of metabolic activation of pro-insecticides. One immediate utility is the formulation of an upregulation oil with organophosphate (thiono-types) insecticides, since they require oxidative activation inside the insect.

AGRO 243

Determining insecticidal mode of action: Terpenes and a new class of natural insecticides inhibit adult mosquito acetylcholinesterase

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Insecticides often act on one or more locations within the insect nervous system, but it can be difficult to determine which target location is responsible for the insecticidal properties. Mode of action (MoA) studies highlight the most efficacious insecticide targets and allow for informed insecticide design. The enzyme acetylcholinesterase (AChE) is responsible for breaking down acetylcholine in nerve synapses. Acetylcholine is an excitatory neurotransmitter and must be maintained at proper concentrations to maintain physiological functions. AChE inhibition, the primary MoA of some synthetic insecticides, leads to overstimulation of the insect's nervous system, causing muscle twitching, convulsions, paralysis, and eventually death. The yellow fever mosquito, *Aedes aegypti*, is a vector of multiple debilitating diseases including dengue fever, Zika fever, and chikungunya. Synthetic insecticides are commonly used to prevent the spread of disease and control mosquito populations. Some mosquito populations have developed resistance to synthetic insecticides, thereby diminishing synthetic insecticide effectiveness and prompting a search for alternatives. Natural products such as terpenes have shown promise as insecticides, but the MoA remains unclear. Here we explore the ability of terpenes and a new class of natural insecticides to inhibit acetylcholinesterase in adult mosquitoes. Enzymes obtained from adult mosquitoes are used to simulate acetylcholinesterase inhibition in a modified version of Ellman's assay. The experimental setup was used to screen various other natural products and known acetylcholinesterase inhibitors for inhibitory properties. We conclude that, while these natural products show some inhibitory potential on mosquito acetylcholinesterase, this is unlikely to be the primary mode of action by which they are active as insecticides.

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Nematode receptor ACR-16 as a target site for natural pesticides

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In developing a novel pesticide to be on the market for a reasonable product lifespan, it is important to identify and exploit unique target sites. The portion of the pest organism that a pesticide interacts with is the target site. Currently used pesticides often target a protein in the nervous system, and mutations to these proteins can render a pesticide less effective, leading to resistance. Having multiple pesticides that target the same protein creates a selection pressure for the pest to develop resistance faster and creates the potential for the resistance to reduce the effectiveness of all of the pesticides that are used against this same target. Luckily,

even similar proteins can present alternative targets for pesticides. The nematode nAChR (nicotinic acetylcholine receptor), ACR-16, belongs to a subfamily of nAChRs which differ from the normal structure of the protein family. This difference in structure has an effect on function and also allows us to use it as a target site distinct from other nAChRs, which have been previously exploited as pesticide targets. These ACR-16 receptors are found in different kinds of parasitic nematodes. We are attempting to express the ACR-16 protein from *Ascaris suum* (a pest of swine) into a model that can be used to test its interaction with pesticide candidates. We plan to transfect the *Asu*-ACR-16 receptor into a mammalian cell line of human embryonic kidney (HEK) cells. This involves transfection of the ACR-16 gene, along with genes for Ric-3 chaperone protein and green fluorescent protein. With the cell line established, we will characterize the individual sensitivities of the protein to natural pesticide candidates. Previous work from our lab has led us to find these natural pesticides among plant secondary metabolites of the monoterpenoid class.

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Sustainability benefits of efficient phosphorus fertilizer: Life cycle analysis and soil incubation studies

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Between 2020 and 2050, the world's population is expected to grow from 7.8 billion people to over 9 billion. Projected increases in arable land do not meet population growth over this same time frame, as an increase from 1.59 to only 1.66 billion hectares is projected. Therefore, the amount of arable land per capita is projected to decrease roughly 10%, from 0.2 hectare/capita to 0.18. Compounding this is the issue of carbon emissions – there is a clear link between the global population and greenhouse gas emissions (GHGs), and agriculture, forestry and other land use constitutes a significant fraction (24%) of GHG emissions associated with human activity. There is an urgent need to meet growing food demand in a more efficient manner, both in terms of land use required, and in terms of greenhouse gas emissions. Actagro Structure® is a 7-21-0 liquid fertilizer that contains a carbon-based component that we believe provides greater nutrient availability in the soil relative to commodity fertilizer sources, and thus allows for lower product use rates in terms of pounds of nutrient required per acre. The goal of this work is to assess the GHG emissions associated with the manufacture of Structure®, relative to a commodity fertilizer, 10-34-0. By using emissions data for chemicals available in the ecoinvent life cycle inventory database, we determined that the emissions associated with producing 10-34-0 are 0.186 lbs CO_2 per pound, and 0.110 lbs of CO_2 per pound for Structure®. This calculation is simply a 1:1 comparison by weight and does not take into consideration nutrien use efficiency benefits of the Structure® formula. Recent work by the Nutrien Ag Solutions R&D team puts into stark relief the quantitative benefit of the Structure® formula vs. commodity sources in improving nutrient use efficiency. Soil incubation studies of Structure® vs 10-34-0 demonstrate a clear benefit in available phosphorus found in calcareous soil. Based on these results, in order to achieve 50 ppm of plant-available phosphorus in calcareous soil requires application of roughly 907 lbs/acre of 10-34-0, and 914 lbs/acre of Structure® are required. The corresponding GHG emissions associated with these use rates are then 517 lbs of CO_2 emitted per acre for 10-34-0, vs only 314 lbs per acre for Structure®, roughly a 40% reduction in emissions.

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Biogeosystem technique for sustainable agriculture, water scarcity overcoming, healthy soil and environment

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Sustainable Agriculture and Public Health goals can't be embodied using the current outdated technological framework. Biosphere and technology conflict leads to poor soil health, scarce plant development, excessive water loss, off-target transport and environment overload of pesticides. The global environmental challenge is a new biosphere technological platform search. We propose the transcendental (not a direct imitation of nature) Biogeosystem Technique (BGT*) methodology. BGT* ensures simultaneously the safe biosphere abundance and expanded sustainable technological development. BGT* implementations are as follows: One time intra-soil milling of the 20–50 cm layer provides soil stable fine multilevel aggregate system for a period of up to 40 years. The high rate rhizosphere and soil biome long-term development ensures soil fertility and plant organogenesis certainty. Plant resistance to stress increases and the need for pesticide reduces. Crop yield is higher and stable. World water scarcity problem solving is the intra-soil pulse continuous-discrete watering. Water consumption is about 5–20 times less compared to standard irrigation. The soil solution matrix potential is provided in the range from −0.2 MPa to −0.4 MPa. At this matrix potential, a stomatal apparatus of plant operates in the regulation mode. Plant transpiration is reduced. The water and nutrient supply is sufficient for plant productive organogenesis due to the stable soil structure and higher soil solution concentration. This decreases nutrients and pesticides off-target transport and soil degradation. The yield is higher compared to standard irrigation. Intra-soil recycling of municipal, industrial, biological (including agricultural, slaughterhouse, hazardous) waste and gasification byproduct in the soil layer of 20–50 cm in the course of this layer milling provides safety of the environment. This intensifies the nutrient turnover, ensures the functioning of humic substances, polymicrobial biofilms, plant stimulants, and plant resistance to phytopathogens. BGT* possibilities are as follows. Soil compaction overcoming; fresh water saving and high level soil solution equilibria control; waste recycling; the biogeochemical barrier for heavy metals; atmosphere N fixation; soil organic matter synthesis; soil-biological reversible C sequestration; medical and veterinary environmental safety; adaptation to climate change. The yield is higher for 50–80% compared to standard technology.

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Biosolarization using Grape Pomace is a pre-planting method to inactivate soil-borne human pathogens

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The consumption of fresh produce and its demand/production has increased during recent years in the United States. As a result, multistate foodborne outbreaks have increased. Besides these challenges, farmers also have to avoid billions of dollars in losses caused by pests and be in alignment with sustainable practices. Biosolarization is a pre-planting disinfestation method that meets these challenges by promoting in-soil fermentation of food waste streams during summer season. Microbial activity, increased temperatures and biomass matrix disinfest soil from plant pests; on the same note, our lab proved that biosolarization is capable of inactivating *Escherichia Coli* using 3 different batches of white grape pomace. In this study, we explore the role of bioactive compounds in the process by testing various pomace treatments and white grape varieties against *E.coli*. We analyzed 5 varieties of grapes, with two of these varieties tested using two isolated batches. These 7 batches were tested after sun drying or sun drying followed by two days at 70C, biomasses were milled to particles sizes smaller than 0.1mm. Finally, we accessed differences between fresh berries and its sundried counterpart. Biosolarization was carried using 50 mL tubes filled with 10g of the respective dry soil mix and wetted with *E.coli* in phosphate-buffer to a final concentration of 10^9 cells/ml. Circadian changes in temperature were mimicked by incubating samples under cycles of 30-50C (12 hours each) for 8 days. Results are expressed as Log Reduction Values (LRV) by subtracting the log of Colony-forming Units (CFUs) of a given sample from the non-amended soil control. For sundried batches, 3 unique varieties had no detectable cells after 8 days. The results from different batches were not consistent. Viognier grapes had no detectable cells for both batches while Verdello grapes showed total inactivation in batch one and LRV close to zero in batch two. The last batch using mixed varieties had a negative LRV (protective effect). Phenolic profiling using revealed compounds associated with the protective effect as well as a group associated with the inactivation. Finally, all grapes kept at 70C had no detectable CFUs after 8 days. HPLC profiling revealed that Maillard compounds might be responsible for the increased inactivation rate for the two varieties. This study is the first step in the search for markers that can predict the efficiency of biosolarization against *E.coli* and human pathogens.

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Bioactive compounds in food waste streams used as soil amendments to inactivate *Escherichia coli* during biosolarization

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Current challenges to farming include (a) how to avoid the billions of dollars in annual losses to world agriculture caused by weeds, pests, and plant pathogens (b) maintaining intergenerational sustainability, and (c) add value to wastes from food processing. Biosolarization is an known method that can help farming to overcome all these challenges; the process couples anaerobic soil fermentation to high temperatures in an environment that is capable of disinfesting soil pre-planting from many pests. The process was proven to inactivate seeds, nematodes, and weed propagules; however, the efficacy against soilborne human pathogens (such as *Escherichia coli)* has yet to be explored. In this study, we quantified the efficiency of 6 different agricultural residues against *E.coli* during biosolarization. We tested Tomato Pomace, White Grape Pomace, Almonds hulls, Almond Shells, Coffee and Sugarcane bagasse as the pesticide chloropicrin. To simulate field conditions, 50 mL tubes were filled with proper soil mix. *E.coli* in phosphate-buffer saline was used to inoculate the bacteria (10^9 cells/ml) and wet the soil. Tubes were incubated under cycles of 30 C-50 C (12 hour each) for 8 days to mimic circadian changes in temperature. Log Reduction (LRV) of *E. coli* was calculated by subtracting the log of Colony forming units (CFUs) of a given treatment from the respective soil control. LRV results showed three different patterns: total inactivation; zero effect; and mitigation of the inactivation rate. CFUs from biosolarization with tomato pomace were much higher than the control while Grape pomace and chloropicrin decreased CFUs by nearly 4 logs representing total inactivation. Sugarcane, spent coffee, and both almond residues had no statistical significance to the control. Our results suggest that the presence of bioactives working synergistically with temperature to inactivate or protect *E.coli*. Moreover, the different combinations of bioactives polyphenols within grapes or coffee are key element for *E.coli* inactivation. The high fermentability of tomato pomace might be the responsible for the protective effect by triggering acid-resistance mechanisms.

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Fluorescent endophytic *Pseudomonas* spp. isolated from *Agave palmeri* promote root growth, root branching, and fungal pathogen resistance in crop plants

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Agave palmeri is a large succulent native to the Sonoran and Chihuahuan Deserts of North America. Some of the bacterial endophytes present in *Agave palmeri* seeds have been identified as fluorescent *Pseudomonas* spp. Fluorescent bacteria from the genus *Pseudomonas* are known to be plant growth promoters and potent biocontrol agents of plant pathogens. When introduced into the plant rhizosphere, fluorescent *Pseudomonas* have been shown to enhance plant resistance to fungal pathogens. The objective of this study is to assess the root growth promotion and biocontrol capabilities of agave endophytic *Pseudomonas* spp. when inoculated into carrot, coriander, and rice seeds. Antifungal activities of *Pseudomonas* spp. were examined through a dual culture assay with one of two plant pathogenic fungi: *Fusarium oxysporum* or *Cercospora* spp. Every strain of *Pseudomonas* spp. inhibited hyphal growth, though the extent of inhibition varied depending on the strain. Strains P3A-W, P3B-W, AY2, and WCY also colonized fungal mycelia, resulting in hyphal degradation in a manner reminiscent of cell lysis. Three strongly antifungal strains (P3A-W, AY2, WCY) and one slightly antifungal strain (WO for carrot and coriander, 3A2 for rice) were used in *in-vivo* tests on crop

seeds. Surface-sterilized crop seeds were inoculated with individual *Pseudomonas* strains and placed into sterilized soil that was either treated with water or with *F. oxysporum* suspension. Seedlings inoculated with *Pseudomonas* spp. show faster growth, enhanced root growth, greater root branching, increased root exudate production, and increased soil aggregate formation around the root compared to non-inoculated controls. When grown in soil inoculated with *F. oxysporum, Pseudomonas*-inoculated seedlings showed less signs of disease in addition to enhanced root growth and root exudate production. This effect also occurs in seeds inoculated with a simulated *Pseudomonas* microbiome composed of all four strains. These preliminary results indicate that endophytic fluorescent *Pseudomonas* from *Agave palmeri* have the potential to be used as both a biostimulant and a bioprotectant in a non-host-specific manner in crop plants.

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Ion chromatography analysis of food-based three-component fruit fly lures

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The Mediterranean fruit fly, *Ceratitis capitata* (Wiedmann) (Diptera: Tephritidae), is an agricultural pest of great economic importance worldwide. It affects, citrus, guava, papaya and coffee, among a long list of host crops. Throughout the years, several lures have been developed to monitor and control this fly. Currently, the most widely used is a three-component food-based dry lure (3C cone; Scentry Biologicals, Inc.) which contains ammonium acetate, trimethylamine hydrochloride and putrescine dihydrochloride (AA, TMA and PUT, respectively). A crucial part of lure effectiveness in the field is having a known initial amount and a controlled release of each component. Therefore, quality control necessitates quantification of all chemical components, which sometimes requires complex analytical methods. This presentation discusses a revision to an existing method used by APHIS-PPQ for the analysis of 3C cones. Under the current method, liquid chromatography with mass selective detection (LC-MSD) is used to analyze TMA and PUT, and ion chromatography with a conductivity detector (IC) to analyze AA. With the revised method, all three analytes can be quantified using only IC. Results show that the new method is suitable for the full analysis of all components in these lures. The improved method also confers added benefits, including a shorter preparation time, lower volume of organic solvent, simplified analysis and reduced overall costs. Concentration range, as well as linearity, were revised for each analyte in the 3C lures, and the method was validated.

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Production and use of selenium nanoparticles as soil fertilizers

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Selenium is a trace indispensable element for the most living creatures functioning. The laser ablation in water technology was proposed for Se zero-valent state nanoparticles producing as fertilizers and antioxidants. The main Se nanoparticles mass part shifted from 800 nm correspondingly the laser fragmentation time to the size less than 500 nm. The resulting nanoparticles were monodisperse in size and mass. The Se nanoparticles water suspension was introduced into the soil. The soil Se nanoparticles concentration was of 1, 5, 10, and 25 μg kg^{-1}. The experiment was carried out in a climate chamber in two series. 1.) Growing plants in soil imitating the standard organogenesis environment conditions: illumination of 16 hours per day, temperature of 22 °C, soil humidity of 25% SDW, experiment duration of 30 days. 2.) Growing plants in soil under changing conditions environment conditions of organogenesis. The standard environment conditions for the first 10 days: illumination of 16 h day^{-1}, temperature of 22 °C, soil humidity of 25% SDW. The plants stress for 5 days: hyperthermia of 40 °C. The standard environment conditions for the next 15 days: illumination of 16 h day^{-1}, temperature of 22 °C, soil humidity of 25% SDW. At standard organogenesis, the plant leaf plate surface area was of 30 ± 2 cm^2 in control option, and at the Se nanoparticles doses correspondingly: 1 μg kg^{-1} of 32 ± 3 cm^2, 5 μg kg^{-1} of 37 ± 2 cm^2; 10 μg kg^{-1} of 38 ± 3 cm^2; 25 μg kg^{-1} of 28 ± 4 cm^2. The hyperthermia stressed plants growth has been studied. The highest plant growth rate was in Se nanoparticles concentration of 5 and 10 μg kg^{-1} options. The eggplant grown on the soil with Se nanoparticles addition at a concentration of 10 μg kg^{-1} leaf plate surface area was twice compared the eggplant grown on untreated soil. The same was for tomato plants. The cucumber plant leaf plate surface area grown using Se nanoparticles was by 50% higher compared control option. The Biogeosystem Technique (BGT*) non-imitating a nature processes methodology was proposed of the 20–45 cm soil layer intra-soil milling for soil multilevel aggregate system formation and the intra-soil pulse continuous-discrete watering for controlled soil water regime. BGT* provides the Se nanoparticles better function in real soil ensuring a synergy effect on soil fertility of intra-soil mechanical processing, intra-soil water saving pulse continuous-discrete moistening, humics, polymicrobial biofilms, and nanoparticles.

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Activated potassium phosphate fertilizer solution for agricultural plants growth stimulation

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Current obsolete agriculture, irrigation, remediation technologies leading to negative environmental effects are incapable to ensure the agrobiogeosystem sustainable response to plant stimulation. This causes soil matter degradation, restricts the role of polimicrobial association as the starters of soil biological process. The anti-stress regulators are necessary for successful agricultural practice. Crop stimulation and protection are to be based on a thorough dosed environmental impact of physical factors. The signaling crosstalk between H_2O_2 and NO, H_2O_2 and Ca^{2+} influence plant developmental and physiological processes. The high-frequency low-temperature plasma generated via cathode high-frequency glow discharge provides electrolysis of the low concentration solution of the strong electrolyte KH_2PO_4. The activated solution was named Plasmolite. Oscillogram of voltage, current, and plasma glow on the active electrode) supports the fact that electrolysis of the strong electrolyte KH_2PO_4 aqueous solution can proceed actively at a low concentration of 0.01M. The maximum of the Plasmolite solution Raman (red) scattering spectrum of 1640 cm^{-1} was associated with hydrogen atom vibrations.The scattering spectrum bands of 875 cm^{-1}, 930 cm^{-1}, 1050 cm^{-1}, and 1123 cm^{-1} were associated with the aqueous electrolyte solution plasma treatment. The goal H_2O_2 concentration was of 100 uM. Seeds were germinated in the Plasmolite based 2×10^{-5}M KH_2PO_4 moisturizing medium for three days. Spring spelled seeds (grade "Gremme") sprouted for 92 % compared 56 % in control option. Spring rye seeds (grade "Onokhoyskaya") sprouted for 90 % compared 75 % in control option, and the seeds sprouted percentage with a root length of more than 6 mm was 80% compared 50 % in control option. The Plasmolite is promising for the production of activated H_2O_2 for plant stimulation and protection, focusing on K and P_2O_5 content. We proposed the Biogeosystem Technique (BGT*) methodology to provide a non-conflict implementation of high level chemical technology into the biosphere. The BGT* componentse: intra-soil robotic system for the fine multilevel soil aggregate system formation, intra-soil pulse continuous-discrete watering. BGT* provides Plasmolite H_2O_2, fertilizes, humic substances, polymicrobial biofilms starters synergistic effect on plant physiology and soil-biological process. The plant protection, high rate soil biological process, soil health and productivity are possible.

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Plant derived intrinsically disordered proteins carbon nanotube conjugates as biofunctional materials towards plant stress tolerance

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Intrinsically disordered proteins (IDPs) have become increasingly examined for their role in plant physiology. These proteins defy typical molecular biology paradigms by imparting stress tolerance to plants and influencing plant development without having a well-defined hydrated secondary structure. Despite their disorder, IDPs exhibit unique biophysical properties such as the formation of secondary structure upon stress stimuli such as low temperature, which may allow them to stabilize membranes and proteins. Furthermore, IDPs form membrane-less compartments upon liquid-liquid phase separation in living plant cells to partition biomolecules for controlling protein expression and enzyme activity. Orthogonally, nanomaterials have been shown to interact with plants with chemically-tunable precision. These unique physiological and physical properties make plant IDPs attractive biological ligands to conjugate to nanoparticles and synthesize IDP-nanoparticle (IDP-NP) hybrids that can deliver bioactive proteins to plant or mammalian tissues, or may yield constructs with unique physical and stimuli-responsive properties. In a prospective study, we recombinantly expressed two membrane associating plant IDPs from *Arabidopsis thaliana* in *E. coli* and conjugated them to single walled carbon nanotubes (IDP-CNT). CNT were chosen as hosts for IDP conjugation due to their unique photophysical properties, ease of preparation, and high propensity for uptake by both plant and mammalian cells. The resulting IDP-CNT constructs are highly dispersible in water, are near-infrared (NIR) photoluminescent, and demonstrate adsorption to human HEK293T cells. Importantly, these data suggest that IDP-CNT preparations may retain some unique IDP functions and encourage further investigation into their characteristics and applications in plants. Subsequent research will also explore IDP delivery applications for enhancing plant abiotic stress tolerance as well as *in vitro* cryo- or xero- preservation of cells.

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Properties and use of water activated by plasma of direct piezo-discharge

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Plasma activated media and, in particular, plasma activated water have good prospects for use in medicine, food industry, and agriculture. In this paper, distilled water was exposed to a cold atmospheric plasma (CAP) generated by the piezotransformer based source. The CAP liquid media treatment was carried out using a six-well plate methodology. The exposure time was from 30 s to 10 min. The emission

spectrums of piezo-discharge were obtained. Physical and chemical changes in CAP activated water were observed for 8 days. Basing on the optics methods, quantitative assessment was made of the nitrite and nitrate ion concentration change over time after treatment. The processes were investigated of reactive oxygen and nitrogen species (RONS) generation. The processes were investigated of reactive oxygen and nitrogen species (RONS) production. The dependence of the RONS production on the exposure time and the RONS lifetime in the treated liquid were determined using absorbance spectra. The medium conductivity and redox potential varied linearly depending on exposure time. The pH value change correlates with nitrate anion concentration. The concentration of peroxide hydrogen was measured immediately after CAP water treatment. The concentration of peroxide hydrogen increased linearly depending on CAP water treatment duration. The data showed that CAP water treatment provides a significant change of the liquid media properties. Biogeosystem Technique (BGT*) provides CAP activated water agriculture synergistic effect. Plant stimulation is closely linked to the plant protection from pathogens. A CAP activated water higher synergistic capability was shown for providing plant phitopathogens resistance, humic substances functioning, and healthy soil environment basing on the preconditions of BGT* methodology. We proposed new transcendental system for the plant protection via biological drug and CAP activated water synergy apply to the soil. The method envisages the liquid biological drug based on CAP activated water pulsed intra-soil discrete injection and followed pulsed intra-soil discrete watering with CAP activated water. The method provides improved conditions for the applied biological objects development inside the soil, reliable pathogens prevention, high quality biological production, and soil health.

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Assessment of *in vitro* cell cultures as a comparative metabolism model to provide additional information for OECD 501 and 502 data read across

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Metabolism data is generated from crop studies performed according to the OECD 501 (Primary crop) and 502 (confined rotational crop) guidelines for the registration and re-registration of active ingredients. The crops selected are representative of one of five crop groups (root vegetables, leafy crops, fruits, pulses and oilseeds, and cereals), usually three crops are selected that are representative of the target use. The data generated is taken to be representative of other crops of the same nature, for example a leafy vegetable may be lettuce, and this data would be read across for spinach. There is potential for the use of an active ingredient to change or to be used on additional crops after the initial registration. At this point there may be a data gap where the metabolism has not been investigated in two or more of the five crop groups and, as a result additional crop metabolism studies may need to be conducted in additional species, which can be costly and time consuming. Plant cell cultures have been shown to reliably produce metabolites that are representative of the whole organism; this presentation evaluates the feasibility of performing a crop species comparative metabolism study using *in vitro* cell cultures to generate data for the missing crop groups that may potentially fill the data gap.

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Effects of organic, integrated and conventional production practices on the quality of solanaceous vegetables

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With the improvement of the living standard of the public and the enhancement of health awareness, consumers' demand for organic vegetables is gradually increasing, and determining the influence of different vegetable production systems on vegetable quality is the key to produce high-quality nutritious vegetables. Based on the long-term positioning experiment of three different vegetable production systems (organic, integrated, and conventional) at Quzhou experimental station of China Agricultural University, which began in 2002, compared with the conventional tomatoes, the contents of soluble sugar, soluble protein and vitamin C in organic tomatoes were significantly increased ($p < 0.05$) by 25.35%, 133.33% and 80.45%; the content of nitrate was significantly reduced by about 50%; the contents of total phenol, β-carotene and lycopene were significantly increased by 166.89%, 176.6% and 155.38%. Phenylalanine ammonia-lyase activity, a key disease-resistant enzyme regulating phenolic synthesis in organic vegetables, was about 1.5 times as high as that in conventional and comprehensive systems ($p < 0.05$); the organic vegetables had higher total antioxidant capacity, superoxide dismutase, ascorbate peroxidase, and catalase activities. Organic production systems can improve the nutrition of solanaceous fruit vegetable quality, functional quality, more ecological function (oxidation resistance and disease resistance function), and this may be due to organic farming system restricted conditions management to a certain extent, inspired the vegetables oxidative stress reaction, improve related enzyme activity, and thus promoted accumulation of the oxidation activity substances such as vitamin C, total phenol and flavonoids, beta-carotene and lycopene. In this paper, entropy weight method and comprehensive evaluation method were combined to evaluate the quality of vegetables under different production modes. Both vegetables presented the conventional comprehensive evaluation results of organic >integrated > conventional, which indicated that the comprehensive quality of fruit vegetables under an organic production system was much better.

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Responses of soil microbial communities and organic carbon from long-term organic, low-input and conventional vegetable farming

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Soil microbial communities may be strongly influenced by agricultural practices which change the soil environment. We investigated differences in soil organic carbon of organic, low input, and conventional vegetable production systems from two depths, 0-20 and 20-40 cm, and differences in microbial biomass carbon (MBC), and phospholipid fatty acid (PLFA) composition. Three production patterns of long-term test significantly enhanced soil organic carbon in 0-20 and 20-40cm soil. Organic system was significant to improve soil organic carbon. MBC and total PLFA of organic farming

system was the highest in the three systems. With the increase of soil depth, soil microbial biomass carbon decreased. Despite these differences, the change trends of soil organic carbon and microbial communities in the three vegetable production systems were similar. So, using organic fertilizer would increase carbon availability to microorganisms. Soil organic carbon, microbial biomass, and community structure were influenced significantly by long-term different practice in farming system.

AGRO 258

Determination of 111 pesticide residues in lettuce and Chinese chives with sin-QuEChERS nano cleanup using GC-MS/MS and LC-MS/MS

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In this study, a gas and liquid chromatography-tandem mass spectrometry multiresidue method, with sin-QuEChERS Nano cleanup procedure, based on acetonitrile extraction is developed and validated in lettuce and Chinese chives, with a large scope that includes pesticides of different chemical classes. Average recoveries of most pesticides were in the range from 73% to 118% with the relative standard deviation <20% at both validation levels. The limits of quantification were 0.3-8.4 μg/kg. Matrix-matched calibrations were performed with the coefficients of determination more than 0.99 between concentration levels of 10-500 μg/kg. The developed method was successfully applied to the determination of pesticide residues in market samples. The study demonstrated that sin-QuEChERS Nano cleanup method could be used as a rapid, convenient and high-throughput cleanup method for analysis of pesticide residues.

AGRO 259

Biomimetic synthesis and herbicidal activity of tenuazonic acid derivatives

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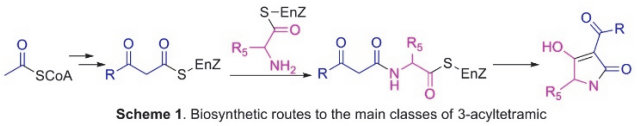
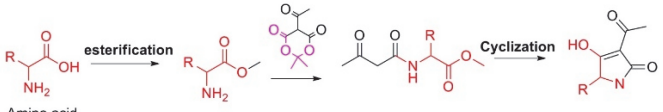
The heterocyclic core of tetramic acids (*i.e.*, pyrrolidine-2,4-diones) has attracted significant attention over the years due to their occurrence in naturally bioactive products isolated from bacteria, moulds, algae, fungi, lichens, and sponges. Tenuazonic acid, is a unique pyrrolidine-2,4-diones that exhibits herbicidal activity by inhibits photosynthesis by blocking photosystem II (PSII) electron flow from QA to QB. In order to discover new, effective and safe agrochemicals for weed control in crops, a series of tenuazonic acid analogues in which the isobutyl was replaced with substituents have been synthesized via biomimetic synthesis with the aim of obtaining molecules with various bioactivities. The target compounds were evaluated their herbicidal activity, and the preliminary bioassay data showed that some of the compounds exhibited good herbicidal activity against dicot *Arabidopsis thaliana* at a concentration of 10 mg/mL. As the skeleton of these derivatives belong to the triketones, a commercial herbicide motif acting as an HPPD inhibitor. Most of these compounds indeed exhibited similar plant symptoms to sulcotrione or other HPPD inhibitors, although their mode of action has not been confirmed. Based on studies with D1-mutants of green alga *Chlamydomonas reinhardtii*, No., 256 amino acid was found to play a key role in its binding to the QB-niche, tenuazonic acid and its derivatives should be considered as a new type of PSII inhibitor.



Scheme 1. Biosynthetic routes to the main classes of 3-acyltetramic



Scheme 2. Synthetic routes to 3-acyltetramic derivatives

AGRO 260

Establishment of import tolerance for thiacloprid in strawberry with several residue-field trials

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For export promotion, import tolerance of thiacloprid in strawberry was proposed using OECD Maximum Residue Limit (MRL) calculator after conducting three different field trials. Pre-harvest interval (PHI) of residual pattern and degradation dynamic of thiacloprid in strawberry were determined using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Samples were extracted with acetonitrile and a mixture of salts, and dilution was performed for purification. A six point matrix matched calibration curve was constructed which provided excellent linearity with coefficient of determination (R^2) ≥ 0.9998. Detection and quantification limits were 0.003 mg/kg and 0.01 mg/kg, respectively. The method was validated in quintuplicate at three different concentrations and resulted acceptable recovery ranging from 80.86~101.71% with relative standard deviation (RSD) ≤ 6.50 among the three filed sites. The developed method was applied to the field treated sample harvested at different intervals. In PHI trial, the thiacloprid residues were ranged 0.24~0.70 mg/kg in the field site 1 (Nonsan), 0.16 ~ 0.50 mg/kg in the field site 2 (Sunchang), and 0.36 ~ 0.50 mg/kg in the field site 3 (Sacheon). Whereas, in the degradation trial, the observed residues were 0.03 ~ 0.81 mg/kg in the field site 1, and 0.02 ~ 0.48 mg/kg in the field site 2. Consequently, the import tolerance of thiacloprid in strawberry using OECD MRL calculator was proposed to 2 mg/kg, which is exactly same as the MRL established by Republic of Korea. In conclusion, the residue study supported to propose 2.0 mg/kg as a MRL of thiacloprid for strawberry.

AGRO 261

Dissipation pattern and pre-harvest residue limit (PHRL) of chlorfluazuron in cabbage

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The dissipation and residue pattern of chlorfluazuron in cabbage was studied under greenhouse condition. The pesticide was sprayed at recommended standard rate under two different fields. Samples were collected at 0, 1, 2, 3, 5, 7, and 10 days after last spraying. Analytical method was developed using UHPLC-MS/MS. Method limit of quantitation was 10.0 ng/mL. At the fortification levels of 0.01 and 0.1 mg/L, recoveries ranged 93.1~97.7% and 99.9~104.0%, respectively. The biological half-lives of chlorfluazuron in field 1 and field 2 were 3.1 and 2.9 days, respectively.
Based on the dissipation patterns of chlorfluazuron under two fields, the PHRL of chlorfluazuron was suggested as the guidelines to make the pesticide residue under the MRL on harvest day.

AGRO 262

Use of metallic ion complexes with natural products to protect food crops from plant diseases such as peach leaf curl

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Metallic ions such as Copper II and Zinc II when complexed with Apple Cider Vinegar provide an effective and immediate cure for Peach Leaf Curl. These constituents are combined with minute quantities of simple detergents, then applied to the shoot system of the crop to every plant part from the ground up. This Treatment Protocol includes applying this mixture to all plant surfaces including the top and bottom sides of leaves, branches, trunks, and fruit.
This treatment will immediately block the spread of Peach Leaf Curl so that new leaf growth is completely free of Peach Leaf Curl so that new leaf emergence can immediately start that will support the maximum production of fruit without further fruit loss.
Similar results were obtained with several different species of fruit trees, ornamental trees and shrubs, tomatoes, red peppers, eggplant, and squash.
Further details will be presented during the Poster Session.

AGRO 263

Strategies for the synthesis of biologically active organohalogenated natural products

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To date, close to 6,000 organohalogenated natural products have been isolated and structurally characterized, yet only a small fraction of these compounds has succumbed to chemical synthesis and biological study. In reported syntheses, carbon–halogen bond-forming steps are often plagued by low selectivity. We are actively engaged in developing new strategies and methods for the selective installation of chlorine- and bromine-bearing stereogenic centers in complex molecule-relevant settings. A historical account of our work and recent progress will be discussed. Furthermore, motivated by the lack of understanding of how organohalogens interact with biological systems, we are pursuing follow-up biological evaluation and mechanism of action studies to explore the therapeutic potential of natural organohalogens and analogues.

AGRO 264

Novel pyrazolyl *N*-aryloxazolidinone herbicides for *Amaranthus* and grass control in corn and soybeans

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Amide and carbamate-substituted *N*-aryl thiazolidinones, pyrrolidinones and oxazolidinones were discovered at Zeneca in the 1990's as a new class of cellulose biosynthesis inhibiting herbicides where oxazolidinone as the central core provided optimum activity. Structurally-related *N*-linked pyrazolyl *N*-lactams were later explored at DuPont in the early 2000's as corn herbicides with activity against both *Amaranthus* and grass weeds. Further work at that time was not pursued due in part to the dominant position of glyphosate in the marketplace. However, renewed interest from more recent weed resistance concerns, prompted exploration of a new chemotype where the pyrazole substituent was linked to the central lactam ring *via* a C-C bond. Here, the synthesis and preemergence herbicidal activity of pyrazole-substituted *N*-lactams will be reported, with an emphasis on the herbicidal activity of analogs with an oxazolidinone as the central ring.

AGRO 265

Unique picolinamides for broad spectrum disease control

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Research efforts on the UK-2A natural product has led to the development of two new fungicidal active ingredients from Corteva Agriscience: Inatreq™ active (fenpicoxamid) and Adavelt™ active (florylpicoxamid). During subsequent structure-activity relationship investigations, an unexpected byproduct was found. This observation led to the identification of a new picolinamide scaffold possessing unconventional stereochemical features. Details on the synthesis and attributes of this novel picolinamide series will be reviewed.

AGRO 266

Synthesis, biological activity and structure-activity relationships of a new broad spectrum fungicide

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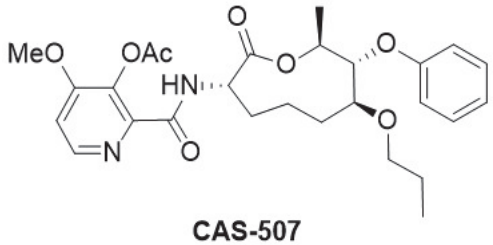
Fenpicoxamid, a semi-synthetic derivative of the natural product UK-2A, has recently been developed for the control of wheat and banana diseases. It was found that derivatization of UK-2A allowed for relatively limited exploration of the key binding elements necessary for activity. As a part of a program around the identification of new broad spectrum fungicides, the total synthesis of a chiral-pool derived macrocyclic analog of UK-2A was completed. This presentation will detail the synthetic exploration around a new type of macrocyclic fungicides represented by CAS-507, and provide an overview of the general structure-activity trends observed.



CAS-507

AGRO 267

Regulatory perspective on exposure task forces

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Several task forces over the last few decades have generated data for pesticides which are routinely used in U.S. EPA risk assessments for evaluating occupational exposures and exposures in the general population. Outputs include unit exposures for pesticide handlers which relate exposure to the amount of pesticide handled for specific conditions (equipment, PPE use, clothing, etc.) or transfer coefficients which relate exposure to activities which occur in previously treated areas (vegetable harvest, tree pruning, etc.). Data call-in authority under FIFRA sparked the development of these groups. EPA and other regulatory agencies provided input for each task force during their lifecycles on several topics: program scope, study development, statistical design, SOP development, peer review, regulatory compliance, and data analysis. The resulting data from these efforts are used broadly on a global basis, and they represent one of the largest dermal sampling databases in existence across all chemical space. The task force approach has many benefits. It provides a forum for impacted stakeholders to coalesce in order to develop information that can be used broadly for regulatory purposes in a uniform manner. This allows for more consistent regulatory decision making. It also allows for collaborative input from all involved including regulators and impacted stakeholders through all phases of the data development process which makes it more efficient and well documented. The U.S. EPA completes hundreds of risk assessment actions for pesticides each year. Task force data play a significant role in each of these which involve non-dietary exposures. These factors will be addressed in the presentation.

AGRO 268

Overview of the outdoor residential exposure task force (ORETF)

Jennifer Thomasen*, jennifer.thomasen@bayer.com. Bayer, Ellisville, Missouri, United States*

The Outdoor Residential Exposure Task Force (ORETF) is a consortium of over 30 agricultural chemical companies formed in 1994 in preparation for a scheduled Data Call-In (DCI). The ORETF members jointly developed data on exposure of homeowners and professional lawn care operators during the application of pesticides as well as exposure to individuals who enter a residential turf area following a pesticide application. The database of exposure data generated by ORETF is being used by United States Environmental Protection Agency (USEPA), California Department of Pesticide Regulation (CDPR), and Canada's Pest Management Regulatory Agency (PMRA) to assess exposure potential and to conduct risk assessments for outdoor residential turf, garden and ornamental pesticide products. In addition to exposure data, the ORETF developed a single standardized technique for measuring product-specific turf transferable residues (TTR), the Modified California Roller. This presentation will provide a broad overview of the ORETF including study methods, design, and conduct employed to develop the ORETF database.

AGRO 269

Agricultural reentry task force (ARTF): History, data development, and data use

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The Environmental Protection Agency (EPA) issued a Data Call-In (DCI) on October 18, 1995, requiring that pesticide manufacturers submit data on the persistence of chemical residue on crop foliage (termed Dislodgeble Foliar Residue or DFR) and also on dermal and inhalation exposure to pesticides during reentry into any pesticide-treated agricultural site. The intention was for the data to be used to estimate exposure to agricultural workers when they enter fields that have been treated with a pesticide, and to calculate the restricted entry intervals that appear on product labeling. The Agricultural Reentry Task Force, L.L.C., (ARTF) was organized as a "joint data development task force" in which pesticide manufacturers worked together to develop a database that met EPA's requirements. ARTF worked very closely with EPA, Canada's Pest Management Regulatory Agency (PMRA), the United States Department of Agriculture (USDA), and the California Department of Pesticide Regulation (CDPR) in developing the data and database. The Task Force had a Joint Regulatory Committee where representatives from these groups conferred with task force members on the regulatory direction of the project. ARTF's general plan for conducting an exposure study was approved by EPA, PMRA, USDA, and CDPR. ARTF data began to be used in government risk assessments when the database was put on-line in August 2000. This presentation will cover the

establishment and structure of the ARTF, the design and performance of the studies completed to generate the necessary data, and the development of a database to allow regulators and registrants to access and use that data.

AGRO 270

Agricultural Handler Exposure Task Force: Generic data development to generate exposure information for agricultural workers performing mixer/loader and applicator tasks

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The Agricultural Handler Exposure Task Force (AHETF) was established in 2001 to generate agricultural pesticide handler exposure data meeting EPA data requirements for registrations. EPA had explicitly imposed data requirements on several pesticide registrants through data call-in notices, reregistration requirements, or through consultation with applicants regarding prospective registrations. These companies, and eventually many others, agreed to form AHETF and jointly develop the reliable generic data necessary to support the continued EPA registration of most products approved for agricultural uses. The scientific question addressed by AHETF was: "What is the expected magnitude and (statistical) distribution of worker exposure to pesticides during distinct occupational pesticide handling scenarios?" The primary AHETF goal was to collect worker exposure monitoring data for a wide variety of pesticide mixing and loading scenarios (such as mixing/loading different formulation types and mixing/loading systems), application scenarios using different types of application equipment (such as ground boom, airblast, aerial, and hand-held sprayers), and engineering controls (such as enclosed cabs). These pesticide handler exposure data replaced data from PHED (Pesticide Handler Exposure Database, EPA 1998) that had been found to suffer from various scientific deficiencies by EPA and other regulatory agencies. AHETF submitted reports and a database (AHED®, Agricultural Handler Exposure Database) to EPA, CDPR, and PMRA (collectively called the Joint Regulatory Committee, or JRC) that was involved with the overall development of the testing program. This presentation will cover the establishment and structure of the AHETF and technical aspects of the AHETF monitoring program including; how AHETF designed, conducted, and reported exposure data for a variety of pesticide mixing, loading, and application scenarios.

AGRO 271

Overview of the pet care product task force

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The Pet Care Product Task Force (Bayer Animal Health, Central Garden and Pet, Elanco, Hartz, and PetIQ) was established in 2019. The objective of the Task Force is to work in collaboration with EPA's OPP, per the Agency's request, and in lieu of a data call-in, to develop a refined, scientifically robust set of post-application pet care product exposure assessment methods that can replace the EPA's existing methods (EPA 2012 Office of Pesticide Programs Standard Operating Procedures). The Task Force member companies have provided chemical/ formulation-specific transferable residue data (petting simulation studies), and have done an evaluation of existing, alternative methods (and underlying data that are referenced). The existing methods include EPA 2012, PMRA 2018, EMA 2010 and 2018. We will share our positions on the use of these alternative methods as appropriate for pet care product exposure estimation and evaluation.

AGRO 272

Recommended approaches for data sharing within a task force

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The successful implementation and actions of a task force can strengthen resources and data for regulatory action or response. Task forces may form to address regulatory action for similar active ingredients (*e.g.*, pyrethroids), or to address specific risk assessment topics (*e.g.*, pollinator risk assessment or endangered species). Despite the task force objectives, proper management of data-sharing efforts is critical for all task force efforts. Maintenance of proper registrant data anonymity must be considered for confidentiality, while appropriate traceability is preserved. In addition to anonymity, consistency of data compilation is a critical step in task force data sharing efforts. This presentation will present recommended approaches for data sharing efforts within a task force to optimize efficiency, leveraging registrant resources wisely, and implementing stakeholder confidentiality appropriately.

AGRO 273

Regulatory use of exposure task force data: The Canadian perspective

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Health Canada's Pest Management Regulatory Agency (PMRA) is responsible for the federal regulation of pesticides in Canada. According the Pest Control Products Act, companies intending to sell pest control products in Canada must provide data demonstrating the safety of these products to both human health and the environment, and also that these products have value when used according to label directions. Only pest control products that have acceptable risk are registered for use in Canada. PMRA data requirements for human health risk assessment include mammalian toxicology studies to characterize the hazard profile of a pesticide and studies to assess the degree and nature of exposure to sentinel human populations, such as workers and children. As the science of risk assessment has evolved, so too have the requirements for exposure studies. For example, industry task forces, working in conjunction with regulatory agencies and researchers, have developed the scientific methodology that allows exposure studies conducted with one pesticide be used for the assessment of many pesticide active ingredients in a generic manner. While the protocols of chemical-specific studies are typically required to adhere to the appropriate regulatory guidelines, variations between studies has limited

the ability to use the data in a more generic fashion. Furthermore, differing policies on the requirements and use of these studies has sometimes led to differences in interpretation between regulatory agencies. The formation of exposure task forces has also enabled the standardization of protocols and the generation of human exposure data that is applicable to a wide variety of pesticide scenarios and submissions. Overall, the collaborative and scientific approach used by the exposure task forces, which includes attaining regulatory input throughout the process, has resulted in a large, comprehensive, and modern collection of data, and has been an efficient mechanism for developing data required for pesticide submissions.

AGRO 274

Council for the Advancement of Pyrethroid Human Risk Assessment (CAPHRA): Broad collaboration and scientific innovation to reduce uncertainty in pyrethroid human risk assessment

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In 2010, the United States Environmental Protection Agency (US EPA) issued a request for proposals to evaluate the potential differential sensitivity between juvenile and adult rats exposed to pyrethroid insecticides. A consortium of industry registrants organized with the objective of not only addressing the observations in rats, but also to determine the relevance of those findings to humans (*i.e.*, are children more sensitive to pyrethroid toxicity than adults?). This consortium (*i.e.*, the Council for the Advancement of Pyrethroid Human Risk Assessment; CAPHRA), working closely with experts across regulatory, industry, academic and contract research organizations, undertook a multi-year, multi-million dollar effort to design a research program to provide to the US EPA (and other regulatory agencies) the knowledge to reduce these uncertainties and suggest innovative methods for risk assessment for the entire class of pyrethroid insecticides. Novel toxicokinetic, toxicodynamic and metabolic ontogeny data were developed using *in vivo*, *in vitro*, and *in silico* approaches. Finally, a fully parameterized, life-stage PBPK model was developed for rats and humans that enabled the generation of human-relevant regulatory endpoints, which can be used to assess risk without the need for between-species uncertainty factors. The results of this work confirmed that the observed age-related sensitivity of rats to pyrethroids at high doses is due to differences in kinetics and that those differences are not manifest in humans. Based on these findings, the US EPA reduced the age-related uncertainty factor for pharmacokinetics for pyrethroids as a chemical class by 3-fold, representing a significant advancement in human health risk assessment methodology. It also remains a definitive example of industry consortia working collaboratively to strengthen the scientific basis for animal to human extrapolation thereby reducing the uncertainty associated with risk-based regulatory decisions.

AGRO 275

Task force data generation for risk assessment

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The Coalition of OP Registrants has ADAMA, AMVAC, Bayer Animal Health, FMC and Gowan as members. It was formed a few years ago to address a U.S. EPA Health Effects Division Memorandum titled "Literature Review on Neurodevelopmental Effects and FQPA Safety Factor Determination for the Organophosphates." The memorandum was a proposal for how EPA would utilize a number of epidemiology studies to create uncertainty and the reapplication of an FQPA 10x Safety Factor in human health risk assessments for organophosphate pesticides. The Coalition worked with third party scientists and industry experts to demonstrate the scientific flaws in these epidemiology studies and the conclusions in the Literature Review. The Coalition has not generated specific data but has and continues to provide evaluations of existing epidemiology studies and human and animal toxicity data. We will share our positions on use of epidemiology studies versus human and animal toxicity data in human health risk assessments for the organophosphate pesticides.

AGRO 276

Use of CARES NG to Conduct a Cumulative Dietary Exposure Assessment for Chlorotriazine Compounds: A Case Study

Jason E. Johnston*, jej8641@gmail.com. The Acta Group, Washington, D.C., United States*

Estimation of dietary exposures to pesticide residues in agricultural and livestock commodities is critical in regulating the safe use of pesticide products. The U.S. Environmental Protection Agency (EPA) has used the Dietary Exposure Evaluation Model - Food Commodity Intake Database (DEEM-FCID) successfully for such purposes for many years. Increasingly complex assessments are now sometimes required, however. A particular example is the cumulative dietary assessment for the chlorotriazines (atrazine and simazine), for which four-day rolling average dietary exposures are needed. In the 2018 cumulative assessment for chlorotriazines, EPA used point estimates for residues and 100 percent crop treated in combination with two-day average food consumption data to approximate four-day rolling average dietary exposures for both atrazine and simazine separately. EPA then summed these two estimates to produce a conservative approximation of cumulative chlorotriazine four-day rolling average dietary exposures. Both atrazine and simazine are registered for use on corn, and EPA's cumulative approach implies that all corn commodities are treated with both compounds. The dietary model in the Cumulative and Aggregate Risk Evaluation System - Next Generation (CARES NG) permits calculation of *n*-day rolling average exposures. CARES NG also permits creation of a residue input file that preserves EPA's assumption of 100 percent crop treated for corn commodities while accounting for relative market share of atrazine and simazine in corn applications. This case study illustrates the impact of these two features in CARES NG on estimated cumulative dietary exposures for chlorotriazines. For example, CARES NG produces a 95th percentile four-day rolling average dietary exposure for children 1-2 years old,

the most highly exposed subpopulation, that is approximately 30 percent lower than EPA's approach and yet is conceptually as conservative as EPA's assessment.

AGRO 277

Spray drift task force (SDTF)

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In the 1980's, EPA required product-specific spray drift data. However, it was recognized that drift was predominately a function of application parameters such as droplet size, application height, and wind speed rather than the product being sprayed. Therefore, the Spray Drift Task Force (SDTF) was formed in 1990 to generate generic databases, allowing development and validation of spray drift models applicable to most products. The SDTF included an Administrative Committee that handled the business, financial, and legal aspects of the SDTF and a Technical Committee providing expertise in a wide range of scientific disciplines. Contract labs and consultants performed the technical activities under the direct supervision of the SDTF, allowing input from the best scientists in the industry worldwide and Agencies working together on a common objective. The regulatory agencies in the U.S., Canada, and California were involved throughout the testing program, which subsequently underwent a peer review and EPA Science Advisory Panel (SAP) review. SDTF developed methods and conducted studies on the effects of tank mix physical properties on droplet size formation; droplet size spectra formed by a wide range of nozzle characteristics, and field drift studies under differing release heights, wind speeds, droplet sizes, and crop canopies. Data were generated for applications by aircraft, ground boom sprayers, airblast sprayers, and chemigation systems. SDTF conducted surveys to ensure the protocols reflected actual field use conditions and published several scientific papers and educational booklets. The SDTF was the first major generic task force of this kind and served as a template for subsequent exposure task forces. The SDTF clearly showed that the generic task force approach is the most efficient and cost-effective means for generating non-product-specific (*i.e.*, generic) data for use by both the industry and the Agencies.

AGRO 278

FIFRA endangered species task force: Dealing with unusual challenges and multiple agencies to address pesticide and endangered species data needs

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The FIFRA Endangered Species Task Force (FESTF) was formed in December of 1994 to develop data required by EPA of applicants or registrants relative to the potential impacts of pesticides on species listed by the Endangered Species Act as "threatened" or "endangered" ("listed species"). FESTF was organized to develop information sufficient to address FIFRA data requirements imposed on registrants. The work product to be accomplished by the Task Force has provided necessary information for many pesticide product registrations and re-registrations in the United States, but via a path not initially anticipated for task force data development. The Task Force covers a wide cross-section of pesticide registrants that benefit from this endeavor and has submitted multiple datasets to EPA and the Services. FESTF has also developed a customized information management system that allows access to and comparison of data in a spatial and tabular software environment. The original mission of the task force was envisioned to be addressed through a Cooperative Research and Development Agreement (CRADA) for the purposes of EPA's state by state assembly of data for listed species. However, the CRADA proved unworkable, and rather than EPA being burdened by the task of data assembly, FESTF took on the effort and commitment to support data aggregation and software program development. This presentation will explore the challenges that required unique approaches to data provisioning and will assess how 24 years of operations, constantly changing data, shifting policy and technology have been managed to address the complex data interactions brought about by FIFRA Registration Review and subsequent consultation under the Endangered Species Act.

AGRO 279

Pollinator research task force - Contributing to the science of pollinator risk assessments for pesticides

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In 2012, North America regulatory agencies published a new framework for pollinator risk assessments for pesticides, and in 2014, the agencies published their final guidance for assessing pesticide risk to bees. This resulted in enhanced regulatory testing and science-based risk assessments for pesticide products in North America. However, scientists from both the regulatory and regulated communities recognized several uncertainties in the new guidance including estimation of residue levels in pollen and nectar of a treated crop, pollen and nectar consumption estimates, the relevance of routes of exposure other than direct contact or dietary, and the use of the honey bee as a surrogate for native bee species. In addition, the guidance contained new data requirements, including some studies without fully validated protocols. To help address these uncertainties, U.S.-based crop protection companies established the Pollinator Research Task Force (PRTF). The mission of the PRTF is to review existing knowledge and develop new data to identify and fulfill generic data gaps, improve and validate test methods, and improve the risk assessment process. To date, the PRTF has conducted a diverse series of projects covering laboratory test design, field design and population modeling, exposure assessment, and toxicity evaluation. PRTF work products are submitted to US EPA and, as appropriate, submitted for publication in scientific journals.

AGRO 280

Separation, isolation and identification of metabolic biotransformation products of a novel *radio-labeled* small molecule into proteins and peptides in a metabolism study

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In an animal metabolism study, *14C-labeled compound X* was orally dosed to lactating goats in a dry feed. It was determined that once absorbed, *compound X* was rapidly and extensively metabolized. In the milk and edible tissues such as liver, kidneys, muscle, and fat, the primary *compound X* related *14C* metabolites were determined to be physiologically relevant moieties. The water extracts of liver, kidney, and muscle contained high concentration of proteins, and further characterization using *SDS-PAGE* indicated that radioactive moiety derived from labeled *Amine or labeled-Allyl* labels of *compound X* was incorporated into natural constituents such as peptides and proteins, which were separated into radioactive protein bands on *SDS-PAGE* gel. The organic extracts of milk and pooled fat contained lipid-type of radioactive residues, such as triglyceride derivatives, which were converted into fatty acid methyl ester after transesterification. Three *compound X* structure related small molecule metabolites were observed in the *radiolabeled Amine* label of liver, kidney, and muscle, which were identified by *LC-MS/MS* to be small peptide conjugates. This presentation will reveal challenges that our metabolism team had to overcome to determine the fate of *compound X* in physiological environment, its conversion to directly related chemical-based metabolites, and its biotransformation into physiologically relevant moieties.

AGRO 281

***In silico* tools for the generation and identification of novel metabolites**

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In silico metabolomics is an emerging branch of metabolite profiling that combines cheminformatics and machine learning to help generate novel structures and identify novel metabolites. In this presentation I will describe recent developments that our lab has undertaken to advance the field of *in silico* metabolomics. I will first describe two new software tools that we developed (CypReact and CypBoM) that use machine learning to identify cytochrome P450 substrates and reaction sites. I will then show how these tools have been integrated into the latest version of BioTransformer, an open access, web-based biotransformation predictor. I will also describe some of the latest developments with BioTransformer and will introduce BioTransformerDB, a database of more than 5 million predicted biotransformation products generated from known starting compounds (drugs, pesticides, food chemicals, and human metabolites). Finally, I will show how machine learning is being used by our group to develop software that can accurately predict the retention indices (RI), collisional cross section (CCS), NMR chemical shifts and MS/MS spectra of these predicted compounds. Together, these resources should make the identification of novel metabolites much easier and much faster in both agrochemical and drug discovery research.

AGRO 282

Pilot soil/sediment metabolism testing and metabolite identification

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Soil and sediment metabolism studies can sometimes yield results that are not entirely expected. When possible, it can be helpful to run a short pilot test to get an idea of how the study will go before diving headfirst into a 100+ day long study. A few items that can be parsed out in a pilot test are degradation rates affecting what sampling intervals make sense to use in the full test, determining predicted amounts of volatiles and therefore what trapping needs will be, extractability affecting what solvent extractions are used, troubleshooting LSC analysis and concentrating issues, and metabolites affecting instrumentation and results. Metabolites showing up in the pilot test can allow for a head start in including them in the instrumentation method and metabolite identification. Determining metabolites early for new compounds can allow for acquisition of reference standards ahead of time. Learning how the study will go and working out issues beforehand allows for a smooth study once the full test is started.

AGRO 283

One-stop metabolite shop: Employing multiple tools for accessing metabolites of drugs and agrochemicals

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Often several strategies are needed to access all key metabolites of drugs and agrochemicals, especially where chemical synthesis is not straightforward. Hypha has developed a "one-stop metabolite shop" scheme, which utilizes a combination of biological and chemical techniques in order to fulfil requirements to access any type of metabolites. The one-stop metabolite concept offers a parallel or sequential screening step to identify the most productive and cost-effective method to produce target metabolites. Depending on the type and quantity of metabolite required, a combination of chemical synthesis, mammalian S9, microbial biotransformation and recombinant phase I enzymes can be employed. Once the optimal production system is identified, the method can be scaled up to provide up to tens of grams of purified metabolites. Important to this suite of techniques are an extended panel of PolyCYPs® enzymes, microbial CYPs cloned from some of Hypha's actinomycete strains and expressed in *E. coli* together with redox partners. These enzymes are effective for scalable synthesis of CYP-derived human and other mammalian metabolites. For conjugated metabolites, a proprietary late-stage chemical screen has been devised, consisting of five robust conditions with deprotection strategies suitable for acyl glucuronides and some unstable *N*-glucuronides. The chemical screen has proven effective for producing at least one glucuronide from 90% of a panel of commercially available drugs from which glucuronides are known to be formed. New to Hypha's capabilities are glycosylating enzymes, under investigation for

the scalable production of *O*- and *N*-glycoside conjugates of agrochemicals. These enzymes have been derived from some of Hypha's strains talented at conjugating small molecules. The synergy of using the one-stop shop concept to access sets of metabolites will be exemplified using recent case studies.

AGRO 284

Challenges involved in predicting metabolites and their properties

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Anticipating what metabolites will be generated from a xenobiotic when it is introduced into a new organism or environment can be very challenging, in part because the enzymes responsible for the biotransformations involved are inherently somewhat promiscuous. They have evolved to process a wide range of substrates, which means that they tend to have large, flexible active sites that are difficult or impossible to address reliably using structure-based modeling methods. On the other hand, many of them retain key metabolic roles related to specific endogenous substrates. The latter point is especially true for plants, with their rich and often idiosyncratic secondary metabolisms. This talk will explore those themes and discuss how they relate to some tools successfully developed and applied for modeling human and mammalian metabolism of drugs and agrochemicals, though the underlying principles also apply to other animals as well as to insects, plants and fungi.

AGRO 285

Metabolomics revealed system-wide changes of physiology of *Botrytis cinerea*

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Gray mold fungus, *Botrytis cinereal,* is one of the most important pathogens, causing tremendous economic loss in agrobusiness. Their phytotoxic symptoms are mainly derived from secreted virulence proteins and secondary metabolites. Azole fungicides are common choices to control the disease. However, their biochemical effects other than sterol biosynthesis were not documented, especially pathogenesis-related metabolites. In this study, selected azole fungicides were treated *in vitro* and *in vivo* conditions. Comparative profiles of primary and secondary metabolites were assessed to discern the metabolic consequences. The results have shown that extensive metabolic differentiation was induced by azole fungicides both in intra and extracelluar metabolomes. Some azoles can definitely reduce the phytotoxic activities via reduction of toxin productions. According to instrumental analysis and bioassay, toxin reductions by azoles can be attributed to multiple factors. For example, several key primary metabolisms were impaired by azoles, including TCA cycle and aromatic amino acid catabolism. Cellular lipid profiles were altered drastically by azoles, which causes extensive changes of metabolite influx/efflux between cytosols and external environments. In addition to toxin productions, defensive metabolite productions were also down-regulated by azoles. Overall, metabolomics investigations have shown that crop-protective effects of azoles are derived from system-wide changes of whole metaboloms.

AGRO 286

Using monitoring data and model evaluations to support mitigation efforts to address pesticide concentrations in surface waters

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Pesticides are widely recognized as contaminants of concern in surface waters receiving urban runoff inputs. Extensive statewide monitoring conducted by the California Department of Pesticide Regulation (CDPR) Environmental Monitoring Branch provided a robust dataset which identified fipronil and pyrethroid insecticides as contaminants of ecological concern. Concentrations are often at levels that are potentially toxic to sensitive aquatic species. To address ecological concerns surrounding fipronil and pyrethroids from urban runoff, CDPR developed regulations and label use restrictions for products containing pesticides of concern. CDPR worked collaboratively with several stakeholders including product registrants, university researchers, and professional applicators to develop application restrictions that minimize the mass of pesticide applied to the landscape, while maintaining effective pest management. Predictive modeling was used to estimate the runoff concentrations resulting from different application scenarios including variations to active ingredient concentration, application interval, and application to impervious surfaces. The modelled runoff concentrations resulting from the restricted pesticide application scenarios were found representative of empirical data obtained during experimental field trials. Continued statistical evaluation of spatial and temporal trends in environmental pesticide concentrations provides a feedback loop for regulatory agencies to inform potential future mitigation designs.

AGRO 287

Data-driven interpretation of fipronil surface water monitoring data

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The assessment of surface water quality using monitoring data alone is a challenging task, particularly given that the data have highly intermittent and irregular sampling frequency in both temporal and spatial scales. To overcome this challenge, we propose a new assessment framework that compensates for the shortcomings of surface water monitoring data with other data collected at more refined and regular temporal and spatial scales. Those data sources provide information on a large number of possible factors that can affect the fate and transport of pollutants in the environment, including pesticide use information, watershed characteristics (land use, topography, soil property, etc.), climate, demographics, and management practices (irrigation practices, land management, mitigation, regulation, etc.). Such data are known as attributes. We will demonstrate how

the attributes data are re-scaled to catchment and/or watershed level and used to develop and refine data-driven geospatial models. The Random Forest Model (RF) will be used to interpret fipronil concentrations in California's surface water. RF is an ensemble machine learning method for classification, regression, and other tasks that operates by constructing a multitude of decision trees at training time with random sampling of training data points and attributes, and then outputting the consensus relationship observed from all trees. It is less prone to overfitting the unrepresentative monitoring data, and it can accommodate a complex relationship among the attributes as well as non-linear relationship between the attributes and the observed pesticide concentration. The model is able to identify the most influential factors that can be targeted in mitigation, estimate concentration at under-monitored places to support a more comprehensive risk assessment, and forecast trends or outcomes for future scenarios.

AGRO 288

Monitoring, modeling, and mitigation to support reductions in dissolved copper concentrations in California Coastal Marinas

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Copper antifouling paints (Cu-AFPs) are used to prevent biofouling of aquatic vessels. Copper is the most commonly used biocide in California marinas due to copper's broad-spectrum biocidal properties. Recreational boat marinas are particularly susceptible to pollution from Cu-AFPs because they tend to have poor hydrodynamic exchange and are densely packed with sporadically-used recreational vessels that have Cu-AFPs applied to their hulls. Historically, measured concentrations of dissolved copper (DCu) in marinas have exceeded the California Toxics Rule (CTR) acute (4.8 µg/L) and chronic (3.1 µg/L) criteria for protection of aquatic life. The CTR criteria exceedances led the California Department of Pesticide Regulation to develop regulations aimed to reduce DCu concentrations in California marinas. The modeling of different scenarios, ranging in marina dimensions and number of boats, were used to determine leach rates necessary to achieve DCu concentrations less than the CTR chronic criterion. DPR's leach rate cap regulations, which took effect in July 2018, were based on both risk assessment and pest management efficacy requirements. A new monitoring study was initiated in 2019 to assess DCu concentrations in eight representative saltwater marinas. Large (>1000 vessels) saltwater marinas were prioritized for monitoring because previous studies have indicated that larger saltwater marinas have relatively high DCu concentrations. Cu-AFPs are on boats for years and boatyard capacity for turnover is limited, thus results presented here are considered a reference point to evaluate long-term trends. Results from this study showed median DCu concentrations between 2.98 and 10.32 µg/L, with 79% of marina samples above the CTR chronic criterion and 52% above the CTR acute criterion. Waterbody characteristics (*e.g.*, marina dimensions, water chemistry parameters) that significantly correlate with DCu concentration trends will be discussed with a focus on spatial trends within and between the marinas.

AGRO 289

Evaluation of a large wetland for removing urban-use insecticides

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Water treatment and reuse initiatives are essential to combat declining water supplies in a changing climate, especially in arid and semi-arid regions such as California. Pollution of water resources intensifies the search for strategies to provide water for potable and non-potable reuse that mitigates detrimental ecological and human health effects. Fipronil and synthetic pyrethroids are common urban-use insecticides that have aquatic toxicity at trace levels and have been often found in urban surface streams. In this study, we systematically evaluated the spatial and temporal occurrence and distribution of fipronil, fipronil degradates and pyrethroids in the Prado Wetlands - the largest constructed wetland system on the west coast of the U.S. Concentration-based removal rates and changes in mass flux were calculated to determine the efficacy of constructed wetland treatment. Observed water concentrations were further used to calculate toxicity units for invertebrates *Hyalella azteca* and *Chironomus dilutus*. Pesticide concentrations in water, sediment, and plant samples consistently decreased as the water passed through the wetland cells at all time points. Removal rates for fipronil desulfinyl, fipronil sulfide, fipronil, fipronil sulfone, bifenthrin, and cyfluthrin were 100%, 99.7-100%, 57.8-88.1%, 75.6-100%, 74.7-100%, and 36.6-82.2%, respectively, and there was a general net attenuation of pesticides by the wetland. Toxicity units decreased in every instance for both aquatic invertebrates. The results of this study further indicated that settling of contaminated particles, adsorption to sediment, plant uptake or adsorption, and subsequent on-site degradation contributed to the effective removal of these urban-use insecticides. These findings highlight the potential of constructed wetlands for protecting urban water quality by removing trace contaminants.

AGRO 290

Monitoring pesticides in wastewater – with an eye on modeling and mitigation goals

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Pesticides in treated wastewater effluent are commonly discharged to surface water at concentrations that may pose a risk to aquatic organisms indicating available treatment technologies do not adequately reduce pesticide loads. Until recently, the pesticide data generated to characterize wastewater (including influent, effluent, and biosolids) have been obtained through one-time studies that lack a comprehensive analytical suite, consistent analytical methods, and systematic spatial or temporal coverage. In 2019, the California Department of Pesticide Regulation (DPR) initiated a permanent statewide monitoring program to evaluate pesticide concentrations in wastewater. The primary goals are to gain a better understanding of pesticide sources and fate in treatment, evaluate spatial and temporal variability, and evaluate the potential risk posed by pesticides in treated wastewater effluent to the surface waters receiving discharge. To date, 25 participating wastewater treatment plants have contributed influent, effluent, and biosolids samples, which

have subsequently been analyzed for 23 pesticides and pesticide degradates. Pyrethroids, fipronil and fipronil degradates were frequently detected in all influent samples. Regional variations were observed in influent concentrations: several pesticides were detected at higher frequencies and concentrations in Southern California samples. Future monitoring efforts will focus on collecting sub-sewershed samples to characterize potential intensive pesticide sources (*e.g.*, pet grooming operations, nurseries including cannabis, commercial laundry). DPR's monitoring results will refine predictive models used to evaluate pesticide products for registration in the state of California. Gaining a better understanding of pesticide sources, pathways, and fate in wastewater systems will not only improve DPR's ability to prevent registration of potentially problematic products, but will also inform development of potential mitigation approaches to address future water quality concerns.

AGRO 291

Assessing pesticide uses with potentials for down-the-drain transport to wastewater in California

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Pesticides and degradates are ubiquitously detected in municipal wastewater influent in the United States. However, little detailed information exists on their sources. The aim of this study is to investigate California Pesticide Use Report (PUR) and sales data to identify pesticide uses with a high potential for indoor down-the-drain (DtD) transport. DtD transport of pesticides could result from direct applications to drains and sewers or through indirect activities such as washing pets, treated textiles, laundries, and cleaning surfaces treated with pesticides. An initial screening on pesticide products registered in California with DtD potentials showed that fipronil, imidacloprid, and seven pyrethroids were pesticides of concern due to relatively high sales in DtD use patterns and high toxicity to aquatic organisms. Use and sales data of products containing the selected AIs were analyzed for mass of AIs applied with various DtD pathways and by different user groups. Professional uses were retrieved from PUR and consumer uses were estimated by comparing PUR to sales data. Overall, approximately 38.6 thousand kg fipronil, 44.6 thousand kg imidacloprid, and 240.5 thousand kg pyrethroids were used annually in California from 2011 to 2015 with some likelihood of DtD transport. About 56% (cypermethrin) to 98% (cyfluthrin) of the total mass was applied by professional applicators in and around structures and for some pesticides (imidacloprid and permethrin) on landscapes as well. The remaining mass was from consumer use, including treatments on pets (fipronil, imidacloprid, and permethrin), textiles (permethrin), other indoor-only uses (cypermethrin), and mixed indoor/outdoor or outdoor-only applications (other pyrethroids). Outdoor applications could become available for DtD transport, but it is expected to be a less important pathway than indoor applications. Results from this study help elucidate relative significance of specific DtD pathways and pesticide occurrence in California waste streams.

AGRO 292

Potential removal of pesticide active ingredients by common water treatment processes

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Tackling the growing concern about plant protection products entering not only surface water but also ground water and drinking water starts at the production site. Identifying the potential for elimination of active ingredients (AI) from waterbodies is a first step towards decreasing the point pollution around a given production site, and also potentially increasing safety for human health. The efficacy of three common water treatment processes (flocculation/coagulation/sedimentation) activated carbon adsorption, and biological degradation) at removing more than 25 AIs from commercial plant protection product formulations was examined.
Flocculation/coagulation/sedimentation with three commercial coagulant products has been tested in a Jar-Test setting. The efficiency of the process was assessed by analyses of dissolved organic carbon (DOC), turbidity, and AI removal. The supernatant (treated clean water) resulting from this process was then used as feed water in the next two processes; activated carbon adsorption and biodegradation. This was conducted in order to simulate commonly applied conventional wastewater treatment processes applied for industrial effluents containing AIs. Activated carbon adsorption has been assessed by deriving adsorption isotherms with five different dosages of commercially available granular activated carbon products. The efficiency of the process was based on DOC and AI analyses. In parallel, the biodegradation potential was assessed in a modified test based on the OECD 302 guidelines using activated sludge from an existing municipal wastewater treatment plant. The extent of the biodegradation was calculated based on three parameters: DOC elimination (which is a sum-parameter including true biodegradation and other physical process *e.g.* adsorption), true biodegradation (mineralization, *e.g.* CO_2 production) and AI removal (analytical determination of the respective AIs). This paper will present the efficacy of treatment processes for specific AI types (fungicides, herbicides, and insecticides). By the use of relatively common and simple laboratory methods, valuable insights are acquired into the treatability of specific AIs/products, enabling fast and more efficient extrapolation to full scale treatment.

AGRO 293

Continuous modeling of vegetative filter strips for pesticide removal under PWC (pesticide in water calculator) scenarios

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Vegetative filter strip (VFS) is a commonly cited agricultural best management practice that has the potential to reduce pesticide pollution to surface water. Use of VFS has been required or recommended for agricultural applications of some pesticide products including pyrethroids and neonicotinoids. While the Vegetative Filter Strip Model (VFSMOD) has been used for hydrological simulation in a VFS,

pesticide simulations are currently based on empirical or semi-mechanistic methods. In this study, a fully mechanistic approach is developed with physically-based modeling on the runoff-soil exchange of pesticide in a VFS. A PWC-VFS modeling system is proposed with the new approach to predict pesticide removal efficiency by a VFS for a wide range of chemical and soil properties, and facilitate continuous modeling for long-term (30 years) mitigation effects under the PWC scenarios. The new system is demonstrated in case studies with 4 widely-used pesticides (bifenthrin, chlorpyrifos, imidacloprid, and permethrin) under 14 crop scenarios in California. The results of model validation recommend a runoff interaction fraction of 0.4 (*i.e.*, 40% runoff will interact with and extract pesticide from the soil mixing layer), which generates the best modeling performance.

AGRO 294

Joint removal of pesticides and nitrate in woodchip bioreactors: Elucidating mechanisms and the influence of reactor design

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Woodchip denitrification bioreactors are a best management practice (BMP) for nitrogen removal from agricultural tile drainage. Assessment of a field-scale woodchip bioreactor system installed in California's Central Coast region indicated that woodchip bioreactors may also remove pesticides in tile drainage. We constructed continuous-flow benchtop woodchip bioreactors (4.3 L/day) to evaluate and optimize pesticide removal efficiency alongside nitrate reduction under relevant field conditions. Imidacloprid and diuron were selected as initial targets because they were frequently detected in field reactors, and they represent pesticides with high and medium solubilities. Benchtop reactors achieved, on average, 79.8% removal of imidacloprid and 81.4% removal of diuron per day. Additionally, batch kinetic tests (40 mL) were used to assess the relative contribution of sorption and microbial degradation on pesticide removal under denitrifying conditions. Adsorption was the main mechanism of removal for both diuron and imidacloprid, with removal capacities up to 753.3 ng/g-woodchip removal of diuron and 621.1 ng/g-woodchip removal of imidacloprid. We then tested a second reactor configuration, a sequencing-batch (fill, drain then refill) design, to evaluate the impact of redox conditions on pesticide and nitrate treatment efficiencies. A sequencing-batch reactor design inherently operates in a 'draining and refilling' regime, which can increase denitrification efficiency by preventing the formation of preferential flow paths. The sequencing-batch reactor design achieved 89.2% nitrate removal with half the hydraulic retention time (HRT) that the continuous-flow design took to reach similar reduction (12- and 24-hour HRT, respectively). Analysis of pesticide removal by the two reactor designs is underway. This work provides insight for optimizing woodchip bioreactors as a BMP to reduce the exportation of both nitrate and pesticides from agricultural sites.

AGRO 295

Large-scale regional ecological risk assessment of esticides and other stressors in the upper San Francisco Estuary using bayesian networks

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A key question is how to conduct a cumulative risk assessment for pesticides and other chemical stressors in large scale systems. In this study we are demonstrating a method by which this question can be addressed. An ecological risk assessment to be incorporated into an adaptive management framework is now being conducted for the Upper San Francisco Estuary (USFE) using the Bayesian network-relative risk model (BN-RRM). The area has multiple sources, stressors, habitats and endpoints, making it tailored for our tool set. The study area is defined in part by the range of the Delta smelt, the confluence of the Sacramento and San Juaquin Rivers, and Suisan Marsh. These rivers drain the Central Valley of California. The water that passes through the study site also accounts for 70 percent of the drinking water of the state. As of now we have identified 6 risk regions and have built a dataset derived from the California Environmental Data Exchange Network (CEDEN) and Surface Water Database (SURF) repositories of 100,000 observations from the last 10 years. A geographic information system (GIS) structure is used to put the observations into a geographical context. Pesticides include numerous organophosphates, pyrethroids, and metals such as mercury and selenium. Our endpoints are Delta smelt, Chinook salmon and macroinvertebrate community structure. Among the tools being used to asses exposure-response include regression models and case learning. The presentation will summarize the status of the conceptual model, the designation of the risk regions, the building of the Bayesian networks, and a preliminary assessment.

AGRO 296

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 (OPP) 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 U.S. Geological Survey developed the SEAsonalWAVEQ with EXtended capabilities (SEAWAVE-QEX) model to impute missing or censored pesticide concentrations from surface water monitoring data using daily streamflow as a covariate. OPP evaluated SEAWAVE-QEX and presented the results to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP). The Panel suggested that OPP evaluate the accuracy of the model results for pesticides that have multiple potential use windows or have sporadic use. OPP investigated the Panel's

suggestion through a case study using available surface water monitoring and pesticide usage data.

AGRO 297

Perspective of target-site synergism for insect and vector control

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Insecticide synergism plays an important role in alleviating the impact of pest resistance. The rotation or the combination of insecticides with different modes of action can mitigate the impact of resistant arthropods. Resistance diminution is particularly relevant in the control of insect vectors; these organisms have wide-spread resistance issues and are limited by their modes of action used for their control. The modulatory effects of G-Protein-Coupled Receptors (GPCRs) can aid in synergistic activity. For instance, mixtures containing a formamidine insecticide that activates the physiologically important octopamine receptors, enhances the toxicity of some insecticides (*e.g*. neonicotinoids). Further, we have shown that neuroexcitation produced by octopamine, and the octopaminergic signal transduction pathway, can be blocked by atropine, a muscarinic acetylcholine receptor (mAChR) antagonist. The muscarinic system plays an important role in the regulation of acetylcholine at the synaptic level. Pyrethroid insecticides and organophosphates, which result in an excess of acetylcholine in the synapse, will activate a feedback mechanism that can influence the synergistic toxicity. Crosstalk between the muscarinic and the GABAergic systems has also been shown to affect the physiology in *Caenorhabditis elegans*. Using toxicological bioassays with *Drosophila melanogaster,* we found that the mAChR agonist, pilocarpine, can significantly enhance the toxicity of GABAergic insecticides (fipronil, lindane and dieldrin); with synergistic ratios as high as 32-fold. Finding insecticides that work alone at GPCRs may be the incorrect approach, as only one insecticide class (formamidines) is currently on the market. However, the neuromodulatory role of GPCRs may provide better target-site synergists in agrochemical development.

AGRO 298

Translating volatile pyrethroids into the field for mosquito abatement

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Spatial repellents are largely composed of pyrethroid active ingredients that have some volatile characteristics. Mosquitoes are a principle target of spatial repellent compounds, and a range of deleterious effects have been recorded among vector insects. This presentation covers a swath of research intended to transition spatial repellents into clearly insecticidal tools for managing mosquito populations. Whether as a solitary a.i. or supporting ingredient, trans-chrysanthemate volatile pyrethroids appear to facilitate mosquito control. Additionally, the effects are evident against both adult mosquito and egg collections when targeting container-inhabiting *Aedes* mosquitoes. Some correlates are drawn with sub-lethal effects work in prior findings (and confirmed in the present data), but this presentation focuses on the on-site management of mosquitoes rather than larger meta-population analysis with hypothetical intervention mechanics (*e.g*. prior talks on fecundity loss, oviposition deterrence, sterility, etc.).

AGRO 299

Target site mechanism of action of resistance-breaking natural products

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Insecticide resistance is an ever-growing concern of vector control agencies throughout the world. Natural products represent promising alternatives as future insecticides and synergists of currently available compounds. They act via numerous mechanisms of action, and in some cases can circumvent insecticide resistance in wild field populations. These characteristics, in addition to their accelerated pathway for EPA registration, make them ideal candidates for future pest control technologies. Thus, we investigated extracts of veratrine (V), aconitine (A), Sichuan pepper (SP), and black pepper (BP) for biological activity, since they are known to affect voltage-sensitive Na^+ channels, at binding sites different from that occupied by pyrethroids. Extracts were topically applied to *Aedes aegypti* adult females for evaluation of their potential lethality alone and in concert with natural pyrethrins (NP) to assess the degree to which they could synergize this natural mosquitocide. A significant increase in the toxicity of natural pyrethrins was observed after 24-hr co-exposure with plant extracts. To further elucidate if this synergism was mediated via changes in metabolism or directly at the site of action, we applied extracts alone and in combination with natural pyrethrins directly to dissected mosquito ventral nerve cords. All extracts significantly enhanced natural pyrethrins on the mosquito central nervous system of the pyrethroid-susceptible strain. Interestingly, only two extracts were capable of synergizing on the pyrethroid-resistant strain via this route of exposure. Further studies were also performed to better characterize the mechanisms of this potent synergism. These findings demonstrate the potential of natural extracts to synergize pyrethroids directly on the nervous system. Moreover, differences in synergism on a pyrethroid-resistant and pyrethroid-susceptible strain may elucidate chemicals that are capable of reversing kdr-type resistance when applied in combination with pyrethroids.

AGRO 300

Citronella oil derivatives and their repellent properties

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The field of mosquito control has long been searching for molecules that can be used as mosquito repellents to fight against the spread of vector-borne diseases. A breakthrough

was made while researchers were examining the essential oil associated with lemongrass (*Cymbopogon nardus*). Citronella (Ceylon) oil was extracted from the plant and was proven to be an effective oil for repelling mosquitoes, especially in short-term evaluations. The multiple components of this oil have also been extracted and screened for their ability to repel mosquitoes. Citronellol, a major component of citronella oil, has proven to be quite effective when used as a spatial repellent, although its volatility is high, and it has a very short residual time. Our lab has now used citronellol as a parent molecule to which we have been able to make a series of less volatile derivatives. Certain of these biorational derivatives have shown high levels of efficacy when screened against multiple genera of mosquitoes in our laboratory spatial repellency assays in both short and long-term situations.

AGRO 301

Vapor phase delivery of plant oils alters pyrethroid efficacy, detoxification activity, and metabolic rate in mosquitoes

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The use of synthetic insecticides to limit the spread of mosquito-borne disease faces significant challenges, including insecticide resistance, environmental impact of widespread insecticide use, and slowed development of new insecticide chemistries. One important alternative to broadcast insecticides is the use of personal protection strategies to limit contact with vector species, including the use of spatial repellents that can employ synthetic pyrethroids or plant-derived oils. A currently underexplored area of research involves the investigation of plant-derived oils for their potential to serve as insecticide synergists when delivered as a vapor. This presentation will describe the development and implementation of plant-derived oils delivered as a vapor for enhancement of deltamethrin efficacy in pyrethroid-susceptible and –resistant strains of the vector mosquito species *Aedes aegypti*. Deltamethrin efficacy was significantly increased following exposure to vapors of cinnamon, tagetes, and sage oils, while deltamethrin efficacy was significantly decreased following exposure to amyris oil vapor. Biochemical assays suggest these effects may be mediated by changes in cytochrome P450 monooxygenase activity. Flow-through respirometry not only shows significant differences in metabolic rate between the mosquito strains, but provides evidence for plant-derived vapors to disrupt physiological homeostasis. This work will demonstrate that plant-derived oil vapors are capable of increasing deltamethrin efficacy similar to classical synergists, but also elicit changes in metabolic rates, offering an alternative approach for the enhancement of insecticide efficacy using natural product chemistries.

AGRO 302

Targeting Kir channels to reduce vector competency of mosquitoes

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Vector competency is determined by the ability of the mosquito to acquire, amplify, and horizontally transmit the virus, thus mechanisms to inhibit these processes are likely to have deleterious consequences to arbovirus transmission. We employed structurally diverse chemical modulators of inward rectifying potassium (Kir) channels to test the hypothesis that Kir channels are a critical ion conductance pathway in the mosquito salivary gland that enables saliva secretion and vector competency. Our data clearly show that multiple pharmacological modulators of Kir channels reduce the ability to ingest blood by up to 97.3% in a concentration-dependent manner. To determine if the failure to feed was due to inhibition of salivary gland function, we quantified secreted saliva 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. To determine if Kir-mediated inhibition of the salivary gland altered vector competency of *A. aegypti*, we studied the acquisition and dissemination of Dengue virus 2 (DENV2). Data show DENV2 mRNA was not detected in any (n = 120) individual mosquitoes that fed on infected blood meals with Kir modulators, yet control mosquitoes had an infection rate of 30% and a mean DENV2 titer of 4e4 pfu/mL. In addition, horizontal transfer of DENV2 was reduced by 99% when exposed to Kir channel modulators, presumably due to reduced saliva secretion. Taken together, data suggest chemical modification of Kir channels in the salivary gland represents a viable mechanism to reduce mosquito blood feeding and vector competency.

AGRO 303

Experimental releases of genetically engineered *Aedes aegypti* in Florida: The long and winding road to federal approval

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Genetically engineered *Aedes aegypti* that carry a lethality trait for female larvae have been approved for experimental release in Florida. Given the steadily expanding range of *Aedes aegypti* as a result of climate change, this new technology could result in significant public health benefit in the United States. This presentation will discuss the long road to approval for experimental release of this genetically engineered *Aedes aegypti*, and the process ahead for full regulatory approval.

AGRO 304

Sodium channels and pyrethroids: An interesting journey of adventures and opportunities

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Modern agriculture and human health rely heavily on natural and synthetic chemicals as part of an integral approach to control insect pests and infectious disease vectors. Voltage-gated sodium channels are critical for electrical signaling in the nervous system and are prominent targets of many naturally-occurring and synthetic neurotoxins, including pyrethroid insecticides. During my graduate school years in Dr. Jeffrey Scott's laboratory at Cornell University, I became fascinated by the idea that one type of insect resistance (knockdown resistance; kdr) to pyrethroids may be linked to mutations in the sodium channel gene. Over the past 25 years at Michigan State University, I have had the great privilege to work with talented students and collaborators and dive deep into the molecular mechanisms of pyrethroid action and resistance, molecular basis of pyrethroid selectivity, the

molecular biology, electrophysiology, and pharmacology of insect sodium channels, and more recently, the mechanisms of pyrethroid repellency. Looking back, it has been an interesting journey of many adventures, challenges, and opportunities. In this presentation, I hope to reflect on what we have accomplished and share our current and future research endeavors.

AGRO 305

Repellency and insecticidal activity of halogenated aromatic amides against anopheline mosquitoes

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Vector control is critical for suppression of the transmission of malaria parasites. It is important to identify safe repellents that can provide a long and reliable protection from arthropods and prevent arthropod-borne diseases. In the present study, the spatial repellent activity and toxicity of two novel halogenated aromatic amide derivates (**4-6c** and **4-6d**) were evaluated against *Anopheles albimanus*, *Anopheles quadrimaculatus*, and *Anopheles gambiae*. DEET and transfluthrin were used as standards against which to compare the new compounds. In addition, repellency synergism with a constituent of transfluthrin, transfluthrin acid (TFA), and the repellent 2-undecanone was also examined. The electrophysiological response of the mosquitoes to all the compounds was also assessed via electroantennography. DEET demonstrated weak spatial repellency and toxicity, while transfluthrin had potent activity as both a repellent and toxicant against these *Anopheles* mosquitoes. In behavioral bioassays, the novel amide, **4-6d**, was predominantly more effective than DEET and comparable to transfluthrin in some *Anopheles* species. Significant synergism was observed in the mixture of transfluthrin acid and the repellent 2-undecanone. EAG responses provided insight to the olfactory response of the *Anopheles* mosquitoes to the novel compounds and currently available chemical repellents. All the tested compounds produced significant antennal response with the exception of transfluthrin in *An. gambiae*. Overall, these studies provide insight for further synthesis of alternative compounds for use as spatial treatments.

AGRO 306

Quantifying the levels of resistance conferred by different *kdr* alleles in the yellow fever mosquito, *Aedes aegypti*

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Recent outbreaks of dengue and Zika have emphasized the importance to effectively control *Aedes aegypti,* which vectors the viruses causing these diseases. Pyrethroid insecticides are primarily used to control adult *A. aegypti*, especially during disease outbreaks. However, pyrethroid resistance in *A. aegypti* is an increasing problem. Mutations in the *voltage-sensitive sodium channel* (*Vssc*) are a common mechanism of pyrethroid resistance. Two common resistance alleles are V410L+V1016I+F1534C and F1534C. However, the levels of resistance conferred by these alleles is unknown. We isolated congenic strains (410L+1016I+1534C and 1534C) which are closely related to the susceptible Rockefeller (ROCK) strain, but are homozygous for the *Vssc* putative resistance allele. We determined resistance levels against eight insecticides that target the VSSC: six pyrethroids, DDT and DCJW (the bioactivated metabolite of indoxacarb). We will report on the levels of resistance each allele confers and compare these results to those obtained using oocyte expression systems. Our results provide useful information for resistance management, specifically the levels of resistance conferred by the most common *Vssc* mutation in *A. aegypti*.

AGRO 307

Spatial repellents for the control of mosquito-borne disease: Updates from clinical trials and status of WHO recommendations

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Following decades of natural observations, experimental studies, and proof-of-concept trials indicating spatial repellent products to reduce mosquito biting and potentially prevent mosquito-borne human diseases, such as malaria and/or dengue, several clinical trials have been conducted to support formal data assessment requirements by the WHO Vector Control Advisory Group (VCAG) regarding the public health value of a spatial repellent product category. This presentation will provide historical context on the envisioned role of spatial repellents in disease prevention strategies, and describe outcomes from a recent large-scale, randomized-cluster trial conducted in Iquitos, Peru, to evaluate the protective efficacy of a spatial repellent against *Aedes*-borne viruses (dengue, Zika) with a summary overview on the current status towards attaining full WHO policy endorsement for the use of spatial repellents in global public health programs.

AGRO 308

Gene regulation by terpenes and plant essential oils explains synergism of insecticides in *Aedes aegypti* mosquitoes

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Traditional synergists, including piperonyl butoxide, are thought to act primarily by binding to CypP450-dependent monooxygenase enzymes that detoxify many xenobiotics in the mosquito body, including pyrethroid insecticides. Our lab has shown that certain plant essential oils and certain of their constituent terpenes also are active as synergists with synthetic pyrethroids, while a few other oils actually antagonize their efficacy. However, the oils that enhance potency of pyrethroids decrease the efficacy of organophosphate insecticides (OPs), and there are no commercial synergists for OPs, since PBO also antagonizes OPs' potency. We have now utilized RNA-sequencing (RNAseq) to explore the effects of oils and terpenoids on gene expression, to determine whether upregulation or downregulation of the expression of Cyp genes is involved in

the enhancement or antagonism of insecticide efficacy. We determined that a few of the plant essential oils were able to enhance the efficacy of OP insecticides, which is logically not due to an interaction with Cyp detox enzymes. The RNAseq results showed that certain oils and certain terpenoids are capable of upregulating expression of Cyp detox genes, leading to greater oxidative capacity in the insect, which in turn, activates OPs from their thion form to their oxon form, which is orders-of-magnitude more potent as an inhibitor of acetylcholinesterase, their target site. The experiment also revealed that some oils and some terpenoids downregulate the expression of certain detox genes, adding one more factor to our understanding of detoxification processes in mosquitoes. Chlorfenapyr is also an insecticide that requires oxidative activation (O-dealkylation) to a more potent state in the insect in order to exact its lethal effect; the plant oils and terpenoids that activate OPs also are effective at enhancing the potency of chlorfenapyr against *Aedes aegypti*. This represents another case where selection of the appropriate plant oil can lead to a significant synergism of that insecticide. The more we understand the basics of detoxification of different classes of insecticides used on mosquitoes, the better we can select the correct natural synergist for them.

AGRO 309

Using semiochemicals to control disease vectors

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Few organisms on the planet have had a more devastating impact on human health and development than bloodfeeding arthropods, such as mosquitoes, lice, and ticks. Pathogens transmitted by these organisms cause some of the world's deadliest diseases and, in numerous cases, have changed the course of world history. Decades of intervention efforts have demonstrated that combatting tick and mosquito borne diseases is a long uphill struggle. Progress requires a suite of vector control tools embedded in and tailored to local contexts. Today, areawide management uses almost exclusively conventional chemical pesticides. Sustainable alternatives are needed. Among these are semiochemical based tools, none of which have been scaled beyond monitoring and intervention trials. Currently, use of most attractant baits requires mechanical devices, which are operationally difficult to deploy in areawide interventions. Alternatives that do not require mechanical devices facilitate adoption of areawide vector control programs. One such method is attract and kill. This technique includes using semiochemicals to lure pests to a control agent, thus increasing contact incidence and duration, reducing sublethal exposure and the development of resistance. Recent examples in the field have shown their potential: in modulation of odor of nonhuman hosts to attract and kill host seeking adult mosquitoes, in combining oviposition pheromones and larval attractants in formulations laced with phagostimulants and larvicides targeting control of immature stages. The use of spatial repellents is another method that has shown much promise. These repellents are highly volatile compounds that diffuse through the air in treated regions deterring host seeking female mosquitoes from entering homes with susceptible individuals. Each of these novel interventions target behaviors in different stages of the vector's life. As complementary techniques, semiochemical based technologies can provide sustainable, low tech and cost effective alternatives or additions to increase the effectiveness of vector control strategies. Here we will provide an abridged overview of the status and potential of these tools.

AGRO 310

Avoiding *Silent Spring*: Can phenotypic screening platforms deliver new, safer chemistries for vector control?

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In the next five to ten years, scientists must deliver multiple new technologies to control insecticide-resistant mosquitoes. Yet, 50 years after *Silent Spring*, we still employ "blunt hammer" pesticides that kill helpful, as well as harmful species, concentrate in the environment, and affect human health, inspiring substantial public controversy. An emphasis on screening for neurotoxic molecules that produce lethal end points has stagnated insecticide discovery and contributed to a limited number of chemical classes of insecticides. Our research program is pursuing the identification of new, safer classes of insecticides that operate via novel modes of action (MoA). Here, we will present the development of an innovative High Content image-based Screen (HCS) to rapidly explore chemical space, detect novel phenotypic endpoints, and recover unique insect-active molecules. Screen validation was achieved using the Library of Pharmacologically Active Compounds1280 ($LOPAC_{1280}$) against *Aedes aegypti* L1 stage larvae. The screen detected conventional pesticides, and differentiated toxic "hits" from chemistries associated with atypical morphological and developmental phenotypes. The toxicity/phenotype associated with a subset of novel chemistries from the pilot screen was subsequently confirmed in secondary, and larval and adult dose-response assays. An additional assay series revealed an impact of chemistries, including at sub-lethal dose, on a variety of entomological parameters tied to behavior, development, and fecundity/longevity. These data suggest that empirical screens focused on mortality as a readout, may overlook chemical scaffolds with potential for insecticide development. To explore the natural product (NP) chemical space, HCS of an NP-derived library was performed. Multiple novel toxic "hits" and chemistries associated with atypical phenotypes were identified, providing several potential pharmacophores for further evaluation. In this presentation, we will discuss the concept of targeting "exotic" phenotypes for insecticide discovery and mosquito control, MoA studies to implicate molecular targets, and environmental considerations associated with developing chemical control technologies.

AGRO 311

Reduced susceptibility and neural sensitivity to pyrethroids in the absence of the *kdr* genotype

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The fall armyworm (FAW), *Spodoptera frugiperda*, is a global pest of multiple economically important row crops and thus, significant efforts have been placed on investigating the molecular and genetic mechanisms of organophosphate and pyrethroid resistance. We employed neurophysiological

recordings to quantify neuronal sensitivity to neurotoxic insecticides and assessed toxicity across field and lab FAW populations. Topical toxicity resistance ratios (RR) in field collected FAW was 767-fold compared to lab strains and importantly, we observed a 1750-fold reduction in potency of l-cyhalothrin in neurophysiological assays. Field collected FAW were found to have a RR of 12 to chlorpyrifos when compared to the susceptible strain and was 8-fold less sensitive in neurophysiological assays. Surprisingly, no point mutations in the voltage-gated sodium channel known to cause pyrethroid resistance were identified in this study. For *ace*, 80% of individuals possessed nucleotide sequences consistent with susceptible strains for A201S and F290V, yet 40% of the tested population was heterozygous for the G227A mutation. These data indicate point mutations did not contribute to the high level of pyrethroid resistance and nerve insensitivity in this population of field collected FAW. These data suggest the knockdown resistance (*kdr*) genotype explains only a portion of heritable variation in FAW resistance and indicates *kdr* is not the only predictor of dramatically reduced neural sensitivity and high levels of pyrethroid resistance. Thus, phenotypic assays, such as toxicity bioassays or neurophysiological recordings, against field-collected populations are necessary to reliably predict resistant phenotypes and product failures.

AGRO 312

Enhancing the potency of GABAergic insecticides through chemical and genetic inhibition of K^+/Cl^- cotransporter

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The role of K^+/Cl^- cotransporter (KCC) in insects remains understudied. Thus, we aimed to test the hypothesis that KCC is an essential ion transport system that mediates proper neurotransmission in the insect CNS by driving the efflux of chloride ions to facilitate γ-aminobutyric acid (GABA)-receptor-chloride-channel (GRCC) complex GRCC function. Data show that inhibition of KCC with VU0463271 resulted in neuroexcitation of *Drosophila* CNS with an EC_{50} value of 479 nM. Interestingly, pretreatment of the *Drosophila* CNS with 3 mM VU0463271 followed by 5 mM GABA reduced the average firing rate to 16 ± 5 Hz from an average baseline of 28 ± 11 Hz whereas 5 mM GABA alone reduced average firing rate to 0.5 ± 0.2 Hz. These pharmacological data were validated through genetic ablation studies and taken together, indicate KCC provides a critical role in chloride homeostasis that drives the functionality of GRCC. The ability to interact with GABA-mediated inhibitory signaling pathways suggests perturbing the function of KCC could synergize the activity of LGCC directed insecticides. Indeed, co-treatment of an EC_{10} of dieldrin, a known GABA-R inhibitor, and an EC_{10} of VU0463271 significantly ($P<0.001$) increased the firing rate in a multiplicative manner with a 110% increase over baseline at 5-10 minutes post-exposure. Lastly, we aimed to determine the toxicity of VU0463271 to mosquitoes and found microinjection induced dose-dependent lethality (ID_{50}: 39 ng) to susceptible and pyrethroid-resistant strains. These data support the notion that insect KCC is a critical transport pathway in insect neural systems and is toxicologically relevant.

AGRO 313

Synergism of fipronil, lindane and dieldrin by the muscarinic acetylcholine receptor agonist pilocarpine in *Drosophila melanogaster*

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Neuronal insect muscarinic acetylcholine receptors (mAChRs) have various physiological functions that could be exploited for insecticide development. Insecticide mixtures are an alternative strategy for pest control. We have used the non-selective mAChR agonist, pilocarpine, to investigate the role that mAChRs play in target-site synergism. mAChRs have been associated with crosstalk between the octopaminergic and GABAergic system. In the *Drosophila melanogaster* wildtype strain (CSOR), pilocarpine (alone) does not result in high mortality. However, the mixture of pilocarpine (6,000 ppm that results in 20-40% mortality) increases the toxicity of insecticides that modulate GABA-gated chloride channels (fipronil, lindane, and dieldrin), with synergistic ratios between 4.3 and 9.6. The observed synergism for fipronil, lindane, and dieldrin were all significantly higher than the theoretical additive values. However, when pilocarpine was tested against the 1675 *D. melanogaster* strain, which is resistant to GABAergic insecticides through a point mutation in the GABA receptor (*rdl*), the synergism observed with pilocarpine in the susceptible strain was lost. To explain this loss of target-site synergism, extracellular electrophysiological studies using *D. melanogaster* were performed. Fipronil (10 μM) resulted in a strong neuroexcitation followed by a decrease in the firing rate. Pilocarpine (10 μM) dampened the neuroexcitation caused by fipronil in the CSOR-strain. Similar experiments in the 1675 strain indicated that pilocarpine did not have the same dampening effect on fipronil, which through a yet identified mechanism may explain why there is no target-site synergism in the *rdl* strain. Collectively, the results indicate the potential for target-site synergists.

AGRO 314

Biorational baits and their ability to control dipteran pests

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Dipteran pests are capable of causing many different types of problems in many situations. Multiple species of flies such as house flies and stable flies are capable of causing not only nuisance issues, but also they can bite livestock and function as mechanical vectors for diseases in stockyard situations. Mosquitoes are also able to vector lethal parasites and viruses such as: malaria, yellow fever, zika, and chikungunya. These diseases account for hundreds of thousands of deaths a year and are the reason mosquitoes are known as the deadliest animal in the world today. Dipteran control strategies are usually based around insecticidal sprays and repellents. Our lab has been researching the potential for the deployment of biorational baits (synthesized from monoterpenes) and their potential to control dipteran populations. We have designed molecules that can be deployed as either a solid bait or in a liquid form. These compounds have been shown to be potent

against dipterans as well or better than commonly used insecticidal baits. We believe these baits could become integral parts of any dipteran control strategy.

AGRO 315

Inward rectifier potassium (Kir) channels are an integral component of mosquito vector competency

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Inward rectifier potassium (Kir) channels are emerging in the field of insecticide science as an important ion conductance pathway, yet these channels remain uncharacterized in mosquito feeding biology. The goal of the proposed research was to test the ability to alter vector competency of Dengue virus (DENV) by *Aedes aegypti* through modulation of Kir channels. Data show Kir channels are critical for proper salivary gland function and two Kir channel agonists, pinacidil and VU0071063, reduce salivary secretions by 15- and 13.3-fold when compared to control mosquitoes, respectively. This reduction in salivary secretions was correlated to an inhibition of blood feeding as we observed no blood ingestion in 97% of mosquitoes. To assess the influence of reduced feeding to competency to vector DENV, we employed an artificial host system and combined Kir channel modulators with DENV in the blood meal and tested the ability to acquire and amplify the virus. Data show control mosquitoes had high levels of DENV mRNA in the body and disseminated to the legs at 13 days post infection, yet mosquitoes exposed to Kir modulators had no detectable DENV mRNA in the body or legs. These data suggest the lower volume of blood ingested prevented acquisition of enough DENV virions to generate a competent vector. Together, these data provide insights into the role K^+ ion channels have in blood feeding biology and vector competence of *A. aegypti* and begin to validate Kir channels as a molecular target to interrupt the viral lifecycle in the vector.

AGRO 316

Dual-target mechanism of bioallethrin repellency in *Aedes aegypti* mosquitoes

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The use of pyrethroid insecticides is a pivotal strategy for mosquito abatement in the world. Commonly known for its insecticidal activity by acting on voltage-gated sodium channels and consequently leading to mortality, some pyrethroids also have been used as insect repellents. For example, volatile pyrethroids such as bioallethrin and transfluthrin are commercially used in mosquito coils, emanators, and other vaporizers to repel mosquitoes. However, the mechanism(s) underlying pyrethroid repellency remains unclear. Here we confirmed that bioallethrin elicits non-contact repellency (*i.e.*, spatial repellency) in *Aedes aegypti*, a major vector for yellow fever, Dengue, and Zika viruses. Using electrophysiological and behavioral approaches, we found that bioallethrin mediates repellency by targeting two distinct pathways. Firstly, bioallethrin activates a specific type of antennal sensillum neuron and its repellency is reduced in $Orco^{-/-}$ mosquitoes (which are defective in an obligate olfactory co-receptor). These results demonstrate a critical role of the mosquito olfactory system in bioallethrin repellency. Secondly, we assessed the contribution of the voltage-dependent sodium channel to bioallethrin repellency. For this, we used a pyrethroid-resistant kdr strain, which carries two well-defined mutations in the sodium channel gene that are responsible for pyrethroid resistance. We found that bioallethrin repellency was also reduced in kdr mosquitoes. Collectively, our study demonstrated that repellency by bioallethrin is the result of a dual-target mechanism involving voltage-gated sodium channels and an odorant receptor(s) present in a specific olfactory sensory neuron. Our results not only contribute to the understanding of the modes of action of volatile pyrethroids in spatial repellency, but also provide a framework for developing new repellents based on the dual-target mechanisms revealed.

AGRO 317

Spatial repellency, oviposition deterrence, and development inhibition of *Aedes aegypti* mosquitoes exposed to cajeput oil chemistries

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The yellow fever mosquito, *Aedes aegypti*, is a major concern to human health. While insecticides are highly effective for managing mosquito populations, the emergence of insecticide resistance is an ongoing threat to current management strategies. Previous studies have shown plant-derived oil vapors to enhance pyrethroid insecticide efficacy to mosquitoes. This current study investigated cajeput oil (*Melaleuca cajuputi*) and three primary fractions (eucalyptol, d-limonene, and o-cymene) as spatial repellents and oviposition deterrents for mosquitoes. It was found that cajeput oil and fractions have significant spatial repellent and oviposition deterrent activity to mosquitoes. Additionally, it was observed that cajeput oil and fractions significantly affected the development of mosquito larvae and pupae resulting in the reduced emergence of adults. This work not only demonstrates plant-derived oil vapors of cajeput to be candidate repellent chemistries for mosquitoes, but also interrupts egg laying and development activities, offering alternative approaches for the management of mosquito populations.

AGRO 318

Novel approach for insect control: E3 ligase ligand library for potential degradation of vital insect proteins

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Proteolysis-targeting chimeras (PROTACs) are emerging tools for therapeutic intervention by eliminating disease-causing proteins. This novel strategy can be extended to agriculture as a new generation of pesticides overcoming resistance. PROTACs are bifunctional heterodimers that bind simultaneously to an ubiquitin E3 ligase, and a protein to be degraded (POI), where the two small molecule ligands (E3 ligase ligand and "warhead") are connected by a chemical linker. The close vicinity of the POI and the E3 ligase triggers the polyubiquitilation of the target protein (POI) leading to its degradation. Inhibition of apoptosis proteins (IAPs) are efficient ubiquitin E3 ligases, therefore, IAPs are potential degrading units for PROTACs. IAPs are involved primarily in cell death regulation in humans (vertebrates) as well as in invertebrates including insects. IAPs inhibit caspases (and apoptosis), however, binding small molecule mimetics of the N-terminal tetrapeptide to binding region of IAPs may act as antagonists of IAPs' facilitating the apoptosis. Furthermore, the interaction with the BIR domains (BIR2 and BIR3) may also induce auto-degradation of the protein through ubiquitination (Ub). In humans, the binding anchor of IAP antagonist protein (called Smac/Diablo) is an N-terminal tetrapeptide: AVPI (Ala-Val-Pro-Ile). Insects (*e.g*. in *Drosophila melanogaster*) have similar apoptosis inhibitor proteins DIAP1, DIAP2 (Death-associated inhibitor of apoptosis) with three tetrapeptide binding motifs identified as: Reaper (AVAF: Ala-Val-Ala-Phe), Grim (AIAY: Ala-Ile-Ala-Tyr) and Hid (AVPF: Ala-Val-Pro-Phe). DIAP1 or DIAP2 act also as E3 ligases, similarly to the human IAPs via binding by their corresponding tetrapeptides to BIR2 domain, which also induces ubiquitination and degradation. There are various proteins in insects, mites etc. that can be interacted with small molecules such as enzymes (*e.g.*, AChE, proteases), receptors (ryanodine, olfactory, hormone - *e.g*., juvenile, diuretic). Such small molecules may act as potential "warheads" linked to E3 ligases DIAP1 and DIAP2 forming insecticide PROTAC conjugates. In the present poster we report the 2D/3D design of AVPF mimetic libraries as potential E3 ligase ligands. In PDB database there are several crystal structures available for DIAP binding to Reaper, Grim, or Hid IAP antagonist proteins, which allowed us building 3D models suitable for structure-based virtual screening and compound design.

AGRO 319

Mechanism of transfluthrin repellency in *Aedes aegypti*

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Volatile pyrethroid insecticides, such as transfluthrin, have received great attention in recent years for controlling human disease vectors due to their highly insecticidal and repellent activities. It is well understood that the insecticidal activity of pyrethroids is caused by prolonged activation of insect voltage-gated sodium channels, which results in the disruption of electrical signaling in the nervous system. In contrast, the mechanism of pyrethroid repellency remains not well understood and controversial. Here, we show that transfluthrin vapor elicits spatial repellency in a hand-in-cage assay at concentrations as low as 1 ppm in *Aedes aegypti*, a primary vector of dengue, Zika, chikungunya, and yellow fever viruses. Sodium channel mutations that reduce the potency of transfluthrin on sodium channels decreases transfluthrin repellency. We found that transfluthrin does not evoke an olfactory response from mosquito antennae or impair mosquito's ability to detect attractants and other repellents. Furthermore, the inactive 1S, cis isomer of transfluthrin, which does not activate sodium channels, could not elicit repellency. These results suggest that sodium channel activation is the principal mechanism of transfluthrin repellency. Our results clarify the molecular basis of a major insect repellent and provide a new framework for developing new repellents to combat human disease vectors.

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Inhalation risk assessment for crop protection products – the past, the present, and the future

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Inhalation is an important non-dietary route of exposure. Lately, it has gathered further attention in the area of regulatory risk assessments for crop protection products. In 1994 EPA-ORD published a comprehensive publication on inhalation exposure. Regulatory authorities take different approaches to assess inhalation risk. Although these approaches seem disjointed, they can be viewed as tiered approaches, ranging from simple to sophisticated. The adverse effect from inhalation may be caused due to systemic effects or through the portal of entry (irritation) effects. There may be chemicals that have the lungs as the target organ for adverse effects. This poster will highlight the most widely adopted approaches to inhalation risk assessment for agricultural pesticide handlers. In all these cases, the inhalation exposure estimate comes from standard worker exposure studies. Statistical models were fitted to measured data and are used for estimating the magnitude of inhaled dose for a specific activity. The simplest risk assessment approach uses the toxicological endpoint derived from a 28-day or 90-day subchronic oral toxicity study. The uncertainty

in the risk assessment could be high and generally accompanied by a weight of evidence to justify that this approach is protective of human health. EPA generally uses a 10X safety factor in addition to the standard 100X uncertainty factor resulting in a Level of Concern (LOC) of 1000. The second approach is to use the toxicological endpoints from a repeat dose inhalation study conducted on animals, usually rats. Using inhalation dosimetry models, Human Equivalent Concentration (HEC) can be derived from the study endpoints. Alternately, some regulatory authorities only derive Animal Equivalent Concentration (AEC) and use it in risk assessment. Usually, the risk assessment LOC for an AEC is 100, whereas for an HEC it is only 30. The third approach is based on a New Approach Method (NAM) presented at an EPA-SAP (December 2018). This approach eliminates the use of the animal model by using a combination of *in vitro* testing for effects and a Computational Fluid Dynamics (CFD) model that precisely predicts the deposition of inhaled particles in different parts of the respiratory tract. Deriving the endpoint from this is a complex process but results in less uncertainty and supports the use of a LOC of less than 30.

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New metabolites from *Alternaria alternata* ZHJG5, and their antibacterial activities against *Xanthomonas oryzae* pv. *oryzae*

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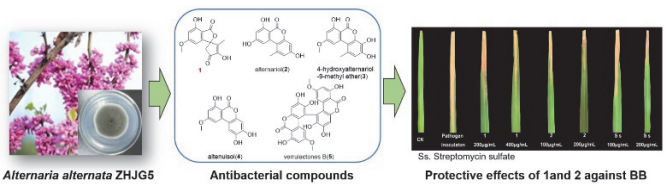
Plant diseases such as rice bacterial blight (BB) caused by *Xanthomonas oryzae* pv*. oryzae* (*Xoo*) result in immense crop as well as economic losses worldwide. However, sustained use of the commercial bactericides such as streptomycin causes serious drug-resistance, which reveal the urgent needs of highly effective and environment-friendly new bactericides. Screening of microbial natural products with antibacterial activity is an effective approach for the discovery of novel bactericides. In our previous work, we isolated 138 kinds of endophytic fungi from 19 medicinal plants (which were collected from Nanjing Botanical Garden, Memorial Sun Yat-Sen.) and found that endophytic fungus *Alternaria alternata* ZHJG5 showed effective antibacterial activity against *Xoo*. After the fermentation and a series of separation techniques, twelve metabolites including five new ones were isolated from this fungus. In these compounds, compounds **1-5** had significant antibacterial activities against *Xoo* with minimum inhibitory concentration (MIC) values from 2 to 16 μg mL^{-1}. In addition, alternariol (**2**) displayed a protective efficacy of 59.4% against BB at 200 μg mL^{-1}, compared to that of streptomycin sulfate (66.0%). The result suggested that these metabolites from *A. alternata* ZHJG5 has potential to be used as lead compounds for the development of new bactericides.



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Structural optimization of isoorientin as glycogen synthase kinase-3*β* inhibitors for potential Alzheimer's disease relief

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Alzheimer's disease (AD) is a worldwide disease that causes memory loss, cognitive decline, and eventually death due to severe brain degeneration with no cure yet. Increasing evidence suggests that glycogen synthase kinase-3*β* (GSK-3*β*), playing a key role in tau hyperphosphorylation, is a therapeutic target for AD. Isoorientin, a flavonoid widely present in foods and fruits, selectively inhibits GSK-3*β* and can induce tau hyperphosphorylation and neurotoxicity in the neuroblastoma SH-SY5Y cells. Structural optimization of isoorientin is expected to increase the potencies against GSK-3*β* and develop new candidates for the AD relief. Considering the potential druglikeness and potency improvement of the compound, the pocket-based optimization strategy, organic synthesis and molecular docking were used to design new inhibitors (half maximum inhibition concentrations 0.6-5000 μM versus 180 μM by isoorientin). The hydroxyl groups of isoorientin were replaced with other functional groups to improve absorption, distribution, metabolism and blood-brain barrier penetration. The hydroxymethyl binding at the hydrophobic site of GSK-3*β* was replaced with hydrophobic groups to form stronger Van der Waals forces, thus making the binding of inhibitors with GSK-3*β* more stable. Molecular docking studies suggest that the designed molecules are more stable in binding to the GSK-3*β* with lower energies than isoorientin, and exhibit stronger inhibitory activities against GSK-3*β* than the parent isoorientin. The newly synthesized compounds can serve as novel chemical probes to study AD treatment and as drug lead candidates for further drug development.

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Anti-neuroinflammatory effects of GSK-3β inhibitor TFGF-18 in LPS-activated SIM-A9 microglial cells

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Alzheimer's disease (AD) is the most common neurodegenerative disorder. AD and dementia associated with several fatal clinical disorders is a considerable social, economic, and medical challenge. Neuroinflammation is a key factor of Alzheimer's disease (AD) and other neurodegeneration conditions. It has been reported that glycogen synthase kinase-3β (GSK-3β) mediates the release of proinflammatory cytokines and promotes inflammatory responses in peripheral immune cells. Microglia are the resident mononuclear immune cells of the central nervous system (CNS). Here we investigated the anti-neuroinflammatory effects of a semisynthetic flavonoid GSK-3β inhibitor [(2S,3S,4R,5R,6S)-6-(2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-4-oxo-4H-chromen-6-yl)-3,4,5-trihydroxy-N-((S)-1,1,1-trifluoropropan-2-yl)tetrahydro-2H-pyran-2-carboxamide; TFGF-18] in lipopolysaccharide (LPS) activated SIM-A9 microglial cells. The results showed TFGF-18 significantly inhibited LPS-induced production of nitric oxide (NO) and pro-inflammatory cytokines such as TNF-α and IL-

1β. The migration of activated SIM-A9 cells was also greatly reduced with pretreatment of TFGF-18 in a scratch assay. Analyses in signaling pathways demonstrated that TFGF-18 led to the suppression of LPS-induced GSK-3β activation and p65/NF-κB activity. Furthermore, the co-culture of SIM-A9 with SH-SY5Y neuroblastoma cells showed that TFGF-18 suppressed the microglia-mediated neurotoxicity *in vitro*. Taken together, TFGF-18 inhibits LPS-stimulated microglia activation through collective regulation of GSK-3β and p65/NF-κB signaling. The results indicate a potential role of TFGF-18 in neuroprotection via its anti-neuroinflammatory effect. Therefore, modulation of microglia responses may provide a therapeutic target for the treatment of AD and other neurodegeneration conditions.

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Developmental deformities in zebrafish embryos by the treatment of pyraclostrobin: Molecular biological approaches to understand malformation of hearts

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Pyraclostrobin, which is widely used in agricultural environments, is a strobilurin-based fungicide that inhibits the mitochondrial respiration of fungi. The application of fungicides to crops could potentially contaminate adjacent water environments. To evaluate the toxicity to aquatic organisms, a representative model animal, zebrafish (*Danio rerio*), was used. The acute and developmental toxicity caused by pyraclostrobin was evaluated. Changes in survival rate, morphological malformation, and hatching rate were determined to understand acute toxicities of pyraclostrobin. When exposed to pyraclostrobin up to 96 hours after fertilization, the zebrafish embryos had an LC50 value of 77.75 ppb. A phenotype with severe edema was observed and accompanied by a decrease in hatching rate. In addition, during the observation period, it was confirmed that the developmental delay of zebrafish embryo occurred at a concentration of 150 ppb or less. As treated with pyraclostrobin on the genetically engineered Tg (fli1a: EGFP), which can be used for fluorescence observation of blood vessels, the value of the intersegmental vessel did not show any difference between controls and the treated groups. Changes in the mRNA levels of zebrafish exposed to pyraclostrobin were found in cyp1a1, cyp21a1, cyp24a1, cyp3a1, and cyp7a1. This study shows that pyraclostrobin causes acute toxicity and developmental disorders in fishes.

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Nature Inspired - natural products and crop protection

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Natural products (NP) have been long been tools to crop protection, both as a source of crop protection agents, and models and inspiration for an array of NP-based products. A significant percentage (7%, 15%, 33%) of existing herbicides, fungicides, and insecticides, respectively, have their origins in NPs from a variety of sources. Owning to the evolving needs of agriculture and the desires of consumers, reflected in ever more stringent regulatory requirements, new pest control tools, options, and approaches remain a critical need. As such, NPs continue to be an important source of ideas and inspiration for these required new pest control solutions. Importantly, a large proportion (~50%) of the known modes of action are defined or predicted by NPs, further highlighting their importance. Further value from NPs is also possible through the coupling of precision delivery systems with advanced plant genetics. These and other aspects will be discussed.

PICOGRAM V. 98

Abstracts

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