AGRO DIVISION

AGRO 1

Dr. Toshio Narahashi, the light traveling through ion channels

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With his discovery of the sodium channel blocking action of tetrodotoxin, Dr. Toshio Narahashi established the field of pharmacology and neurotoxicology of ion channels, and was widely considered the founding father of that field. He also pioneered the study of insecticides on ion channels by identifying the voltage-gated sodium channel as the target of DDT and pyrethroids, and continued to make important contributions to the field throughout his long career. In six decades of research, Dr. Narahashi published 324 papers, 148 reviews and book chapters and 376 abstracts, edited 11 books, and received numerous awards. Toshio Narahashi received an undergraduate degree in Agriculture from the University of Tokyo in 1948 and started his lifetime scientific career in the study of the mechanism of action of insecticides, receiving a PhD from the University of Tokyo in 1960. In 1961, he moved to the University of Chicago to work as a postdoctoral research associate. In 1963, he was recruited to Duke University as an assistant professor and was promoted to Associate Professor in 1967. He was promoted to Professor in 1969 and was appointed Vice Chair of the Department in 1973. He was recruited as Chair of Department of Pharmacology at Northwestern University Medical School in Chicago in 1977, and served for 17 years. Dr. Narahashi was known and admired not only for his talent and capacity for hard work, but also for his warmth and kindness that shed a light on his colleagues. As a mentor and educator, he helped illuminate the career paths of generations of scientists. Dr. Narahashi trained close to 140 graduate students and other professionals, many of whom are still active in academia, government, and at global chemical and pharmaceutical companies.

AGRO 2

Ion channels as insecticide targets

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"What works is clever." In the case of insecticides, what works is the disruption of ion channel function in the nervous system. The process of insecticide discovery, stretching across centuries from the earliest botanicals to contemporary chemistry, has converged on the exploitation of only a handful of targets. Modern insecticides that act directly on ion channel targets account for approximately 66% of the world insecticide market. If one adds organophosphorus and methylcarbamate acetylcholinesterase inhibitors, which can be considered indirect activators of acetylcholine receptors, the disruption of only four ion channel targets accounts for 85% of the insecticide market. This clever convergence on a handful of targets was not intentional but rather the result of the empirical discovery of insecticidal activity that works, with the identification of targets typically lagging behind. This presentation will provide an overview of ion channels as insecticide targets and consider the implications of the convergence of discovery efforts on a small number of targets on the search for new insecticides and the stewardship of current insecticides and targets.

AGRO 3

Molecular mechanisms of action of pyrethrum and pyrethroid insecticides

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Pyrethrum is a botanical insecticide extracted from dry flowers of certain *Chrysanthemum* species. Pyrethroids are synthetic analogues of pyrethrum. Pyrethroids have a long history of extensive usage in the control of arthropod pests and human disease vectors due to their great insecticidal activity and low mammalian toxicity. It is well-established that these compounds exert toxic effects by modifying the function of voltage-gated sodium channels which are critical for electrical signaling in the nervous system. However, besides the insecticidal activity of pyrethroids, behavioral observations indicate that pyrethrum and pyrethroids exhibit spatial and/or contact repellency. In fact, spatial repellency of several volatile pyrethroids is now being evaluated as an alternative vector control strategy. Surprisingly, the molecular basis of the spatial repellency of pyrethrum and pyrethroids remains unknown. To understand the mechanism of spatial repellency, we examined olfactory responses of adults of *Drosophila melanogaster* to these compounds using electroantennogram (EAG) and single sensillum recordings (SSR). Our results show that fly antennae could detect these compounds and that olfactory neurons/receptors in specific basiconic sensilla are activated by these compounds in a dose-dependent manner. Our identification of pyrethrum/pyrethroid-responsive olfactory receptors provides a new paradigm for understanding the modes of action of pyrethrum and pyrethroids and a new platform for screening effective mosquito repellents.

AGRO 4

Relative activity on ion channels and mechanism of action of bifenthrin

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Bifenthrin is a pyrethroid insecticide with an *o*-Me biphenyl alcohol in place of the more traditional (α-CN) 3-phenoxybenzyl. Bifenthrin is referred to as a Type I pyrethroid because it lacks a α-CN group in the alcohol moiety (Type II). In the intact, electrode-implanted cockroach it failed to cause prolonged repetitive firing in sensory nerves, an effect typical of Type I pyrethroids. A paper by the Pyrethroid Working Group (*Neurotoxicology*, 2009), used by EPA in 2012 for pyrethroid cumulative risk assessment, concluded that pyrethroid mechanism of toxicity fell into two main types. This was based on in vivo (various clinical signs) and in vitro data (effects on voltage-gated channels for Na^{+}, Ca^{++} and Cl^{-}). Exceptions that did not clearly fit into either group were bifenthrin and fenpropathrin, an α-CN pyrethroid known to cause both Type I and II effects in insects and mammals.. An in vivo assay commonly used to characterize pyrethroids is the Acoustic Startle Response (ASR) in the rat. Type I pyrethroids cause an increase in ASR whereas Type II cause a decrease. In a bifenthrin Developmental Neurotoxicity study, it reduced the ASR, dose-dependently, in female pups at PND20, the effect reversing at PND60. Effects were correlated with bifenthrin concentrations in blood/milk of dams and pup blood. The precise mode of action of bifenthrin remains unclear but human VGSC experiments by ChanTest suggest that bifenthrin's effects may be more similar to Type II than to Type I pyrethroids. Furthermore, it appears that pyrethroids may be less active on human than on rat VGSCs, confirming work published by Soderland. Molecular modeling data on the bifenthrin alcohol vs. Type I and II alcohols will be presented.

AGRO 5

Functional reconstitution of sodium channels in vitro for studies of insecticide action

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The ability to reconstitute sodium channel function and pharmacology *in vitro* using cloned subunits of known structure has greatly enhanced our understanding of the action of insecticides at this target and the structural determinants of resistance and interspecies selectivity. However, the use of reconstituted channels raises three critical questions: (1) Which subunits and subunit combinations should be used? (2) Which heterologous expression system is preferred? (3) Which combination of subunits and expression system best represents the function of native neuronal channels in the organism of interest? Here, we consider these questions from the perspective of the action of pyrethroid insecticides on mammalian sodium channels. Using the rat $\mathrm{Na_v}1.6$ sodium channel a subunit isoform and the pyrethroid insecticide tefluthrin, we compare the impact of the rat β1 and β2 subunits on channel function and insecticide response when channels are expressed in either *Xenopus* oocytes or stably-transformed HEK293 cells. We also compare the effects of tefluthrin on these reconstituted channels its effects on the heterogeneous sodium channel population expressed in rat embryonic cortical neurons in primary culture. These comparisons provide new insight into the modulatory effects of sodium channel auxiliary subunits on the action of pyrethroids and the relative fidelity of the *Xenopus* oocyte and HEK293 cell expression systems for studies of channel function and pyrethroid action.

AGRO 6

Mapping insecticide receptors in two lipid-exposed domain interfaces of sodium channels

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Pyrethroid insecticides and DDT are sodium channels agonists. Understanding atomistic details of their action may help develop new insecticides. Kv1.2-based homology models of open insect sodium channels are elaborated with two pyrethroid receptors, PyR1 (O'Reilly et al., 2006) and PyR2 (Du et al., 2013), which are located in domain interfaces D1/D2 and D2/D3, respectively. The complementary models differ in the number of contributing transmembrane helices, orientation of the bound ligands (e.g., deltamethrin), and the depth of their penetration in the domain interfaces. We employed our PyR2 model to elaborate a revised PyR1 model. Computational docking of two deltamethrin molecules in PyR1 and PyR2 predicted that each ligand binds between three transmembrane helices (S5 and two S6s) and a linker helix S4-S5. A common feature of pyrethroids and DDT is a bulky hydrophobic moiety. We docked DDT into interfaces D1/D2 and D2/D3 and found that its trichloromethyl moiety binds at the same locus as the dimethylcyclopropane moiety of pyrethroids. The model-driven mutagenesis unveiled seven new pyrethroid-sensing residues and nine new DDT-sensing resides in PyR1 and PyR2. Taken together with early published data, our results support the following conclusions. (i) Two DDT receptors overlap with PyR1 and PyR2. (ii) The sodium channel is activated due to simultaneous binding of agonists in interfaces D1/D2 and D2/D3. (iii) Pyrethroid-sensing residues are located in analogous positions of domain interfaces D1/D2 and D2/D3 indicating rotational symmetry of PyR1 and PyR2. (iv) Pyrethroids bind to PyR1 and PyR2 in similar orientations, deeply penetrating into domain interfaces, with dibromoethenyl and diphenylether moieties oriented, respectively, in the intra- and extra-cellular directions. (v) Helix IIS6 contains residues that contribute to both PyR1 and PyR2. Our study elaborates the concept of dual pyrethroid-receptor sites and provides a structural background for rational development of new insecticides. Supported by NIH and NSERC.

AGRO 7

Potassium channels as under-exploited targets for insecticide design

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Potassium channels exist in a variety of molecular configurations, display a range of voltage-dependent properties, and are important for regulation of electrical excitability of nerve and muscle, as well as other functions. Moreover, nerve and muscle potassium channels are the targets of several structural classes of synthetic compounds and natural toxins, which can serve as leads for insecticide development. Early work by Dr. Toshio Narahashi showed effects of pyrethroids on potassium currents, though not as important in the expression of toxicity than effects on voltage-sensitive sodium channels. Current efforts in our laboratories include ongoing research into blockers of the Kv2 (delayed rectifier) potassium channels of the malaria mosquito, *Anopheles gambiae*. Initial compounds showed toxicity to insects and blocked potassium channel currents, albeit at concentrations > 100 micromolar, *in vitro*. However, additional structural series with greater potency have recently been identified. Effects of these compounds on potassium ion channel currents will be presented, along with their insecticidal activity against mosquitoes, in the context of evaluating potassium ion channels as possible new targets for insecticidal molecules.

AGRO 8

Insect ryanodine receptors as molecular targets for diamide insecticides

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Ryanodine receptors (RyRs) are a distinct class of ligand-gated calcium channels controlling the release of calcium. They are located on the sarcoplasmic reticulum of muscle and the endoplasmic reticulum of neurons. Diamides belong to a new chemical class of insecticides (MoA Group 28) that act as conformation-sensitive activators of insect RyRs, releasing calcium from intracellular stores, and causing an irreversible paralysis. All diamide products on the market today contain the active ingredients flubendiamide (a phthalic diamide), chlorantraniliprole or cyantraniliprole (anthranlilic diamides). Flubendiamide, discovered by Nihon Nohyaku and co-developed with Bayer, became available for crop protection applications in 2008. Chlorantraniliprole and cyantraniliprole were developed by Dupont who, in partnership with Syngenta, commercialized these products in 2009/2013. Unprecedentedly, diamondback moth (DBM) resistance to diamides was recorded in Bang Bua Thong, Thailand within 18 months of the launch of the highly effective lepidopteran control agent flubendiamide, due to over-reliance on this one product by cruciferous vegetable producers in that region. DBM resistance has subsequently been recorded in 10 other countries, including the Philippines, Taiwan, India, China, Brazil and the USA. High level resistance to diamide insecticides in this species is thought to be due to a target-site mutation conferring an amino acid substitution G4946E, located within the trans-membrane domain of the RyR. Diamides have also been extensively used to manage the tomato leaf miner *Tuta absoluta*, an invasive pest of tomato. High resistance levels of up to 2,414-fold have been detected in populations originating from Sicily, suggestive of a similar target-site insensitivity being present in this species. Testing to date indicates that insect populations resistant to any one diamide product are cross--resistant to all the other diamide actives. In this talk we will review the structure of the insect RyRs characterized to date and address the exquisite specificity of the phthalic acid and anthranilic diamides for certain pest insects. Our investigations on the effect of the G4946E mutation on diamide efficacy will also be briefly reported.

AGRO 9

Accounts table, a tool for structuring the integration and interpretation of evidence regarding causation of toxic effects from chemical exposure

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It is a challenge to create a process for integrating diverse lines of toxicological evidence in a way that is both sufficiently specified that consistency and accountability can be assured and also sufficiently flexible that the unique issues that arise in particular cases can be evaluated with appropriate scientific scrutiny. Here, I propose a method – the generation of "Accounts Tables" in the context of the Hypothesis-Based Weight-of-Evidence (HBWoE) approach – that may prove helpful. As Bradford Hill reminded us, we have before us a set of facts (the observations plus or our knowledge of the agent and the underlying biology it may affect), and the causality-assessment challenge is to determine how compelling a proposed causal role of the agent may be compared to other ways of explaining the same array of outcomes. In HBWoE, an "Account" is the sets of hypothesized explanations for how and why particular observed results occurred – in short, a collection of tentatively considered reasons for each of Bradford Hill's "facts before us." Depending on need and possibility, the explanations may be detailed or general, invoking specific processes or just empirical consistency with other experience, keyed to specific indicators or simply *ad hoc*. An Accounts Table is a formal tabulation of the key facts, with each one accompanied by the explanations proffered by competing alternative Accounts. The Accounts Table then permits an assessment of how the facts are most plausibly explained with and without invoking the causal role of the agent in affecting toxicity. The weight of evidence can be evaluated by judging whether and to what degree the Account with the agent's role incorporated is superior in plausibility, parsimony, and reasonableness of the assumptions. The method is illustrated with examples.

AGRO 10

Integration of mechanistic and epidemiologic evidence in the identification and classification of human carcinogens

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Traditional epidemiology provided critical evidence for 85 of 107 human carcinogens identified in volumes 1-100 of the IARC Monographs Program. For another 11 agents, including 9 biological agents, full-scale epidemiologic studies based on biomarkers were instrumental in the classification. In the case of a further 10 agents, epidemiology fell short of providing conclusive evidence, and transitional (mechanistic) biomarker-based studies were used to upgrade the overall classification (see Table; for one agent, neutrons, the classification of human carcinogen was based exclusively on mechanistic data). In most instances, the biomarkers that were critical to the cancer hazard identification were technically simple and easily applicable to large-scale population studies. Biomarker-based epidemiology also has provided critical contributions to the development and evaluation of cancer prevention strategies. Newly developed classes of biomarkers have the potential to further extend the current understanding of the causes and mechanisms of human cancer and contribute to its prevention. This contribution, however, depends on the characteristics of the biomarker in terms of validity, reproducibility, and applicability to large-scale studies, as well as on the understanding of its role in the process of carcinogenesis. Methods for integrating evidence from these and other lines of scientific inquiry are needed, and hold great promise.

AGRO 11

Weight of evidence and quantitative data integration using multicriteria decision analysis

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"Weighing" available evidence in the process of decision-making is unavoidable, yet it is one step that routinely raises suspicions; what evidence should be used, how much does it weigh, and whose thumb may be tipping the scales? This presentation will evaluate the current state and future roles of various types of evidence for hazard assessment as it applies to environmental health. In its recent evaluation of the US Environmental Protection Agency's Integrated Risk Information System assessment process, the National Research Council committee singled out the term "weight of evidence" (WoE) for critique, deeming the process too vague and detractive to the practice of evaluating human health risks of chemicals. Moving the methodology away from qualitative, vague, and controversial methods towards generalizable, quantitative, and transparent methods for appropriately managing diverse lines of evidence is paramount for both regulatory and public acceptance of the hazard assessments. The choice of terminology notwithstanding, a number of recent Bayesian WoE-based methods, the emergence of Multi Criteria Decision Analysis for WoE applications, as well as the general principles behind the foundational concepts of WoE, show promise in how to move forward and regain trust in the data integration step of the assessments. We will offer our thoughts on the current state of WoE as a whole and while we acknowledge that many WoE applications have been largely qualitative and subjective in nature, we see this as an opportunity to turn WoE towards a quantitative direction that includes Bayesian and Multi Criteria Decision Analysis. Application of quantitative WOE in environmental management and toxicology will be discussed.

AGRO 12

New model to track strawberry harvester activity and predict pesticide exposure

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Strawberry harvesters hand-pick fruit and are exposed to pesticides primarily through contact with foliage. Currently, dermal exposure is assessed by dividing the amount of pesticide detected on skin by work hours to the calculate exposure rate. This method assumes that foliage contact is proportional to harvester work time. However, among harvesters working in the same field and at the same time, the exposure rates may vary up to 10 fold. This variation may affect exposure modeling used for mitigation efforts. Our study pointed to one possible cause of the variation by showing that given the same work time, foliage contact time may vary by as much as 50% amongst harvesters. We found that the amount of strawberry picked correlates well with harvester foliage contact time. To pick one kilogram of strawberries, a harvester would be in contact with foliage for an average of 34 seconds. By using general linear model and pairwise comparison we further found among harvesters 1) from different crews, 2) of varying years of experience, 3) using a variety of hand protection and 4) regardless of gender, the foliage contact time per kilogram of picked strawberries was not statistically different (p=0.05). We developed an exposure prediction model based on past monitoring studies and found good correlation between the amount of strawberries picked and pesticide exposure on hands. The *Pearson*'s r were 0.11-0.91, and the positive correlation was significant for 4 out of 5 farms (p<0.05). Compared to the previous model using the work time, the exposure rates predicted by our model were closer to the pesticide amounts found on harvesters' skin. We are conducting an exposure monitoring study to test the model's applicability under current agricultural conditions.

AGRO 13

Consideration of the weight of evidence in local antipesticide initiatives: The Montgomery County, Maryland experience

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Chemical regulation is data dependent. At the federal level, regulatory scientists are very thorough in reviewing analytical, animal, and human data from many studies. The large volume of data with frequently contradicting results for the same chemical, underscore "…the need for a robust, consistent, transparent, and objective analytical framework for such evaluations" (Lutter et al., 2014, Risk Anal. 2014 Dec 16. doi: 10.1111/risa.12277. [Epub ahead of print]. Improving Weight of Evidence Approaches to Chemical Evaluations). A robust consideration of the weight of evidence, as well as application of the Hill criteria, is critical for federal regulatory scientists. Unfortunately, at the local level, our experience is that there is a significantly less robust approach to data review. Montgomery County, Maryland is proposing to ban the use of most pesticides for applications to lawns and athletic fields. Many supporters of the proposed ban allege that pesticides cause multiple human health effects. The allegations apply to individual chemicals, as well as "pesticides", in general. The putative health effects target children and the elderly, and range broadly from obesity and cancer, to Alzheimer's disease and heart disease. In most cases, these allegations ignore the risk assessment reviews by federal scientists and are made without a weight of evidence analysis, or even simple considerations of the Hill criteria. Public statements for the anti-pesticide initiative will be analyzed from a scientific perspective.

AGRO 14

Pesticide use on medical marijuana: An emerging crop that has no EPA-registered plant protection agents

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Growing medical marijuana is now a reality in nearly half of the states in the US, and use of pesticides for this crop is creating a conundrum for growers and state regulators. Since pesticide use is regulated by the U.S. Environmental Protection Agency, and growing marijuana is illegal, no pesticides have been registered for this crop. In addition to the use of medical marijuana, three states, Washington, Colorado and Alaska permit recreational use, and other jurisdictions, including the District of Columbia, Nevada and Oregon are planning to have recreational marijuana legal in the next several months. A variety of diseases, insects and mites have been observed on marijuana, and use of registered pesticides is commonly observed, although this use is not consistent with federal regulations. A review of the various state regulations indicates a wide variety of issues, including the lack of data on residue dissipation on pesticides, the lack of consistent testing of medical marijuana for residues, the lack of appropriate certification of laboratories, and the inconsistent enforcement of federal regulation of pesticide use. In many of the states that allow medical and/or recreational use of marijuana, the conflict between state and federal regulations is untenable and is posing a threat of differential regulation of pesticide use on federally legal agricultural crops and marijuana. Ultimately, this conflict will be resolved only by federal regulatory changes that will probably require congressional action.

AGRO 15

Pesticide dose – a parameter with many implications

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Like pharmaceuticals, pesticides can have many unintended effects, even when used at the proper dose. For pesticides, the possible effects are even more diverse because the chemicals are released immediately into the environment. Even when properly applied at the recommended dose, the actual dose reaching microenvironments within the field to which they are applied can vary widely. Subtoxic doses can cause stimulatory effects on many physiological and growth parameters (hormesis). Low, but usually lethal doses, favor evolution of resistance, often through enhanced degradation of the pesticide. High doses favor evolution of target site resistance. Most pesticides are directed at a range of pest species that vary in the effective dose for best management. Therefore, recommendations are often based on the dose that effectively kills the most tolerant species for which the product is recommended. Furthermore, to insure that the least sensitive species for which the product is recommended is killed, the recommended dose is one that is effective under most all conditions. When a pesticide is applied in the environment, organisms on site and off site, as well as organisms that appear in the sprayed field at times after the pesticide application can be influenced by a range of doses. Controlled release formulations of pesticides can alter the kinetics of dose exposure to targeted and non-targeted species. Technologies such as smart spray systems for weed management have the potential to greatly reduce the amount of herbicides used per unit of land area. Since herbicide use dwarfs the use of other pesticides in agriculture, such technologies can have significant impacts on total pesticide dose per unit area. These are but a sampling of the complex and far-ranging implications of pesticide dose.

AGRO 16

Herbicide dose: A relative and not an absolute term

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In the wake of the increasing number of cases of herbicide resistance and, in particular, the many cases of non-target site resistance (NTSR) in recent years the question of low versus high herbicide doses has attracted renewed attention. Several studies have shown that sub-lethal herbicide doses can select for NTSR biotypes and this has led to that farmers are now recommended to stick to the 'recommended doses' and not to use reduced doses. The objective of this presentation is to discuss whether this simple and undoubtedly very easy to understand message is a simplification of real life that could eventually result in an unnecessary use of herbicide. Two issues are important to address to answer this question. One is the fact that in weed management in agricultural and horticultural crops we are nearly always targeting a population of species in contrast to pest and disease management. The term 'recommended rate' therefore makes little sense as it is determined by the least susceptible of the weed species claimed to be controlled on the label, i.e., the majority of the other weed species controlled by the herbicide is more susceptible. For some herbicides more than 90% control can be obtained on a range of weed species with 50% or less of 'the recommended dose.' Moreover 'recommended doses' can vary significantly even between neighbouring countries due to marketing consideration or national legislation further questioning the use of this term. The other important issue is to understand the dose response relationship and the fact that there exists no unequivocal relationship between reductions in herbicide rate and reductions in herbicide efficacy. In this context particularly the asymptotic properties of herbicide dose response curves are important to appreciate. Besides that 'sticking to the recommended dose' may not always be the correct recommendation the dose discussion tend to have diverted the focus from what is more important managing herbicide resistance namely diversification be it in control methods or crop rotation. The lack of it is the cause of most cases of resistance rather than the applied herbicide dose.

AGRO 17

Pesticide dose: Using conceptual models of exposure to understand risks

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The dose of the pesticide that reaches the target cellular components in the pest is the determinant of the ability to control the pest. It is also the determinant of effects in non-target organisms (NTOs). What differentiates a pesticide that can be safely used with little or no effects on non-targets from a non-selective chemical is the extent to which the pathways of exposure and the target system(s) differ between the pest and the NTO. Differences in sensitivity at the target system of the pest or the NTO relate to the toxicodynamics of the pesticide, however, the pesticide must travel from the sprayer, hopper, or seeder to the target system before any response will occur. The pathway of exposure relates to the toxicokinetics of the chemical in the environment and in the affected organisms. Differences in these pathways between target and NTOs can provide selectivity or result in harm. Thus, in assessing risks of pesticides, the exposure pathways must be understood to correctly characterize risks. Even in an ecologically simple agroecosystem, there are thousands of possible pathways of exposure and it is necessary to narrow these down to the few that are primary determinants of the dose in the pest or the NTO. This is best achieved through the use of conceptual models that consider the methods of application, the inherent physical and chemical properties of the chemicals and/or the formulation, and the environment in which they are used. Starting with all possible pathways, the properties of the pesticide can be used to eliminate those that less likely and focus the most important ones that can then be tested with risk hypotheses. Examples of conceptual models for several pesticides will be used to illustrate how they are used.

AGRO 18

Drift, dose, and non-target organisms

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New chemical weed management technologies, particularly the use of synthetic auxin herbicides on genetically modified auxin herbicide-tolerant crops, are increasing the focus on potential spray drift problems among farmers, researchers, government regulators, and the public. The new technologies are based on two of the first synthetic herbicides that were developed 50-70 years ago and have had a long history of drift and damage to non-target plant species. Companies claim their new formulations of these auxin herbicides will perform better and the improved application methods mandated on herbicide labels will greatly reduce drift, but the inherent problem is that these herbicides will be used in new ways and on much more area that is nearby very sensitive non-target plants. This presentation will review spray drift factors that need to be mitigated, as well as the rather intangible "no effect" dose criterion. These factors include the rate of herbicide being applied, plant sensitivity, tank mix partners, formulations and adjuvants, spray application parameters, and meteorological conditions. The U.S. Environmental Protection Agency is implementing measures to reduce the potential for drift with these products, but some will not accept any approach based on thresholds and think any chemically or biologically detectable off-target movement is unacceptable. These concerns will be discussed in the context that Paracelsus, the father of toxicology, established 500 years ago when he observed that the dose makes the poison.

AGRO 19

Variations in pesticide doses under field conditions

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Pesticide doses are mostly expressed by the volume or weight of pesticide applied to one acre or hectare. However, many plants, insects or microbes can complete their life cycle in environments of only a few cm² or mm². In the field, and in such a small scale, pesticide rates are not uniform, causing some target organisms to survive because

they do not receive enough pesticide. Highly variable rates within a field also can contribute to selection of resistant biotypes and for some target organisms to receive rates low enough to show hormesis. Aiming at a better comprehension about the effects of pesticide dose variation under field conditions, a total of 36 applications of pesticides or tracers were studied. In all experiments, spray solution deposition was expressed in uL/cm² of leaf or target surface. The information was arranged to produce data sets with 30 to 600 replications, and frequency distribution models were fitted to the data. Spray solution deposition in 600 individual citrus leaves ranged from 7.7 to 422% of the mean. In peach orchards, six applications of tracers added to fungicides were studied. The spray solution deposition was evaluated in a total of 300 individual peach leaves for each application, and the broadest dose range observed had 2.1 and 256% of the mean for the minimum and the maximum values. The deposition of pre-emergence herbicides was studied using concentric circular targets with diameters ranging from 0.32 to 10.16 cm and 117 replications. For the smallest and largest targets, spray solution deposition ranged from 0.63 to 262% and from 68 to 128% of the mean, respectively. A total of 20 field applications of pre-emergence herbicides sprayed to sugarcane fields were studied. The evaluations were carried out with 30 to 52 replications, using 10 x 20 cm glass plates as targets. Herbicide dose in each plate ranged from 33.7 to 172% of the mean. The results agree with information available in the literature showing high variation of pesticide deposition or concentration in individual leaves, plants (crops or weeds) or soil samples.

AGRO 20

Use of intermittent sprayers for automatic thinning of direct seeded lettuce

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Lettuce has traditionally been seeded and thinned to desired stands by a hand weeding crew with hoes. However, decreasing labor availability and increasing costs for lettuce hand thinning has resulted in need for labor saving technologies. Recently, commercial machines capable of robotic lettuce thinning have been developed to machine-thin lettuce to the desired final crop density, helping growers reduce the ~$40 million/year spent to hand thin the crop. These systems typically utilize machine vision technology to detect plant location and accurately direct herbicidal sprays, such as carfentrazone to thin crops to desired stands. The lettuce thinners typically treat 13% of the surface area of a lettuce field spraying an intermittent band 10 cm wide with two plant lines per 1 m wide raised bed. Within the length of the plant line, about 30% is left unsprayed to preserve the “saved” lettuce plants. However, the current state-of-the-art in this technology cannot distinguish crop from weed plants, but depends upon recognition of row patterns to detect the crop row, and rudimentary object detection for selection of unwanted crop plants for thinning. Research in CA and AZ on these systems show, that while they work well in weed-free fields, their performance is limited in weedy fields, which obscure the row pattern. The machine vision system has been tested for selective application of fungicides or insecticides to the saved plants with a second applicator system mounted on the lettuce thinner. In selective fungicide/insecticide application to the saved plants about 7% of the field is treated, compared to 100% for a broadcast application. The potential for machine-vision guidance systems to reduce pesticide dose applied to a field is just beginning to be appreciated.

AGRO 21

Brief introduction to maximum residue levels (MRLs) and challenges in harmonization

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Maximum Residue Levels (MRLs) are the highest level of a residue from use of a crop protection chemical legally allowed to be on or in food or animal feedstuff. These levels are set by national regulatory authorities and correspond to residues found after use of the product according to the Good Agricultural Practices, or use directions, found on the label of the crop protection chemical. Companies that register these chemicals must generate data about the residue left by use of the chemical for the regulatory authority's review. Countries may share and discuss their findings together in a Joint Review, which contributes to harmonization efforts. Regulatory authorities, as well as the non-regulatory body, Codex Alimentarius Commission, a joint effort of the WHO and FAO, can each interpret data differently from the others, which may contribute to differences. Companies that sell crop protection chemicals also face other challenges in harmonizing MRLs to serve their customers. Issues such as registration timing and the reality that certain crops are just not grown in every country (especially tropical fruits), in addition to different use patterns that address different pest problems, make the task that much harder. MRLs serve as trade standards around the world. The lack of harmonization may cause trade irritants or trade barriers for those selling and shipping commodities from one country to another. These issues are very complex, but feeding the world requires the use of pesticides and their MRLs!

AGRO 22

Challenges in complying with multiple MRLs

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With production on six continents and 21 countries, Driscoll’s is a global enterprise exporting fresh berries to many different countries. Export MRL compliance is essential to maintain this business and further expand into new markets around the world. We’ve developed a number of processes to ensure regulatory compliance in export markets as well as tools that enable us to comply with MRLs globally. A continuing challenge is the determination of pre-harvest intervals required to meet some of the more restrictive MRLs. This is especially true when an agency has a policy of setting default MRL values at the limit of detection or 0.01 ppm. There are clear regulatory policies and also grey areas that need to be better understood in order to maintain compliance. As issues arise we work with various regulatory agencies to resolve the particular issue and to improve our processes. Efforts to harmonize global MRLs, a clearer understanding of regulatory policies, alignment with growers, customers, regulators and registrants all contribute to our ability to participate in the global market place and maintain good standing with regulatory authorities.

AGRO 23

U.S. grower priority project: Establishing import maximum residue levels (MRLs) in Taiwan and South Korea

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Taiwan and South Korea are important markets for US agricultural exports. According to data from the United States Department of Agriculture's Foreign Agricultural Service (USDA/FAS), South Korea is the United States' sixth-largest agricultural export market closely followed by Taiwan as the seventh-largest export market. In 2014, US agricultural exports to South Korea was at a record level of $6.9 billion, up 32 percent from 2013. Likewise, U.S. agricultural exports to Taiwan were valued at $3.5 billion in 2014, reflecting a 10 percent increase from 2013. On a per capita basis, Taiwan is one of the world's leading purchasers of U.S. food and agricultural products. Maximum Residue Levels (MRLs) for pesticides registered and used in the United States must be established in Taiwan and South Korea in order for commodities to be exported to these countries. Often newer, softer pesticide chemistries are registered in the US but cannot be used on exported commodities because of a lack of a MRL. US commodity groups and pesticide developers together with USDA and the Environmental Protection Agency (EPA) are spearheading efforts to obtain Import MRLs in these two key agricultural export markets. Recent progress has resulted, and further efforts are underway.

AGRO 24

U.S. forage export market MRL challenges

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U.S. forage exports parallel the growth of global agriculture trade and have developed from the Pacific Northwest timothy hay trade to horse racing stables in Hong Kong and Japan to forage products for dairy cows along the Pacific Rim. The development of containerized transportation, a growing demand for animal protein in developing countries, and water resource management in the United Arab Emirates has led to an increase in demand for U.S. forage products. U.S. hay exports have almost doubled from 2004 to 2013 with Japan being the most important export market for U.S. grown hay followed by China, Korea and the UAE. One of the goals of the USFEC is to remove trade obstacles to allow formation of new and larger markets for U.S. forage and complying with foreign market Maximum Residue Levels (MRLs) sometimes set at different levels than those established in the U.S. growing region, is the latest challenge. Pesticides are an important tool used to meet the increasing forage demand and grow a healthy crop. A key focus for our forage growers is determining the MRL or Import Tolerance (IT) of the pesticides in export markets. In the past, using a pesticide per the US EPA label was enough to ensure the crop would meet worldwide MRLs but with the move toward developing their own national food standards programs in the Pacific Rim, it has become more challenging to identify whether a pesticide used in the U.S. will be acceptable in an export market. According to the USDA FAS Global Agricultural Trade System (GATS) the top 4 export markets for US hay (Japan, China, Korea and UAE) represent approximately $1B and about 3 billion MT in 2014, so knowing the MRLs/ITs in these markets is vital to U.S. agricultural trade.

AGRO 25

Regulation of animal feed import tolerance MRLs in Japan

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Both the Japanese consumer and the Japanese livestock industry rely heavily upon imported foods and feeds from overseas. The so-called Positive List system containing MRLs (Maximum Residue Limits) for residues of agrichemicals in human food commodities was introduced by Japan's Ministry of Health, Labour and Welfare (MHLW) in May, 2006. However, the Positive List does not apply to livestock feed items, such as alfalfa hay and fodder, or dried grasses. Another Japanese authority, the Ministry of Agriculture, Forestry and Fisheries (MAFF) is responsible for setting MRLs for livestock feed items in order to ensure that agrichemical residues in animal commodities derived from livestock (i.e., eggs, milk, meat, offal, etc.) comply with the MHLW-established MRLs for these commodities. When the Positive List was introduced by the MHLW, the MAFF established feed MRLs for about sixty pesticides that can potentially leave residues in animal commodities. Key points of the regulation affecting the need for and establishment of MRLs in animal feed items in Japan will be covered in the presentation.

AGRO 26

Conclusions and follow-up from 2014 IUPAC ACS MRL workshop

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Growers, regulators, international reviewers and registrants met at the 2014 IUPAC ACS meeting to increase communication and to discuss the complexities of MRLs and their impact on trade of agricultural commodities. A goal of the workshop was to recognize the obstacles and brainstorm new strategies or solutions/remedies. Trading standards established by national authorities can be as unique as the nation itself. Meeting to strategize avenues towards increasing trade of food around the world benefits human kind. The results of the brainstorming session will be provided as documentation of the situation as it currently exists and what the best minds in agriculture, regulation and the pesticide industry recommend for progress in the trade of food from growing regions to those who desire diverse, fresh and delicious food.

AGRO 27

Harmonized risk assessments to support acceptance of another country's MRLs for imported foods

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Harmonization of global MRLs is important to allow trouble-free trade of food around the world. As part of the import MRL approval process, dietary risk assessment practices used to established the safety of pesticide uses must be harmonized to allow for quicker evaluation and more uniform conclusions regarding the acceptability of pesticide usage and the MRLs associated with each use pattern. In particular, harmonization of the underlying assumptions and acceptable refinements to dietary risk for pesticides would allow for greater MRL harmonization and therefore would eliminate barriers to smooth global trade of food commodities. This presentation will demonstrate through case study, the applicability of residue data from a major exporting geography to support establishment of import MRLs globally. Furthermore, incorporation of refinements to the dietary assessment, including the usefulness of trade data as a possible refinement will be explored. Following completion of a favorable dietary risk assessment based on local consumption information, MRLs from exporting countries can be adopted unchanged by importing countries, thereby maintaining global harmonization and allowing for easy global trade of food commodities.

AGRO 28

USDA's Pesticide Data Program – a residue monitoring program for foods

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The Pesticide Data Program (PDP) was initiated in 1991 to collect data on pesticide residues in food, with an emphasis on commodities highly consumed by infants and children. PDP data are used by the U.S. Environmental Protection Agency to prepare realistic pesticide dietary exposures for continuing pesticide re-registration activities in accordance with the 1996 Food Quality Protection Act and ongoing registration review responsibilities. PDP data are used by the U.S. Department of Agriculture's Foreign Agricultural Service to facilitate the trade of U.S.-grown products. The data are shared with trading partners to demonstrate the safety of U.S. exports and compliance with international MRLs.
PDP is a Federal-State partnership. There are currently ten cooperating sampling and testing States – California, Colorado, Florida, Maryland, Michigan, New York, North Carolina, Ohio, Texas, and Washington. Together these States represent approximately 50% of the U.S. population. To date, PDP has tested 112 commodities and includes over 400 pesticides and metabolites in its testing profiles. In 2015, PDP is testing apples, cherries, cucumbers, grapes, green beans, lettuce, nectarines, oranges, peaches, peanut butter, pears, potatoes, spinach, strawberries, sweet corn, tomatoes, and watermelon using multiresidue methods.

AGRO 29

FDA pesticide residue program

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The FDA pesticide program must meet the challenge of analyzing thousands of pesticides and industrial chemicals at extreme (ppb → ppm) levels in essentially infinite matrices with complexities ranging from water to feeds/foods/herbals; and, in most cases, the analysis must be completed within day for as many as 50 samples per day. To meet this challenge the FDA pesticide program incorporates both targeted and screening analytical approaches. Using the targeted approach, pesticides are analyzed and quantitated against standards for over 20 compounds by LC-MS/MS in 15 minutes, and more than 200 pesticides by GC-MS/MS in 20 minutes. Each sample is additionally screened to detect over 600 pesticides using full scan GC-MS coupled with spectral deconvolution against standardized MS spectra and retention indices. Looking to the future, FDA is working to expand pesticide and toxin coverage by additionally screening with LC coupled with high resolution mass spectroscopy. Also, under evaluation is a procedure to streamline import sample processing by prescreening pesticides and toxins on the surface of raw agricultural commodities using ambient pressure desorption with high resolution mass spectrometry at the port of entry.

AGRO 30

Multiple nicotinic acetylcholine receptor subtypes are insecticide targets

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Nicotinic acetylcholine receptors (nAChRs) are the major receptors mediating fast excitatory neurotransmission in the insect central nervous system and are among the most valuable insecticide target sites. Two very different nAChR subtypes have been identified with electrophysiology: desensitizing (nAChD) subtypes, which are the targets of neonicotinoids, and nondesensitizing (nAChN) subtypes, which are selectively activated by spinosyns. Several radioligands have been used to measure binding affinities of competitive agonist insecticides to nAChD receptors, but these can measure different nAChD subtypes depending on concentration and radioligand. Subtypes of nAChD receptors can also be recognized by the differential effects of selective insecticides on various kinetic components of ACh-evoked currents in voltage clamp studies on insect neurons. While the picture is not complete, it is now clear that different types of competitive agonist insecticides are selective for different nAChD receptor subtypes.

AGRO 31

Molecular mechanisms for diverse actions and selectivity of neonicotinoids

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Neonicotinoids with mammalian safety and plant systemicity are used intensively for plant protection. However, possible adverse effects on beneficial insects and insectivorous birds led to European bans of several neonicotinoids. Thus a more detailed understanding of MOA and selectivity mechanism on their target nicotinic acetylcholine receptors (nAChRs) is needed. Voltage clamp electrophysiology was used to investigate how structural diversity of neonicotinoids affects their agonist/antagonist actions on nAChRs. Most neonicotinoids are partial agonists. However, imidacloprid shows antagonist actions on a class of nAChRs expressed in native insect neurons, while a clothianidin analog shows a higher agonist efficacy than acetylcholine. Neonicotinoids show a higher affinity for insect over vertebrate nAChRs. Studies using voltage clamp electrophysiology combined with receptor mutations and modeling identified determinants of the nACR-neonicotinoid interactions. The author will review these findings and provide a perspective on future research in this research field.

AGRO 32

Modes of action of meta-diamide insecticides and ivermectin on the RDL GABA receptor

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Meta-diamides [3-benzamido-N-(4-(perfluoropropan-2-yl)phenyl)benzamides] with high insecticidal activity are a distinct class of RDL GABA receptor noncompetitive antagonists. The site of action of the meta-diamides was suggested to be distinct from that of conventional noncompetitive antagonists (NCAs) such as fipronil. NCAs act at the 2′ and 6′ residues in the pore formed by membrane-spanning region M2s of the RDL GABA receptor. In contrast, meta-diamides were suggested to act at or near G336 residue in M3 of the *Drosophila* RDL GABA receptor. Binding studies showed that binding of the meta-diamide to housefly RDL GABA receptor was facilitated by fipronil, but was inhibited by ivermectin completely. These results suggest that binding site of ivermectin overlaps with that of meta-diamides, but that of fipronil does not. Using membrane potential assay, we observed that ivermectin acted as an allosteric agonist of the *Drosophila* RDL GABA receptor. G336A, G336S, and G336T mutations had profound effects on the allosteric agonist activities of ivermectin, and a G336M mutation abolished the activity of ivermectin. These results suggest that G336 in M3 of the *Drosophila* RDL GABA receptor is important for the binding of ivermectin, as is the case with meta-diamides. Then, we compared the effects of mutations around G336 and A 2’ mutations in *Drosophila* RDL GABA receptor on the activities of ivermectin and meta-diamide 7. The effects of mutations on the activity of meta-diamide 7 differed from those on ivermectin. Molecular modeling studies showed that ivernectin docked in the intersubunit pocket near G336 in the *Drosophila* RDL GABA receptor in the open state. In contrast, meta-diamide 7 docked into the *Drosophila* RDL GABA receptor in the closed state. This suggests that the modes of action of ivermectin on the wild-type *Drosophila* RDL GABA receptor differ from those of meta-diamides.

AGRO 33

Molecular pharmacology of homomeric UNC-49B channels from southern root-knot nematodes

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Root-knot nematodes are important plant parasitic nematodes, which cause serious damage to a wide range of crops. Fumigants and non-fumigants targeting acetylcholinesterase are currently used to control the nematodes. To examine whether γ-aminobutyric acid (GABA) receptors serve as a target of nematicides, we cloned an *unc-49B* orthologue from the southern root-knot nematode (*Meloidogyne incognita*) and investigated the ligand responses of homomeric UNC-49B channels expressed in *Xenopus* oocytes using a two-electrode voltage clamp technique. The extracellular orthosteric binding site and four transmembrane domains are conserved in *M. incognita* UNC-49B (Mi-UNC-49B), whereas a long second intracellular loop is characteristic of this protein. Mi-UNC-49B channels expressed in oocytes were less sensitive to GABA compared with other invertebrate GABA receptors. Picrotoxinin and fipronil inhibited GABA-induced currents in Mi-UNC-49B channels with micromolar and submicromolar IC_{50}s, respectively. These channels became ~20-fold more sensitive to both picrotoxinin and fipronil by a mutation (G2’A) in the second transmembrane domain. A mutation (T6’M) in the same domain diminished the potency of picrotoxinin. Two nematicidal compounds, samaderine B and 4-cyclohexyl-3-isopropylbicyclophosphorothionate (SF-14), inhibited GABA-induced currents with micromolar and submicromolar IC_{50}s, respectively. Essential oils (α-terpineol, thymol, and methyleugenol) and an anthelmintic macrolide alone (ivermectin) failed to activate Mi-UNC-49B channels. However, these compounds potentiated currents induced by low concentrations of GABA, whereas they antagonized currents induced by high concentrations of GABA. The unique pharmacology of Mi-UNC-49B channels should provide opportunities to develop specific nematicides.

AGRO 34

T345M, an additional mutation associated with insecticide resistance in the *Anopheles gambiae* GABA receptor, Rdl

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Cys-loop ligand gated ion channels contain the molecular targets for insecticides, including the γ-aminobutyric acid (GABA)-gated chloride channel Rdl (‘resistance to dieldrin’). In *Anopheles gambiae* Rdl the A296G mutation is associated with insecticide resistance. We report a further mutation, T345M, identified in insecticide resistant *An. gambiae* mosquitoes. An *An. gambiae s.s* strain containing phenotypic resistance to dieldrin was colonised using wild material collected from the Democratic Republic of Congo. Samples of adult females were exposed to 4% dieldrin and were sorted according to resistant or susceptible phenotype 24 h post exposure. From both phenotypes the complete coding sequence of Rdl was amplified. In all resistant samples, two mutations were identified, A296G, the classic mutation within the second transmembrane domain, and a novel T345M mutation, present in the third transmembrane domain. Susceptible samples did not contain either mutation. This mutation has also been identified in *Drosophila simulans* (T350M), whereby both mutations also appeared simultaneously. Two-electrode voltage-clamp electrophysiology was applied to *Xenopus laevis* oocytes expressing *An. gambiae* Rdl with the single and double mutations. Results showed that GABA sensitivity was not significantly altered in the presence of the mutations, however sensitivity to the insecticide fipronil was significantly reduced in the A296G and double mutation, from that of the wild type. This study provides insights into the mechanisms of insecticide resistance. Also, functional expression of mosquito specific Rdl isoforms may provide a useful tool for testing compounds on a validated target, in the search for novel pesticides for major disease control.

AGRO 35

Action of pymetrozine, pyrifluqinazon, and flonicamid on chordotonal neurons requires TRPV channels

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Pymetrozine, pyrifluquinazon and flonicamid are selective insecticides that disrupt feeding of homopteran pests by unknown mechanisms. Pymetrozine is known to specifically stimulate specialized stretch receptors in insects known as chordotonal organs, without affecting other types of mechanoreceptors, and we have found that flonicamid and pyrifluquinazon have the same effect. Furthermore, 4-trifluoromethylnicotinamide, the bioactive metabolite of flonicamid, as well as the de-acetylated metabolite of pyrifluquinazon, are much more potent than the parent compounds in stimulating chordotonal organs, indicating that the compounds are prodrugs. Johnston's organ of the fruit fly *Drosophila melanogaster* is a chordotonal organ in the second antennal segment that is specialized to perceive sound, wind and orientation with respect to gravity. Johnston's organ is perturbed by feeding of pymetrozine, pyrifluquinazon or flonicamid, resulting in increased intracellular Ca2+ in the chordotonal neurons and disruption of hearing and gravity perception. While examining the dependence of chordotonal organs’ insecticide sensitivity on ion channels known to be expressed in them, we determined that the action of pymetrozine, pyrifluquinazon and flonicamid on chordotonal organs required the two transient receptor potential vanilloid (TRPV) channels Nanchung (Nan) and Inactive (Iav), which co-assemble exclusively in chordotonal neurons to form a heteromeric channel that is critical to mechanosensitivity. These results indicate that pymetrozine, pyrifluquinazon and flonicamid stimulate chordotonal organs by activating insect TRPV channels either directly or indirectly.

AGRO 36

Pymetrozine and pyrifluquinazon activate heterologously-expressed insect TRPV channels

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Two commercial insecticides are shown to target two transient receptor potential vanilloid (TRPV) ion channels, Nan and Iav. Nan and Iav co-expressed in Chinese hamster ovary cells (CHO-K1) conferred cellular responses to pymetrozine, and pyrifluquinazon by promoting calcium uptake and membrane depolarization. Pyrifluquinazon underwent spontaneous deacetylation in aqueous solution and the deacetylated derivative was two orders of magnitude more potent that the parent in activating the TRPV channels, confirming that pyrifluquinazon is a prodrug. In insects, Nan and Iav co-occur exclusively in chordotonal stretch receptor neurons, and, similarly, their response to insecticides in the heterologous system also required the simultaneous presence of both Nan and Iav. Formation of functional Nan-Iav complex was directly confirmed by co-immunoprecipitation and FRET. This study establishes TRPs as novel insecticide targets, and defines the first specific agonists of insect TRPs. Like pymetrozine and pyrifluquinazon, flonicamid and it's more potent bioactive metabolite, 4-trifluoronicotinamide, stimulate chordotonal neurons in a manner that is dependent on expression of Iav and Nan, but neither flonicamid nor 4-trifluoromethylnicotinamide activated heterologously expressed Nan-Iav heteromers, indicating that these TRPs are not the target of flonicamid.

AGRO 37

Ion channel screening for insecticide discovery

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Ion channels comprise the molecular basis of diverse physiological functions such as membrane excitability, neurotransmitter secretion, and muscle contraction. They are also a key target class for pest control in crop protection as well as in animal health. Active ingredients in roughly 70% of current insecticidal market products act on ligand- or voltage gated ion channels. To identify new compounds acting on insect ion channels, libraries containing up to millions of compounds have to be tested on the respective target. The gold standard to directly record ion channel activity is the patch clamp technique, which was invented in the 1970s by E. Neher and B. Sakman and revolutionized ion channel research. Unfortunately, patch clamping is very laborious and has a limited throughput. Therefore, other assay formats like radioligand competition binding studies on native receptors or fluorescence based screening of cell lines heterologously expressing the target protein were driven to a higher throughput or were newly established. Although these techniques allow much higher throughput, they have some weaknesses compared to the patch clamp technique. Binding studies are limited to known target binding sites and handling of radioactive compounds is not desirable. Fluorescence readouts with calcium- or voltage sensitive dyes on target-transfected cells include compound effects on all endogenously expressed proteins, necessitating control experiments in follow-up experiments. Furthermore, lack of adequate membrane voltage control can lead to false-negative results, e.g. if compounds have channel state dependent affinity. To circumvent these problems, different attempts were made to automate the patch clamp technique. Now, after a decade of progressive improvement of automated patch clamp systems, electrophysiological screening with reasonable throughput and high data quality becomes feasible and is routinely applied for ion channel research in pharmaceutical and crop protection industry.

AGRO 38

Specialized exposure analysis techniques for aquatic and terrestrial animal drug

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As part of the pre-market approval process for new animal drugs, the Food and Drug Administration (FDA), Center for Veterinary Medicine (CVM), evaluates whether significant environmental impacts would occur pursuant to the National Environmental Policy Act (NEPA). Under FDA regulations, many agency actions, including the approval of new animal drug applications, may require the preparation of an environmental assessment (EA). A key component of an EA is the environmental exposure assessment. The exposure assessment considers such information as the use pattern of the drug (e.g, species treated, dose, frequency, route), the animal husbandry practices, and manure management practices. The exposure assessment is generally conducted using a tiered approach to determine the predicted environmental concentration (PEC) of veterinary pharmaceuticals in animal feces, soil, water, and sediment. It begins with a conservative screening level analysis followed as needed by higher tier and more realistic analysis. This presentation will discuss examples of exposure assessments used to evaluate the potential environmental impact of aquaculture and terrestrial animal drugs. The examples will include antimicrobials used for therapeutic claims (e.g., oxytetracycline) and steroid hormones used for production purposes (e.g., estradiol and trenbolone). Higher tier exposure assessment techniques used to derive PECs will be discussed, including specialized laboratory and/or field testing, probabilistic exposure analysis, and environmental fate modeling.

AGRO 39

Geospatial technologies for characterizing veterinary medicine exposure in the watershed and placing exposure into context

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The application of geospatial technologies to exposure assessments (EA) is tightly woven in the U.S. Environmental Protection Agency (USEPA) pesticide registration process. From adjusting the percent of crop in a watershed treated with pesticides to characterizing the diversity of soils, climate, and hydrology in the landscape, Geographic information systems (GIS) are commonly used in higher tier exposure assessments. In this presentation, a case study in which spatial approaches were applied to characterize the potential for aquatic exposure from the excretion of beef cattle treated with veterinary medicines will be demonstrated following techniques similar to Tiers 3 and 4 of USEPA's aquatic exposure assessment process.
GIS was used to establish high versus low risk regions of

exposure potential across the US. Multiple regions representing a variety of beef cattle characteristics and climatic conditions were identified as having the highest vulnerability potential. From within each region, a single vulnerable watershed was selected for watershed-scale modeling. The watershed selection places modeling results into national context and promotes confidence that the results represent a realistic intense-use scenario that can be applied to other U.S. beef regions. For each watershed modeled, local factors relevant to simulating veterinary medicines in surface water were identified using spatial data on feedlot densities, pastured cattle lands, and croplands treated with manure. While fate and transport models ultimately calculate the concentration of drug in the environment, GIS measures the distribution of landscape factors influencing exposure and places the concentrations into the larger national risk perspective. The workflow to achieve a representative watershed for higher tier modeling will be presented. Examples using geospatial data to define vulnerability at the region and watershed scales will be discussed. The coupling of geospatial with modeling to characterize exposure is a framework suitable for other commodity groups grappling with the challenges of higher tiers EAs.

AGRO 40

Guidance for the consideration of unextracted residues in laboratory soil and water metabolism studies for pesticides

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Considerable amounts of unextracted, unidentified residues are present in soil and aquatic degradation studies submitted to the United States Environmental Protection Agency's Office of Pesticide Programs by pesticide companies to support pesticide registration. These residues may be bound to the soil matrix, or they may be unextracted due to inadequate extraction methods. In September 2014, EPA implemented new guidance for the review and consideration of unextracted residues in these studies. It is expected that this guidance will provide a more consistent and robust approach for use and consideration of unextracted residue data in exposure and risk assessments. The new guidance identifies steps to more consistently identify when residues can be assumed to be bound, and methods for considering unextracted residues in fate and exposure assessments when they cannot be assumed to be bound. As such, the guidance should result in submitted studies with more fully characterized residues.

AGRO 41

Higher-tier surface water exposure modeling approach at watershed scale of veterinary pharmaceuticals administered to beef cattle

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Watershed scale exposure modeling of veterinary pharmaceuticals in surface water was conducted following the U.S. Environmental Protection Agency's (USEPA) Tier-2 drinking water pesticide exposure modeling approach. Three potential sources of veterinary pharmaceuticals administered to beef cattle were modeled –feedlots, agricultural fields applied with manure collected from the feedlots, and pasture. Runoff and erosion from these sources were modeled and combined and inputted into a waterbody. The Pesticide Root Zone Model (PRZM) used in the FOCUS Surface Water and Groundwater model software (winPRZM) was modified to simulate a feedlot and pasture. PRZM 3.12 was used to model an agricultural field. EXAMS was used to model the waterbody. Enhancements to winPRZM included having options to model timing of entry and exit of beef cattle on the feedlot and pasture and model constant mass of active ingredient in feedlots if degradation rate in manure in unknown. The landscape metrics were derived from the GIS analysis of watersheds to estimate percent watershed area contributing to feedlots, pasture, and agricultural land applied with manure to model the watershed level index reservoir. The "application rates" of active ingredient to feedlot and pasture were estimated based on daily release rate of the active ingredient of the pharmaceutical in manure. The application rate of active ingredient in manure applied to agricultural land was based on daily release rate and phosphorus requirement of corn grain/silage. The feedlots with less than 1000 head cattle were included in the calculations since feedlots greater than 1000 head are not permitted to release wastewater following the USEPA's Clean Water Act. USEPA's standard Tier-2 crop scenarios and weather files were used as such. The 90th percentile concentrations for peak, 4-day, 21-day, 60-day, 90-day and annual average exposure durations were estimated based on 30-year daily model runs following the USEPA's tier-2 modeling methodology

AGRO 42

Screening level environmental risk assessment (ERA) of cosmetic ingredients in the USA and beyond

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The Personal Care Products Council's (PCPC) environmental program recently began the development of a new ERA framework for cosmetic ingredients in North America. Initially, 7000 ingredients were identified as being used in cosmetic formulations. These ingredients were divided into a number of broad chemical categories. This allowed the PCPC to assign screening level assessment strategies to each group of ingredients. One of these groups, discrete organic ingredients (both neutral and charged) was chosen to undergo a series of aquatic screening level assessments. As this group is widely used and disposed of chiefly *via* a "down the drain" exposure scenario, prioritizing discrete organic materials for aquatic screening was justified. Within this assessment, a network of *in silico* models was developed to estimate the potential removal of cosmetic ingredients from wastewater treatment. The models were based on the work of Salvito *et al*. (2002). Concurrently, acute aquatic toxicity was conservatively predicted by ECOSAR models (USEPA's EPISuite toolbox). Cosmetic usage was estimated using a "market forensics" approach (DeLeo *et al*., 2011) and industry survey data. From these combined approaches, an MST (maximum sustainable tonnage) value was derived for each ingredient. This represents the total concentration of an ingredient which can be present in the environment without causing an adverse ecological effect. This value was then compared to total usage (of an individual ingredient) and a risk ratio (similar to a traditional PEC: PNEC) could be calculated. Other screening methods were developed to evaluate other ingredient types, such as polymers. Once a screening level assessment is conducted ("tier I"), certain ingredients then enter a second tier of assessment. In addition, a number of other prioritization schemes were developed, such as use of VCRP (Voluntary Cosmetic Reporting Program) data.

AGRO 43

Modeling the soil binding affinity of positively charged organic chemicals

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Using our own consistent experimental data set for 70 bases, we examined how the sorbent properties, sorbate properties and medium properties, affect the sorption affinity to several individual soil components and natural soils. The positively charged species of these bases, including commonly used pharmaceuticals, biocides and a wide series of model amine structures, strongly sorb to negatively charged sites in natural organic matter (NOM) as well as to negatively charged phyllosilicate clays. This sorption process appears to occur mainly through an ion-exchange process with other sorbed cations, such as sodium or calcium. The affinity of organic cations for both NOM and clay increases (i) if salt concentration decrease, (ii) more for lower sodium concentrations than for calcium concentrations, and (iii), for organic cations with larger nonpolar side chains. Thus, the ion-exchange affinity of organic cations on soils involves both ionic and nonionic interactions. Current risk assessment sorption models for organic cations are poorly verified, assume only sorption to NOM, and are based entirely on Kow and as a function of pKa. For a proper risk assessment and for transport modeling of organic cations in soils, it is highly desirable to predict the influence of variable soil properties (NOM and clay content), as well as the influence of ionic and nonionic interactions from the molecular structure. Under fixed medium conditions (pH, ionic composition), we showed that specific sorption coefficients to 1 reference NOM (micronized pahokee peat) and 1 reference clay mineral (illite) can be summed to predict sorption coefficients to natural soils (Eurosoils 1+5). We further examined several published sorption data for organic cations that were also present in our data set to test a more general applicability of this approach. Sorption to the clay fraction appears to be a dominant process for most soils and most organic cations, often a factor 10 stronger than sorption to organic carbon, but within acceptable uncertainty margins this can be accounted for with straightforward parameters.

AGRO 44

Application of passive dosing to maintain constant aqueous exposures of sparingly soluble, difficult-to-test compounds

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Regulatory requirements continue to increase for high quality environmental fate and effects data to support environmental assessments and fill existing data gaps. Aquatic fate and effects laboratory testing of petrochemical substances is particularly difficult due to their inherent hydrophobicity and susceptibility to both biotic (biodegradation, metabolism) and abiotic (sorption, volatilization, photolysis) loss mechanisms. The application of traditional laboratory dosing techniques in aquatic test systems such as static/semi-static renewals, flow-through systems and use of co-solvents frequently results in variable exposure concentrations and uncertainty associated with inconsistent and irreproducible fate and effects outcomes. Passive dosing offers an intelligent and practical approach to maintaining aqueous concentrations of difficult-to-test compounds including PAHs, multi-ring cyclic alkanes, lower olefins and other constituent hydrocarbons present in refinery streams. A variety of passive dosing formats have been applied and will be presented demonstrating the flexibility of this technique in overcoming many of the common obstacles encountered in laboratory fate and effects testing of hydrocarbons. The presentation will also include a discussion of the potential application of passive dosing to other neutral, nonpolar compounds.

AGRO 45

Simulation studies to evaluate surfactant biodegradation rates and their degradation pathways in sewer systems

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Limited data has been published using the OECD 314A test method, a simulation test that assesses the biodegradability of chemicals in sewer wastewaters. This research used the OECD 314A method to obtain biodegradation rate and pathway information on a suite of high volume surfactants including homologues of alkyl ethoxylate (AE), alkyl ethoxysulfate (AES), and linear alkyl benzene sulfonate (LAS). In this test, trace levels of ^{14}C labeled test materials were incubated in raw wastewater at 15 ^0C while the dissolved oxygen in the system was maintained at 0.5 mg/L DO. Test results provided first-order kinetic rates for primary degradation and pathway information based on RAD-TLC analysis of metabolites. Comparisons of different alkyl chain lengths for the same surfactant were used to evaluate the impact of chain length on biodegradation rates and metabolite formation. Kinetic data for AE showed that parent half-lives in sewer wastewater ranged from 6 minutes for $C_{12}E_3$ to 1.5 hours for the longer alkyl chain length $C_{16}E_3$. Homologs with ^{14}C labels in different positions were used to elucidate biodegradation pathway information. For example, a comparison of AES homologs with ^{14}C labels in different locations of the molecule showed that biodegradation begins with fission of the central ether followed by rapid mineralization of the resulting alcohol through both ω and β-oxidation and slower mineralization of the resulting sulfated polyethylene glycol. This test method and the data it generates can be used to improve exposure assessments for down-the-drain chemicals including those in personal care products and pharmaceuticals.

AGRO 46

Critical clay content in defining sorption behavior of pesticides in soil

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Sorption behavior of a pesticide in soil is currently defined by measuring sorption parameters in soils with a range of organic carbon, clay, pH or other soil properties. The correlation of sorption with soil properties is then examined by regression and used to predict sorption behavior in soils. For a majority of pesticides, sorption is dependent on organic carbon. Thus, the normalized parameters K_{oc} or K_{foc} are used in exposure modeling. However, for some pesticides, it has also been observed that sorption is dependent on clay content in soils. In this study, we have measured sorption parameters for one pesticide in 21 soils and the other pesticide in 56 soils. For both pesticides, we observed a critical clay content of 8%. For the first pesticide, at > 8% clay, sorption is significantly dependent on clay content, but displays distinctively different correlations with clay contents in sandy soils and silty soils. At < 8% clay, sorption is dependent on organic carbon. For the second

pesticide, sorption is linearly dependent on organic carbon at < 8% and > 8% clay, but sorption increases faster with organic carbon in soils with >8% clay. This critical clay content seems to result from the fact that the surface area of clay particles is equivalent to that of sand and silt particles in sandy soils with 8% clay. At >8% clay, the surface area of clay particles is dominant and also coated with organic matter. This combination can magnify the effect of organic carbon on sorption behavior and can lead to clay dependency of sorption. At < 8% clay, sorption is dependent on organic carbon due to disappearance of such a magnifying effect. The study demonstrates the importance of organic-mineral interaction in determining sorption behavior of a pesticide.

AGRO 47

Catch 22: All doses select for resistance - the questions are when this may happen and how to delay evolution

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Whereas crop and pesticide rotations are typically invoked as major mechanisms for delaying pesticide resistance, dose usage has a very important role. In many cases crops cannot be easily rotated, e.g. with trees or paddies, lessening possibilities. High doses typically select for target site resistances but ultra-high doses can delay by requiring ultra-low frequency homozygosity (if they do not kill the crop). Low doses typically select for multi-factor resistance by any of/or mixtures of the following: metabolism of pesticide - often by more than one or sequentially modified enzymes; multiple allelic changes and/or target gene amplification that stepwise enhance target site resistance and/or numbers of target molecules, increases of sequestration or expulsion of the pesticide, etc. There are many instances where this has been seen in the field with the same pesticide and pest; e.g. Lolium in low dose using Australia has widespread metabolic resistance to diclofop where only target site resistance is seen in high dose California with the same weed and herbicide. Uneven low doses result in some pests receiving sub-lethal doses that are stressful, but do not kill. Stress typically increases mutation rates, quickening the rate of evolution of both target site and multi-factorial resistance. Models suggest that alternating high and low doses can delay evolution much more than either alone. Pesticide mixtures can only delay resistance when they fulfill all of the following criteria: both pesticides kill the same pests, when they act on different targets, when they are degraded by different mechanisms, and when they dissipate at the same rates - otherwise they are not truly mixtures vis a vis resistance management. Thus a Bt that kills Lepidoptera mixed with a Bt killing Coleoptera, or a mixture of a grass killer with a broadleaf herbicide, or a mixture of a contact fungicide with a systemic one, or a residual pesticide with an environmentally ephemeral one, are not mixtures vis a vis resistance, even if they meet all other criteria. Mixtures meeting these criteria have lasted decades longer than single pesticides; e.g. triazine herbicide resistance has been rampant for three decades but has not evolved when triazines were always mixed with very-long chain fatty acid inhibitors, even though the latter alone are not good broadleaf weed killers. Doses can be lowered when there are synergistic mixtures that meet the above criteria, and still delay evolution.

AGRO 48

Reduced fungicide doses in cereals: Which parameters to consider?

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A long tradition for using reduced fungicides rates in cereals has been exploited successfully in Denmark for more than 20 years and a similar trend has also been seen in other Northern European countries. The use of reduced and appropriate dose rates has been driven by field trials with several dose rates, where data often have shown sufficient control and better net yield responses with reduced rates. This has encouraged farmers to go for reduced rates in order to minimize their cost for disease control. Optimal dose rates have been shown to depend on the active ingredient, the pathogen, disease pressure and timing of treatments. Certain diseases are known to require higher input (40- 75% rates) for achieving satisfactory control – this is the case for Septoria leaf blight, Rhynchosporium net blotch and Fusarium head blight, whereas most rust diseases generally have been found to be controlled successfully at rates down to quarter rates. Fungicides, in general, show highly variable dose responses for specific diseases, which is important to know when specific advice is given. Preventive treatments generally require less input compared with treatments during the latent period or later on well-established disease attack. Rates also have to be adjusted according to canopy structures. Early timing on a small canopy requires less fungicide than a full canopy around heading. The knowledge gathered concerning fungicide rates has led to great focus on the importance of optimizing timing of application. Most farmers are capable of monitoring the risk for diseases hereby ensuring that treatments are carried out at low disease pressure. In winter wheat the economically optimal fungicide input per season would require between 1-3 treatments and a total dose between 0.5 and 1.5 times the recommended dose. In spring barley the economically optimum input can vary from 0-2 treatments with a total fungicide use equivalent to 0.25 to 0.5 times the recommended dose. Applying reducing rates should never result in significant inferior control and economical yield losses. A recent review concluded that reduced fungicide rates select less for fungicide resistance and can help to delay development of resistance.

AGRO 49

Hormesis: Adaptive responses in biology and medicine

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This presentation provides an assessment of hormesis, a dose-response concept that is characterized by a low-dose stimulation and a high-dose inhibition. It will trace the historical foundations of hormesis, its quantitative features, mechanistic foundations, and its biomedical/clinical applications. It will be argued that hormesis is the most fundamental dose response, significantly outcompeting other leading dose-response models in large-scale, head-to-head evaluations used by regulatory agencies such as the EPA and FDA. The hormetic dose response is highly generalizable, being independent of biological model, endpoint measured, mechanism, chemical class, physical agent (e.g., ionizing and non-ionizing radiation) and interindividual variability. Hormesis also provides a framework for the assessment of chemical mixtures, incorporating the concept of additivity and synergism. Because the hormetic biphasic dose response represents a general pattern of biological responsiveness, as well as quantitatively defining the limits of biological plasticity, it is expected that it will become

progressively more significant within toxicological evaluation and risk assessment practices as well as having numerous biomedical applications, some of which will be emphasized in this presentation.

AGRO 50

Occurrence and significance of pesticide-induced hormesis in insects

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High amounts of stress are harmful to organisms, but in low amounts may stimulate certain biological processes. This biphasic response to a stressor, termed ‘hormesis’, has been seen in many insect taxa following mild exposure to stressors, including pesticides. Pesticide-induced hormesis in insects is most often observed as stimulated reproduction, although stimulatory effects on other physiological and behavioral processes have also been reported. Given that insect pests in agricultural settings are often exposed to sub-lethal doses of pesticide, the ramifications of pesticide-induced hormesis for pest resurgences and insecticide resistance development may be significant. On the other hand, there may be opportunities to use hormetic principles to improve commercial production of insect natural enemies, or to better understand how beneficial insects like pollinators respond to low doses of pesticide.

AGRO 51

Chemical hormesis on plant pathogenic fungi and oomycetes: What we know

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Although the current knowledge about responses of fungal strains exposed to subtoxic fungicide doses is limited and few studies have focused on fungicide hormesis, evidence of stimulation of plant pathogens due to chemical hormesis is accumulating in the scientific literature. Unintentional exposure of plant pathogens to low doses of fungicides could potentially reduce crop productivity. The most economically relevant plant pathogens are fungi and fungus-like organisms that belong to the kingdoms Fungi (Ascomycetes and Basidiomycetes, among others) and Stramenopila (Oomycetes). The diseases they cause are overall referred to as fungal diseases, and can affect underground and aboveground plant parts. Under optimal environmental conditions, fungal diseases result in severe crop losses. Even though disease resistant cultivars are used when available, management of fungal diseases is largely done by combining cultural practices with chemical control. Growers face several challenges when selecting fungicides for the cost effective management of diseases affecting their crops. Since the physiologies of true fungi and oomycetes differ substantially, most fungicides are specific to either group and a limited number of chemistries are effective against both groups of microorganisms. Pesticide use adds to crop productions costs, and in an attempt to minimize chemical control inputs growers may use reduced doses of individual fungicides; sometimes they may apply the same fungicide repeatedly, against expert recommendations. While intensive use of some chemistries without proper mode of action rotation often results in selection of fungicide resistant strains, exposure to low doses of certain fungicides increases the tolerance of fungal strains that previously were not resistant to them. Selection of fungicide resistant strains by improper use of fungicides in agricultural systems increases the risk of exposure to subtoxic doses. The lack of awareness among growers, extension educators, and the general public about this risk is a widespread threat to crop productivity. An update of our current knowledge about fungicide hormesis will be presented with particular emphasis on selected ascomycetes (*Sclerotinia, Fusarium*), basidiomycetes (*Rhizoctonia*) and oomycetes (*Pythium*).

AGRO 52

Herbicide hormesis: What do we know about the mechanisms leading to low dose growth increases?

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Low dose herbicide stimulation of growth in plants is frequently observed. Despite the general acknowledgement of its existence, however, the physiological mechanisms governing the induced growth is still an enigma. We have studied glyphosate hormesis in several species with the aim of elucidating the mechanisms behind the low dose growth increase. Methods ranging from explorative microarray screenings of gene expression, qPCR analyses for specific target genes, metabolic screenings of sugars and aminoacid compositions, enzyme activity assays, ^{14}C-pulse-trace experiments, photosynthetic measurements and growth trials under variable nutrient, CO_2 and light conditions have been used to test hypotheses on proposed mechanisms. In this presentation we will show our results, answer some of the hypotheses and propose new ones for further investigation. Growth hormesis is clearly a complex issue. Understanding the mechanisms can provide new insight into the basic physiology of plant growth regulation, which is as yet far from fully understood.

AGRO 53

Low dose effects of glyphosate on plant reproduction in *Arabidopsis thaliana*: A biological and transcriptomics approach

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Glyphosate is a slow-acting, non-selective herbicide widely used in conjunction with transgenic herbicide-resistant crop varieties. While wide spectrum weed control is usually achieved at relatively high doses (*ca.* 1 kg a.e. ha^{-1}), low doses of glyphosate can produce alteration of flower phenology and hormetic effects, such as increased growth. The basis for these phenomena was investigated by observing biological and transcriptomic responses in *Arabidopsis thaliana* exposed to 0, 0.18, 0.6, 1.8, 6, 18, 60 and 180 g a.e. ha^{-1} glyphosate. Seedlings were at their 6-7 rosette leaf stage in the first experiment and 4-5 leaf stage in the second experiment. The number of days to bolting, first floral bud, first flower and first silique during the entire development was determined for each plant. Samples were collected at each measurement time for transcriptomic analysis. In both experiments, low doses of glyphosate reduced the time of bolting, bud formation, flowering and silique formation of *A. thaliana* by 2-3 days. The optimum effect was observed at 6 g a.e. ha^{-1} glyphosate in the first experiment and at 1.8 g a.e. ha^{-1} in the second experiment. Seedlings also consistently had greater numbers of flowers and siliques formed at these sublethal concentrations of glyphosate. The effects were most pronounced, with up to 300% more flowers and siliques than controls, during earlier developmental stages. In both experiments, the most effective glyphosate concentration in causing these hormetic effects corresponded to the portion of the dose-response curve between the minimum dose for injury and that causing 20% visual injury. Insights into these mechanisms may be

useful for agricultural biotechnology applications such as seeking improvement in commercial crop production.

AGRO 54

Canadian perspective on MRLs

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What progress has made on harmonizing Canadian MRL's with US-EPA as main trading partner and other related countries? What is the current status-quo of MRLs needs supporting Canadian import and exports? Discussion of possible options to continue building blocks towards the ideal MRL-harmonization scenario from the Canadian perspective.

AGRO 55

JMPR and Codex MRLs: Roles, responsibilities, and challenges

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As the scientific arm of the Codex Committee on Pesticide Residues, the Joint Meeting on Pesticide Residues provides data-driven recommendations for MRLs and dietary exposure/risk estimates to Codex. The FAO/WHO Joint Meeting consists of an international collection of toxicologists (WHO) and pesticide residue experts (FAO) who provide evaluation of new compounds and new uses for new MRLS, as well as periodic evaluation of existing MRLs. The talk provides an insider view of JMPR and the challenges associated with dossier evaluation and MRL recommendations.

AGRO 56

Global field residue data supporting harmonized MRLs and exchangeability

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Crop field residue data is highly variable given diversity of crop varieties, agro-ecological conditions, and agricultural pratices. A new methodology has been developed to prove the similarity of residue results across climatic or geographic zones. The method has been validated on 73 crops, 46 pesticides, and 3,600 field trials from 2-4 global zones. The conclusion is similar to initial one produced by OECD/FAO 2003 Zoning Project which stated that more than 70% of the variability comes from local use patterns and not from region/zone. This validation enables exchanging crop residue data between countries and setting global-MRLs for independent crops based on combined global dataset, or setting crop group MRL's based on combined sets across crops.

AGRO 57

Working toward a global regulatory program for minor uses

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IR-4 is recognized by the international community as the model program that helps specialty crop growers obtain legal access to safe and effective pest management technology. Over the years, IR-4 has developed numerous partnerships with other countries to share the burden of developing regulatory data to register products for minor uses and to create other "IR-4 like" programs. Specifically, the governments of Australia, Brazil, Canada, Costa Rica, New Zealand, South Korea, and Taiwan have been involved. IR-4 and its partners realize that this cooperation is critical in finding solutions to the "Minor Use Problem." IR-4 has also been a principal educator in capacity building projects in the ASEAN region, the sub-Sahara Africa and in Latin/South America. It is anticipated that these partnerships, which work with government personnel on conducting regulatory studies to address minor use needs, will hopefully lead to future collaborative research projects to address minor use needs. Finally, IR-4 is working in these collaborations to also develop and implement specific extrapolation proposals and models (Crop Groups/Subgroups) that allow data developed on certain representative crops to be used to support registration on multiple crops within a group, which will further assist minor crop growers. Specialty crop producers around the world want access to the newest and safest pest control tools while also having access to international markets without the concern of residues being a barrier to trade.

AGRO 58

EU MRL regulation and import tolerance application procedures

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In the EU residues of pesticides in or on food and feed are legally controlled by Regulation (EC) 396/2005. The Regulation replaced previous EU legislation dating from the 70s and 80s, and became fully applicable in September 2008. One of the key drivers for bringing in this legislation was to fully harmonize the Maximum Residue Levels applicable in the EU, as previously individual countries within the EU were permitted to set their own MRLs, causing trade frictions within the EU, and complications for third parties wishing to trade with the EU. As a result of the harmonization exercise, the number of MRLs within the EU was reduced from 500,000 to approximately 100,000. The Regulation aims to establish a high level of consumer protection, sets out key definitions, and in its annexes lists: the crops and animal commodities for which MRLs are required, the MRLs themselves, substances for which no MRL is needed, and will list agreed processing factors when these are available. The Regulation also provides the framework procedure by which applicants must seek to obtain an MRL in the EU, or an Import Tolerance for cases where no appropriate EU MRL exists. In practice, the timelines from application to approval of an MRL have proved to be longer than anticipated, and the process more complex than envisaged for both applicants and regulators. The MRL Regulation is separate from but operates in parallel to the EU pesticides authorization Regulation (EC) 1107/2009, and a disconnect has emerged between the mandated timeline of one year to obtain an authorization vs. 1.5-2 years to obtain the MRLs needed to support uses. Debate is underway on how best to optimize the MRL setting process, and minimize potential trade disruption.

AGRO 59

Delivering safe and effective advice on pest control in developing countries through the Plantwise programme

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Plantwise is a gateway to online and offline actionable plant health information, including diagnostic resources and pest management advice. Plant doctors are trained within the Plantwise programme to offer sustainable plant health management advice to farmers following the principles of Integrated Pest Management which involves the use of cultural, biological and mechanical methods, alongside interventions with pesticides when justified. Locally created pest management decision guides help plant doctors deliver the most appropriate pest preventive and curative management options. Where the use of pesticides is necessary, plant doctors are advised to recommend only locally-registered and available pesticides and to give advice to keep pesticide usage to the lowest effective level, resulting in minimal residues and low risk to human health and the environment. The knowledge bank acts as a central store for information being collected from the plant clinics so that local experts can analyse the data to assess any needs for new pesticide registration, quality of advice given and implications for the reduction of MRL problems.

AGRO 60

Finding potential solutions for growers' needs in the field of pests and diseases by searching for existing solutions in other countries

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Homologa contains detailed information about registered crop protection products and its MRLs for about 70 countries. The data from the different countries is harmonized so that it is possible to search by individual crop and pest across all countries. The presentation shows, with a few examples, how this database can be used to find solutions to grower's needs in making knowledge accessible that exists already in other countries. The tool is not limited to crop/pest- and invasive species needs but it also can be used for finding solutions to MRL issues like differential MRLs or missing MRLs.

AGRO 61

Biological control agents for sustainable urban agriculture, safe water, and soil health

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Use of bioagents must be encouraged in urban agriculture as the use of chemicals inversely impacts urban population and natural resources. This paper gives a wide variety of biological agents being used in India for various pests in different crops. Studies can further be extended to use these bioagents in urban turf and ornamental pest control. Neem based bio-insecticide is used at a concentration of 5% against Diamondback moth, Plutella xylostella in cabbage management. Farm yard manure (FYM) enriched with *Trichoderma harzianum* (4 g/kg) is used to control thrips, mites and soil borne diseases and *Pseudomonas fluoresence* is used 5 g/litre for inducing systemic resistance in Hot peppers. *Beauveria bassiana* alone or in combination with BT have been used to control soil insects including potato beetles. The isolates of *Trichoderma* spp. have been characterized for biopriming, plant growth promoting characteristics, reduction of disease incidence and corresponding yield increase in cabbage, cauliflower, mustard and field pea at 5-10 g/kg seed. *T. harzianum* in the concentration of 2×10^8 cfu/g of soil and *P. fluorescens* 1×10^{12} cfu/g of soil was said to be best towards management of root knot nematode (Meloidogyne incognita). Application of *T. harzianum* (250 g) + *P. fluorescens* (250 ml), and FYM (25 kg) + *T. harzianum* (250g) + *P. fluorescens* (250ml) against Fusarium wilt, mites and root knot nematode has been found promising in cucumber. Above bioagents can also be successfully used for the control of urban turf and ornamental plants by conducting field trials to maintain ecological balance, saving public health and environment.

AGRO 62

Lysimetric studies to access the groundwater contamination through unlined drain

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The identification of proxy indicator for groundwater infiltration of chemical and bacteriological contamination is an important step in managing groundwater resources and hazards assessment. Lysimetric based approaches in groundwater systems in such highly dynamic process leads the depth of knowledge of the relationship between the variable of interest in groundwater infiltration systems. Lysimetric studies were conducted to know the leaching potential of sewage waste from unlined drains, accessing the leachate quality in turn of physic-chemical parameters as pH, total dissolve solid, electric conductivity, biological oxygen demand, chemical oxygen demand, total hardness, calcium hardness, nitrate, nitrite and turbidity whereas the microbiological parameters as spread plate count, pour plate count, total coliform, faecal coliform and faecal streptococci. The soil and drain samples were collected from the selected location of overloaded drain and also the groundwater of the site. Most of the water parameters of drain leachate for the chemical and bacteriological qualities were higher than leachate of groundwater samples and also the prescribed level of WHO standard for drinking water. Lysimetric experiments indicates that while leaching the drain sample from soil profile, the maximum population of microbial flora as well as the chemical pollutants which have been adsorbed on the soil particles (holding capacity) leached down to the aquifer from a point source and diluted to the entire ecosystems. The concentration of chemical or population of microbial leachate also indicates the saturation of soil profile by allowing the movement of contaminants. The unlined drain (non concrete) and unsanitary environmental conditions could be additional risk factor for groundwater contamination.

AGRO 63

Microalgal agriculture: An integrated approach to remediate the wastewater for irrigation use and production of biodiesel and manure

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The integrated approach of wastewater treatment and microalgal biodiesel production based on phycoremediation is a promising process. Phycoremediation is the process of employing algae to get rid of the excess nutrient load from wastewater and subsequently diminish the pollution load and eutrophication. The experiments were set up to study the remediation potential of three microalagae viz. *Chlorella minutissima,* Scendesmus spp., Nostoc *muscorum* as well as their mixed consortium was used to remove the pollution load from primary treated IARI's (Indian Agricultural Research Institute) wastewater and tertiary treated CETP (Common Effluent Treatment Plant) wastewater and evaluation of biomass for biodiesel and biomanure after harvesting. The physical and chemical parameters of wastewater quality such as nitrate, phosphate, potassium, EC, TDS, BOD, COD, etc., were studied. C. *minutissima* removed about 90-98% TDS, 70-80% N, 60-70% P and 45-50% K from the wastewater within 12 days. The level of BOD and COD were reduced by 60 and 75%, respectively. The algal biomasses were harvested for biodiesel extraction. The highest specific lipid productivity for CETP and IARI wastewater was 0.171 and 0.132 g-lipids $\text{g-cell}^{-1}\ \text{day}^{-1}$, respectively. The oleic acid has increased 59.6% in CETP wastewater in comparison to IARI wastewater. The maximum biomass were observed in *Scendesmus* and *C. minutissima,* i.e. 0.79 ± 0.02 and 0.78 ± 0.01 g L^{-1}, respectively, while the percentage of nitrogen and phosphorus was highest in *C. minutissima* i.e. 3.46 ± 0.27 and 1.15 ± 0.03 respectively. The results of this study concluded that algal strain *C. minutissima* is not only a means for remediation of pollutant load, but it can also be used as a potential driving force for biodiesel production. The outcome from research showed that this integrated approach of microroalgal agriculture in urban region is a successful mean for treating wastewater, mitigating the pollutants, recycling and reuse of the wastewater for the irrigation and producing valuable product biodiesel and biomanure.

AGRO 64

Physical methods in wastewater treatment

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While a number of biochemical processes for waste water treatment exist, they have inevitably an adverse biological impact. In sludge, various substances and agents collect in the form of aggregates and flakes, including bacteria, viruses, cellulose and starch. Ultrasound is a known physical tool and has a remarkable effect on sludge solubilisation, reduction of the sludge volume, increases biogas production, flock size reduction and cells lyses. The ultrasonic irradiation has improved the process of anaerobic disintegration, solubilization, biological degradation, cell disruption of stabilized sludges, disintegration of bio-solids and the reduction of weight and volume. Ultrasound treatment also promote hydrolysis – the rate-limiting stage during sludge treatment.Ultrasound has an advantage that it can be generated at abroad range of frequencies 25, 35 and 130 KHz and acoustic intensities. Ultrasound breaks the bacterial cell walls, the bacterial cells release iso-enzymes that biocatalyst in hydrolytic reactions. This results in acceleration in the break down of organic material into smaller readily biodegradable fractions. Many micro-organism servive in waste water. These organism may cause different types of diseases, such as- Cholera, Typhoid, Hepatitis etc. Further treatment with silver nanoparticles can break bacterial cell wall and impart desired purification of water for human consumption. Clean water so obtained can be tested by measuring different parameters such as, COD, BOD, Total Phosphorus, Total Nitrogen etc. It is concluded that the above mentioned technique is inexpensive and can be adopted for domestic use. In urban water reuse, this technology can be used effectively.

AGRO 65

Residues of pesticide in Ghaggar River flowing through urban cotton cropping area

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Cotton in India is an important cash crop. About 50% of all pesticides used in India go for cotton cultivation. As cotton is a water intensive crop, it's the quality of the water that is most affected by its cultivation, due to leaching and run off chemicals in ground and surface water. Therefore a study was undertaken to analyse the by pesticides usage in cotton growing area and subsequent contamination of water resources. Water samples were collected from eight tubewells of cotton growing area and from river Ghaggar (Punjuwan, Sirsa, and Haryana). Samples of river were taken from nearby surface, 2km, 2km bottom and 5km.

According to the result organochlorine pesticides residues analysed ranged from 0.007- 5.391 µg/l and 0.005- 1.055 µg/l in surface and groundwater samples. Organophosphate pesticide residues ranged from 0.013 to 3.015µg/l and 0.004 to 4.364µg/l in surface and groundwater samples respectively. However, all the synthetic pyrethroids were found Below Detectable Limit (BDL), while the herbicide residues analysed ranged from 0.165 to 0.471µg/l and 0.012 to 0.248µg/l in surface and groundwater samples respectively. Therefore the results are alarming and show that either the use of banned pesticides is still continued or they are present in water samples as they are very persistent. On the contrary, synthetic pyrethroids, owing to their biodegradable property, were found Below Degradable Limit and hence can be considered as safe.

AGRO 66

Utilizing reduced risk pesticides and IPM strategies to mitigate golfer exposure and hazard

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There is growing concern over human exposure to pesticides following their application for the management of turf environments. This research evaluated the exposure of golfers to chlorpyrifos, carbaryl, cyfluthrin, 2,4-D, MCPP, imidacloprid, chlorothalonil, azoxystrobin, carfentrazone-ethyl and halofenozide following 242 rounds of simulated golf. Hazard quotients ranged from a high of 0.6 (chlorpyrifos) to a low of 8.4 x 10^{-7} (carfentrazone-ethyl). All exposures were below the USEPA hazard quotient (HQ) of 1.0, indicating that adverse effects are unlikely. Dermal, airborne and dislodgeable foliar residues were determined by dosimetry, personal air samplers, and California roller, respectively, and compared to concurrently collected biomonitoring samples whenever possible.

Dermal absorption was determined to be the principle route of exposure to golfers for chlorpyrifos, carbaryl, imidacloprid, 2,4-D, MCPP, azoxystrobin, carfentrazone-ethyl and halofenozide. When pesticide applications were followed by irrigation, 43-75% of the residues were found on pants, lower legs and upper socks. When applications were not followed by irrigation 41-71% of the residues were detected on the hands. It was also determined that a 1 hour reentry interval generally reduces exposure.

Reduced risk pesticides (azoxystrobin, carfentrazone-ethyl, and halofenozide in this study) were shown to reduce the risk to golfers. The HQ established for the herbicide carfentrazone-ethyl (8.4 x 10^{-7}) was more than 80,000-fold less than that determined for 2,4-D (0.07). The HQ determined for halofenozide (0.07) was about an order of magnitude less than that determined for chlorpyrifos (0.6). The HQ calculation for azoxystrobin (0.001) is the same as that determined for chlorothalonil (0.001). The use of azoxystrobin does not greatly reduce golfer hazard in this case.

AGRO 67

Attenuation of pesticide-laden runoff using vegetative filter strips

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Pesticides in runoff can degrade water quality and has led to the use of mitigation strategies, including vegetative filter strips (VFS). Five plant species that degraded pesticides in a greenhouse study were planted in VFS, each with a 5% slope. Three replicated VFS were planted in either successions (shortest to tallest) or random mixtures and compared to plots that were unvegetated or were planted with turfgrass cut to three heights that increased in the downslope direction. Pesticides were applied in runon water during a simulated 1 year rain event. Runoff was collected during the irrigation event, while soil cores and subsurface water samples from lysimeters were collected at discrete intervals post-application. All samples were chemically analyzed for the pesticides of interest and quantified by GC/MS or LC/MS/MS. All vegetated treatments were more effective in decreasing runoff volumes and the pesticides in runoff compared with unvegetated plots, and turfgrass was the most effective of the three vegetated treatments examined. Vegetated treatments also reduced pesticide concentrations in soil samples taken from VFS post-application. The lowest pesticide concentrations in soil were seen in the turfgrass VFS. Of the five tested pesticides, only imidacloprid was consistently found in soil samples, while pendimethalin, propiconazole, chlorpyrifos, and propiconazole were found in higher concentrations in the runoff water. The highest percent of total pesticide applied found in vegetated VFS were 0.38% for pendimethalin in runoff, and 4.3% for propiconazole in soil. Results to date indicate that the turfgrass treatment is most effective at reducing runoff water and the pesticides associated with runoff.

AGRO 68

Roles of conjugated double bonds on electron-donating capacity of sorghum grains

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Sorghum bicolor is cultivated worldwide as a staple food and forage, and is receiving a renewed interest as a bioenergy crop. The objective of this study was to understand the relationships between the function (redox and metal complexation) and structural properties (aromaticity and ionizable functionalities) of (poly)phenolic pigments in the pericarp of sorghum grain that will ultimately control its antioxidant and bird/mold resistance behaviors. Compared to white seeds lacking condensed tannins, brown seeds (with and without pigmented testa) contained (i) 0.08-0.01 wt% cyanidin equivalent condensed tannins, (ii) higher aromaticity (that will decrease the reduction potential of polyphenols), and (iii) as much as 6-fold greater Fe^{III} reduction capacity. The degree of aromaticity was determined by (i) UV absorbance at 360 nm and (ii) fluorescence excitation-emission (EEM) peak position. Basic (0.1 M NaOH) extracts of all seeds contained EEM peaks (230/330 and 280/330) attributable to protein. Addition of Fe^{III} resulted in a new aromatic EEM peak (320/440) only for the brown seeds that could be used as a fingerprint for the redox and coordination chemistry of sorghum grain pigments.

AGRO 69

Overview of seed treatment in North America, 2015

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Abstract: The seed treatment industry in North America continues to grow both in terms of its market value and improvements in treatment technology. Seed treatments have evolved from having a focus on crop performance specifically related to emergence, stand establishment, and yield, to incorporate protection from seed and soil-borne pathogens. Coating technologies include nutritional benefits for seed development, chemical protection from pests and disease, enhanced safety, and reduced dustiness delivered in an attractive package. Has the technology in seed treatment kept pace with the good stewardship practices including human, environmental and pollinator health?

AGRO 70

Seed enhancement evaluation

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Each organism has evolved through time growing alongside surrounding species and adapting to environmental conditions. Plant seed is no different. Plant seed, however, carry with it all requirements for early stage growth. This includes not only the genetic material to allow development of the newly emerging plant, but also the nutrients for the first few weeks of life. This packet of energy, the seed's genetic delivery mechanism, is essentially a treasure trove for opportunistic pathogens. In some cases the seed still contains its natural defenses against attacking pathogens, but in other cases the natural defenses have been stripped away during processing to allow for easy handling. Additional advancements in seed stock, both traditional breeding practices and genetic modification, have increased the seed value, generating a greater desire to protect the seed. Recent trends to utilize seed as the delivery system for biological and chemical improvements has lead to some interesting challenges. With seed-applied technologies becoming more prevalent and the mounting need to protect the seed from environmental stresses and pathogen attack, multiple evaluations must be preformed to determine the usefulness of the compound on the seed. These evaluations must consider several variables: the ability to load the product on the seed, if the chemical can be applied to the seed at the desired rate, if the product is safe to use on the seed, and if the compounds being placed on the seed are compatible with each other. Additional important factors must also be considered including environmental effects and hazards to seed handlers. Various evaluation techniques can be employed to evaluate the effects to the seed including phytotoxic evaluation utilizing standard germination and vigor evaluations, mechanical evaluations such as flowability, dusting and plantability; as well as treatment loading rate analysis. These methods of analyzing seed-applied enhancements' effects will be reviewed.

AGRO 71

Achievement and measurement of soil anaerobicity during conduct of anaerobic transformation studies

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Biodegradation studies are required for registration of agrochemicals and pharmaceuticals which are directly applied to soil, or which are likely to reach the soil environment (OCED 307 or OPTTS 835.4100/4200). These studies are typically performed either under aerobic or anaerobic conditions. Since a complete absence of oxygen rarely occurs in the natural environment, anaerobic conditions are defined in practice as conditions with redox potential of less than -100 mV. Such conditions are generated in laboratory settings by flooding test soil with nitrogen-purged water and are maintained by flushing the system with nitrogen gas. Achievement of anaerobic conditions is difficult due to the buffering effect of soils. The aim of this study was to evaluate ways of measuring anaerobic conditions (on open bench and in nitrogen-filled bag) and to assess the effect of alfalfa amendment on facilitating anaerobicity. For assessment, one US agricultural soil type was tested. After a 30-day incubation under aerobic conditions, one group of test soils was flooded with nitrogen-purged water, while a second set was flooded with nitrogen-purged water and amended with an appropriate amount of alfalfa. The third set of test systems served as controls and

was flooded with water not purged with nitrogen. Each group was incubated for 120 days in the dark at 20 ± 2℃, and the following parameters were monitored in duplicate samples for both water and soil phases: pH, oxygen concentration, total organic carbon and redox potential. Microbial biomass was determined by fumigation extraction at the start, mid-point and end of the incubation period. Initial observations indicate that measuring redox potential on an open bench tends to generate values that are artificially high due to the exposure to air. The experimental results will be discussed, and recommendation on measuring parameters in anaerobic systems will be given.

AGRO 72

Estrogen conversion in poultry litter by liquid chromatography mass spectrometry

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Confined animal feeding operations (CAFOs) of poultry generate significant amounts of litter which is used to fertilize croplands. Poultry litter can be applied in the composted, pelletized, or raw form; however all types of applications contain naturally excreted estrogens and their metabolites which may contaminate surface waters through runoff. Previous studies have shown that the primary form of estrogen present in the poultry litter is estrone (E1). Although E1 is less estrogenic than estradiol (E2), endocrine disrupting effects are still observed in fish exposed to poultry litter runoff. The kinetics of the conversion of E2 to E1 has been well studied, yet little is known about the conversion of E1 to E2 in poultry litter runoff. Mass labeled E1 was used to determine if E1 present in the poultry litter is converting to the more estrogenic E2 through photolytic, abiotic, or biotic pathways. In order to elucidate the primary conversion pathway, time course experiments were carried out over 30 days. Experiments took into account the following variables: sterile/non-sterile, dark/light, presence/absence of poultry litter. Water aliquots were taken on multiple days for the analysis of E1 and E2. Samples were concentrated using solid phase extraction and derivatized to increase ionization efficiency. Results were determined using liquid chromatography mass spectrometry. Preliminary results show that the presence of poultry litter in non-sterile environments facilitates the conversion of E1 to E2.

AGRO 73

Microtransplantation of rat brain neurolemma into *Xenopus laevis* oocytes to study the effect of environmental toxicants on endogenous voltage-sensitive ion channels

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Microtransplantation of mammalian neurolemma into *Xenopus* oocytes has been used to study ion channels in terms of their function and structure in the central nervous system. Use of microtransplanted neurolemma is advantageous in that tissue can be obtained from various sources, ion channels and receptors are present in their native configuration and they can be used to evaluate numerous channelpathies caused by environmental toxicants. Here we show that *Xenopus* oocytes injected with rat brain neurolemma successfully express functional native ion channels in their own plasma membrane. Using a high throughput two electrode voltage clamp (TEVC) electrophysiological system, we were able to detect currents that were sensitive to tetrodotoxin (TTX), ω-conotoxin MVIIC, and tetraethylammonium (TEA) indicating the presence of multiple voltage-sensitive ion channels (voltage-sensitive sodium, calcium and potassium channels, respectively). In this current research, we conducted a “proof-of-principle” experiment in which we were able to successfully isolate TTX-sensitive voltage-sensitive sodium channel (VSSC) currents. VSSCs are known sites of action for 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT) but not for its non-toxic metabolite 1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene (DDE). DDT results in slowing of sodium channel inactivation kinetics whereas DDE has no effect. Our current results showed a differential sensitivity of DDT and DDE on TTX-sensitive sodium current in neurolemma-injected oocytes. DDT elicited an increase in depolarization-dependent, TTX-sensitive sodium current while DDE had no significant effect. These results are consistent with those obtained using heterologous expression of single isoforms of rat brain VSSCs. Once fully characterized, this approach can be expanded to study the role of environmental toxicants and contaminants on target tissues (e.g. neural, reproductive, developmental, etc.), including but not limited to those from humans and knockout mice.

AGRO 74

Effect of glyphosate formulations on two species with different leaf surface properties

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Glyphosate has physical-chemical properties that make it difficult to penetrate leaf surfaces. The efficacy of glyphosate, therefore, strongly depends on its formulation combined with the properties of the plant leaf surface.
In this greenhouse study we tested two glyphosate formulations (A and B) containing different surfactants and compared their potency on *Brassica napus* and *Sinapsis alba* in a dose-response assay against the formulation without surfactant (C). We harvested biomass after 15 days and assessed mortality of the plants after 22 days to compare two end points for the same bioassay.
For *B. napus,* biomass ED_{50} values (95% confidence intervals in parenthesis) showed that potencies relative to the formulation without surfactant (C) were 1.5 (1.1-1.9) for formulation A and 2.5 (1.8-3.2) for formulation B.The formulations containing surfactant were thus more potent than the formulation without surfactant (formulation C). For *S. alba* neither the relative potency of formulation A, 0.64 (0.22-1.04), nor formulation B, 0.79 (0.29-1.2), were different in potency relative to the formulation C without surfactant. Relative potency based on LD_{50} (mortality) for *B. napus* confirmed biomass results. For formulation B, *B. napus* relative potency was 8 (4.1-12). For *S. alba* 1.5 (1.1-1.9) for formulation B but for formulation A the relative potencies were not significantly greater than 1.00.
In conclusion, for easily wetable leaves (*S. alba)* the addition of surfactants did not in all instances significantly affect the efficacy of glyphosate, while surfactants significantly increased the efficacy of glyphosate in plants with a typical “hard-to-wet” leaf surface (*B. napus*).

AGRO 75

Reduction of lignin levels in mutant sorghum lines developed for saccharification leads to increased production of insecticidal compounds in stalk pith

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Production of material for biomass that can be converted to energy sources such as ethanol is impeded by the presence of lignin that limits saccharafication. Lines of crops such as sorghum have been developed with reduced levels of lignin that have promise for use in bioenergy production due to enhanced rates of saccharification. Two of mutant lines developed, that have *bmr6* (disrupted cinnamyl alcohol dehydrogenase) and *bmr12* (disrupted caffeic acid O-methyl transferase), have been converted into lines near isogenic with normal lignin line BTx623. Laboratory and field trials have so far indicated no consistent increases in insect or disease susceptibility in the low lignin lines, and often increased insect resistance. The most resistant tissue to caterpillars is the pith, which in some cases has killed nearly 50% of first instar corn earworms, while only about 6% of those feeding on normal lignin pith died. Initial chemical analysis after acid hydrolysis indicated increased levels of some phenolic acids obtained by acid hydrolysis, some of which caused reduced insect growth when incorporated into diet made from sorghum leaves. HPLC and accurate mass spectrometry indicated an increase in the levels of ferullated sugars including di-feruloyl-sucrose and feruloyl-glucose. Large scale separation and purification of pith extracts from the most resistant bmr6 line simple fractionation by reverse-phase flash chromatography resulted in a fraction that was greatly enriched in the feruloyl-sugars which was also shown to have the most impact on insects in controlled feeding studies.

AGRO 76

Possible glyphosate tolerance mechanism in pitted morningglory (*Ipomoea lacunosa* L.)

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Natural tolerance of *Ipomoea lacunosa* to glyphosate has made it problematic in the southeastern U.S. since the adoption of glyphosate-resistant crops. Experiments were conducted to determine (i) the variability in tolerance to glyphosate among accessions, (ii) if there is any correlation between metabolism of glyphosate to aminomethylphosponic acid (AMPA) and/or sarcosine and the level of tolerance, and (iii) the involvement of differential translocation in tolerance to glyphosate. Fourteen *I. lacunosa* accessions had GR_{50} values ranging from 58 to 151 g ae/ha glyphosate, a 2.6-fold variability in tolerance to glyphosate. There was no evidence of the most tolerant (MT) accession metabolizing glyphosate to AMPA more rapidly than the least tolerant (LT) accession. Metabolism to sarcosine was not found. ^{14}C-glyphosate absorption was similar in the two accessions. LT accession translocated more ^{14}C-glyphosate than MT accession at 24 and 48 hours after treatment. Differential translocation partly explains glyphosate tolerance in MT accession.

AGRO 77

Drug discovery and optimization using computer generated molecules with predicted potencies

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Contour®, a proprietary drug discovery platform is a de novo drug design program which grows and scores molecules within a protein binding site. Application of Contour® has enabled the discovery of drug-like small molecule enzyme inhibitors, and receptor agonists and antagonists. An overview of the program and examples of its application in drug discovery and compound optimization will be presented.

AGRO 78

It takes a team: Reflections on select insecticide discoveries, toxicological problem approaches, and enjoying the unexpected

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There isn't much rational debate as to the benefits that modern Agrochemicals deliver, but there is still debate and multiple approaches to their discovery and optimization. Many Agrochem companies run compounds "through a process" which provides a framework with which to characterize/prioritize agriculturally active leads quickly and efficiently. However every lead compound series poses different and unique problems on the commercialization path, and the ability of the team to expeditiously solve those problems, some of which may fall outside of ongoing processes, can determine ultimate success. This talk will reflect on 3 insecticide classes which teams were able to discover, chemically optimize and eventually register as commercial products. Each involves a different primary molecular target site (all novel at the time of discovery), very different chemistries, and importantly very different issues regarding metabolism and bioavailability of the toxin to the pest target site as opposed to nontargets/the environment. Ultimately each class also achieved moderate to very high selective pest insect toxicity via different mechanisms, and each also contributed to fundamental scientific understanding regarding their target site families, sometimes leading to unexpected positive outcomes. Finally, the talk will discuss the fundamental science which made these discoveries possible, often emanating from academic labs not devoted to agrochemical research as their primary mission. It is hoped that this presentation and the associated symposium on applying "ADME-like studies" to agrochemical discovery will stimulate reflection on how to go about finding novel, valuable new crop protectants in the future. A mix of structured discovery processes with appropriate organizational and personal flexibility, as well as judicious application of new technologies, may be beneficial.

AGRO 79

Fast, structured, adaptable approach to screen bioformulation amendments and stabilizers

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A major technical challenge facing the biopesticide industry is long-term room temperature storage stability of microbial active ingredients. Liquid formulations that are compatible with traditional agrochemical products are also highly desired. Developing room-temperature stable bioformulations has largely been trial and error by industry. Several different amendments (e.g., carriers, sugars, dispersants, surfactants, etc.) are used in practice, and each may individually or in combination have an impact on long-term stability of the microbial active ingredient. Here we present a rational approach for evaluating formulations of microbes using *Pseudomonas fluorescens* as an example. Our approach uses a partial factorial statistically structured screen that lends itself to mass screening of numerous amendments and combinations in parallel. The semi-high throughput screening platform can be adapted to evaluate different formulations for microbes or consortia of microbes of interest in order to determine suitable formulations that retain desirable active ingredient properties (e.g., viability). Here, the viability of *P. fluorescens* in various formulations was measured after storage at room temperature for two, four, and eight weeks using a 96-well assay that measures microbial growth via optical density. Viability results were then analyzed using a statistical approach that involved fitting models to the data and determining the amendments, combination of amendments, and interactions of amendments that showed the highest correlation with desired output (i.e., *P. fluorescens* stability). Such information can be used to remove non-useful amendments and "dial in" on a formulation that provides optimal stability. Using this structured approach, it was found that unique combinations containing ingredients such as trehalose, molasses, alginate, and arginine provided enhanced *P. fluorescens* stability compared to control formulations.

AGRO 80

Are pharmaceutical enhanced solubilization technologies useful in agriculture?

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The Biopharmaceutics Classification System (BCS) categorizes drug compounds based on their solubility and permeability. BCS II/IV compounds have generated a range of technologies and a number of companies around the idea of enhancing the solubility of pharmaceutical compounds in these quadrants. The number of New Molecular Entities (NME) that utilize an enhanced solubility technology is relatively small, but the trend is increasing. The same technologies used in the pharmaceutical industry, in principle, can be applied to pesticidal active ingredients. The simplest case for consideration is a foliar applied insecticide for a chewing pest, such as a caterpillar. The material is sprayed onto a plant, the caterpillar eats the plant, and the toxicant is absorbed as the plant material is digested, a scenario that is representative of an oral pharmaceutical. Enhancing the rate of dissolution of an insoluble insecticide could lead to enhanced activity. Some approaches and their limitations will be examined in this talk.

AGRO 81

Ultrahigh resolution MS and label-free MALDI molecular imaging: A novel approach for the study of plant biosynthesis and metabolism

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For addressing the complex chemical challenges associated with plant systems, accurate molecular identification is a necessity. However, identifying which species are present is only the first step to understanding the underlying biology. Mass spectrometry imaging (MSI) is a technique that is successfully used to directly measure compounds such as drugs, metabolites, lipids, peptides and proteins in tissue without the need for fluorescent or radiolabels. While the majority of MSI development has previously focused on mammalian tissue, plant biologists have also begun utilizing this technique to solve complex biological mechanisms through the direct mapping of spatial and temporal localization of active components directly from roots, stems, leaves, seeds, and more, providing a better understanding of the mechanisms of biosynthesis, metabolism, and disease progression. The coupling of MSI to ultra-high resolution mass spectrometry (UHR-MS) has served to increase the number of mass channels that can be interrogated in a single experiment, allowing for unparalleled throughput and specificity on the basis of accurate mass measurement. Recent advances in UHR-MS now allow for measurements with far higher mass resolving power than previously possible. New workflows that combine this ultra-high mass resolving power with statistical profiling and isotopic fine structure matching now allow for faster and more sensitive small molecule and metabolite identification, which can provide even greater insight into complex biological mechanisms using a completely untargeted approach. This presentation will highlight the use a UHR-MS platform, alone or in conjunction with MSI, applied to the study of plant and bacterial systems with examples detailing the latest cutting edge advances in improving the small molecule ID pipeline and the development of spatial metabolomics approaches.

AGRO 82

Visualization of small molecule distributions in plant, insect, and mammalian tissues by mass spectrometry imaging

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Mass spectrometry imaging (MSI) is established as a valuable tool for imaging metabolite distributions in mammalian tissues and to an increasing extent also in plant tissues. The technique utilizes MS to map the spatial distribution of small molecules across the surface of a tissue sample, and is therefore characterized by the usual virtues of MS such as sensitivity, selectivity and identification capacity. From an experiment of some hours' duration, a wealth of selective images of endogenous and exogenous compounds may be generated from one sample. Unlike many other imaging techniques, MSI enables even structurally very similar compounds to be distinguished, also in complex mixtures, without any use of labelling, and images of endogenous compounds may be used for tissue classification. Furthermore, MSI provides structural information, allowing confirmation of known compounds and identification of unknowns. Over the last decade, the research in MSI of the vast and highly complex and diverse plant metabolome has expanded rapidly, as the instruments have become commercially available. The structural information obtained by MS analysis enables discovery of new compounds, such as unknown conversion products of known molecules. MSI therefore has great potential for becoming an important standard method for studying e.g. biosynthetic and detoxification pathways in plants, but also distribution and detoxification/activation of xenobiotics in insects. This presentation will provide an introduction to the two most widely applied techniques for MSI in biological samples, DESI-MSI (Desorption Electrospray Ionization-MSI) and MALDI-MSI (Matrix Assisted Laser Desorption Ionization-MSI). Examples from our own lab and other labs will be given on the use of the techniques for imaging distributions of molecules representing the chemically different compound classes flavonoids, terpenoids, alkaloids and the labile cyanogenic glucosides, in very different plant species and tissue backgrounds, and for imaging distributions of drugs in locusts and mammalian tissues. Examples will also be presented of results from a new MALDI-MSI instrument which enables imaging at cellular resolution of plant and animal tissue. Several of the results have contributed to biological understanding of the organisms in question and prompted new research based on the findings, clearly demonstrating the potential of MSI for metabolite research.

AGRO 83

RNA interference in agriculture: Today and tomorrow

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Modification of gene expression is an established strategy for delivering safe and effective agrochemical products. Landmark efforts have successfully protected several crops from insect pests by expression of exogenous toxins. Another strategy takes advantage of gene expression machinery to alter mRNA levels in both the host and related dependent species. To date, RNA interference has been used to modify several crop traits and offer protection from viruses. Currently marketed RNAi technologies are delivered via incorporation into the host plant; what are the delivery strategies of the future?

AGRO 84

Review of the practice and potential for global horizon scanning and research prioritization exercises in narrowing the environmental science-policy gap

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A gap between the data generated by researchers and the information required by policymakers is widely recognized. In an effort to bridge the gap between science and policy, more than 30 horizon scanning and research question identification and prioritization exercises have been conducted in the fields of conservation biology, agriculture, ocean science, and environmental management. Here we present a brief summary of the scope and impact of those exercises, focusing on the development of a common methodology for engaging scientists, policymakers, and other actors that utilize scientific evidence. In addition, we detail the potential for synthesis across national and sectoral exercises using an example from a recent ocean science research question prioritization. That effort examined how 2187 physical, ecological and social scientists from 94 countries (representing some 36000 person-years of research experience) perceived their role as scientists working at or near the ocean science-policy interface. Results showed that scientists held different perspectives about their appropriate level of engagement at the ocean science-policy interface and the relative primacy of science versus politics in formulating ocean policy. Three clusters accounted for 94% of the sample and members of those clusters showed substantial consistency in their top research priorities. This alignment of priorities among scientists from different regions, disciplines, and sectors should increase our level of confidence that research priorities identified via various bottom-up big question and horizon scanning exercises are highly relevant for global ocean management and enjoy support from all segments of the ocean research community, irrespective of discipline, geographic location, or professional background. Those lessons are relevant for the new SETAC-sponsored horizon scanning and question idenfication exercise currently in progress for global environmental quality.

AGRO 85

Formalizing the identification of high prioirity research needs: A case example with pharmaceuticals and personal care products

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Identification of research needs that are both societally important and scientifically relevant is critical to meaningful progress in the field of environmental toxicology and chemistry. Too often prioritization of these needs is based solely on responses to short-term demands/concerns (e.g., anticipated regulations) that, while sometimes seemingly critical, do not allow the type of integrative consideration needed to develop coherent long-term research strategies to support environmental sustainability. This type of planning requires balanced input from a variety of stakeholders which include both scientists and science clients. One approach to achieve a deliberative, integrated, multi-stakeholder approach to research prioritization is via the "Big Question" exercise whereby input is initially solicited from a very broad community and subsequently is synthesized and ranked by committees of relevant experts. This presentation will provide an overview of such an exercise focused on identifying research priorities to support assessment of the risks of pharmaceuticals and personal care products (PPCPs) in the environment. An initial effort (Boxall et al. 2012; Environ Health Perspect 120:121), which focused primarily on PCPP issues in North America and Europe, identified a ranked list of research questions/needs that fell in seven categories ranging from hazard and exposure assessment to risk management. A follow-up analysis (Rudd et al. 2014; Integ Environ Assess Manag 10:576) expanded the scope of the exercise in terms of international representation, and demonstrated clear regional differences relative to research priorities for PPCPs. This presentation will provide an overview of the PPCP Big Questions exercise not only with regard to methodologies and specific outcomes, but relative to "lessons learned" in the context of a broader, ongoing Horizon Scanning Project for environmental research needs supported through the Society of Environmental Toxicology and Chemistry.

AGRO 86

Environmental fate and physical-chemical properties of dicamba, 3,6-dichloro-o-anisic acid

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Dicamba was introduced into the market in 1967 as a broadleaf foliar applied herbicide. Despite many years of use as a widely applied product, misconceptions persist regarding dicamba's environmental fate and physical chemical properties. This summary will focus on the compounds environmental fate and physical – chemical properties based on laboratory studies supporting the registration and reregistration of the molecule. Comparisons will be made with other herbicide guideline study results as well. Results from multiple registrant guideline studies indicate that dicamba exhibits favorable behavior in the environment.

AGRO 87

Pesticides in soils: Correct kinetics and flawed parameters

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Industrial scale agriculture feeds billions of people. That requires large tonnages of pesticides. To manage the impact on soil and water, about 40 pesticide fate and transport models have been developed. The Zhang group in China published the earliest known attempts to introduce kinetics into one more than 15 years ago. Shortly thereafter the S. O. Prasher group of McGill University showed experimentally that replacing the distribution coefficient k_D with oversimplified chemical kinetics reduced prediction errors by an order of magnitude. More than a dozen years ago Sitea, and Yeh et al. reviewed all of the empirical parameters and also concluded that they were scientifically unsound and caused errors. The 40 hydrology engineering models clearly need to be upgraded. Experimentally revealed chemical kinetics and mechanisms could now replace the incorrect kinetics descriptions and parameters criticised by the Sitea and Yeh research groups. The nature and scope of this problem has to be faced next.

AGRO 88

Buffers as potential catalysts of hydrolysis and halogenation during agrochemical fate experiments in bench-scale reactors

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Buffers are commonly included to minimize changes in solution pH during bench-scale investigations of the environmental fate of agrochemicals. The potential influence of buffers on transformation rates of agrochemicals is, however, frequently overlooked. Herein, we discuss two case studies in which buffers significantly influenced kinetic experiments of two commercial herbicides (isoxaflutole and dimethenamid). Isoxaflutole is an oxazole pro-herbicide that must undergo hydrolysis to generate the active herbicide (a diketonitrile). Rates of isoxaflutole hydrolysis are known to increase with increasing hydroxide concentration (via specific-base catalysis). Our results indicate, however, that several common pH buffers (including phosphate, MOPS, and borate) can enhance rates of isoxaflutole hydrolysis. The extent of buffer catalysis increased as the basicity of the buffer species increased. For example, the inherent ability of phosphate species to catalyze isoxaflutole hydrolysis increased in the order: $H_2PO_4^- < HPO_4^{2-} < PO_4^{3-}$. Our findings suggest that in reactors at pH 7.2 containing 0.10 M total phosphate, 73% of the reactivity previously ascribed to hydroxide ion is likely due to reactions with buffer components (ostensibly via general-base catalysis). Dimethenamid is a chloroacetamide herbicide that has been detected in drinking water. Dimethenamid is readily chlorinated in solutions containing free chlorine (the most common disinfectant of drinking water). In solutions containing free chlorine + bromide, dimethenamid can also undergo bromination. Rates of dimethenamid chlorination did not change appreciably as a function of the total concentration of buffers (including phosphate and borate). Rates of dimethenamid bromination, however, increased linearly as a function of total phosphate concentration, but were insensitive to changes in total borate concentration. As pH decreased, the degree to which phosphate species catalyzed dimethenamid bromination increased, suggesting general-acid catalysis as a putative mechanism.
Overall, these studies demonstrate the importance of evaluating the influence of buffers when measuring transformation rates of agrochemical in model systems.

AGRO 89

Evaluating unextracted pesticide residues in laboratory environmental fate studies

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The ambiguity and inconsistency in the evaluation of unextracted pesticide residues (URs) in laboratory environmental fate studies has been challenging for researchers and regulators. The newly released Guidance for Addressing Unextracted Residues in Laboratory Studies from EPA[i] has provided some clarification and outlined the laboratory approaches to address this issue. Based on this guidance, additional extraction procedures with different solvents are applied after initial extractions have been conducted on soil/sediment samples. Aliquots from the same soil/sediment samples with over 10% unextracted residues are extracted using organic solvents with varying polarities from low to high utilizing identical extraction procedures. Characterizations of the resulting residues are attempted with extracts containing over 5% unextracted residues. In addition to the exploration of the effect of solvent polarities on the extraction of soil/sediment unextracted residues, several laboratory extraction techniques, such as room temperature mechanical shaking, accelerated solvent extraction and high frequency sonication will also evaluated for extraction efficiency. The extractability of the ^{14}C-labelled residues using different methods will be compared side by side.
[i] Guidance for addressing unextracted residues in laboratory studies. Environmental Fate and Effects Division, Office of Pesticide Programs, United States Environmental Protection Agency, September 12, 2014.

AGRO 90

Estimation of veterinary drug concentrations in Canadian soils: Do the PECs MEC sense?

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Veterinary drugs administered to food animals primarily enter ecosystems through the application of livestock waste to agricultural land. A means to predict environmental concentrations of veterinary drugs in soil (PECsoil) is an important component of a directed testing approach for environmental assessment of veterinary medicinal ingredients (VMIs). A science-based and transparent methodology to calculate PECsoils for Canada, based on an approach used by the European Medicines Agency under VICH, has recently been developed. To validate the conceptual basis of the PECsoil approach and the newly developed Canadian production categories and defaults, measured environmental concentrations (MECs) of VMIs in agricultural soils from 24 North American and European studies were compared with PECsoil values calculated with the appropriate defaults for the respective region. A total of 57 MECs for 16 different VMIs commonly used in pig, poultry and cattle production were included in this analysis. MECs of VMIs found in North American agricultural soils that had received applications of pig manure were also contrasted with PECsoil refinements that account for target animal metabolism and degradation during manure storage. Comparison of PECsoil values with MECs demonstrates that the Canadian and European PECsoil models provide conservative screening level estimates for environmental exposure assessments of VMIs. The results of this study will be discussed in the context of differences in production systems and manure management practices for cattle, swine and poultry in Canada and internationally.

AGRO 91

Does the incorporation of vegetative filter strip mass balance and degradation processes affect the long-term pesticide environmental exposure assessments?

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Implementation of Vegetative Filter Strips (VFS) is one of the most widely adopted management practice for limiting pesticide transport from adjacent fields, such as agricultural areas, to receiving water bodies. The long-term VFS efficacy is dependent on site-specific factors related to soils, weather, land use, vegetation and maintenance. Previous research has proposed a modeling approach that links the U.S. Environmental Protection Agency's (EPA's) PRZM/EXAMS higher-tier environmental exposure framework with a well-tested process-based model for VFS (VFSMOD). However, this previous framework assumed that pesticide mass stored in the VFS was not available for transport in subsequent storm events. The objectives of this study were to develop and apply a mechanistic VFSMOD pesticide mass balance component to estimate surface pesticide residue trapped in the buffer at the end of a runoff event, and its degradation between consecutive runoff events. Using this updated PRZM/VFSMOD/EXAMS modeling framework, this research studies the effect of the addition of surface mass balance and degradation processes on acute (peak) and chronic (60-d) aquatic environmental exposure concentrations (EECs) and percent reductions across three distinct 30-yr US EPA scenarios:: Illinois corn, California tomato, and Oregon wheat. Global sensitivity analysis (GSA) was used to assess the relative importance of adding or removing mass balance and degradation processes in the context of other important input factors like VFS length (VL), pesticide organic-carbon sorption coefficient (*Koc*), and half-lives in both water and soil phases. Considering degradation in the VFS was not relatively important if single, large events were controlling the transport process, as is typical for the higher percentiles considered in standard exposure assessments. Degradation processes become more important when considering percent reductions in acute or chronic EECs, especially under scenarios with lower pesticide losses like for California tomato and Oregon wheat scenarios. Importantly, the mechanistic component allows elucidating the internal VFS pesticide dynamics for all events during the long-term assessment period.

AGRO 92

Emerging contaminant soil fate modl subroutine development for developmnt for the USDA soil water assessment tool

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A new soil module to update and expand the current one, targeted specifically onto pesticides presently in SWAT, is the subject of an ongoing development project at the US Department of Agriculture-Agriculture Research Service Grasslands, Soil and Water Research Laboratory, Temple, TX USA. The objective of the project is to formulate a comprehensive, process-based mass-balance theory, advection-diffusion transport model with reaction applicable to pesticides as well as emerging contaminants (EmCons) which include antibiotics, hormones, metals, polychlorinated biphenyls, nano-particles, etc. All these and more may arrive at or be directly placed onto or under shallow surface layers of soils on watersheds. A novel modeling approach built around the strongly layered structure of surface soils in the meter+ depth was named the G-Box. The conventional, simple, transparent and useful box-modeling approach popular in environmental chemical fate modeling was adopted but, selectively modified so as to handle the multitude of in-layer diffusive-like transport processes needed in a realistic soil chemical model. Hence the letter "G" for gradient [diffusive], in the model name. The principal of "continuity of flux" was used to mathematically connect the adjacent boxes and maintain the law of conservation of mass. It allows for easy numerical changes, with depth, of soil layer process parameters. A series of interconnected, transient ordinary differential equations is the result. They are soluble by the well know 4th order Runge-Kutta numerical integration method. The HRU-focused, layered soil boxes receives EmCon inputs from the adjoining atmospheric boundary layer, has lateral seepage out the sides, plant water uptake, downward water infiltration to a deeper infinitely thick sub-layer sink. A total of eighteen processes are currently included. Mirex, a historic pesticide with well established physico-chemical properties, will be used to illustrate some simulation capabilities of the new module.

AGRO 93

Modeling transport of a controlled release larvicide through catch basin systems

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Larvicides are commonly applied in urban catch basins systems for controlling mosquitoes in larval and/or pupal stages. The controlled released devices (CRD) are a formulation of larvicides that extend the effectiveness of such chemicals into several months, thus providing long-term mosquito control. However, the temporal release rate of CRD can be variable depending on various water characteristics (e.g. pH, organic matter, temperature, etc.) in catch basin and intensity of storm events. To better understand the effect of this variability on CRD fate and transport to receiving waters, a modeling approach was tested. The Soil and Water Assessment Tool (SWAT) hydrological and water quality model was customized to mimic 30 linked catch basin system that drained a 14-ha area. Inputs to the SWAT model included local 30-year weather, elevation, soils, land-use information, catch basin routing, and environmental fate properties of the CRD. A preliminary CRD release curve was developed as a time-varying point source load, based on a review of literature and a pilot study conducted using Novaluron 0.2G mosquito larvicide. Parameter adjustments were made to simulate flow rate and reasonably represent larvicide concentration at the outfall and in a receiving waterbody measured during a 57-day period. The point-source input files were then varied to represent a range of CRD release characteristics to capture the variability in water conditions. The SWAT model was simulated for a 30-year period to account for variability due to precipitation and temperature. The resulting estimated environmental concentration (EEC) will be presented probabilistically to demonstrate the uncertainty in CRD release and transport. Results from this modeling exercise will be helpful in understanding the exposure pathways of CRDs in urban water systems.

AGRO 94

Evaluating ecological risk of a controlled release larvicide applied to catch basin systems that drain directly into natural waterbodies

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Municipal stormwater systems were selected, among labeled use sites, as a "worst-case scenario" under which non-target aquatic macroinvertebrates inhabiting natural waterbodies could potentially be exposed to the controlled release larvicide, novaluron. Two types of receiving waterbodies, ponds and streams, were chosen to evaluate the potential impact on different types of macroinvertebrate communities. Novaluron was applied to catch basins in the stormwater systems at the labeled rate. Monitoring stations equipped with area-velocity probes and autosamplers, were constructed at each system outfall to record flow volumes during runoff events and collect flow-weighted samples for analysis of novaluron residues. Secondary "tethered" autosamplers collected concurrent samples from the receiving waterbodies during runoff events. Samples were also periodically collected from the catch basins through the field season. In addition to residue samples, macroinvertebrate samples were collected from the receiving waterbodies before product application and twice more during the field season for future community sensitivity analyses. Preliminary analysis indicates that novaluron residues near the detection limit were present in storm discharge only during initial runoff events, with little to no residues detected in subsequent events. Residues were detected only once in receiving waterbodies following a single storm event that registered among the highest precipitation totals during the study. Data collected from one of the sites during the field season was used to calibrate a mathematical model. The model will be used for simulating long-term transport of the larvicide through stormwater systems and in receiving natural waterbodies under a range of infrastructure and storm event conditions.

AGRO 95

Novel application of HPLC core-shell column technology: The successful separation of three small molecule conformational isomer plant metabolites of EPTC by LC-MS/MS

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EPTC (*S*-ethyl dipropyl(thiacarbamate)) and 3 of its hydroxylated plant metabolite residues have previously been determined by GC-NPD (EPTC) and GC-MSD (metabolites). EPTC has more recently been determined by LC-MS/MS (California Department of Food and Agriculture). For the purposes of this project, it was desirable to determine the parent and three metabolite compounds by LC-MS/MS. Using an iterative approach manipulating different gradient settings, temperatures, and column types; a set of chromatographic conditions were developed to resolve and detect all 4 compounds within a single LC-MS/MS run using reversed-phase chromatography. This is significant due to the structural similarity of the 3 conformational isomer metabolites. The conditions were developed using a core-shell column from Phenomenex. Use of core-shell column technology coupled with an iterative approach to method development can potentially yield chromatographic resolution of other small molecule conformational isomers. The need to analyze for small molecule conformational isomers often arises from the degradation of pesticide, herbicide, and fungicide active ingredients. This approach can simplify the otherwise difficult process of analyzing these types of metabolites and degradates.

AGRO 96

Identification and application of matrix components for analyte protection during the GC/MS analysis of current use pesticides in snail tissues following the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method

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The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method was developed to streamline sample preparation through the removal of matrix components such as sugars, organic and fatty acids, and polar pigments while simultaneously extracting a broad range of target analytes. However, preliminary attempts to use this method have revealed matrix effects for some current-use pesticides during GC/MS analysis. Although matrix matched standards are ideal for removing matrix effects, analysis of environmental matrices sometimes prohibits the use of matrix matched standards due to limited availability of uncontaminated matrices. For instance, clean tissues may be unavailable for field-collected organisms used as biomonitoring tools. Under these circumstances, the identification and use of matrix constituents causing matrix effects or analyte protection can be a viable alternative to matrix matched standards. Although the QuEChERS method is effective at removing many constituents from the tissue matrix, sterols (i.e. cholesterol) are not removed. These sterols seem to have a matrix stabilizing effect and therefore have the potential to be used as analyte protectants. Based on the retention times of the matrix constituents, it is believed that they are providing inlet stabilization of target analytes rather than on column stabilization. QuEChERS extraction coupled with utilizing cholesterol as an analyte protectant is a promising approach for measurement of current-use pesticides in tissue and may be important for analytes that are sensitive to inlet or column degradation.

AGRO 97

Ultratrace determination of neonicotinoid insecticides in pollen, anthers, and nectar using high-throughput sample preparation and liquid chromatography with tandem mass spectrometry detection

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Colony Collapse Disorder (CCD) is the term used to describe the abrupt disappearance of worker bees from European honey bee (*Apis mellifera*) colonies. Possible mechanisms of CCD include pathogens, parasites, poor nutrition, immune deficiencies, loss of habitat, bee handling practices, genetic factors, exposure to neonicotinoid insecticides, or a combination of factors. The potential of neonicotinoids as a causal agent has resulted in a rush of studies to determine the level of exposure of honey bees to the insecticides in agricultural settings. These "pollinator health studies" involve the collection and analysis of samples from crops treated with insecticides to determine the magnitude of residues in various flower structures and nectar. Common to these sample matrices are the extremely small sample sizes. Pollen, nectar and anther samples collected from flowers are typically 100 mg or less, necessitating the need to extract the entire sample. Study requirements for limit of quantitation (LOQ) are also very low (≤ 1 ppb). Small sample sizes and low LOQ requirements present challenges in the analytical laboratory. Liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) is the only current, commonly available and accepted technology sensitive enough to achieve the method LOQ requirements. Since the small sample sizes dictate extraction of the entire field sample, high-throughput sample preparation techniques should find utility in analytical methods for a variety of insecticidal residues. This research provides bridging data for two representative neonicotinoid insecticides; thiamethoxam and clothianidin. The use of a bead mill extraction apparatus and 96 well plate solid-phase extraction (SPE) demonstrated acceptable accuracy and precision, and results were comparable to previously validated conventional extraction and SPE cleanup techniques. Recovery from laboratory fortified samples at the LOQ and 10x LOQ levels using high-throughput techniques were in excess of 80% with a relative standard deviation of <20% for each sample matrix. Significant time savings without loss of accuracy or precision can be realized using the high-throughput equipment. Utilization of these techniques will help laboratories keep up with the expected sample volumes from an ever increasing number of pollinator health studies to be conducted over the coming years.

AGRO 98

Multiresidue anticoagulant residue method using novel surrogate compounds

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Rodenticides are safely used for commercial and commensal rodent control all over the world. However, non-target and secondary exposures may occur from their use, especially when applied improperly. Concern over the potential hazard from these exposures has created a demand for more sensitive and selective analytical methods. To answer this need, we developed a liquid chromatographic method to simultaneously quantify warfarin, coumatetralyl, coumachlor, difenacoum, brodifacoum, pindone, dipahcinone, chlorophacinone, bromadoilone, and difethialone residues in carcass, liver, and kidney tissues using solid phase extraction and spectrophotometric detection. Fortification with novel surrogate compounds resulted in excellent recovery and reproducibility for replicate analyses. The method was applied to field-collected samples that were concurrently analyzed by a very sensitive LC-tandem mass spectroscopy method to confirm the observed results. Results from the two techniques compared very favorably.

AGRO 99

Rapid screening of herbal supplements and their extracts for pesticides utilizing a direct analysis of solid phase microextraction (SPME) fibers by DART-based ambient ionization mass spectrometry

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Rapid screening of herbal supplements and their extracts for pesticides is demonstrated utilizing a direct analysis in real time mass spectrometry (DART-MS) method. Method development involving solid phase microextraction (SPME) and direct desorption of chemicals from the SPME fiber in order to assess the safety of these products. Potential deployment of QuEChERs-based extraction methodology to prepare samples for DART-MS are also examined as a means to isolate the pesticides more efficiently in incurred samples. Operation of the DART with different temperature gas is utilized to increase the range of pesticides detected on the same SPME fiber. Utilization of full scan high resolution mass spectrometry (HRMS) and tandem mass spectrometry (MS/MS) for confirmation was completed. Various pesticides are detected alone or in mixtures in supplements including Ginkgo Biloba, Pomegranate, Ginseng, St. John's Wort and other over the counter products. The use of statistical analysis to investigate the occurrence of these materials in food products at lower levels will be discussed.

AGRO 100

Determination of multiple rodenticides in avian tissues using a modified QuEChERS technique and LC-APCI/MS/MS detection

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Rodenticides are used world-wide to control rodent populations in agricultural and urban settings. A sensitive method was developed to assess exposure of non-target avian species to seven rodenticides (warfarin, diphacinone, chlorophacinone, bromadiolone, brodifacoum, difethialone, and desmethyl bromethalin). The QuEChERS (quick, easy, cheap, effective, rugged and safe) methodology was modified for use with avian liver, kidney and carcass tissues. Homogenized tissues were fortified with surrogate standards and extracted with a water/acetonitrile solution followed by addition of excess NaCl. An aliquot of the extract was cleaned-up dispersive solid-phase extraction (dSPE) using PSA and C18 sorbents and magnesium sulfate. Extracts were analyzed by liquid chromatography coupled with atmospheric pressure chemical ionization tandem mass spectrometry (LC-APCI/MS/MS) operated in negative ion mode. Analyte recovery from quail tissues fortified with seven rodenticides was on average 103(±9) %. Detection limits were less than 10 ng/g for six of the seven rodenticides.

AGRO 101

IR-4 program for registration, efficacy testing, and development of organic products and biopesticides

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The primary objective of the IR-4 Biopesticide Research Program is to further the development and registration of biopesticides for use in pest management systems for specialty crops, or for minor uses on major crops. IR-4 has developed and approved research protocols. The program has also funded small and large scale field efficacy trials and residue trials, as needed. Depending on the stage of development, IR-4 has assisted in obtaining Experimental Use Permits from the EPA. IR-4 regularly prepares and submits petitions and registration documents to the EPA to support clearances. In 2014, IR-4 changed the Biopesticide Grant Program from a research proposal based program to one based on grower priority needs. The top project needs for 2015 include spotted wing drosophila, aflatoxin management on figs, fireblight in organic apple production, American Chestnut Blight, varroa mite in honeybees, RNAi based technologies, bacterial diseases of tomato, and downy mildew on basil. Among IR-4's recent successes in registration include natural products, microbials, and most recently, transgenic products. A discussion of specific products will be reviewed during the presentation.

AGRO 102

Plant/plant allelopathy for herbicide and bioherbicide discovery and development

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Plants produce phytotoxins that are used by the producing plant to suppress growth of competitors. In some cases, this process (allelopathy) is pronounced. Allelopathy can be used in two ways to discover and develop bioherbicides. The first is through discovery of phytotoxins that might be used directly as biochemical bioherbicides or as templates for synthetic herbicides. The only biochemical bioherbicides from phytochemicals available are crude mixtures of fatty acids and essential oils. In most cases, these very high use rate compounds have not been implicated in allelopathy. The natural triketones apparently are allelochemicals and are moderated effective when used as herbicides. However, only synthetic triketone analogs inspired by natural triketones have been commercialized as herbicides. Few reported cases of allelopathy have been properly studied to determine the compounds responsible weed competitor suppression in the field. Bioassay-guided isolation and identification of phytotoxins using soil-based bioassays, along with determination of total activity according to S. Hiradite, is the best procedure for discovery of allelochemicals and herbicides from plants. A transgenic approach can be used to enhance or impart allelochemical production by crops as plant incorporated protectants (PIPs). PIPs are considered biopesticides by the USEPA. With modern biotechnology, this approach to weed management is highly attractive, in that it would have many of the environmental and economic advantages of *Bt* toxin crops. Allelopathy can provide much needed new tools for weed management.

AGRO 103

Insect pest-fungal spore mutualism: A potential source of new biopesticide products?

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Recent investigations into the development of a host plant volatile-based attractant for a major insect pest of almond orchards led to the discovery of a key attractant that was produced by fungal spores on damaged or hull split almonds. Results from further work regarding this phenomenon have suggested that the fungal spores are signaling host plant vulnerabilities to the insect pest. This production of key semiochemicals by fungal spores may be an important avenue for the development of new naturally occurring compounds that can be used in lures or attractants of agricultural insect pests. Discussed will be recent results for the generalist navel orangeworm moth and its corresponding host plants.

AGRO 104

Insect control with specialized pheromone and lure application technology (SPLAT®)

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SPLAT (ISCA Technologies, www.iscatech.com) is a biologically inert matrix for the sustained release of insect semiochemicals, phagostimulants, plant volatiles, biological control agents, insecticides and countless other compounds used for pest management in agricultural, urban and forest ecosystems. SPLAT formulations can be tailored to deliver species-specific control through a variety of mechanisms including attract & kill, mating disruption, repellency and mass trapping. The amorphous and flowable quality of the SPLAT matrix allows for flexible methods of application including disposable syringes, electric and pneumatic grease guns, tractor and gator-driven systems and aerial applications. Another benefit is the ability to vary the placement, density and size of chemical-emitting point sources in the field to optimize control of the insect target. Point sources can be applied to almost any application surface and will remain in place, releasing active ingredient over a period of weeks to months. Most formulations become rain-fast after three hours of drying, forming a protective skin that maintains the shape of the point source and offers protection of the active ingredients from UV rays. Numerous studies have shown that SPLAT is retrievable, non-phytotoxic and safe for the environment. A US EPA science review has certified all inert ingredients in SPLAT to be "suitable for food use," with several formulations labeled as organic. We will discuss the use, as well as the regulatory aspects of these new of SPLAT formulations designed for the management different insect pests and vectors of importance.

AGRO 105

Challenges in applying boric acid as a toxicant for managing spotted wing drosophila

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Choosing an appropriate insecticide to use for integration with attractant traps, or perhaps SPLAT technology, for managing spotted wing drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae), is an urgent challenge. Boric acid has many advantages for this purpose: (1) it would likely be organically acceptable, (2) it is exempt from tolerance by U.S. EPA, (3) it has a biochemical mode of action different from commercially used fruit insecticides, and (4) it is not subject to photodegradation. The challenges in its use are (1) finding a potential registrant: it is inexpensive, readily available at high purity, and may not be patentable, (2) as a micronutrient of plants, it is probably unsuitable for use in broadcast sprays, and (3) field responses of SWD demonstrate that it is a relatively slow acting toxicant and is selectively toxic to male flies, unless we can trick the flies into ingesting a larger dose.

AGRO 106

Development of botanical-based biopesticides and repellents against biting flies on livestock animals

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Biting flies are the most important insect pests leading over billions of dollars losses in livestock industries. The attack by those biting flies has caused significant losses in animal production and increases in food contamination and disease transmitting. The present presentation reports our recent findings on the development of botanical-based biopesticides and repellents against these biting flies (stable fly and horn fly). We have further explored plant-based antimicrobial agents from several plant essential oils that are demonstrated with antimicrobial activities, which can be used to manipulate microbial community in the fly larval development and oviposition location. Novel botanical-based antibacterial agents were identified acting as larval growth inhibitors and oviposition deterrents. The further development using these findings in novel fly control strategy will be discussed.

AGRO 107

Exploring the toxicity and synergism of chalcone analogs as biologically-based alternatives to control insects

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Mosquitoes are hematophagous insects that are both nuisance pests and significant vectors of human and animal diseases. The mainstay for controlling mosquito populations, and therefore disruption of mosquito-borne diseases, is the use of chemical insecticides. The widespread use of chemical insecticides, some having similar biochemical mechanisms of action, has resulted predictably in mosquitoes developing insecticide resistance. There is a continued need for the identification and development of new chemistries that have either a novel mechanism of action or enhance the toxicity of insecticides that are already available. Initial toxicity screening of chalcones was performed against *Aedes aegypti,* using a first instar larval assay, and *Drosophila melanogaster* using an adult feeding assay. Generally, chalcone analogues containing a 2-thiophenyl group showed higher toxicity compared to chalcones analogues containing a 2-furyl group, indicating that the 2-thiophenyl group is important for toxicity. Low chalcone toxicity was not due to cytochrome P450s, as determined by the absence of any effect by pre-treatment with piperonyl butoxide (PBO). A select number of chalcone analogues were screened against *Ae. aegypti* to determine if the analogues were capable of synergizing the toxicity of carbaryl. These studies were performed by pre-treating adult female *Ae. aegypti* with 500 ng (per mosquito) of the chalcone analogue for 4 hr prior to treating each mosquito with 2.5 ng of carbaryl (the approximate LD_{25}). Three chalcone analogues showed promising activity; however, none of the tested analogues displayed better activity than PBO.

AGRO 108

Novel strategy for selective determination of dicamba residues in raw agricultural commodities by paired ion electrospray ionization (PIESI) mass spectrometry

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The often low concentrations of pesticides in raw agricultural commodities (RACs) require the development of analytical approaches that allow for the rugged and robust quantification of pesticides with high sensitivity and selectivity. In this work, a novel strategy named paired ion electrospray ionization (PIESI) mass spectrometry was employed for the quantification of dicamba residues in RACs. This approach utilizes structurally optimized ion-pairing reagents to associate with certain anionic pesticide residues, forming positively charged analyte/ion-pairing reagent complexes. Consequently, these anionic complexes are monitored and quantitated in the positive ion mode. In contrast to the negative ion mode, the positive PIESI mode detection often reduces or eliminates previously observed interferences and background, allowing more robust quantitation at low analyte concentrations. This is partially due to the fact that it moves the detection of analyte away from a higher noise, low m/z region to a relatively lower noise, high m/z region. PIESI-MS improved the detection selectivity for dicamba residues, as compared to the conventionally used negative ion mode detection method. The developed method was successfully validated for determination of dicamba and its key plant metabolites, 5-hydroxy dicamba and 3,6-dichlorosalicylic acid in raw agricultural commodities.

AGRO 109

Effect of lanthanum on amino acid composition of soybean seedlings under supplementary UV-B radiation stress

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The effect of spraying lanthanum among different concentration (30 $mg\cdot L^{-1}$、90 $mg\cdot L^{-1}$ and 150 $mg\cdot L^{-1}$) on amino acid content and composition of soybean seedlings in soybean early flowering stage exposed to high level of UV-B radiation and low level of UV-B radiation(simulated ultraviolet radiation increased by 12.80% - T2 and 4.33% - T1 compared with natural light of Harbin in heilongjiang province) was studied by using soybean Dongnong 42 with planting in the pot.The results showed that: 1. Compared with that of the control, supplementary UV-B radiation caused 5.56% (T2) and 13.41% (T1) decrease in total amino acid of soybean seedlings respectively; Spraying rare earth lanthanum can make the total amino acid of soybean seedlings increased in UV-B treatment. In addition, spraying $30mg\cdot L^{-1}LaCl_3$ made the content of amino acid reach a maximum value, increased 6.37%(T2) and 12.37%(T1) compared with the control. 2. The content of amino acid of soybean seedlings were decreased 0.97%(Met) and 18.20%(Arg) except Cys and Pro which were increased 1.60% and 20.41% in T2 treatment respectively. In the essential amino acids, spraying rare earth lanthanum made the amino acid content increased, while Met in the T2 and T1 treatments and Cys in T2 treatment decreased. Spraying $30mg\cdot L^{-1}LaCl_3$ made the content of amino acid reach a maximum value, which Ile content increased up to 12.85% (T2) and 13.30% (T1).The results show that the effect of amino acid content and composition of soybean seedlings under low level of UV-B radiation is bigger than high level. Spraying rare earth lanthanum in optimum fertilization concentration can alleviate the adverse effects and the best dosage is 30 $mg\cdot L^{-1}$.This study provides reference for alleviating the negative impact on soybean seedlings under Supplementary UV-B radiation stress and improving the quality of soybean through spraying the best appropriate concentration of rare earth lanthanum.

AGRO 110

Isolating trace impurities for structural elucidation in a commercial fungicide formulation using preparative supercritical fluid chromatography (SFC)

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Trace impurities in synthetic products that interact with human end users or may have an undesirable environmental fate are regulated by various government agencies such as the FDA and the EPA. As a result, impurity separations and their structural identification is an important research area in many different industries, including pharmaceuticals, agrochemicals, foods, cosmetics and consumer products. Unknown impurities present in amounts greater than the regulatory limits may be required to undergo toxicology studies. For this reason there is great motivation to identify all of the trace impurities, which now being categorized as known impurities, have higher limit thresholds that will

trigger the need for further study. Some structural information can be gleaned *in situ*, with spectroscopic methods such as UV and MS, for the chromatographic separations of the trace impurities that are achieved on the analytical scale. However, UV, MS and MS/MS studies alone are often insufficient to unambiguously identify a compound structure, especially in the case of isomers. Elucidating full structural assignments often necessitates obtaining the isolated pure compounds of interest using purification procedures for further structural studies. Various 2D NMR techniques, which are best applied to isolated milligram amounts of the pristine purified material, are capable of giving much more structural information for these impurities.
In this presentation we will present an example using the fungicide propiconazole as a compound having trace impurities, including regional and stereoisomers, where the full structural assignments are made on isolated materials obtained using orthogonal preparative SFC techniques. The pristine materials garnered in this manner were appropriate for several 1D and 2D NMR studies that unambiguously gave the full assignments for the isomers.

AGRO 111

Enantioseparation and detection of triazole fungicides in wheat grain and wheat straw using ultraperformance convergence chromatography and MS/MS detection

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In the agricultural chemicals manufacturing industry extensive studies are carried out in support of product registration. The studies ensure that any risks associated with using the product are characterised and properly understood so that it can be safely applied to the field. When a crop protection active ingredient (AI) contains one or more stereogenic centers in its structure the enantioselective behaviour must be studied, as it is known that enantiomers can exhibit different bioactivities.[1,2] Analytical methods used to evaluate the influence of stereochemistry on the degradation dynamics, environmental fate and final residue levels help to establish a more accurate risk assessment of crop protection products. The detection sensitivity and specificity offered by MS/MS is advantageous for determining trace levels of pesticides in complex matrices like field crops or soil. In this study UltraPerformance Convergence Chromatography (UPC^2) and tandem quadrupole mass spectrometry were used for the trace level enantioanalysis of triazole fungicides in wheat grain and/or wheat straw. A QuEChERS (quick easy cheap effective rugged and safe) extraction modified for dry commodities was performed followed by solid phase extraction using Oasis® MCX. Enantioseparation of the target fungicides was carried out using CO_2 as the primary mobile phase and methanol with 0.1% formic acid as the co-solvent at a flow-rate of 2.0 mL/min. An amylose chiral column 3.0 x 150 mm, 2.5-µm provided optimum separation of the enantiomers. The outlet backpressure was 137 bar. Detection was by positive ion electrospray mass spectrometry (MS) using a tandem quadrupole mass spectrometer. MassLynx 4.1 was used for data acquisition and TargetLynx application manager was used for data processing. Chiral resolution of the racemic triazole fungicides in wheat grain and wheat straw was performed in less than 3.5 minutes. The results from the chiral UPC^2-MRM analysis show that trace level detection can be achieved with good precision and accuracy using this technique.

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2. J, Ye, M, Zhao, L, Jing, W, Liu. Enantioselectivity in environmental risk assessment. Environmental Pollution. 2010,158, 2371-2383.

AGRO 112

Analysis of fungicide body residues in tissue via the QuEChERS (quick, easy, cheap, effective, rugged and safe) method and use of a real matrix component for analyte protection

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Protocols that streamline extraction and cleanup of environmental samples (specifically tissues) offer obvious benefits for keeping pace with the latest developments of agriculture chemicals and new legislation. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method was developed for this purpose; providing the removal of matrix components such as sugars, organic and fatty acids, and polar pigments while simultaneously extracting a broad range of target analytes. However, preliminary attempts to use this method have revealed some matrix effects that buffer the breakdown of some analytes (namely the fungicide pyraclostrobin) during GC/MS analysis. Without the use of matrix matched standards, breakdown of the parent molecule changes the quantitation by a factor of two, thus preventing proper calibration between extracted samples and standards lacking matrix constituents. Although the QuEChERS method is effective at removing many constituents from the tissue matrix, sterols (i.e. cholesterol) are not removed. These sterols seem to have a matrix stabilizing effect on pyraclostrobin and therefore have the potential to be used as analyte protectants. Based on the retention times of the matrix constituents, it is believed that they are providing inlet stabilization of target analytes rather than on column stabilization. QuEChERS extraction coupled with utilizing cholesterol as an analyte protectant is a promising approach for measurement of pyraclostrobin in tissue and may be important for other analytes that are sensitive to inlet or column degradation. Although matrix matched standards are ideal for removing matrix effects, analysis of environmental matrices sometimes prohibits the use of matrix matched standards due to limited availability of uncontaminated matrices. Under these circumstances, the identification and use of matrix constituents causing matrix effects or analyte protection can be a viable alternative to matrix matched standards.

AGRO 113

Photolysis of herbicides absorbed to plant surfaces: Imazethapyr on corn and soybean waxes

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Most herbicides are sprayed onto fields during application. As the droplets dry, non-volatile lipophilic herbicides spread into the epicuticular waxes present on the leaves and any fruit or vegetables present. Herbicides accumulate in these wax layers before migration to the active biological site, but the herbicides accumulated in or adsorbed on the epicuticular wax are subjected to solar light and may undergo photochemical transformation while in/on this layer. Examining photolysis on plant leaves is complex, since diffusion and transmission of light make experiments on the heterogeneous surfaces of the leaves difficult. In our study of the photolysis of imazethapyr on corn and soybean plants, we removed the epicuticular wax from plant leaves to study the waxes separately. Plants were grown in the Gustavus Adolphus College greenhouse growth chambers allowing the control over factors such as growing temperature, relative humidity, light conditions, and the age of the plants. A design of experiment (DOE) methodology was used to develop a set of experimental conditions to test these growing factors and to build a model for the response variable (photolysis rate) based on the growing factors. Imazethapyr was placed on the epicuticular waxes collected under the various growing conditions, and then samples were irradiated in a Q-Sun Solar Simulator for up to 48 hours. HPLC and LC-MS were used to record photolysis rate constants and identify photoproducts. Initial results suggest that the composition of the waxes do vary as a function of the growth conditions and these physical and chemical changes result in difference in the photodegradation of the absorbed herbicides. In addition to the photochemistry work, microscopy and spectroscopy were used to examine the waxes grown under each set of experimental growing conditions. For comparison and as controls, experiments were also conducted on glass petri dishes and in aqueous solution.

AGRO 114

Uptake and accumulation of endosulfan isomers and its sulfate metabolite in lettuces grown on contaminated soil

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Residual amounts of endosulfan isomers (α- and β-) and its sulfate metabolite in lettuces grown on artificially contaminated soils were assessed to understand plant uptake patterns of soil-persisting pesticides. Endosulfan formulation including α- and β-isomers in ratio of 7:3 was treated with concentrations of 10 and 20 mg kg^{-1} in soil, and the uptake experiments of lettuces were conducted at both indoor-growth chamber and outdoor-greenhouse conditions. Residual amounts of endosulfan isomers and its metabolite in samples collected at different times were analyzed using a gas chromatography-mass spectrometer. For the indoor tests, half-lives, DT_{50} of the α-isomer (17.7-19.6 d) in soil were shorter than those of the β-isomer (36.1-56.3 d), and dissipation rates of both isomers in 20 mg kg^{-1}-treated soil were faster than those in 10 mg kg^{-1}. Uptake rates of lettuces in the indoor tests were 5.4-9.3% for the isomers and 8.6-13.2% for the metabolite, and the most residues of absorbed endosulfan were present in roots. For the outdoor tests, the DT_{50} of the isomers in the 10 mg kg^{-1}-treated soil was longer over 2 times than that of the 20 mg kg^{-1}. Compared with results of the indoor tests, the uptake rates of lettuces obtained from the outdoor tests were lower as 0.2-1.8% for the isomers and 3.9-6.2% for the metabolite. Furthermore, for the absorbed amounts, 81.0-91.0% of the α-isomer was detected in roots throughout the outdoor test, and 74.8-96.6% of endosulfan-sulfate was accumulated in leaves. For all the tests, the accumulation of the α-isomer was predominant in lettuces due to its high content in endosulfan formulation used. In conclusion, we have shown that results of the indoor tests may give major information to understand plant uptake patterns of pesticides in actual agricultural fields.

AGRO 115

Encouraging the use of drift reduction technologies in the United States

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The Environmental Protection Agency's Office of Pesticides Programs (OPP) recently announced a voluntary program to verify the effectiveness of pesticide spray application technologies on reducing spray drift. The new voluntary Drift Reduction Technology (DRT) Program encourages manufacturers to test their technologies (such as nozzles, spray shields, and drift reduction chemicals, etc.) under a standard protocol to verify the quantitative drift reduction potential of the tested application technology. Use of DRT technologies offers the potential for on-target application of pesticides that can improve efficacy and potentially reduce off-site drift and adverse effects on human health and the environment. As a risk mitigation measure option, the Agency will encourage pesticide manufacturers to label their products for use with DRT technologies. EPA envisions that verified DRTs will be included in future pesticide risk assessments and in consideration of risk management decisions and application restrictions for pesticide product labels.

AGRO 116

Persistence of oxadiazon residues in soil and grains in an upland rice (*Oryza sativa*) field

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Weeds are a major constraint to increasing productivity in direct-seeded rice systems. One agronomic approach to achieve a sustainable weed control practice is to combine appropriate herbicide mixtures with optimal application timing and herbicide rotation. Oxadiazon is a pre-emergence herbicide that controls grasses, broadleaf weeds, and sedges, which are present in aerobic rice systems. The World Health Organization has estimated the risk of exposure from consuming food and drinking water containing pesticide residues, and set a maximum residue limit for oxadiazon in polished rice at 0.05 mg kg^{-1}. An HPLC method using a C18 column, 80% acetonitrile mobile phase, and a UV detector were used to determine the concentration of oxadiazon in soil and rice grain samples collected from a field experiment, wherein some plots were treated with oxadiazon while others were not. Results from method validation gave a Limit of Detection of 0.01 mg L^{-1} and a Limit of Quantitation of 0.03 mg L^{-1}, with percent recoveries greater than 80%. Preliminary results showed that oxadiazon persisted in the soil after more than 20 days of application, with average concentrations of 0.10, 0.13, and 0.017 mg kg^{-1} in the three sites. Oxadiazon was also detected in grain samples that have maximum concentrations of 0.54 mg kg^{-1} and 0.18 mg kg^{-1} in the hulls and in brown rice, respectively. Detectable amounts of oxadiazon were found in the grains (hulls only) in non-treated plots, and this was attributed to contamination either from spray drift or irrigation water seepage. Comparison of the grains between treated and non-treated plots showed that the average concentration of oxadiazon in the grains (hulls + brown rice) in non-treated plots was 0.05 mg kg^{-1} and is statistically different from those of the treated plots, with concentrations of 0.45 mg kg^{-1} (treatment 2) and 0.58 mg kg^{-1} (treatment 3).

AGRO 117

Occurence and formation of insecticide degradation products in urban environments

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Pesticides are used in urban environments to control the populations of undesirable organisms such as ants and termites. The most intensive urban pesticide use occurs in areas like Southern California with a large human population density and a climate that allows pest species to be active almost year-round. For example, over 3.5 million pounds of pesticide active ingredients were applied to urban environments in California during 2012. In California, urban insecticide use is dominated by pyrethroids and the phenylpyrazole fipronil. While the occurrence of the parent compounds has been widely documented in urban watersheds, the occurrence and formation of their degradation products has largely been ignored, even though the common pyrethroid metabolite 3-phenoxybenzoic acid (3-PBA) is a known endocrine disrupting compound, and the fipronil degradation products have equal or greater toxicity than fipronil. In this study, the formation of degradation products was investigated by spiking concrete slabs with different formulations containing permethrin or fipronil and measuring 3-PBA, fipronil sulfide, fipronil sulfone, and fipronil desulfinyl in runoff water. The occurrence of degradation products in urban waterways was also determined by collecting runoff samples from 4 locations in southern California, as well as in urban streams. Rapid transformation of concrete applied permethrin to 3-PBA was observed with 3-PBA levels in runoff exceeding 100 µg L-1 even 3 months after the initial application. Similarly, the occurrence of 3-PBA in urbanized waterways was found to be widespread. These findings suggest that concrete surfaces may play an important role in the transformation of urban-use pesticides, and that the inclusion of pesticide degradation products should be considered in monitoring and risk assessment in the future.

AGRO 118

Uptake of triclosan and triclocarban by vegetables from soils and biosolids-amended soils

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Biosolids are extensively used in agriculture as fertilizers. However, studies showed ubiquitous occurrence of pharmaceuticals and personal care products (PPCPs), such as triclosan and triclocarban, in biosolids, with levels up to the ppm range. Amending soils with biosolids may pose a human health risk through dietary intakes since these micropollutants may enter human food chain via plant uptake in biosolids-amended soils. Uptake of triclosan and triclocarban by root vegetables (i.e. carrot and radish) in soils and biosolids-amended soils was investigated in a greenhouse study. Accumulation of triclosan and triclocarban was from 0.96 to 15.8 times higher in roots, especially root peels, as compared to the leaves. The BCFs of triclocarban in the radish root peel were much greater than other tissues. For example, in a sandy soil, the BCFs in radish root peels, core and leaves were 2.2, 1.0 and 0.48, respectively. However, amendment of biosolids decreased the respective BCFs to 0.4, 0.28 and 0.1. Biosolids application consistently inhibited the uptake and accumulation of triclosan and triclocarban by root plants, indicating reduced bioavailability of triclosan and triclocarban in biosolids-amended soils.

AGRO 119

MixTox SW – a software tool for mixture-toxicity exposure assessments in FOCUS surface water scenarios

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The registration of plant protection products in the EU requires a standard risk assessment for aquatic organisms, where toxicity endpoints are compared to PEC_{SW} (Predicted Environmental Concentrations in surface waters). The FOCUS model calculations use realistic worst-case scenarios, where pesticides can enter surface waters via spray drift, runoff or drain flow. Simulations result in complex exposure patterns with multiple peaks over long periods. An aquatic risk assessment for mixture toxicity is required for formulations that contain two or more active substances. A common known approach is to evaluate combined effects based on Toxicity/Exposure Ratios for all actives. This approach evaluates the cumulative risk and conservatively assumes a simultaneous occurrence of exposure peaks. However, the FOCUS surface water scenarios often show exposure patterns where occurring maximum concentrations differ in time for active substances, when applied with identical crop/scenario/application timings. A detailed analysis of the 'realistic worst-case' exposure profiles for each of the actives is a promising refinement option for critical scenarios. A software tool was developed that allows the user to perform a step-wise evaluation of mixture toxicity in FOCUS surface waters scenarios for formulations containing up to three active substances. The scenarios can be evaluated individually or simultaneously in a first step, resulting in detailed results and visualizations. If scenarios fail the first evaluation step, an in-depth scenario analysis can be performed in a second step, resulting in the identification of relevant key characteristics (*e.g.* maximum peak duration) for the scenario-compound combinations. Detailed results and professional visualizations provide a profound basis for decision-making in higher-tier risk assessment. An example for a time-dependent additive mixture toxicity assessment is presented introducing the step-wise approach and explaining the results.

AGRO 120

Residue Patterns of Insecticides Applied on Perilla Leaf Belonging to the Minor Crop in Korea

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It is important to know degradation pattern of pesticides applied on the crops for safety. If residual patterns of pesticides in crops can be predicted during the cultivation period, it will assist in the production of safe agricultural products. In this study, residue patterns of bifenthrin and chlorfenapyr applied on perilla leaves belonging to the minor crop in Korea were investigated. Bifenthrin and chlorfenapyr were sprayed 2 or 3 times at a week interval prior to harvest. The recovery rates for residue analysis of bifenthrin and chlorfenapyr spiked on perilla leaves were ranged from 81.9 to 104.8%. The variation of residual amounts of bifenthrin and chlorfenapyr during cultivation period was expressed with first order kinetic model. Exponential equations of bifenthrin were $y=0.4177e^{-0.0724x}$ for 2 times application and $y=0.5522e^{-0.0535x}$ for 3 times application, respectively, while chlorfenapyr were $y=9.4124e^{-0.0948x}$ and $y=10.1052e^{-0.0821x}$ for each application, respectively.

According to calculated dissipation constant, half-lives of bifenthrin and chlorfenapyr on perilla leaves were 9.6-13.0 and 7.3-8.4 days, respectively. Pre-harvest residue limit(PHRL) was calculated using dissipation constant obtained by the statistical processing. Proposed PHRL indicates that if residual amounts of bifenthrin and chlorfenapyr in perilla leaves were 13.9 and 10.5 mg/kg at 10 days prior to harvest, their residual amounts would be below than their maximum residue limits at harvest day.

AGRO 121

Biopesticidal potential of fungi from tropical regions of Mexico

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Fungi are widely recognized as a very important resource of secondary metabolites with a tremendous structural diversity and useful activities for agricultural and pharmaceutical purposes. However, most of the fungal metabolites have been obtained mainly from macro and micro-fungi isolated in temperate regions. In Mexico, there are very few reports of the biological and chemical profile of the native tropical fungi. Therefore, the objective of this work has been to isolate and characterize the mycobiota of some special zones and substrates, including endophytes, from the southeast of Mexico to build a library of fungal isolates. We have also carried out the biological screening of fungal extracts against crop pathogens and pests in order to evaluate their biotechnological potential. We have identified several strains with promissory repellent effects on *Bemisia tabaci*, *Myzus persicae, Rhopalosiphum padi*; nematotoxic activities against *Meloidogyne incognita*, *Meloidogyne javanica*; and antagonistic and antimicrobial effect on several important bacterial and fungal plant pathogens. Among these, some fungal extracts have been submitted to bioassay-guided fractionations, such as *Acremonium* sp. XH4*, Beltraniella japonica, Beltraniella portoricensis, Gliomastix masseei*, *Lasiodiplodia* sp. MF11, *Verticillium* sp. TH28. Novel natural products have been characterized from some of these fungi.

AGRO 122

Insecticidal effects of pantropical nodulisporic acid producing endophyte (Hypoxylon pulicidum) against *Spodoptera littoralis* larvae

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Nodulisporic acids (NAs) are indole diterpenes exhibiting potent systemic efficacy against blood-feeding arthropods, e.g. bedbugs, fleas and ticks, via binding to arthropod specific glutamate-gated chloride channels. Intensive medicinal chemistry efforts employing a nodulisporic acid A template has led to the development of N-tert-butyl nodulisporamide as a product candidate for a once monthly treatment of fleas and ticks on companion animals. The natural source of the NAs is a monophyletic lineage of asexual endophytic fungal strains that is widely distributed in the tropics, which was recently identified as a new species of *Hypoxylon pulicidum* (Ascomycota, Xylariales) (Bills *et al*., 2012). Eleven *H. pullicidum* strains were tested over *Spodoptera frugiperda* cell line (Sf9) using MTT assay. Seven out of them showed interesting *in vitro* activity and were further selected for *in vivo* assays against *Spodoptera littoralis* larvae (oral injection tests). Three strains (one endophyte isolated from the plant *Dorstenia elliptica* and two isolated of the ascomata from a wood) showed post-ingestive toxic effects against *S. littoralis* L6 larvae.

AGRO 123

Endophyte screening from Indo-Spanish medicinal plants: Biotechnological green crop protectants

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Endophytes are microorganisms that live in the intercellular spaces of plant tissues producing bioactive substances that may be involved in the host-endophyte relationship[1]. These microorganisms represent a biotechnological source of bioactive metabolites. As part of our ongoing study of the endophyte potential for the development of biotechnological biopesticides[2], we carried out the screening of endophytes from medicinal plants belonging to the genera *Laurus, Mentha, Artemisia* and *Thymus* present in India and Spain. Our study included the endophyte biodiversity (isolation and identification) of these plants species, the antagonism-based primary selection of isolates for microfermentation and the secondary selection based on their micro-extract activity on selected targets including insect pests, fungal pathogens and root-knot nematodes. From a total of 11 plant species more than 1000 fungal strains were isolated and 700 were selected for microfermentation. In this presentation we will discuss the relative percentage occurrence for each order and the colonization frequency for each fungal species. Additionally the antagonistic effects of these isolates against fungal pathogens (*Fusarium* sp.) and the bioactivity of their microextracts on the target species will be presented.

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AGRO 124

Fungal endophyte diversity and bioactivity in the Indian medicinal plant Ocimum sanctum Linn

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Endophytic fungi isolated from India's Queen of herbs Tulsi (*Ocimum sanctum*) were explored and investigated for their diversity and antiphytopathogenic activity against *Botrytis cinerea*, *Sclerotinia sclerotirum*, *Rhizoctonia solani* and *Fusarium oxysporum*. 86 fungal isolates were recovered from 313 disease – free and surface sterilised plant segments (leaf and stem tissues) in summer and autumn

seasons from three different geographic locations (Delhi, Hyderabad and Mukteshwar) in India. Plant pathogens such as *Fusarium verticillioides, Bipolaris maydis, Chaetomium coarctatum, Rhizoctonia bataticola, Hypoxylon sp., Diaporthe phaseolorum, Alternaria tenuissima and Alternaria alternata* have occurred as endophyte only during summer season in the present study. Isolation rates from stem tissues are found be higher than leaf tissues from Delhi (33.3%) and Mukteshwar (85.7%) in autumn season, whereas it was higher in Delhi (19.1%) and Hyderabad (80%) in summer season. *Fusarium proliferatum* exhibited 0.299mg per ml IC_{50} value against *Botrytis cinerea.*Phylogenetic tree based on neighbor-joining analysis of the rDNA ITS sequences of the endophytic fungal isolates obtained from various tissues of Ocimum sanctum. The bootstrap consensus tree inferred from 1,000 replicates GC MS Chromatogram of hexane extract of Fusarium proliferatum showing presence of major fatty acids

AGRO 125

Residues of pesticide in Hindon River flowing through urban rice cropping area

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Rice is the staple food of Asia and part of the Pacific. Over 90% of the world's rice is produced and consumed in the Asia-Pacific Region. The irrigated rice area currently occupies about 56% of the total area and contributes 76% of the total production. The high water requirements and the heavy pesticide load used in rice in India have resulted in contamination of associated surface water, such as streams, ditches, rivers and lakes. Farmers often spray hazardous insecticides like organophosphates and organochlorine up to five to six times in one cropping season while only two applications may be sufficient. The usual practice of draining paddy water into irrigation canals may cause river and lake contamination. Therefore a study was undertaken to analyse the pesticide residues in river flowing nearby rice growing areas. Water samples were collected from the river Hindon flowing through rice growing area (Badoli Banger, Greater Noida and Uttar Pradesh). Samples of the river were taken from nearby surface, 2km, 2km bottom and 5km. According to the results, organochlorine pesticide residues analysed ranged from 0.007- 2.211µgL^{-1} in surface samples. Organophosphate pesticide residues ranged from 0.011 to 1.919µgL^{-1} in surface samples respectively. However all the synthetic pyrethroids were found BDL while the herbicide analysed ranged from 0.030 to 0.924 µgL^{-1} in surface samples respectively. Therefore the results are alarming and show that either the use of banned pesticides is still continued or the pesticides are present in water samples as they are very persistent. On the contrary, synthetic pyrethroids, owing to their biodegradable property, were found Below Degradable Limit and hence can be considered as safe.

AGRO 126

Chance and design in pro-insecticide discovery

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Many insecticides are inactive on their target site in the form that is sold and applied, needing first to be bioactivated. This stealth strategy has often been achieved by careful design - by systematic derivatization of intrinsically active molecules with protecting groups that will mask their toxic effects until their selective removal in target insects by metabolic enzymes. This pro-drug strategy has often been applied during lead optimization, to alleviate toxicity to non-target organisms. The protecting group might also improve the plant or insect uptake, helping more of the insecticide to enter the insect and reach the target site. In many cases, chance plays more of a role than design in pro-insectide discovery, and insecticides are only discovered after the fact to be pro-drugs, sometimes even after having been on the market for many years. Knowing the active form of an insecticide is essential to mode of action identification, and early mode of action studies on novel chemotypes should take into account the possibility that the compound might be a pro-drug. This talk reviews the strategies for making pro-insecticides and for unmasking pro-insecticides in mode of actions studies.

AGRO 127

Insecticide ADME for support of early phase discovery: Combining classical and modern techniques

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The two factors which determine an insecticide's potency are its binding to a target site (intrinsic activity) and the ability of its active form to reach the target site (bioavailability). Bioavailability is dictated by the compound's stability and transport kinetics, which are determined by both physical and biochemical characteristics. At BASF Global Insecticide research, we characterize bioavailability in the early phases of the pipeline with a pharma-inspired ADME (Absorption, Distribution, Metabolism, and Excretion) approach. Our experimental approach combines both classical and modern techniques to study ADME parameters. For biochemical assessment of metabolism, we purify native enzymes from insects using classical techniques, including the preparation of microsomal membranes from both whole and dissected insects. The more modern approach of recombinant expression of individual insect enzymes is also used for the study of those which are known to be relevant in insecticide metabolism and development of resistance. For analytical characterization of an experimental insecticide and its metabolites, we can conduct classical radiotracer translocation studies when a radiolabel is available. In phase zero, where typically no radiolabel has been synthesized, we utilize modern high-resolution mass spectrometry to probe complex systems for the test compounds and its metabolites. By using these combined approaches, we can rapidly compare the ADME properties of sets of new experimental insecticides and aid in the design of structures with a higher likelihood of advancing in the research pipeline.

AGRO 128

Vssc mutations and insecticide resistance: Understanding the variations

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Mutations in *Vssc* (*voltage sensitive sodium channel*) can cause resistance to pyrethroid insecticides, and many different mutations have been described. Which mutations are found in populations of house flies across the United States varies, presumably as a response to selection with insecticides and fitness costs of the mutations in the absence of insecticide use. In this study we examine strains that are coisogenic, differing only in the *Vssc* allele they contain. We will report on the levels of resistance and the relative fitness cost of different alleles in an attempt to explain the patterns seen at dairies in the United States.

AGRO 129

Discovery, development, and biological characteristics of cyclic keto-enol insecticides

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Cyclic keto-enol insecticides comprise a class of tetramic- and tetronic acid derivatives discovered and developed in the 1990's, as a result of an indication shift within an herbicide synthesis programme. Today three cyclic keto-enol compounds have been commercialised, including two tetronic acid derivatives, spirodiclofen and spiromesifen, and one tetramic acid derivative, spirotetramat. The invertebrate pest profile addressed by cyclic keto-enol insecticides is different between individual members of this chemical class, i.e. spirodiclofen targets pest mites, whereas spiromesifen is active on spider mites and whiteflies. A much broader spectrum shows spirotetramat being active on a wide range of sucking pest insects, including aphids, whiteflies, psyllids, scales and mealybugs. Unless many other insecticides cyclic keto-enol insecticides are non-neurotoxic, interfering with de novo lipid biosynthesis by the inhibition of acetyl-CoA carboxylase, catalysing the rate limiting step in the biosynthesis of fatty acids, i.e. the carboxylation of acetyl-CoA to malonyl-CoA. A new mode of action at the time of development and only addressed by cyclic keto-enol insecticides. The compounds are pro-insecticides which need activation to their enol form by hydrolysis either in planta or – in some cases – after uptake by the targeted pest. The physicochemical properties differ considerably among cyclic keto-enol insecticides and dictate their bioavailability after different routes of exposure. Whereas spirodiclofen and spiromesifen are considered non-systemic due to limited cuticle penetration, spirotetramat is readily taken up and becomes ambi-mobile within the plant vascular system upon activation to its enol form and subsequent deprotonation. The paper reviews the discovery, development and special biological characteristics including mode of action of the cyclic keto-enol insecticides, a chemical class of compounds whose pest efficacy is driven by subtle but notable differences in chemical structure influencing uptake and bioavailability.

AGRO 130

Innovations in discovery: The quest for new fungicidal crop protection solutions

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The quest for new fungicides for the control of *Septoria tritici* continues to be the focus of many agrochemical companies. This is the most devastating disease in the cereal market, and there is a desperate need for a highly robust new mode of action for control of *Septoria tritici*. The aminopyrimidines are novel area of chemistry which are highly active against this commercially important wheat pathogen, *Septoria tritici* and act via a novel mode of action. This talk will focus on the discovery and synthetic strategy the team developed to efficently investigate the structure activity relationship (SAR) in an attempt to further increase activity and/or spectrum, and efforts to identify a backup compound to eliminate a metabolite residue issue while maintaining high *Septoria tritici* potency.

AGRO 131

Search for a systemic anthranilic diamide insecticide: The discovery of cyantraniliprole

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The ryanodine receptor is a non-voltage-gated intracellular calcium channel that plays a critical role in insect muscle contraction. The plant metabolite ryanodine affects calcium release by blocking this channel in the partially open state. With high potency for insect versus mammalian forms of the receptor, anthranilic diamides are a synthetic class of insecticides that bind to an allosteric site distinct from that of ryanodine. The diamide chlorantraniliprole has exceptional activity against a wide range of lepidopteran pests at very low application rates. Although intrinsically active against hemipteran forms of the receptor, chlorantraniliprole is generally less effective for control of sucking-piercing insects, which feed off of the fluids of plants, versus chewing pests. In exploring for analogs with improved aphid activity, a compound with strong contact activity against Myzus *persicae* (green peach aphid) was eventually identified. However, with a log P near 3.0, there was limited systemic activity where plant uptake and translocation were essential for insect availability. Nevertheless, this prompted a search for lower log P derivatives with enhanced systemic properties. Substituting various positions on the anthranilic diamide with a wide range of polar groups generally gave diminished activity. However, a cyano group at one position did lead to encouraging results. Here, the story behind the discovery of cyantraniliprole will be presented along with a discussion of background, synthesis, biology and structure-activity trends for a series of derivatives more polar than chlorantraniliprole. Cyantraniliprole is a second product candidate to emerge from this diamide chemistry class with excellent cross-spectrum activity against a wide range of insects, including lepidopteran, hemipteran and coleopteran pests.

AGRO 132

Development of new tools to advance the estimation of pesticide exposure and effects for listed aquatic and terrestrial species

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The National Marine Fisheries Service, U.S. Department of Agriculture, U.S. Environmental Protection Agency, and U.S. Fish and Wildlife Service have been working collaboratively to develop a unified approach to assess pesticide exposure and effects for federally listed threatened and endangered species. New tools have been created to help facilitate national scale assessments and to assist the agencies as part of the consultation process. Specifically, software programs have been developed to analyze and visualize the available effects endpoints, to automate thousands of aquatic modeling runs and to aid in post-processing the results. In addition to automation tools, substantial work has been done to advance the scientific approaches utilized in ecological risk assessment, including methods to allow for more spatially explicit analyses, incorporation of species-specific life history and probabilistic results. We will present an overview of the tools that have been developed thus far and potential enhancements for the future.

AGRO 133

Ecological risk assessment framework for endangered species assessments

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Issues surrounding potential pesticide impacts to threatened and endangered species (referred to herein as listed species) have grown exponentially from a regulatory policy perspective. Many of the issues have been driven by the differing protection goals of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), administered by the Environmental Protection Agency (EPA), and the Endangered Species Act (ESA), administered by the Fish and Wildlife Service (FWS) and National Marine Fisheries Services (NMFS) (collectively called the Services). The differences between the two pieces of legislation have resulted in different approaches to ecological risk assessment (ERA) used by EPA and the Services. In the 2013 report entitled "Assessing Risks to Endangered and Threatened Species from Pesticides", the National Academy of Sciences (NAS) provided a proposed framework for conducting endangered species assessments under FIFRA with direct consideration of the risk to listed species. The NAS panel recommended the use of refined approaches (e.g., probabilistic methods, population modeling) when conducting risk assessments for listed species. In response to the NAS panel report, EPA, the Services, and USDA released a summary of their implementation plan (often called the 'interagency interim guidance') ahead of a public stakeholder workshop held on November 15, 2013. The interim guidance included discussion of a common approach to risk assessment that addressed the scientific differences between EPA and the Services. Discussion between the EPA and the Services is continuing and final guidance on how to conduct listed species assessments is anticipated. Pesticide registrants individually and through CropLife America (CLA) initiated a project in the fall of 2014 to develop a recommended framework for conducting ERAs for listed species. In January 2015, a workshop was held in Washington D.C. to discuss the and fine tune the framework. This presentation presents the final framework derived during the workshop, and plans for the future development of CLA ecological risk assessment guidance and method development for endangered species assessments.

AGRO 134

Selection and use of data in the assessment of pesticide risk to threatened and endangered species

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Fundamental to any risk assessment process is the appropriate retrieval, selection, review, and use of available data. The National Marine Fisheries Service, U.S. Department of Agriculture, U.S. Environmental Protection Agency, and U.S. Fish and Wildlife Service have been developing an approach for evaluating risk to threatened or endangered species to ensure that all available data are used appropriately and consistently across pesticide risk assessments. Inclusion of data beyond the traditional endpoints of survival and reproduction in particular has resulted in new dialogue in order to achieve common understandings. Here we present an overview of the process being used in the pilot national-level pesticide risk assessments to evaluate quality, relevance, and scientific validity of chemical data, and demonstrate how different types of data may contribute to the overall risk characterization, such as establishing a quantitative threshold, describing pesticide exposure, characterizing adverse outcome pathways, and/or informing lines of evidence.

AGRO 135

Anticipating data needs for endangered species risk assessment under the evolving "interim process" for species assessment

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One of the challenges in FIFRA/ESA national-level pesticide risk assessment is to manage complexity, and deal with data that are constantly changing over time. This forces the risk assessment process towards increased use of authoritative spatial data and representative models. As modeling science, imagery and GIS technologies advance, data quality improves and data abundance multiplies seemingly logarithmically. The national scale of a pesticide registration action by EPA requires that somehow a reasonable snapshot be composed to offer a nationally representative dataset that can be used in risk assessment given the time and resources available to do so. This presentation will explore how to create a usable, national set of information based on data from various sources which must be arrayed in a manner that (1) evens out data portrayal across the nation and (2) provides a uniform platform for data use amongst registrants and EPA-OPP. We will identify additional data sources, disbursed data that might be further aggregated, and how such data would support endangered species risk assessment. We will also address important areas where data are lacking or impossible to aggregate and apply at the national level, and how such data could be sourced through local entities to leverage available resources and expertise.

AGRO 136

Endangered Species Act Section (7) consultation in federal land management agencies

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All Federal agencies are required to ensure that their actions do not harm species listed under the Endangered Species Act. Proposals to use pesticides to control invasive plants or agricultural pests (e.g., grasshoppers, Mormon crickets) within Federal lands, or Federal action to control crop pests on private land (e.g., cotton boll weevil) require ESA Section (7) consultation. Agencies such as the US Forest Service, Bureau of Land Management, and Animal & Plant Health Inspection Service have successfully completed Section (7) consultations on several pesticide use actions, outside the FIFRA registration process, over the last 15 years. We present the process used by the US Forest Service to propose the use of pesticides within National Forests, including the National Environmental Policy Act (NEPA) process, the independent risk assessment process, and the ESA section (7) consultation for project implementation as well as National Forest management plans. We also discuss

the potential for Federal agencies to increase efficiencies in consultation relative to pesticide use and registration.

AGRO 137

Getting over the finish line: Completing pesticide consultations that comply with the ESA

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Although technically daunting, federal government agencies made significant progress in the last three years to complete nationwide consultations on the effects of pesticides on endangered species. Improved analytical methods, combined with better geospatial data, have helped the agencies to address Step 1 issues regarding when pesticides cross the "may affect" threshold and hence require some form of consultation under the Endangered Species Act. One of the remaining challenges is for the agencies to develop a course of action for when a pesticide is likely to adversely affect an endangered species or adversely modify its critical habitat, and when a pesticide jeopardizes a listed species. This presentation will identify several strategies and approaches to answering those questions in a manner that is scientifically defensible, efficient, and consistent with the ESA. In particular, this presentation will address (1) development of pesticide-specific reasonable and prudent measures where the Services reach "no jeopardy" conclusions; (2) development of pesticide-specific reasonable and prudent alternatives where the agencies reach findings of "jeopardy" and/or "adverse modification" of critical habitat; and (3) under what circumstances a compensatory mitigation scheme is, and is not, acceptable under Section 7 of the ESA.

AGRO 138

How should we consider the sources of potential uncertainty inherent in the standard pesticide exposure assessment?

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The standard USEPA exposure assessment scenario is designed to provide initial Aquatic Estimated Environmental Concentrations (EEC) for an Active Ingredient used on a particular crop. The scenario assumes that a 10ha field is 100% treated by air or ground using label-specified use rates and frequencies and that spray drift and rain-driven runoff/erosion both deliver chemical to a 2m deep 1ha pond. These EECs are compared with aquatic ecotoxicity data to determine the potential for aquatic risk. As an approximation to determine whether a more detailed assessment is needed, this is a reasonable worst case scenario. However, to conduct more detailed evaluations of likely aquatic exposures, it is necessary to understand and, where possible, to quantify the relevance and impacts of sources of uncertainty inherent in the standard scenario. We have considered two distinct key regulatory questions, firstly, "What is the probability of occurrence of water bodies as heavily exposed as the standard pond?" and secondly, "When a pond is highly exposed, what is the probability that EECs will be as high as predicted by standard models?" Around 30 potential sources of uncertainty and their potential for co-occurrence have been examined in some detail while many more have impacts that are more difficult to quantitate. Using a range of pyrethroids as examples, the data show that while conditions similar to the standard scenario may occasionally occur, the 90th percentile receiving water body is likely to expose organisms to EECs 1-2 orders of magnitude or more lower than predicted by the standard scenario. Additionally, where a scenario similar to the standard field/pond scenario does exist and a water body may be highly exposed, uncertainties contribute a similar and additive degree of conservatism both for drift and sediment driven loadings.

AGRO 139

Potential impact of modeling assumptions and uncertainties on drinking water concentrations predicted by PRZM-GW for crops and turf

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Sensitivity of various factors including timing of rain storms, type of application, and soil degradation was evaluated with PRZM-GW ver. 1.07 (USEPA's groundwater assessment tool) for Furfural which has a less than 1-day aerobic soil half-life, stable hydrolysis and a low Koc (less than 10 L/kg). Since PRZM-GW estimated drinking water concentrations (EDWC) are averaged over 30 years as opposed to estimating 90th percentile concentrations, timing of heavy (>2 inches) storms have a huge impact on EDWC for compounds with extremely short half-lives as compared to persistent compounds. The presentation will also show that the type of application method, incorporation depth, and wetted-in irrigation amounts can also have a significant impact on EDWCs for such compounds. In addition, various soil degradation schemes were simulated and analyzed for the degradation in soil to the 1-m depth and degradation in soil below 1 m. The EDWC results were reduced 5 fold or to negligible concentrations as compared to results predicted following US EPA PRZM-GW guidance. The impact of these modeling assumptions will be tested in relation to more persistent compounds.
Additionally, the presentation will include an approach to adapting the US EPA standard PRZM-GW scenarios from crop to turf. The results will show a comparison of EDWCs for the standard scenarios versus the PRZM-GW set up as turf.

AGRO 140

Comparison of SCI-GROW and PRZM-GW predicted pesticide concentrations in groundwater with NAWQA observed concentrations

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A key factor in the human health risk assessment process for the registration of pesticides by the U.S. Environmental Protection Agency (EPA) is an estimate of pesticide concentrations in groundwater used for drinking water. From 1997 to 2011, these estimates were obtained from the EPA empirical model, SCI-GROW. Since 2012, these estimates are obtained from the EPA deterministic model, PRZM-GW. The assumptions and chemical environmental fate parameters used in both of these regulatory models are designed to be "conservative", generally resulting in higher predicted pesticide concentrations in groundwater than would be expected to actually occur in the environment for most pesticides. This paper presents a comparison of historical groundwater monitoring data from the National Ambient Water Quality Assessment (NAWQA) Program (1991 – 2014) with predicted groundwater concentrations from both SCI-GROW (version 2.3) and PRZM-GW (version 1.07) for 66 different pesticides of varying environmental fate properties.

AGRO 141

Development of PRZM-GW scenarios for spring and winter wheat-growing areas

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Wheat crops and the major wheat-growing regions of the United States are not included in the six crop and region specific scenarios developed by the Environmental Protection Agency (EPA) for groundwater exposure modeling with PRZM-GW; a groundwater specific user interface to the Pesticide Root Zone Model (PRZM). The goal of this work was to define appropriately vulnerable PRZM-GW scenarios for high-producing spring and winter wheat growing regions. An initial screening was conducted for all wheat areas defined by a multi-year Cropland Data Layer composite (2009-2013). Soil, weather, groundwater temperature, evaporation depth and crop management practices were characterized for each wheat area and used as inputs to PRZM. Vertical profiles of soil characteristics were developed from the Soil Survey Geographic database where map units overlapped wheat areas. Weather data was assigned to wheat areas from the nearest Solar and Meteorological Surface Observation Network (SAMSON) climate station. Groundwater temperature, evaporation depth and crop emergence, maturity and harvest varied at the state level. Additional parameters were set to conservative default values following EPA guidance. Over 150,000 unique combinations of weather/soil/input parameters were simulated with PRZM for an herbicide applied for post-emergence weed control in wheat. The post-breakthrough average herbicide concentrations in the shallow aquifer were ranked nationally and by major wheat states. PRZM-GW inputs resulting in upper-percentile post-breakthrough average concentrations corresponding to significant wheat areas with shallow depth to groundwater formed the basis for wheat scenarios to be used in PRZM-GW.

AGRO 142

Measuring and simulating emissions of 1,3-dichloropropene and chloropicrin after soil fumigation under field conditions

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Soil fumigation is an important agronomic practice in the production of many high-value vegetable and fruit crops. The use of soil fumigant chemicals can lead to excessive atmospheric emissions and information is needed to develop best management practices so that use of soil fumigants does not harm human or ecosystem health. A large-scale field experiment was conducted to obtain volatilization and cumulative emission rates for two commonly-used soil fumigants, 1,3-dichloropropene (1,3-D) and chloropicrin, after soil injection at a depth of 46 cm. Over the course of the experiment, the daily peak volatilization rates ranged from 12 – 30 $\mu g\ m^{-2}\ s^{-1}$ for 1,3-D and 0.7 – 2.6 $\mu g\ m^{-2}\ s^{-1}$ for chloropicrin. Total emissions of 1,3-D and chloropicrin, respectively, were approximately 77 – 168 kg and 1 – 3 kg. This represents approximately 16 – 35% of the total 1,3-D and 0.3 – 1.3% of the chloropicrin applied during the experiment. A soil incubation study showed that the low volatilization rates measured for chloropicrin were due to particularly high soil degradation rates observed at this field site. To test this hypothesis a mathematical model was used to predict total emissions as a function of soil properties, injection depth, shank fracture, soil degradation, and surface resistance to volatilization. The most likely explanation for the low chloropicrin emissions is the soil degradation rate (i.e., $t_{1/2}$ = 2.9 h), which led to predicted total emissions less than 1%.

AGRO 143

Modeling volatilization following pesticide application: Development of a robust pesticide emission model as a stand alone tool

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Recent focus in the atmospheric fate and transport modeling of pesticides has been associated with volatilization following application to both soil and plant surface. Empirical screening tools using linear relationships with pesticides vapor pressure have been used as a first tier screening model to estimate volatilization. However, pesticide volatilization is a complex process that depends not only on vapor pressure but also other physicochemical processes and properties such as water solubility, partitioning and mobility and soil properties (moisture content, organic content, soil temperature and porosity). Additionally, environmental conditions such as ambient temperature, air pressure, wind speed and relative humidity play a major role in determining volatilization flux following pesticide application. Deterministic models attempt to capture all of these processes and properties to estimate pesticide volatilization across a wide range of physicochemical properties and environmental conditions. These mechanistic models however are very complex and require extensive set-up time to configure the model framework to specific application, location and environmental conditions. This presentation introduces the development of a comprehensive deterministic modeling platform as a predictive tool of volatilization from pesticide applications to soil and plant surface. The platform facilitates the use of the pesticide emission model over a wide range of climate, plant type and soil conditions. The model was evaluated using key inputs of climate and soil conditions from California. Good agreement was obtained between the modeled and measured emissions.

AGRO 144

Overview of recent refinements in assessing airborne exposures to pesticide applications: Use of co-variance methods and other field methods and modeling refinements

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Exposure assessments of airborne emissions rely on flux study data and dispersion models to evaluate exposures. The accuracy of such assessments is dependent on the representativeness of the flux data and the ability of the modeling approach to realistically model concentration. This paper stresses the importance of having mutually supportive field study and modeling analyses to promote realistic exposure assessments. Field study needs to

anticipate current and future air quality modeling needs. Models need to make full use of what is learned in the field. An overview is provided of recent advancements in exposure assessment methodology. More simplified screening methods are appropriate, but if necessary, refined modeling needs to be an option. Concepts presented in this paper provide the framework for a refined modeling system to address both acute and chronic exposures, supported by suitable field study data. The objective is to avoid maintaining modeling simplicity that would result in excessive buffer zone restrictions on farming operations. This is true for acute exposures and chronic exposures. A current overview is presented for field and modeling initiatives that are aimed at refining the evaluation of airborne exposures to pesticide applications:

1. Identification of optimal field size and sampling methods for large and small research plots.
2. Maximize data collection through co-variance measurements of temperature and wind speed to compute heat flux on treated field and off - - scaling length theory to best support downstream refined modeling methods.
3. Accurately accounting for nocturnal dilution conditions on irrigated and tarped fields in downstream dispersion modeling (agricultural fields have different heat flux characteristics than unirrigated and untarped surfaces).
4. Methods to address crops in the field (semi-porous obstructions) without model bias.
5. Options for Monte Carlo modeling methods (start times, emissions uncertainty, model input uncertainty).

AGRO 145

Refining the dispersion modeling of airborne flux: Addressing over field nocturnal dispersion

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Evaluating bystander airborne exposures is generally based on dispersion modeling. Dispersion models are designed to address a wide range of source types and conditions. The current state-of-the-art U.S. model that is applicable to localized pesticide exposures is AERMOD. The realistic treatment of worst-case conditions is an important consideration when using AERMOD or any dispersion model. In many cases, maximum exposures occur during nocturnal stable conditions. Even though emissions may or may not be at their peak, nighttime conditions often have much more restrictive meteorological conditions in terms of reduced wind speed and dilution conditions. The use of standard modeling methods assigns dilution conditions (stability) based on conditions at the meteorological monitoring site, which in the case of buffer zone modeling generally is at an airport. Stability is a function of cloud conditions and wind speed conditions, as well as consideration of the land surface type. A limitation of standard modeling methods when applied to agricultural applications, however, is that local on-field dispersion conditions over an irrigated or tarped field can be very different than the idealized surfaces assumed in the modeling. The accuracy of the modeling of agricultural pesticide applications can be refined by providing a more accurate treatment of dilution (stability) conditions for that portion of the plume travel over the treated field.
Over the past 10 years, Sullivan Environmental has collected a large volume of data that describes stability conditions over treated fields (irrigated bare ground, bedded tarped, and broadcast tarped). A common feature in these studies is that during nocturnal conditions, stability conditions over the treated fields are neutral or near-neutral, but not highly stable. This paper presents examples of stability conditions over treated fields and a modeling methodology that can incorporate a refined treatment into AERMOD model runs to refine the modeling of pesticide emissions.

AGRO 146

Use of radiolabeled material to develop, troubleshoot, and radio-validate an analytical method

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Radiolabeled 2,4-Dichloro phenoxy butyric ethylhexyl ester (2,4-DB EHE) was used to develop, troubleshoot and radio-validate a method for its analysis in wheat matrices. The material was labeled uniformly on the ring with carbon fourteen ([^{14}C]-2,4-DB EHE). The proposed procedure was to extract the test material with an organic based solvent, hydrolyze the ester to the 2,4-DB acid, remove the matrix with solid phase extraction, and analyze by LC-MS/MS. This presentation will give an overview of how the use of radiolabeled material validated the extraction technique, monitored the effectiveness of the cleanup and assisted in troubleshooting the loss of 50% of the analyte during the analysis. The interaction of the 2,4-DB EHE with the grain matrix to hinder its recovery during the procedure would have been next to impossible to determine without the aid of a radioactive tracer. However, with the radioactive tracer the time to troubleshoot the method losses was reduced to only 3 days

AGRO 147

Overcoming the analytical challenges of measuring free and total concentrations of nine pyrethroids in sediment, pore water and water column matrices using Solid Phase Micro-Extraction (SPME) and Liquid-Liquid Extraction (LLE) approaches

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In order to measure the adsorption characteristics of a wide range of commonly used pyrethroid insecticides in sediment water systems for refined exposure and risk assessments, it is essential to be able to quantify concentrations in equilibrated sediment as well as free (i.e. bioavailable) and total concentrations in the corresponding aqueous phases. This requires using classical LLE approaches as well as SPME technology and stable isotope labelled internal standards; methods for all of these have already been published in the literature. However, two limitations made adopting these existing methods problematic. Firstly, in order to automate the SPME analyses, the confounding factor of rapid adsorption of pyrethroids to vessel walls had to be addressed; especially for calibration standards. Secondly, the volume required for LLE determination of the total concentration in pore water meant, for certain sediments, that very large samples of sediment were required and, in order to centrifuge those to obtain pore water, over 140 centrifuge vessels (along with the associated transfer steps) were unavoidable. Both problems have been solved. The vessel wall adsorption effect can be obviated by staggered preparation of calibration standards. We have also validated that using stable isotope labeled standards with SPME permits the total concentration to be obtained simultaneously with the free concentration. These results are statistically indistinguishable from those obtained by classical LLE thus reducing the volume or pore water required by around 85%. These automated SPME-based solutions should be applicable to other hydrophobic organic chemical analyses in sediment/soil and water mixtures.

AGRO 148

Fast and easy method for determination of imidazolinone residues in soil by UHPLC-MS/MS

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The variability of soil properties difficult the extraction of pesticide residues of this matrix. Imidazolinones herbicides are widely used to control pre and post-emergence weed crops such as rice, soybeans and other legumes. These compounds are weak acids, with pKa ranging from 1.9 to 3.9. In soils with pH higher than 6, these compounds are weakly sorbed, however, when the soil pH decreases greater amounts of these compounds are strongly sorbed, since the compounds become less dissociated.
The use of ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) allows a rapid and robust analysis of different compounds. The aim of this work was to develop a rapid and simple method for determination of 5 imidazolinone residues (imazamox, imazapic, imazapyr, imazaquin and imazethapyr) in soil samples by UHPLC-MS/MS.
Sample preparation was conducted in a tube using 5 g of soil followed by the addition of 10 mL of an ammonium acetate aqueous solution (0.5 mol L^{-1}), after it was shaken and centrifuged. For clean up step 2 mL of the extract was transferred to a tube containig the sorbent PSA, the tube was shake and centrifuged and the extract was filtered with a 0.2 µm filter and diluted prior injection in the UHPLC-MS/MS system. The analytical curves for the studied compounds showed a linear range between 0.5 and 20 µg L^{-1} with determination coefficient values ≥ 0.99 for all compounds. The LOD and LOQ values of the method were 3.3 and 10 µg kg^{-1}, respectively. Blank samples were spiked at three levels (10, 50 and 100 µg kg^{-1}) and recoveries were evaluated. Satisfactory recovery values (70 to 93%) were obtained for all studied compounds, with RSD values ≤ 14%. Robustness of the method was tested analyzing 10 samples with pH ranging from 4.3 to 7.3; the extraction process was effective, and no imidazolinone residues were found in the soil samples. Because of the complexity of the matrix and the compounds, which strongly depends on the pH for extraction, the developed and validated method proved to be an excellent tool for rapid analysis, with a total analysis time of 20 min, as well as high reliability to be applied in samples with a large range of pH values.

AGRO 149

Development of a matrix imprinted polymer SPE and LC/MS/MS method for the analysis of pyridine herbicides in compost samples

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In the fall of 2012, Dow AgroSciences (DAS) and several Contract Research Organizations (CRO's) received compost samples produced by a commercial compost manufacturer which resulted in "auxin-like" plant injuries when used to enrich planting beds. Analysis of compost samples confirmed the presence of Aminopyralid and Clopyralid residues, but the levels reported varied significantly depending on the facility and the analytical method used. Differences in analytical results from the CRO's have complicated 1) attempts to identify the original source(s) of the herbicide residues (i.e. which inputs in the compost stream contained the herbicide residues) and 2) subsequent label enforcement activities by state agriculture departments. During these product stewardship activities, it became clear that current analytical methodologies were unsuitable for the analysis of the low molecular weight, polar molecules in complex compost matrices at the 1-ppb targeted detection level. This presentation will focus on the method development strategies employed to produce a simple, robust and easily-transferable multi-analyte method for the detection of pyridine herbicides in compost.

AGRO 150

DuPont seed treatment enterprise: Analytical strategies

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DuPont's seed treatment R&D program encompasses numerous pesticide actives across diverse seed types. The verification of active loadings relative to target rates is a prerequisite for the successful staging / interpretation of field trials. Statistical data on loadings variability within treatment batches has proven helpful to applications and formulations development activities as well as assisting biologists when establishing the replication comprising biological screening. Analytical challenges include providing timely and high quality testing across a network of global laboratories, maintaining evergreen analytical procedures facilitating the testing of new actives, and meeting evolving R&D, regulatory and commercial stewardship objectives. This presentation highlights analytical tools, techniques and strategies governing DuPont's seed treatment development programs.

AGRO 151

Optimization of a QuEChERS based method by means of central composite design for pesticide multiresidue determination in orange juice by UHPLC-MS/MS

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The orange juice industry is one of the most successful sectors in the Brazilian economy. The determination of pesticide residues in food matrices is a great challenge especially because of the small quantities of analytes and large amounts of interfering substances which can be coextracted with analytes and, in most cases, adversely affect the results of an analysis. The methods of analysis of pesticide residues basically involve two steps: extraction of the compounds of interest and determination by chromatographic separation. The sample preparation step in complex matrices requires much time, and so is often the limiting factor in the analysis of pesticide residues. In this study, it was compared different extraction procedures based on the QuEChERS method for the determination of 76 pesticides in integral orange juice by Ultra High Performance Liquid Chromatography coupled to tandem Mass Spectrometry. After choosing the best preliminary test it was carried out the experimental design to optimize the same. The parameters evaluated for the validation were: linearity of the analytical curves, matrix effect, limit of detection (LOD), limit of quantification (LOQ), as well as precision and accuracy. The results of the validation were satisfactory, since the method presented recoveries between 70.5 to 117.5% and RSD lower than 19.2%. The method LOD and LOQ ranged from 0.1 to 12.4 µg L^{-1} and from 4.9 to 26 µg L^{-1} respectively, only acephate and deltamethrin compounds were not quantified. Thus, the method was validated for 74 pesticides in integral orange juice. The method developed was adequate for the analysis of pesticide residues in integral orange juice, since all parameters of validation were in according to the guidelines and the sensitivity achieved meets the maximum residue levels (MRLs) established by regulation for food monitoring programs. The results of this study allow concluding that the combination of QuEChERS method and UHPLC–MS/MS is excellent for analysis of pesticide residues due to its quickness and effectiveness.

AGRO 152

Analytical methods for residue analysis: Trends, requirements, and challenges

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Regulatory environments for agro chemicals are changing continuously. Consequently the requirements for analytical methods for residue analysis are also changing globally. In order to meet these regulatory requirements, the crop protection industries are continuously searching for new ways to improve the productivity and efficiency of residue analysis while simultaneously reducing the cost of the analyses. Analytical methods are the main component for residue analysis. It is a challenging task for industry, both from a time perspective and from technical constraints, to develop methods for global use. There are tremendous demands for analytical methods for sample high throughput not only for data generation but also for enforcement purposes. The bigger challenge is to develop/validate methodologies that fulfil guidelines for several countries. The global analytical method requirements including low limits of quantitation (i.e 30 ppt for water method), extraction efficiency, matrix effects, isomeric separation, and enforcement compatibility will be presented and will be discussed in detail.

AGRO 153

Investigation of monoterpenoids and sesquiterpenoids as natural insecticides: Comparisons of activity against mosquitoes and flies

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Terpenes from plant essential oils serve as part of the plants' chemical defense. They can protect against insects and mites, as well as against fungi and bacteria. Most plant essential oils consist of monotepernoids and/or sesquiterpenoids, accompanied by various non-terpeoid plant volatiles. Terpenoids are products of plant biosynthesis via the isoprene pathway (for true terpenes) or the phenyl propanoid pathway (shikimic acid pathway). We have previously conducted numerous investigations of insecticidal properties of monoterpenoids (10-carbon terpenoids); here we will discuss the insect toxicty of sesquiterpenoids and compare their bioactivity to that of monoterpenoids. Lab bioassays have been conducted to evaluate their potency against mosquitoes, house flies and cockroaches. Numerous sesquiterpenoids are excellent insect repllents, but their toxicity to the insects is less impressive.

AGRO 154

Isolation and identification of potential biopesticidal compounds from the North American insect repelling folk remedy plant, sweetgrass, *Hierochloe odorata* (L.) P. Beauv

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Native North Americans used sweetgrass, *Hierochloe odorata*, in a variety of ceremonies and rituals. It has also been used to repel biting insects by making a loop from strands of the grass to be worn around the neck or kept in a satchel hung in homes. This investigation was conducted to evaluate this practice and systematically identify chemical constituents responsible for the mosquito repelling effect by utilizing a mosquito (*Aedes aegypti*) biting deterrent bioactivity-directed purification approach. Fresh steam distilled oil was found to be the most active crude extract generated from sweetgrass and was used in fractionation studies providing 12 distinct fractions. Fractions identified as having activity equivalent to N,N-diethyl-m-toluamide (DEET) contained the compounds phytol and coumarin. *Ae. aegypti* biting deterrent results will be reported for the active fraction and pure compounds.

AGRO 155

Novel biopesticide as piperonyl butoxide-PBO substitute

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Piperonyl butoxide (PBO) is an insecticide synergist, a chemical that is used to make insecticides more potent. PBO is now frequently used, particularly in aerosol products. About 1700 insecticide products contain PBO, 8 percent of the over 20,000 pesticide products registered in the U.S. PBO is often used as a synergist with pyrethrins and the chemically related synthetic pyrethroids. However, it also can synergize a variety of other pesticides, including the insecticides fipronil, parathion,dichlorvos, linalool, and D-limonene, the insect growth regulators methoprene, hydroprene, and fenoxycarb, as well as alpha-naphthylthiourea (formerly used as a rodenticide). Piperonyl butoxide acts as a synergist by slowing the breakdown in insects of certain insecticides. The first step in the breakdown of many drugs, pesticides, and other compounds is oxidation by a family of enzymes called the P450 mono-oxygenases. EPA classifies PBO as a “possible human carcinogen” because it caused liver tumors and cancers in laboratory tests. In a study conducted by PBO manufacturers, PBO caused atrophy of the testes in male rats. Other researchers found behavioral changes (a decrease in home recognition behavior) in the offspring of exposed mothers. PBO affects a variety of hormone-related organs, including thyroid glands, adrenal glands and the pituitary gland. PBO reduces the immune response of human lymphocytes, cells in our blood that help fight infections. There are numerous efforts to develop biobased insecticide synergistic with safer toxicological profile. At USDA we have developed (patent pending) commercially viable biosynergistics. The preliminary results and efficacy of the novel biopesticides will be discussed

AGRO 156

Mosquitocidal constituents from natural sources

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As part of the Deployed War Fighter Protection (DWFP) program in search of environmentally benign mosquito control compounds, natural products and extracts derived from plants and fungi were investigated for larvicide activity. Bioassay guided fractionation of the extracts led to the isolation of two furanochromones from the seeds of *Ammi visnaga*, a member of Apiaceae family that showed larvicide activity. Phomalactone was isolated from ethyl acetate extract of *Nigrospora sphaerica* fungal culture broth*. N. sphaerica* is a plant pathogenic fungus of *Zinnia elegans* and *Hydrangea macrophylla*. Phomalactone showed larvicide and adulticide activities in permethrin susceptible and resistant *Ae. egypti* mosquitoes. Isolation, structure elucidation and biological activities will be discussed.

AGRO 157

Adulticidal and ovicidal activity of two plant-based formulations against the Northern fowl mite, *Ornithonyssus sylviarum*

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The Northern fowl mite (NFM), *Ornithonyssus sylviarum* is an important ectoparasitic pest of domestic fowl with obligate blood-feeding protonymph and adult stages. In laying hens and breeding chickens, NFM can infest birds at numbers as high as 10,000 mites per bird. Current treatments for caged layers utilize organophosphates deployed using high pressure sprayers. Laboratory bioassays were conducted to assess the mortality of two plant-based NFM treatments. Groups of 20 adult mites collected from infested white leghorn hens were directly treated with 201014-JO-6 (1% geraniol) or 080914-JO-6 (1% thyme oil) in filter paper-lined Petri dishes. Twenty-four hours after treatment, 99.1% of mites treated with 201014-JO-6 and 96.2% of those treated with 080914-JO-6 were dead. Mortality in water-treated controls was 1.2%. Current synthetic treatments do not kill or prevent hatching of NFM eggs. Ovicidal activity of 201014-JO-6 and 080914-JO-6 was examined using the methods described above with larval emergence recorded 48 h post-treatment. Emergence in water-treated controls was 70%, while only 1.6% of eggs treated with 080914-JO-6 hatched and no larvae emerged from eggs treated with 201014-JO-6.

AGRO 158

Hop extracts: A safe alternative for honeybee diseases

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Inflorescences of the female hop plant (*Humulus lupulus* L.) are known to contain hard and soft resins (mainly ɑ and β acids) and essential oils. The ɑ-acids (cohumulone and humulone) isomerise to iso-ɑ acids. The β-acids (colupulone and lupulone) do not isomerise and remain unchanged. Besides their use in the beer industry, the β-acids are known to repel and reduce oviposition of the two-spotted spider mite (Tetranychus urticae Koch) and the hop aphid (Phorodon humuli Schrank) (Hampton et al. 2002; Jones et al. 2003; Sopp et al., 1990; Jones et al., 1996). These natural plant-derived acaricides such as hop β-acids are a valuable alternative to synthetic ones and can be applied to other mites such as *Varroa destructor* (Anderson and Trueman). Varroa mites are major pest for the honeybee (*Apis mellifera* L.) and responsible for causing worldwide colony losses and devastation (De Jong et al. 1982; Rosenkranz et al. 2010). A variety of iso-ɑ acids and β-acids were screened against Varroa. The potassium salt of β-acids was selected for laboratory and field studies to determine its miticidal activity against Varroa mites. In vitro studies showed 100% Varroa mortality rate after 21 hours of exposure to 1% potassium β-acids applied topically (De Grandi-Hoffman et al. 2012). The data collection and analysis of numerous studies led to the development of HopGuard™ that contains potassium β-acid as the active ingredient. This delivery system successfully transfers the potassium β-acids onto the honeybee's bodies and distributes it throughout the colony targeting phoretic Varroa without harming the bees. HopGuard™ when applied to either package bees or splits of larger colonies can successfully reduce and maintain low mite population levels (De Grandi-Hoffman et al. 2014). HopGuard™ is considered a natural Varroa treatment and can be applied on organic beekeeping operations.

AGRO 159

Pesticidal principles from the seeds of *Terminalia mantaly H.* and their effect on two pests

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Considering the huge negative impact of conventional pesticides on man and environment, the search for non-toxic alternatives is pertinent. This led to the phytochemical screening, antimicrobial and pesticidal tests of the extracts of *Terminalia mantaly H.* seeds. The stem bark and leaves have been reported to possess antibacterial and antifungal activity but no use has been reported for the prolific seeds produced. Sequential extraction using petroleum ether, ethyl acetate and methanol yielded 5.01%, 6.16% and 6.52% crude extracts respectively. These extracts were subjected to vacuum liquid chromatography and TLC. Phytochemical tests were done by detecting major colour changes of standard methods. Minimum Inhibitory Concentration (MIC) of the seed extracts was determined by a modified broth dilution method. Phytochemical tests revealed the presence of flavonoids, glycosides, steroids, tannins and carbohydrates while anthraquinones, saponnins and alkaloids were absent. Antimicrobial investigations indicated that all test extracts as well as ciprofloxacin showed activity ranging from 18 to 41mm. MIC obtained varied from 0.625 to 2.50 mg/mL for all microbes and 0.625 to 5 mg/ml for *staphylococcus aureus, bacillus cereus, proteus mirabilis, candida pseudotropicalis* and *shigella dysentriae*. Pesticidal tests guarantee results similar to those of *afrostyrax lepidophyllus* where the pet. ether, ethyl acetate and methanol recorded mortality of 96%, 98% and 46% for beans weevil and 100%, 88.67% and 66.7% respectively, for cock roach. H^1-NMR confirms the structure of active fractions. This work points to the potential of *Terminalia mantaly H.* seeds as a veritable bio rational pesticide.

AGRO 160

Metabolism studies of environmental contaminants in plants using plant cell cultures and liquid chromatography-high resolution mass spectrometry

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Plants can extensively transform contaminants taken up via their roots or leaves through phase I and phase II metabolism to a large diversity of products. While plant metabolism is well studied for pesticides and considered in pesticide approval it has received far less attention with respect to environmental contaminants. Hydrophobic contaminants may reach agricultural fields by application of sewage sludge, antibiotics used in animal husbandry via manuring or polar contaminants via irrigation with treated municipal wastewater. In such cases plant uptake may be a path along which contaminants enter the food chain. Contaminant uptake may be significantly underestimated if the plant metabolism reduces the concentration of the parent compound. We have established an approach consisting of experiments with plant cell cultures to study the metabolism of contaminants in plants and LC-high resolution mass spectrometry to detect and to identify the metabolites formed in the cell cultures. Experiments with cell cultures are very fast, reproducible and can easily exclude bacterial degradation processes. However, whole plants are still needed to study translocation of contaminants and their metabolites in the different plant organs including its edible parts. Liquid-chromatography high resolution mass spectrometry using either TOF- or Orbitrap mass spectrometers is ideally suited to detect and to (tentatively) identify phase I and phase II metabolites of contaminants in plants. Target and non-target screening may be employed. To detect the relatively low number of relevant signals out of the huge data sets generated in non-target screening, different strategies of data processing are required which rely either on similarities and differences between samples or between the parent compound and its metabolites. While we first established this approach for metabolism studies of the moderately polar triclosan we are now employing it for more polar contaminants delivered with treated municipal wastewater upon irrigation, such as pharmaceuticals and industrial chemicals.

AGRO 161

Modeling of biological activity for improved efficacy and active compound identification of natural products used in the treatment of human diseases

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The efficacy of the use of natural products to treat human diseases is complicated by the inherent variation in chemical constituents of botanical materials and their interactions with the multiple developmental pathways that lead to the onset of cancers and other diseases in humans. We have developed a modeling approach that exploits this variation in chemical constituents to identify major and minor plant secondary chemical compounds impacting chemoprevention and to identify the impacts of separate chemicals and classes of chemicals on specific disease induction pathways. Our original model used ^{1}H-NMR of crude black raspberry extracts on cancer cell proliferation in HT-29 cell cultures. Our approach is designed to allow examination of biologically active constituents with any type of bioassay, with any type of natural product application. Furthermore, the approach is not limited to NMR applications, but can be adapted to use with LC, LC-MS and GC-MS methodologies.

AGRO 162

Potential for Insect herbivore pest management with chemical ecology

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Plants successfully employ a broad array of chemicals to defend against insect herbivores. If we can discover and understand the chemical and biochemical mechanisms used in natural plant defense systems they may be exploited for crop protection from insect pests. Promising new methods for practical application of chemical ecology for insect pest management are emerging.

AGRO 163

Developing species maps from FESTF's aggregated species location data for EPA's assessment of pesticides and endangered species

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Following the completion of the National Academy of Sciences panel (Panel) report entitled Assessing Risks to Endangered and Threatened Species from Pesticides, the Environmental Protection Agency Office of Pesticide Programs (OPP), the US Fish and Wildlife Service and the National Marine Fisheries Service (the Services), and the United States Department of Agriculture (USDA) developed an interim plan for implementation of the Panel's recommendations. One of the data sources that the Panel pointed to for species information was the FIFRA Endangered Species Task Force (FESTF), including data accessed by FESTF through license from NatureServe. After a number of interactions between FESTF and the Agencies, OPP requested that FESTF provide the spatial extent of FESTF's aggregated species location data on species maps for all federally listed species. Species location data were aggregated from existing government-sourced and publically-available sources, as well as through FESTF's licensed dataset from NatureServe. The maps were delivered in three phases, based on taxonomic group and geographic location, to EPA and the USFWS via the FESTF Information Management System (IMS). This presentation will discuss how the species maps were prepared and delivered by FESTF, including data sources and interactions with the Agencies.

AGRO 164

Examining the crop footprint of organophosphate insecticides when applied to a national level endangered species pesticide risk assessment

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EPA has selected three organophosphate insecticides, chlorpyrifos, malathion and diazinon, as part of a pilot test of its interim approach for conducting national level endangered species risk assessments during Registration Review. This presentation describes the outcome of spatially defining a product-specific crop footprint during Step 1 of the new evaluation process using EPA's proposed crop grouping strategy. Crop footprints are intended to represent the spatial extent of all labeled agricultural use sites of a given pesticide and are intended to define the Action Area (location of pesticide use plus off-site areas where the pesticide is presumed to be present at levels that may be potentially harmful to federally listed species). The first step in defining the crop footprint is to compile a master table representing all agricultural uses of the given pesticide based on EPA-approved product labels. Next, the uses are correlated to crop classes in the Cropland Data Layer (CDL). The crop classes are then grouped according to the 11 crop groups proposed by EPA in their Interim Approach. For each of the CDL crop groups represented by labeled crops, a crop group footprint will be created by aggregating crop classes representative of labeled crops from the most recent years of CDL data available, starting with 2009. After separate CDL crop group footprints are created for each pesticide-specific crop group, they will be aggregated into an overall crop footprint intended to represent the spatial extent of the pesticide's agricultural uses. At this time, it is understood that in using the crop footprints for the Action Area definition, EPA will consider the crop footprint to be a deterministic representation of the use site extent; a comparison between EPA's generic crop groups and more specific footprints that compose them, such as the one presented here for organophosphates, is discussed in an accompanying presentation by the FIFRA Endangered Species Task Force.

AGRO 165

Evaluating the potential impact of grouping CDL crop classes on the spatial extent of pesticide use sites

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In assessing the potential risk of pesticides to endangered species, the US Environmental Protection Agency Office of Pesticide Programs (EPA-OPP) has proposed reclassifying the 134 classes in the Cropland Data Layer (CDL) into 11 crop and 7 non-crop groups. These proposed groups are intended to delineate the spatial extent of labeled pesticide uses, a vital first step in establishing the Action Area for assessment of a given pesticide. Some of the proposed CDL groups include only one crop, such as "rice" but other groups such as "vegetables and ground fruit" include over 40 different crop classes. It is possible that a given pesticide is labeled for use on only one of the crops in a given group and the labeled crop is geographically limited; including the spatial extent of other crops in the corresponding crop group will expand the spatial extent to areas outside where the labeled crop is grown and thus where the pesticide will be used. Additionally, the maximum rates and treatment frequencies used on any one crop within the group have the potential to drive the "worst case" to all crops in that group, even though some crops may have a lower maximum use rate and reduced application frequency. This presentation will explore the FIFRA Endangered Species Task Force's evaluation of the potential impact that the proposed groupings can have on the spatial delineation of the extent of agricultural uses of a pesticide. This analysis will interpret the effect of various grouping strategies on the extent of the Action Area and lead to their impact on the estimation of potential risk. Additionally, the value of state data, where available, will be explored with respect to its impact on refining or clarifying the extent of the Action Area. An example examining the spatial extent of agricultural uses for a specific pesticide is discussed in an accompanying presentation for organophosphates.

AGRO 166

Terrrestrial endangered species assessment for chlorpyrifos: Initial analyses and results

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The US Endangered Species Act (ESA) states that, for listed species and their critical habitat, no harm should come from a Federal action such as registration of pesticides that could jeopardize their survival. Recently, a National Academy of Sciences panel described a 3-step process for conducting endangered species assessments for pesticides being considered for registration or re-registration. The first step is a conservative analysis to determine which listed species and their critical habitats could be at risk from exposure to the pesticide. Step 2 involves more refined analyses to determine if the pesticide of interest is "likely to adversely affect" or "not likely to adversely affect" each listed species and its critical habitat. Step 3 generally involves population modeling to determine if each listed species not screened out in Steps 1 and 2 is in "jeopardy" because of pesticide use. The 3-step process has been employed by the US Environmental Protection Agency and the Services as an interim approach for developing improved mechanisms for pesticide consultations during registration review. However, much method development and guidance is required before the process can be further developed or fully implemented. Those activities are on-going. In the meantime, the interim approach is being used with three "test" pesticides: diazinon, chlorpyrifos and malathion. The regulators are now actively engaged in the biological evaluations of three pesticides. In this presentation, we present our Step 1 analyses for terrestrial species potentially exposed to chlorpyrifos. Our Step 1 analyses began with a screening-level ecological risk assessment (SLERA) to identify listed taxonomic groups potentially at risk for direct or indirect (e.g., effects to habitat or prey availability) effects in treated areas. Those taxonomic groups not at risk were removed from further consideration. The analyses for granular and flowable chlorpyrifos were conducted separately as terrestrial receptors have very different exposure profiles for these formulations.

AGRO 167

Validating datasets representing non-agricultural pesticide use sites for the assessment of pesticides and endangered species

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The FIFRA Endangered Species Task Force (FESTF) has been responding to EPA for nearly 20 years by aggregating, updating, quality checking and validating data to support EPA's endangered species/pesticide assessments under FIFRA. One of the datasets that FESTF maintains is a collection of spatial data representing the location of pesticide use sites in both agricultural and non-agricultural lands. Before a given land use dataset is incorporated into FESTF's collection, an evaluation and validation process is conducted to ensure that the data are spatially representative of the labeled pesticide use. If a dataset is not representative of the corresponding labeled use(s), it could result in an inaccurate portrayal of where a given pesticide is going to be used and in turn lead to an over- or underestimation of risk. This presentation will demonstrate how non-agricultural datasets can be evaluated and then subsequently validated for use in EPA's assessments. For the purpose of providing an illustrative example, the focus will be a land use location dataset recently licensed by the FESTF, and very specific for certain land features such as golf courses. For this dataset, the validation process was relatively straight-forward due to the existence of other sources available to confirm the location of golf courses. Validating other non-agricultural datasets, such as those representing Christmas tree plantations, are not as straightforward and can pose a challenge when the need arises to accurately delineate these uses for assessment.

AGRO 168

Endangered species assessment for chlorpyrifos co-occurrence and proximity analyses: Initial results

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An endangered species ecological risk assessment is currently underway for chlorpyrifos, an organophosphate pesticide, and one of the three case study organophosphate pesticides (chlorpyrifos, malathion, diazinon) being evaluated by the Environmental Protection Agency (EPA). Recently, a National Academy of Sciences panel proposed a 3-step process for conducting endangered species assessments for pesticides being considered for registration or re-registration. The first step is a conservative analysis to determine which threatened and endangered species and their critical habitats could potentially be at risk from exposure to the pesticide. The spatial components of Step 1 include co-occurrence and proximity analyses. The chlorpyrifos labelled use patterns were used to identify the initial action area at the county level using data in the FIFRA Endangered Species Task Force (FESTF) Information Management System (IMS). Three years of NASS AgCensus data (2002, 2007, 2012) were used to establish this initial action area. The co-occurrence analysis identified species with and without spatial data and acted as the spatial starting point for the assessment. The next phase was the identification of species that have sub-county data (elemental occurrences and critical habitat) required to conduct the proximity analyses. Species without spatial data move to more refined ERA approaches. For remaining species, a proximity distance calculator was used to calculate proximity distances for each crop group. The use pattern with the highest application rate/number of applications associated with each custom crop group was used to generate the proximity distance. Inputs to the calculator include the federal family interim approach effects metrics and slopes for each taxon, and spray drift data (AgDrift, RegDISP, or chemical-specific spray drift). The proximity distance calculator calculates the distance off-field at which the predicted exposure drops below the level that would exceed the interim approach effects metric. Both direct and indirect effects are considered in the proximity analyses. Using a customized crop use footprint the proximity distances are used to evaluate the potential for exposure (i.e., the crop footprint will be 'buffered' using the proximity distances and the species spatial data will be overlain to determine where overlap occurs). Relationships between species (e.g. obligate species) taken into account where possible when conducting the assessment.

AGRO 169

Development of generic aquatic habitats for estimating pesticide exposure in threatened and endangered species

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The National Research Council Committee (NRC) recommended that the National Marine Fisheries Service, U.S. Environmental Protection Agency, and U.S. Fish and Wildlife Service adopt common assessment approaches in assessing risk to listed species. A major challenge in deriving accurate estimates of exposure in national- scale pesticide assessments is accounting for the diversity of aquatic habitats that threatened and endangered species occupy or use. To address this issue, NRC recommended that the agencies utilize pesticide fate and transport models to evaluate pesticide concentrations in generic aquatic habitats that are relevant to listed species. Physical characteristics of the aquatic habitats, including volume and flow rate, are key variables influencing both the magnitude and duration of pesticide exposure. This paper will present how these and other considerations were used by the interagency workgroup to establish nine generic aquatic habitats for use in the assessment of pesticide risks to federally listed threatened and endangered species.

AGRO 170

Transformation and fate of veterinary ionophore antibiotics in the water-soil-litter systems

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Ionophore antibiotics (IPAs) are a major group of veterinary pharmaceuticals that constitutes more than one third of the total antibiotic consumption by the livestock industry in the U.S. Our research team has conducted studies to achieve a comprehensive understanding of the occurrence, persistence, transformation, and fate of IPAs from poultry litter before and after applications to the agricultural lands. Based on the common management practices of poultry litter, the potential hydrolysis, photolysis and biotransformation of most commonly used IPAs, including monensin (MON), salinomycin (SAL) and narasin (NAR), were examined under varying conditions relevant to the water-soil-litter systems. The results showed that IPAs are prone to hydrolytic transformation in acidic environments and can undergo direct and indirect photolysis under UV light and sunlight irradiation. The biodegradation potentials of IPAs are highly influenced by water and temperature in the litter, as well as different degraders in soil microcosms. The inhibition and biotransformation potentials of IPAs differ under different redox conditions including aerobic, denitrification, sulfate-reducing and methanogenic conditions. The above knowledge is coupled with results from field-scale litter stacking and rainfall simulation studies to evaluate the environmental fate of IPAs and develop recommendations on litter management and IPA selection to minimize the release and risks of IPAs in the environment.

AGRO 171

Fate and effect of monensin during anaerobic digestion of dairy manure

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There is growing concern about environmental impact of residual antibiotics and feed additives in the manure of treated animals. Monensin, a polyether ionophore coccidiostat, is the only feed additive permitted for use in the U.S. for lactating dairy cows. Previous research has shown that up to 50% of the monensin administered to cattle is excreted in the manure. Anaerobic digestion is an established technology for animal manure and can be affected by monensin residues. The objective of this study was to determine the fate and effect of monensin during the anaerobic digestion of dairy manure. Duplicate plug flow field-scale digesters were operated using dairy manure and dairy manure amended with monensin to 1 and 10 mg l^{-1}. Digesters were operated for 8 weeks at 30 °C, an organic loading rate of 1.4 kg VS m^{-3} d^{-1}, and a 17-day hydraulic retention time. Results show that mean effluent monensin concentrations were 0.5 ± 0.1 mg l^{-1} and 3.3 ± 1.1 mg l^{-1} from the digesters amended with 1 and 10 mg l^{-1} monensin, respectively. Approximately 20% of influent monensin was associated with solids that settled in the digesters. Digesters operated with manure containing 1 and 10 mg l^{-1} monensin produced 12% and 75% less methane, respectively, compared to digesters operated without monensin. These results suggest that anaerobic digestion is not an effective treatment for removing monensin in dairy manure. In dairy manure at concentrations less than1 mg l^{-1}, monensin does not have significant effect on biogas production.

AGRO 172

Evaluation of three manure treatment systems for the removal of common veterinary antibiotics and antibiotic resistance genes

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Confined Animal Feeding Operations (CAFOs), including dairy farms, have been identified as important sources of veterinary antibiotics to the environment. The introduction of antibiotics into areas surrounding dairy operations has become a critical issue in evaluating the spread of antibiotic resistance genes (ARGs) to pathogenic bacteria, and raises concern for future drug efficacy. The removal of widely administered classes of antibiotics from three different dairy waste management practices is examined. This study investigates the relative removal of tetracycline, sulfonamide, and macrolide antibiotics and respective antibiotic resistant genes by treatment systems utilizing natural aeration, liquid separation, and advanced digestion. Ultrasonic extraction and clean up by solid phase extraction (SPE) is performed on both liquid and solid fractions for analysis by liquid chromatography- tandem mass spectrometry (LC-MS/MS). The study demonstrated tetracycline residues are persistent in both liquid and solid fractions. Advanced digestion, however, demonstrated significant differences ($p<0.01$) in the number of ARGs detected for sulfonamide (*sul1* and *sul2*) and macrolide (*ermF*) resistant genes in the absence of antibiotic residues. Tetracycline resistant genes (*tetO* and *tetW*), however, remained unchanged throughout the digestion process in the presence of detectable tetracycline residues. Persistent tetracycline residues and resistant genes indicate that current treatment systems, including advanced anaerobic digestion, is insufficient for the complete removal antibiotics from dairy waste.

AGRO 173

Impact of manure application technologies on the fate of pirlimycin and chlortetracycline in soil

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Antibiotics used in animal agriculture are of increasing environmental concern due to the potential for antibiotic resistance in the environment. On most farms, manure from animals treated with antibiotics is mixed with that of healthy animals and land applied to provide nutrients for crops. Yet, little is known about the impact of different manure application technologies on the environmental behavior of these antibiotics. Therefore, rainfall simulations were conducted to determine the fate and transport of pirlimycin and chlortetracycline antibiotics commonly used in dairy and poultry productions, respectively. Six treatments (surface, injection, and control with no manure) were setup in a randomized complete block design, using dairy manure spiked with pirlimycin and poultry litter spiked with chlortetracycline. Rainfall simulations were conducted immediately after and seven days after application of manure. Surface runoff water samples were filtered and analyzed using LC-MS/MS to determine levels of antibiotics in the aqueous phase. Filtered sediments from runoff water were analyzed to estimate the potential of particulate matter-assisted antibiotic transport via runoff. Soil samples were collected from all plots at two depths (0-5 cm and 5-20 cm). Soil samples were taken from the injection slit and between slits in the plots receiving the injected treatment to determine spatial variability. Preliminary results demonstrated that the levels of antibiotics in the soil follow the trend of Injection slit > Surface > between injection slits. Significantly higher level of antibiotics were detected in surface runoff samples of plots receiving manure surface application compared to subsurface injection. This investigation suggested that manure subsurface injection has the potential of reducing surface runoff of antibiotics to waterways. Thus, subsurface injection could be used as a best management practice to increase nutrient capture and reduce the input of animal manure-associated emerging contaminants to adjacent waterways.

AGRO 174

Antibiotic interactions at the solid-water interface: Implications for understanding sorption to soils and passive sampling of natural waters

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Several antibiotics of concern possess cationic and anionic groups and, therefore, occur as anions (e.g., cephalosporins), cations (e.g. azithromycin), or zwitterions

(e.g., fluoroquinolones) at environmentally relevant pH values. Ionic compounds possess unique driving forces for sorption to solid phases: cationic amine groups, and potentially anionic carboxylic and hydroxyl groups allow for interaction via cation exchange, surface complexation and cation bridging, if the appropriate receptor sites are available on the surface. This works explores how the knowledge of these interaction mechanisms can be leveraged to better anticipate antibiotic sorption to soils and design appropriate passive sampling devices. Our understanding of contaminant sorption and the design of passive samplers has been defined by legacy contaminants, such PAHs and PCBs. The sorption of these compounds to a solid phase is often driven by van der Waals interactions coupled with solvent effects (the so-called hydrophobic partitioning). Hence, the equilibrium sorption constant (K_d) can be predicted from partitioning to organic matter (K_{oc}) and the fraction of organic carbon (f_{oc}) present in the solid matrix. However, the $K_d = K_{oc}.f_{oc}$ paradigm is often invoked to anticipate and understand the sorption of ionic compounds (such as antibiotics), despite several cautionary calls that emphasize that the driving force for a sorption extends beyond hydrophobic exclusion and electron donor-acceptor reactions. This same viewpoint of the dominance of hydrophobic exclusion has also guided the design of passive samplers for polar/ionic compounds; samplers in current use primarily employ hydrophobic matrices. While these passive samplers are successful in monitoring concentrations of polar/neutral compounds, they are unable to account the effects of compound charge. This work demonstrates that accounting for antibiotic interactions with surfaces via cation exchange, surface complexation, and cation bridging can to improve our prediction of antibiotic sorption to soils and the methods of passive sampling in natural waters.

AGRO 175

Development and usage of bacterial bioreporters for monitoring antibiotics used in agriculture

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This study contains the construction of a bacterial bioreporter and the detection of tetracycline contamination with a recombinant *Escherichia coli* sensor strain. Bioreporters are living organisms, which can be used for detection of antibiotics from various types of samples with basic laboratory equipment in only a few hours. In bioreporters detecting antibiotics, the reporter protein genes are under the control of the same regulatory system, which also regulates the encoding of resistance genes in antibiotic resistant bacteria. This way the cells produce measurable signals ("lights on") in the presence of antibiotics. By also using a constitutively luminescent control strain ("lights off"), the interference of solid samples (quenching of the bioluminescent light by color or solid particles and/or toxicity), can be taken into account. We have used bioreporters applying this method for the detection of antibiotics from natural waters, milk, sediment and soil.
A previously constructed bioreporter strain, *E. coli* K-12 M72(pTETLux1), "lights on", and a control strain *E. coli* MC1061(pSLlux), "lights off", were used for the detection of tetracyclines in animal products (kidney and meat) with known tetracycline concentrations. A solid piece of meat sample was incubated in 96-well plate with "lights on" or "lights off" strain cell cultures for 2 h. Luminescence produced by both strains was measured from each well with a luminometer. Tetracycline concentrations were calculated using an external standard curve. The results were in agreement with those reported by Finnish Food Safety Authority (Evira) using chemical methods.
The results suggest that bioreporters detecting antibiotics could be utilized for measuring antibiotic concentrations which can activate the original resistance gene's regulatory system in bacteria. This would possibly give a better estimation than chemical methods of the mircobiological effects of antibiotic pollutants for example in agricultural environments.

AGRO 176

Effects of antibiotic mixture on the metabolism of adult zebrafish

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The presence of pharmaceuticals in the environment has been a growing concern worldwide. Of the pharmaceuticals, antibiotics are probably the most successful family of drugs which have been developed to improve human health. This has caused antibiotics to be released in large amounts in natural ecosystems. Although it is expected that various forms of life are being affected by these low concentrations of antibiotic mixture, it is challenging to quantify the subtle adverse effects on non-target ecological species. The application of environmental metabolomics, which is a study of the interactions of organisms with their environment, has brought limitless possibilities for the better understanding of the implications of antibiotic mixture in a different perspective. In this study, the 100 ppb of antibiotics clarithromycin, sulfamethazine, florfenicol, and their mixture, which did not have any visually observable negative effect on zebrafish, have been evaluated for their metabolic changes on adult zebrafish after 72 hours of exposure. Metabolites were extracted using bligh and dyer method with which the non-lipid layer was considered for analysis. Samples were analyzed using QTOF LC-MS and data from mass spectrometry were statistically evaluated using R. Manhattan plots and hierarchical clustering analyses show a clear separation of metabolites present in the control and the metabolites from fishes exposed to individual and mixture antibiotics. With the aide of metlin database, some potentially affected metabolites were identified. Metabolites such as phosphatidylcholine, arachidonic acid, and adenosine diphosphate were seen to be either upregulated or downregulated in the presence of the antibiotics. The results of this study imply that environmental metabolomics is a promising technology for revealing the subtle changes of ecological life due to the presence of various antibiotics in water systems.

AGRO 177

Scientific basis for GMOs

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Plant domestication is really genetic manipulation.

AGRO 179

Local agencies and GMO regulation

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State laws regulating or limiting GMOs have received significant public attention. The proposals have received mixed support in the state legislatures and at the ballot. So far only Vermont has adopted a statewide law regulating the use of GMOs. Receiving less national attention, local jurisdictions (counties) have also considered regulating GMOs. Since the first local ordinance limiting GMOs was passed by Mendocino County, California in 2005, other counties have likewise considered and debated GMO regulation. GMO ordinances have passed in Jackson County, Oregon, and Marin, Santa Cruz, and Humboldt Counties, California and Maui County, Hawaii. The jurisdictions have acted under their police powers to protect health, safety, and the public welfare. The ordinances, in general, restrict the cultivation, raising, or growing of GMOs within that jurisdiction. Some local jurisdictions, however, have taken the opposite approach, and have expressly permitted or encouraged the growing of GMOs. Some states have responded to local efforts by prohibiting local GMO regulation. This presentation will survey the field of local GMO regulation. It will compare the substantive provisions for both the adopted and failed local ordinances, including the specific manner of GMO regulation, enforcement mechanisms, and penalties. It will also discuss the results of litigation contesting the validity of these ordinances. It will discuss how science was used during the public debate leading up to vote by the local officials or the electorate, and will make suggestions how science may be used more effectively in the future to inform the GMO debate at the local level. Finally, it will show how these ordinances have been implemented and will consider the future of local governments regulating GMOs.

AGRO 180

Intellectual property rights in plants and animals – an overview

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This paper provides historical information about intellectual property rights (IPRs) in plants and animals. Many persons are surprised to learn that IPRs have existed, in various forms, in plants and animals since the 1930s. The paper compares and contrasts these various forms of IPRs in plants and animals. The paper also presents the argument for IPRs in plants and animals – i.e., what justifications exist for IPRs generally and specifically in plants and animals. After presenting the historical information and policy analysis, the paper discusses recent controversies in IPRs in plants and animals. Specifically, those controversies include the following:
a. What is patentable subject matter? Genes and products of nature;
b. Saved seeds and farmers' rights;
c. Expired patents and regulatory issues – generic or off-patent seeds;
d. Public research and IPRs;
e. Open Source discovery and synthetic biology – conceptual ideas to finished products.

AGRO 182

Survey of disputes involving GMO patent rights

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This talk will examine the history of GMO patents in the courtroom. It will involve a discussion of cases in which patent holders enforced their patent rights over farmers, declaration judgments sought by growers, seed selling business and agricultural organizations against GMO patent holders, as well as suits between competitive agribusinesses. The talk will include a discussion, for example, of Monsanto Canada Inc. v. Schmeiser, Bowman v. Monsanto Co., and Organic Seed Growers & Trade Ass'n v. Monsanto Co.

AGRO 183

Molecular breeding, gene editing technologies, and regulatory regimes – past, present, and future?

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This paper discusses the regulatory regimes for agricultural biotechnology that exist in the United States, the European Union, and the international convention of the Cartagena Protocol on Biosafety. The paper explains the impact of these regulatory regimes on agricultural biotechnology in plants and animals, in contrast to pharmaceuticals and food processing aides. After presenting the past and the present, the paper turns its attention to more recent breeding techniques of molecular biology – e.g. site-directed nuclease techniques such as RNAi, ZFNs. TALENs, CRISPR-Cas9. The paper ends with a discussion of a proposal for regulatory reform, giving the reasons for urging regulatory reform and the impact if regulatory reform is not forthcoming.

AGRO 184

Identifying the molecular basis of insecticide resistance in mosquito vectors and agricultural pests

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Insects pose tremendous threats to humans in two main areas: firstly, insect borne diseases cause severe global health problems, and secondly, the sustainability of agricultural output is seriously threatened by insects that devastate crop production. The main way to control insects is the use of insecticides. However, their intense use has resulted in the selection of resistant populations, whose control is extremely challenging. As only a limited number of different types of insecticides are available on the market and new ones are developed very slowly it is getting increasingly important to manage insecticide resistance, in order to ensure the sustainability of current vector control programs. Insecticide resistance management requires however understanding the molecular basis of resistance. We investigate the molecular mechanisms conferring resistance to major vectors of diseases, like *Anopheles gambiae* and *Aedes albopictus* and agricultural pests. Our approaches include transcriptomic studies, functional expression and characterization of recombinant proteins, immunohistochemical stainings, and *in vivo* ectopic expression in drosophila. Understanding the mechanisms responsible for insecticide resistance is leading to the development of new tools to overcome this resistance such as new synergists for improved insecticide formulations and molecular diagnostic tools for resistance management.

AGRO 185

Pyrethroid-resistant head lice: Updated status, lessons learned, and management in the 21st century

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Since the resurgence of human head lice in the 1990s, many cases of pyrethroid resistance known as the knockdown resistance (*kdr*) in global populations of head lice have been reported. This genetically heritable *kdr*, mainly conferred by

three point mutations (M815I, T917I and L920F) in the para-orthologous voltage-sensitive sodium channel gene, is a major attribute of pyrethroid-resistant head lice. Using a tier system of three molecular resistance detection protocols (quantitative sequencing, the real-time PASA and the serial invasive signal amplification reaction), *kdr* frequencies of field populations have been successfully estimated with considerations of sample collection conditions and accuracy of each genotyping method. Recently, quantitative sequencing (QS) suitable for a primary resistance monitoring tool to screen a large number of louse populations has been utilized to generate a detailed *kdr* allele frequency map that will illustrate distribution of *kdr* possessing head lice in the US. For this, our on-going study aims to collect samples from both urban and suburban areas. Preliminary results showed that 104 out of 109 populations were 100% resistant in the MI-TI-LF mutations in states ranging from Maine, Florida, Texas, Minnesota and California. Intermediate levels of resistance were found in 4 states (NJ, NM, OR, and NY). The only completely susceptible population was found in MI.

AGRO 186

Characterizing the physiological role of inward rectifying potassium channels in the insect nervous system

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Novel target sites to control mosquito disease vectors are needed due to the increasing prevalence of insecticide resistance. To combat the growing levels of resistance, our group is exploring inward rectifying potassium (Kir) channels as viable target sites for insecticide development. Although the functions of Kir channels in insects are significantly less understood when compared to mammals, it has been shown that they play important physiological roles in osmoregulation, immunity, development, and ion transport in insect Malpighian tubules. From a high-throughput screen of 75,000 small-molecules, we have identified a number of mosquito selective Kir channel inhibitors that induce mortality, presumably through Malpighian tubule failure. Although the Malpighian tubules represent a viable physiological target for mosquitocide design, the slow onset of intoxication lends the nervous system to be a more attractive target site. In the classical sense, Kir channels are known to maintain the resting membrane potential and regulate the firing threshold in mammalian neuronal cells, yet their role in the insect nervous system is currently unexplored. Therefore, this work aimed to characterize the role of Kir channels in the insect nervous system and determine the potential for the development of insecticides targeting neuronal Kir channels. The known mosquito Kir channel inhibitor, termed VU041, produced lethargic tendencies, uncoordinated movements, and death in *Drosophila* and mosquito adults, suggesting poisoning of the nervous system. Neurophysiological recordings show VU041 to inhibit the spike discharge frequency of the *Drosophila* larval CNS in the low micromolar range but interestingly, no change in the resting membrane potential of the body wall muscle was observed. Lastly, preliminary studies suggest VU041 is a complete inhibitor of the evoked EPSP in the body wall musculature of wandering *Drosophila* larvae. These data suggest Kir channels play a critical role in the insect nervous system and interpretations will be discussed.

AGRO 187

Pyrethroid insecticides elicit olfactory response in *Drosophila melanogaster*

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Pyrethroid insecticides are used extensively in control of human disease vectors as well as agricultural and urbane arthropod pests. Pyrethroids as well as pyrethrum, a natural botanical insecticide, and DDT exert toxic action by disrupting the function of voltage-gated sodium channels. Besides their direct insecticidal activities, it has been long known that these compounds induce behavioral avoidance. In particular, excito-repellency has been documented for mosquitoes in response to pyrethroid-treated bednets for malaria control. Furthermore, volatile pyrethroids, such as transfluthrin, are used extensively in mosquito coils, vaporizer mats and emanators to repel mosquitoes. However, the molecular mechanism underlying the repellency remains elusive. Using electroantennogram (EAG) and a two-choice behavioral assay, we discovered that adults of *Drosophila melanogaster* can smell and avoid pyrethrum and transfluthrin. *Drosophila* mutants lacking the coreceptor of olfactory receptors (ORs), Orco, failed to respond to these compounds. Single sensillum recordings from olfactory receptor neurons (ORNs) in basiconic sensilla on the antenna led to the identification of specific ORs that respond to pyrethroids. Our identification of pyrethroid-responsive olfactory receptors provides a new paradigm for the study of the role of spatial repellency in pyrethroid-treated bednets and a new platform for screening effective mosquito repellents.

AGRO 188

Comparison of immune responses between body and head lice following bacterial challenge

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Human body and head lice are obligatory human ectoparasites. Although both body and head lice belong to a single species, *Pediculus humanus*, only body lice are known to be a vector of several bacterial diseases. The higher vector competence of body lice is assumed to be due to their weaker immune response than that of head lice. To test this hypothesis, immune reactions were compared between body and head lice following infections by two model bacteria, *Staphylococcus aureus* and *Escherichia coli,* and a human pathogen, *Bartonella quintana*. Head lice suppressed the growth of *S. aureus* and *E. coli* effectively, resulting in gradual reduction of bacteria number both in hemocoel and alimentary tract. In contrast, the number of both bacteria steadily increased in body lice. Similarly, orally infected *B. quintana* cells proliferated more extensively in body lice compared with head lice. Although the numbers of *B. quintana* detected in feces from infected lice were almost identical, the viability of the bacteria was significantly higher in body louse feces, suggesting that reduced immune response in the alimentary tract is primarily responsible for increased vector competence of body lice. Transcriptional profiling of representative immune-related genes revealed that some immune genes were up-regulated in head lice following *S. aureus* challenge, whereas no apparent alteration of transcription was observed following either *E.*

coli or *B. quintana* challenge. Nevertheless, the basal transcription levels of major immune genes were lower in body lice than head lice. In addition, body lice exhibited a significantly lower phagocytotic activity against *E. coli* than head lice. These findings suggest that the increased vector competence in body lice is associated with the reduced immune response against Gram-negative bacteria, including human pathogens, which is largely due to the lower constitutive transcription levels of major immune genes and decreased phagocytosis.

AGRO 189

Neural and endocrine disruption of tick reproduction: New perspectives and control approaches

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Transcriptomic and genomic analyses of the tick synganglion and sensory systems along with bioassays has resulted in an improved understanding of tick chemorecption and tick endocrinology associated with reproduction. This work has resulted in a number of unexpected surprises about the systems under study and has resulted in new targets for the development of next generation arthropod repellents and acaricides. The work conducted has also served as the stimulus for the commercialization of control systems in the near term and for the re-examination of the toxicology of compounds already on the market.

AGRO 190

Uptake of three antibiotics and an anti-epileptic drug by wheat crops spray irrigated with wastewater treatment plant effluent

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With diminishing water supplies necessitating water reuse, wastewater treatment plant (WWTP) effluent is often used for the purpose of irrigating agricultural and cropped lands. Due to limited removal of pharmaceuticals and personal care products (PPCPs) during the WWTP process, concern has arisen about the fate of these compounds in the environment, especially with regard to plant uptake. This study's aim was to analyze the uptake of three antibiotics (sulfamethoxazole, trimethoprim, and ofloxacin) and an anti-epileptic drug (carbamazepine) in wheat plants that were spray irrigated with WWTP effluent. These four compounds have limited degradation during WWTP processes and enter the environment. Wheat was collected prior to and during harvest with plants divided into two groups: grain and stems/leaves. Subsets of each group were rinsed with methanol to remove compounds adhering to the outer surface. Prior to chemical analysis, all sets of plant tissue and grain were ground. Samples were, then, analyzed by liquid-solid extraction with an Accelerated Solvent Extraction (ASE) system followed by a solid phase extraction (SPE) cleanup with an Oasis HLB cartridge. Quantification of the compounds was performed by liquid chromatography – tandem mass spectrometry (LC-MS/MS). Residues of each compound were present on most plant surfaces with a concentration range of 0.29 ± 0.16 - 2.9 ± 1.1 ng g^{-1} and an average of 1.3 ± 1.6 ng g^{-1}. Compared to surface residues (2.1 ± 2.7 ng g^{-1}), trimethoprim had a lower plant uptake of 0.09 ± 0.15 – 0.92 ± 1.1 ng g^{-1}, while sulfamethoxazole, ofloxacin and carbamazepine had plant uptake that was similar to or greater than surface residues. Carbamazepine was found throughout the plant at varying levels (0.3 ± 0.06 – 1.3 ± 1.5 ng g^{-1}), while sulfamethoxazole was localized within the grain at an average concentration of 0.45 ± 0.34 ng g^{-1}, and ofloxacin was concentrated within the stems/leaves at 7.1 ± 5.3 ng g^{-1}. These findings demonstrate that PPCPs can be taken up into plant tissues as well as adhere to plant surfaces when WWTP effluent is spray irrigated. The presence of antibiotics and other PPCPs in the tissues and on the surfaces of plants used as food sources could pose potential health risks for humans and animals. Specifically, antibiotics in plants and food sources may select for bacteria resistant to antibiotics used in human and animal health and enable the spread of bacterial infections.

AGRO 191

Analysis of pharmaceuticals in food crops grown in urine- and struvite-fertilized soil by liquid chromatography tandem mass spectrometry

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Human urine is a cost effective, renewable resource that can be used as a valuable source of fertilizer because it is rich in nitrogen, phosphorus and potassium. As fertilizers derived from urine become more widely used, it is important to understand the transport of the pharmaceuticals from urine to the environment. It is known that many pharmaceuticals are excreted from the human body in their native form; hence when urine is used as fertilizer pharmaceuticals can be released as contaminants in the environment. The goal of this study is to develop a sensitive analytical method to measure trace pharmaceuticals in urine, struvite, lysimeter water, soil, and food crops. Percent recoveries range from 50-120% and detection limits range from 0.00089 ppb to 5.7 ppb across all matrices. Analyzing these matrices gives information about the pharmaceuticals present in urine that are carried over into the fertilizer, soil, ground water and eventually crops consumed by humans. This project involved a field study where soil plots were fertilized with urine and struvite prior to planting carrots and lettuce. Lysimeter water was collected after rain events and the soil was tested pre- and post- fertilization. In addition to the urine and struvite fertilized plots, two control plots were monitored, one using synthetic fertilizer, and one with no fertilizer. The carrots and lettuce were analyzed when market ready. All samples were extracted and analyzed via liquid chromatography mass spectrometry (LC-MS/MS). Preliminary results showed there is uptake of pharmaceuticals fertilized by urine in lysimeter water, soil, and food crops at low part per billion levels ranging from 16 ng/L in lysimeter water, to 11 ng/g in lettuce.

AGRO 192

Evaluation of benzylamine and salicylic acid as probes for pharmaceutical sorption to soils

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Fate of contaminants, such as antibiotics, pesticides, and pharmaceuticals, depend on the extent of sorption to environmental solids. Many of these compounds contain cationic and anionic groups and can be categorized as polar and ionic compounds (PIOCs). While predictive capabilities are well-established for non-polar and polar/neutral compound sorption, the same capabilities are lacking for polyfunctional ionogenic compounds. This study evaluates the potential for using benzylamine (BA) and salicylic acid as a mechanism-specific probe that (i) implicitly samples cation exchange and surface complexation/cation bridging sites,

respectively, on soils and (ii) offers a combined measure of both site abundance and a baseline driving force for sorption via these mechanisms. The solid-water distribution coefficient (K_d) for benzylamine, salicylic acid, desipramine and tramadol sorption to 30 well-characterized soils was measured. Our results revealed that $K_{d,BA}$ was a better predictor of the sorption of cationic pharmaceuticals than traditional soil characteristics such as cation exchange capacity (CEC). The extent of cationic pharmaceutical sorption is influenced by the identity and charge of naturally occurring cations (e.g. Ca^{2+} or Na^{+}) initially bound to negatively charged sites on the soil. Our analyses reveal that $K_{d,BA}$ captures the differences in the driving for pharmaceutical sorption to soils with similar CEC values but possessing distinct distribution of exchangeable cations. Similarly analysis of $K_{d,SA}$ values reveal the promise of using salicylic acid as a probe compound for potentially anionic pharmaceuticals.

AGRO 193

Structure based prediction of substituted pyridine cation exchange to soil aluminosilicates: Implications for antibiotics containing pyridine substructures

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Pharmaceutical and antibiotic sorption to soil minerals is an important determinant of environmental fate. Heterocyclic nitrogen moieties, such as pyridyl groups, are important substructures in modern antibiotics and pharmaceuticals. The pyridine moiety within several antibiotics is often positively charged at environmentally relevant pH values. Similar to other cationic amines, cation exchange is the primary mechanism by which pronated pyridine sorbs to soils and sediments. This study evaluates how structural variations affect pyridine sorption, with a final goal of developing a model capable of predicting the equilibrium sorption constant (K_d) for any substituted pyridine. This, in turn, will allow us to anticipate environmental fate of pharmaceuticals with pyridine moieties. Specifically, our study investigated how substituent identity and position on the aromatic ring influenced the K_d values for pyridine cation exchange to Ca-montmorillonite and illite. We find that K_d values for methyl-pyridines is greater than pyridine and increases by substituent location as follows: ortho < meta < para. An investigation of influential structural features revealed that compound hydrophobicity and dipole moment significantly influenced the extent of sorption. A model was built by comparing the experimental K_d values for methylpyridines against the value for pyridine, and quantifying the contribution of the methyl groups in the *ortho*, *meta*, and *para* positions. These substituent contributions (β values) were then used to compare the experimentally obtained K_d value of a dimethylpyridine against the K_d value predicted by this model. The predicted K_d value obtained from β values, for the *meta*-methyl and *para*-methyl substituents, was within a factor of two of the experimental K_d value. Our results point to the potential for expanding our predictive capability for sorption of cationic pharmaceutical compounds.

AGRO 194

Quantification of ionophore antibiotics in chicken litter and identification of their degradation products during different composting procedures

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Ionophore antibiotics (IPAs) are widely used in chicken production to treat coccidiosis and as growth promoters. Some of the top selling ionophores include monensin, salinomicin, lasalocid, narasin and maduramicin. These IPAs are poorly absorbed and not metabolized completely in the animal. Therefore, more than 80% of the ionophores are excreted and found in chicken litter. The land application of chicken litter as fertilizer in agricultural croplands is the main source of contamination by IPAs in the environment. Different composting processes can be applied to treat chicken litter before they are applied in the field, but very little information is known on the fate and behavior of IPAs during composting and land application. Thus, the goal of this study was to develop and validate a sensitive method for the determination of IPAs in chicken litter. It was also aimed to investigate the degradation products formed during different compost processes. Sample preparation was performed based on original QuEChERS method plus ultrasonic bath followed by dispersive solid phase extraction (D-SPE). Analysis was performed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), with electrospray ionization under positive mode (ESI+). External calibration using matrix matched curves were used for quantification of IPAs in chicken litter. Sample procedure was validated considering all the validation parameters. The method was applied to analyze IPAs in various chicken litter samples that have been subjected to different composting procedures.

AGRO 195

Understanding sources of aquatic contaminants of emerging concern

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Contaminants of emerging concern (CECs) have been detected in surface waters worldwide and include biologically active compounds originating from agricultural, residential, and industrial sources that may result in potential ecological and health effects. The objectives of this research were to determine what CECs are associated with specific land-uses, identify indicator compounds to be used as monitoring tools and provide science-based recommendations for more effective monitoring strategies. Water and sediment samples collected from four sub-watersheds of the Zumbro River Watershed, Minnesota, USA, were evaluated for pesticides, pharmaceuticals, antibiotics, phytoestrogens and wastewater contaminants. Agricultural herbicides and non-prescription drugs were more commonly detected than prescription drugs or phytoestrogens. The most commonly detected CECs were atrazine, caffeine, acetaminophen, the mosquito repellent DEET, and metalochlor. The antibiotics erythromycin and sulfamethoxazole were not found as frequently as other compounds, but were found in the greatest concentrations. Detection frequencies of the five most detected CECs were similar across the four subwatershed sites. Urban pharmaceuticals and personal care products (PCPPs) exhibited higher concentrations during low-flow periods, while run-off associated pesticides had higher concentrations during spring and summer high flow periods. PCPPs were significantly elevated in water or sediment at sites with greater population density and percentage of developed land use. We found unique marker compounds that would differentiate agricultural sources from non-agricultural sources. Cotinine, DEET, carbamazepine, erythromycin and sulfamethoxazole were found to be good markers for urban wastewater-derived sources, and atrazine, metolachlor and acetochlor were found to be good markers for agricultural sources. These results can be used to design more effective targeted sampling and monitoring programs. Greater understanding of the occurrence, concentration and sources of these contaminants will guide implementation of appropriate reduction and remediation strategies.

AGRO 196

Phytohormone levels in coconut (*Cocos nucifera L.*) water at three different stages of maturity

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Coconut water (CW) is the colorless liquid endosperm of coconut (*Cocos nucifera L.*) that is commonly used as plant tissue culture media. Phytohormones are of special interest because of the role that it plays in regulating the physical development of plants. Knowing which phytohormones in CW are predominant at a specific maturity stage may be used to determine the appropriate age of the nut to use for tissue culture. No detailed study to date related the age of the coconut nut to the composition and concentration of the different phytohormones in coconut water. Being present in very low concentrations, ranging from 1-100 ng/g fresh plant tissue, phytohormones in CW are difficult to analyze. Liquid chromatography tandem mass spectrometry (LC-MS/MS) then becomes the method of choice due to its high sensitivity and selectivity. In this study six phytohormones (indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA), kinetin (KiN), zeatin (ZEA), and 1,3 diphenyl urea (DPU)) in CW were simultaneously quantified using LC/MS/MS at 3 stages of nut maturity: young (4 months), green (7 months), and mature (12 months). ZEA (1.2 ± 0.3 µg/mL coconut water) was observed to be the predominant phytohormone in young nuts; ZEA is a known growth promoter. ABA (0.37 ± 0.08 µg/mL coconut water) and IAA (0.37 ± 0.09 µg/mL coconut water) on the other hand are observed to be dominant during the green stage while GA (1.3 ± 0.7 µg/mL coconut water) predominates in mature nuts. These results suggest that it is important to consider nut age in deciding about the appropriate CW for plant cell cultures. The presence of phytohormones in mature CW also suggest its potential as additional value to the coconut industry in countries where large volumes of CW waste is generated every day. One drawback of targeted analysis is the possibility of not seeing analytes that are not sought for. Profiling was done using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-ToF) to determine other phytohormones that are present. Among those putatively found are analogues and metabolites of ZEA and IAA, jasmonates, gibberellins, and brassinosteroids.

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Activation, potentiation, and antagonism of *Musca* GABA receptors by ivermectin

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Ivermectin (IVM) exerts insecticidal and antiparasitic effects by activating or potentiating inhibitory glutamate receptors (iGluR). In addition to this action, IVM was shown to act as an allosteric modulator of various receptors including γ-aminobutyric acid receptors (GABARs), nicotinic acetylcholine receptors, $P2X_4$ receptors, and glycine receptors of vertebrates. IVM was also reported to act on *Drosophila* GABARs as an allosteric agonist or an antagonist by different research groups. In this study, we performed a detailed electrophysiological analysis to examine the actions of IVM on housefly (*Musca domestica*) GABARs.
We expressed $\mathit{Rdl}_{\mathrm{ac}}$ encoding a housefly GABAR subunit variant in *Xenopus* oocytes and analyzed GABA- or IVM-induced currents in the expressed GABARs using a two-electrode voltage clamp technique. IVM alone activated *Musca* GABARs, with an EC_{50} of approximately 441 nM. IVM at 30 nM, a concentration that failed to cause GABAR activation, potentiated currents induced by GABA at concentrations below 18 µM (EC_{50}), whereas it inhibited currents induced by GABA at concentrations above 18 µM. Both the potentiation and antagonism became prominent as the IVM concentration increases up to 100 nM. The IC_{50}s of IVM antagonism of 18 µM and 100 µM GABA-induced currents were approximately 156 nM and 36.4 nM, respectively. A mutation (G333A) in the M3 domain markedly reduced the IVM activation and eliminated the IVM antagonism, whereas the IVM potentiation remained unchanged with this mutation. The molecular mechanisms underlying the three different actions of IVM on *Musca* GABARs need to be further explored.

AGRO 198

Evidence of ABC transporter(s) expression in vector mosquitoes

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Mosquitoes affect human health worldwide as a result of their ability to vector multiple diseases. Widespread insecticide resistance is a serious public health challenge that limits the use of high efficacy insecticides to reduce the risk of mosquito-vectored disease. ATP-binding cassette (ABC) transporters are efflux transporter that assists in maintaining the blood-brain barrier interface of insects and may serve as a first line of defense to insecticide exposure. Previous studies have demonstrated the blood-brain barrier of mosquitoes to interfere with target-site delivery and action of anticholinesterase chemistries; however, knowledge of these anticholinesterase interactions with mosquito ABC transporter(s) is unclear. Our previous studies summarize a toxicological and biochemical examination of ABC transporter(s) substrates and inhibitory ligands for the yellow fever mosquito, *Aedes aegypti* (Liverpool Strain). Here, we will summarize a molecular examination of ABC transporter(s) expression in the yellow fever mosquito, *Aedes aegypti* (Liverpool Strain). These data will be discussed with regard to the use of chemomodulators for increased selection of mosquitocides.

AGRO 199

Investigation into the role of PhABCC4 in ivermectin tolerance

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PhABCC4 is a C type ATP binding cassette transporter (ABC-C Type) that utilizes ATP to efflux negatively charged organic substrates across membranes, and is highly related to Mrp1 in *Drosophila melanogaster,* which is known to efflux insecticides. Use of a non-invasive induction assay on human body lice using sublethal concentrations of ivermectin followed by transcriptional profiling led to the identification of PhABCC4 as significantly overexpressed. Injection of dsRNA against PhABCC4 into lice resulted in increased sensitivity to ivermectin, implicating its role in phase III xenobiotic metabolism. Studies done in *Rhipicephalus microplus* showed that ivermectin efflux is sensitive to MK571, implicating the role of ABC-C type transporters in ivermectin tolerance. This current study utilized the *Xenopus laevis* oocytes to express PhABCC4 and directly investigate its role in ivermectin efflux. The *PhABCC4* gene was synthesized by Life Technologies (Carlsbad, CA) using the sequences available on NCBI, and capped mRNA transcribed using the mMessage mMachine kit. *Xenopus* oocytes were defollicated and injected with either *PhABCC4* mRNA or ddH$_2$0, incubated for 3 days to allow for expression, and then injected with [H^3]-PMEA (a known ABCC4 substrate in *Homo sapiens*). The efflux of [H^3]-PMEA was determined over time, and was significantly greater for *PhABCC4* mRNA-injected versus ddH$_2$0-injected oocytes as analyzed by liquid scintillation spectrophotometric analysis, confirming PhABCC4 expression. Using a cold PMEA competition assay, the EC_{50} value for PMEA was determined to be ~75 uM. Co-injection of [H^3]-PMEA and ivermectin in a similar competition assay led to decreased efflux of [H^3]-PMEA, indicating that ivermectin was competing at the same receptor. The EC_{50} value for ivermectin was determined to be ~3 uM. Future directions of this research are to directly measure [H^3]-ivermectin efflux over time in the presence of specific inhibitors such as quercitin, non-specific inhibitors such as verapamil, and efflux inducers such as Mg-ATP.

AGRO 200

Mutations in the inner pore and D3/D4 fenestration of cockroach sodium channel confer resistance to sodium channel-blocker insecticides

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Indoxacarb and metaflumizone belong to a relatively new class of sodium channel blocker insecticides (SCBIs). These insecticides preferably bind to and trap sodium channels in the slow-inactivated state, a mode of action similar to that of local anesthetics (LAs), such as lidocaine. A phenylalanine (i.e., F^{4i15} using a nomenclature that is universal for P-loop ion channels including sodium channels) in the inner helix S6 of domain IV (D4S6 hereinafter) is known to be critical for the action of LAs on mammalian sodium channels. Recently, two mutations, $F^{4i15}Y$ and $V^{4i18}I$, in D4S6 were found to be associated with indoxacarb resistance in several populations of the diamondback moth (*Plutella xylostella*) in China. Introduction of these two mutations into a cockroach sodium channel, $BgNa_v1$-1a, reduced the inhibition of sodium current by indoxacarb, DCJW (an active metabolite of indoxacarb), and metaflumizone in *Xenopus* oocytes. These mutations also reduced the use-dependent block of sodium current by lidocaine, providing the first molecular evidence for overlapping receptor sites of SCBIs and LAs on an insect sodium channel. We built a homology model of $BgNa_v1$-1 using the X-ray structures of an open potassium channel, $K_v1.2$, and a closed sodium channel, Na_vAb, as templates. In this hybrid model, V^{4i18} is found to face the open pore, while F^{4i15} seems to alternate between the pore and a wide fenestration lined by D3S6, D4S6 and D3P1 (P1-helix in domain III). To test this model, we examined five substitutions of three residues, T^{3i18}, L^{4i8} and F^{3p44}, that are located in D3S6, D4S6 and D3P1, respectively. Both $L^{4i8}A$ and $F^{3p44}A$ decreased the channel-blocking activity of DCJW and metaflumizone. However, the effects of other mutations were compound-specific. Mutations $L^{4i8}C$ and $F^{3p44}L$ reduced the inhibition of sodium current by DCJW, but did not alter the inhibitory effect by metaflumizone. Mutation $T^{3i18}A$ reduced the inhibition of sodium current by metaflumizone, but did not alter the inhibitory effect by DCJW. These results support the computational model of the open sodium channel and provide molecular evidence for the involvement of the inner pore and the D3/D4 fenestration in the binding and/or action of SCBIs. In addition, our results reveal potential differences in the interaction of DCJW and metaflumizone with sodium channels at the molecular level. Supported by NIH and NSERC.

AGRO 201

Insecticidal activity of stilbene derivatives and their mode of action on chloride and potassium channels

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Our laboratories have been investigating chloride and potassium channels as targets for the development of insecticidal compounds. In recent studies, we found that some substituted stilbene derivatives showed contact toxicity to mosquitoes. For the toxicological tests, nine compounds were tested on *Aedes aegypti* by using a surface contact (glass test tube) assay. A screening concentration of 3 µg/cm^2 was used, and after 24 hours observation, toxicities ranged from 0 - 100% mortality. The best compound had an LC_{50} of 1.1 µg/cm^2. This same compound was tested on *Anopheles gambiae* susceptible G3 and resistant Akron strains. The corresponding LC_{50} values were 0.82 and 0.92 µg/cm^2, respectively, indicating good activity against the malaria mosquito and no cross resistance from existing mechanisms. PBO pretreatment increased toxicity to *Aedes* mosquitoes about 10-fold, indicating a susceptibility to oxidative metabolism. Further, when the stilbenes were tested on *Drosophila melanogaster*, they did not cause any appreciable toxicity, suggesting unusual insect selectivity. To study the mechanism of toxicity, whole-cell patch-clamp experiments were performed. First, we tested their function on chloride current from Sua1B *Anopheles gambiae* cells. However, unlike DIDS, which could totally block chloride current at 100 µM, other stilbenes did not show any obvious effect below 1 mM. So, we tested the compounds on engineered HEK 293 cell line, which expressed the mosquito Kv2.1 channel, and SH-SY5Y cells, a human derived neuroblastoma cell line. The ability to block K$^+$ current on HEK cells showed some correlation with toxicity to mosquitoes, and activity was reduced on K$^+$ current on Sy5y cells. The implications of these data for development of new insecticides is discussed.

AGRO 202

Toxicity of the isoxazoline fluralaner to larval and adult *Aedes aegypti* mosquitoes

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Mosquitoes, such as *Anopheles gambiae* and *Aedes aegypti,* are important vectors transmitting mosquito-borne diseases. The purpose of this research is to investigate the mosquitocidal activity of isoxazolines, a new chemical class of GABA antagonist. In the first phrase of the study, the insecticidal activity of fluralaner (an oral veterinary parasiticide) was tested by bioassay. Fluralaner was toxic to *Aedes aegypti* mosquitoes in 24 hour exposures, with an LC_{50} (lethal concentration for 50% mortality) and LD_{50} (lethal dose for 50% mortality) values in larval (1.2 ppb) and adult topical assays (1.3 ng/mg), respectively. In adult topical assays, the LD_{50} value of fluralaner was 4-fold higher than fipronil, and 26-fold higher than permethrin. In time course studies, the insecticidal activity of fluralaner to adults increased by a factor of approximately two for each day of exposure, suggesting a slowly developing toxicity. The endpoint for fluralaner toxicity following topical application was about 5 days. The large size and lipophilicity of this molecule might influence its penetration through the mosquito cuticle and into the central nervous system. In adult contact paper assays, a high concentration of 11 micrograms/cm^2 of fluralaner could only kill around 17 % of the tested mosquitoes, which is much less activity than that observed on glass (LC_{50} = 14 ng/cm^2). The data suggest that fluralaner does not have exceptional toxicity to mosquitoes in typical exposure paradigms.

AGRO 203

Characterizing the range of sensitivities of aquatic and terrestrial plants to 2,4-D: A quantitative approach to selection and evaluation of data

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2,4-dichlorophenoxyacetic acid (2,4-D) is an herbicide used for post-emergence, selective control of broadleaf weeds in the United States since 1945. In consideration of its upcoming registration review, an effort is being made to reevaluate the state of knowledge pertaining to the range of sensitivities of terrestrial and aquatic plants to 2,4-D. In order to capture this range, a species sensitivity distribution (SSD) will be derived for relevant combinations of plants and endpoints. An SSD, which is an alternative to using a point estimate for the measure of effect (i.e., most sensitive species), is a statistical distribution derived from available toxicity data and visualized as a cumulative distribution function. SSDs have the advantage of making more use of the available toxicity data for a larger array of species, and describe the range of sensitivity rather than focusing on a single (e.g. lowest) value. The use of SSDs has been proposed for evaluating the risk of mortality to animals in pesticide endangered species assessments, and should also be scientifically appropriate for plants. As a first step in the derivation of SSDs characterizing 2,4-D toxicity to terrestrial and aquatic plants, a comprehensive literature review was conducted. Next, it was necessary to evaluate the reliability and relevance of data prior to consideration for use in the SSDs. A systematic approach was developed that includes identifying databases and other sources for locating data, developing reliability and relevance categories according to pre-determined validity criteria, and scoring the information using a tiered system to select key data for use in deriving the SSDs. This data evaluation process increases the overall transparency, clarity, and objectivity of the risk assessment process.

AGRO 204

Phage display based nanobodies and peptides in analysis of environmental chemicals by immunoassay

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Bio-recognition elements are the key to affinity based bioanalytical technology. Nanobodies or peptides obtained from phage display technology have drawn much attention in recent years due to their promising advantages in gene engineering. Camelid single domain antibodies (sdAb, nanobody or VHH) are increasingly popular due to their small size, high stability, ease of genetic manipulation, and ability for continuous manufacture. We have shown their use has resulted in assays for pesticide metabolites and industrial chemicals that are comparable to polyclonal or monoclonal antibody-based assays. Phage displayed peptides derived from random peptide libraries can be used to increase the sensitivity and safety of competitive assays by using them as coating antigens in heterologous competitive assays that are more sensitive than their homologous counterpart. Also, phage displayed peptides can be found that bind the complex of antibody and pesticide, thus uniquely creating a sandwich method for a small molecule. Several examples of nanobody and peptide-based assays for small molecules and proteins will be presented.

AGRO 205

Biological validation of enzyme-linked immunosorbent assays for detection of Bt Cry proteins in the environment

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The use of transgenic crops expressing genes for the production of crystalline (Cry) proteins for protection against pest insects continues to increase; however, concerns that these proteins may have detrimental effects on non-target organisms persist. Accurate detection of these proteins is essential to determine possible exposure of non-target organisms. . Enzyme-linked immunosorbent assays (ELISAs) are widely used for detection of Cry proteins in the environment. However, their results are not typically validated biologically to ensure that only bioactive Cry proteins are detected. Therefore, concentrations of the bioactive protein may not be accurately represented in ELISA results. This could potentially lead to overly conservative risk assessments and unnecessary regulation. Thus, there is a need for standardized detection methods that are biologically validated in order to properly study Cry

proteins in environmental matrices. This research project will improve methods of detection for Cry proteins in environmental matrices by developing a framework for biological validation of ELISA procedures. The first objective is to develop laboratory model systems to degrade Cry1Ab. These systems attempt to mimic degradation occurring in the environment and generate solutions of environmentally relevant Cry1Ab fragments. The second objective is to analyze aliquots of these solutions using ELISAs and bioassays. Results of ELISAs and bioassays will be compared to determine data anomalies, i.e., if the fragments are detectable by ELISA but have no bioactivity, or vice versa. Finally, specific guidelines will be suggested for the biological validation of ELISAs. Four model systems have been identified for generating fragments of Cry1Ab protein; 1) a chymotrypsin model system, 2) a proteinase K model system, 3) an acidic buffer model system, 4) a photodegradation model system. Results from ELISA and bioassay studies utilizing these fragments will be presented.

AGRO 206

Nasonov pheromone actives as repellents for pollinator-pesticide exposure

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The European honey bee, *Apis mellifera* L., is an economically and agriculturally important species that generates billions of dollars annually. Honey bee colony numbers have been declining in the United States and many European countries since 1947. A number of factors play a role in this decline, with pesticide usage being one of the most prominent. In order to mitigate pesticide exposure, new methods and regulations need to be created. One option is the use of chemicals that repel honey bees from recently sprayed crops. Consequently, this study was undertaken to look at the effects of botanical repellents. Synthetic Nasonov pheromone and its components geraniol, citral, and nerol, while toxic to bees, were found to inhibit feeding by honey bees in a laboratory setting. A choice assay and electroantennography were used to determine the lowest effective concentrations and detection limits of the compounds in order to facilitate screening as repellents.

AGRO 207

Discovery of resistance-breaking chemistries for varroa mite management

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The ectoparasitic varroa mite is a major pest of the honey bee and is a primary driver for the periodical losses of bee colonies. The varroa mite requires bees for food and reproduction and, in turn, elicits physiological deficiencies and disease transmission that compromise the health of bee colonies. The varroa mite nervous system is an established target site for existing acaricide chemistries. However, these acaricides not only have adverse health effects on honey bees, but resistance to these chemistries limits their use to reduce varroa mite infestations and disease transmission in honey bee colonies. The voltage-gated chloride channel is involved in the maintenance of nerve and muscle excitability in arthropod pests, which suggests that this target site might be exploited for novel acaricide chemistries. Here, we will report a toxicological analysis of a natural stilbene product, and related analogs, against acaricide-susceptible and -resistant varroa mite populations. These data will be discussed with regard to the target-site discovery and development of novel chemistries for varroa mite management.

AGRO 208

Toxicodynamics of the pesticide inert *N*-methyl-2-pyrrolidone and its impacts on honeybees

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N-methyl-2-pyrrolidone (NMP) is an inert formulant commonly found in pesticide formulations. As an inert, it is exempt from many of the testing requirements for registering pesticides, though there is very little publicly available data regarding its toxicity to invertebrate nontargets. Recent work has revealed harmful effects of NMP when administered to honeybee larvae in lab and field bioassays, and further investigation into the abundance and persistence of NMP in honeybee foraging areas will add perspective to these findings.

Using liquid chromatography coupled to a mass spectrometer, residues of NMP in the pollen and nectar of apple flowers will be determined following treatment with the NMP containing pesticide formulation, Rimon 0.83EC. Additionally, the results of laboratory bioassays exploring the toxicity and metabolism of field relevant doses of NMP to adult and larval honey bees will be presented. This research will elucidate the toxicodynamics and safety of NMP to *Apis mellifera* when it is used in pesticide formulations and will provide insight to regulators and researchers concerning the potential hazards of this inert formulant.

AGRO 209

Comparative analysis of herbicide-induced oxidative stress on honey bees

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Honey bee (*Apis mellifera* L.) colony failure is a problem that demands attention from the scientific, apiculture, and agriculture communities in Virginia. The decline of honey bee colony numbers in recent years presents an economic and ecological threat to agricultural systems and the services provided by these pollinators. One outstanding threat to honey bees is the unintended exposure to agrochemicals. Our previous studies have demonstrated that herbicide exposures affect mitochondrial electron transport and antioxidant activities in beneficial insects and, in turn, elicit oxidative stress responses that compromise the health of these insects. Here, I will summarize a comparative analysis of oxidative stress responses in honey bees following laboratory- and field-based exposures to current-use herbicides. These data will be discussed with regard to the potential health effects of agrochemical exposures and the failure of honey bee colonies in Virginia.

AGRO 210

Mode of action of insecticides

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The most widely used insecticides and acaricides are small molecules with a molecular weight under 1000 that act at specific target sites affecting either nervous system function, energy metabolism or growth and development. Many of the insecticides developed in recent years are highly selective for certain groups of pest insects and are remarkably safe to pollinators. Others act less specifically but can still be used in a pollinator-safe manner. This talk will give an overview of insecticide mode of action and the standard classification of insecticides and acaricides that is published and maintained by the Insecticide Resistance Action Committee (IRAC).

AGRO 211

Review of laboratory test procedures with the honey bee, *Apis mellifera* L., following current regulatory guidelines

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The various acute and chronic laboratory test procedures involving honey bee (*Apis mellifera* L.) larvae and adults for regulatory submission will be summarized. The appropriate tests based on the mode of action of the compound will be discussed as well as key points from each guideline. The different treated matrices, which include sucrose solutions, royal jelly diet, pollen, proteins, and organic solvents along with storage requirements or special permitting will also be presented.

AGRO 212

Survey for neonicotinoid insecticide residues in bee bread and comb wax from colonies in Washington State

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Laboratory and semi-field studies wherein honey bee workers are provided with contrived neonicotinoid insecticide-spiked solutions have shown sublethal behavioral effects. Field scale studies with colonies given access to field-treated crops tend not to support the hazards seen in the "no-choice" feeding studies. Reconciling perceptions of high hazards to bees and policies aimed to restrict neonicotinoid use depends on risk assessment of colony level effects associated with field exposures. However, too few studies of actual residues exist to adequately characterize risk from realistic field exposures. We hypothesized that analyzing for neonicotinoids in bee bread (stored pollen) from colonies located at multiple field locations could serve as a surrogate for regional field scale exposures of what bees might be exposed to during foraging. Honey bee colonies in 149 locations distributed among five regional areas of Washington State were sampled to collect comb packed with bee bread. About 90% of the sample locations were colonies managed by hobbyists and beekeepers not engaged in pollination services. Three commercial beekeepers engaged in pollination services were also included in the sampling program. An analytical enforcement method using LC/MS/MS was modified to achieve LODs of 2 µg/kg (2013 samples) or 0.5 µg/kg (2014 samples) for dinetofuran, thiamethoxam, clothianidin, and imidacloprid in wax and bee bread. Corresponding LOQs were 5 and 2 µg/kg in 2013 and 2014, respectively. Imidacloprid residues were confirmed in bee bread from one location during 2013. In 2014 detectable residues at non-commercial colony locations (#) were dinetofuran (1), thiamethoxam (3), clothianidin (2), and imidacloprid (1). Thiamethoxam and clothianidin only were detected in bee bread at levels <2 µg/kg at four and 12, respectively, of the different colony locations managed by the three commercial apiaries. Overall, residue data are consistent with a conclusion that non-commercial colonies are not likely to be exposed to and thus affected adversely by neonicotinoid residues. Commercial colonies faced a greater probability of exposure to thiamethoxam or it's insecticidal metabolite clothianidin, but the residue levels are arguably too low to make any conclusions about the likelihood of adverse sublethal effects at the colony level. Use of bee bread as a surrogate for pollen exposure may be useful for characterizing risk of exposure and adverse effects at the colony level.

AGRO 213

Assessing the potential risk of chlorothalonil to honey bees using the new risk assessment guidance for the United States and Canada

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Chlorothalonil is a broad-spectrum fungicide used to control various fungal diseases on many crops and can be applied during bloom to prevent diseases that could impact fruit development and potential yield. Due to the application timing, foraging bees have the potential to be exposed through contact or dietary routes. In this assessment, chlorothalonil toxicity data from adult acute oral and contact studies, adult chronic feeding studies, and larval feeding studies conducted with the honey bee, *Apis mellifera* L., were compared with chlorothalonil pollen and nectar residue data reported in the literature to determine the potential risk to bees using the methods outlined in the EPA/PMRA/CDPR Guidance for Assessing Pesticide Risks to Bees. Acute risk quotients based on adult contact and dietary and larval dietary exposures were below the established level of concern (0.4). Chronic risk quotients for adult and larval dietary exposures were also below the chronic level of concern (1.0). These results indicate that chlorothalonil is of minimal risk to honey bees via direct contact or ingestion of potential residues in pollen and nectar under acute or chronic exposure scenarios and current use patterns.

AGRO 214

Formulation composition makes the pollinator poison

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Assigning major factors for pollinator loss is constrained by lack of defined benchmark criteria for a healthy bee and undisclosed components that may be key to assess risk. Honey bees are sensitive to widespread co-formulants used in agrochemicals, and evaluation of the role of these 'inerts' in pollinator decline has only begun. Effects include learning impairment for adult bees and oral toxicity for larvae and adults. Billions of pounds of formulation ingredients from all uses are released into US environments, making this an important component of the chemical landscape to which bees are exposed. Most inerts are generally recognized as safe, have no mandated tolerances, and their residues are unmonitored. We have found 100% of co-formulants analyzed for in beehive samples, while only 70% of pesticide active ingredients searched for have been detected. Lack of disclosure of formulation ingredients in major products, and no or inadequate methods developed for their analysis, prevents the risk evaluation of total chemical load and agrochemical exposures for bees. Most studies to document pesticide effects on terrestrial non-targets like honey bee are performed without the formulation or other relevant spray adjuvant components used to environmentally apply the toxicant. Formulations are generally more toxic than respective active ingredients, particularly fungicides, by up to 26,000-fold based on published literature. Some 'inert' candidates for future risk assessment for pollinators include the organosilicone surfactants and the co-solvent N-methyl-2-pyrrolidone. Honey bees as a model terrestrial indicator organism reveal that the formulation composition and not just the dose of active ingredient makes the pollinator poison. We will continue to encourage industrial and regulatory scientists, and ecotoxicologists to include all formulations, and not just active ingredients, in studies to document the safety and low risk for pollinators of agrochemical products prior to registration and use, and to make this data publically accessible.

AGRO 215

Current-use pesticides in native bees collected from varying land cover areas in Colorado, USA

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Much attention has been given to the impact of pesticides on honeybees; however, less research has been conducted with native bees. There is little information on the potential exposure of native bees to pesticides in agricultural landscapes. To better understand interactions between native bees and pesticides, native bees were collected from Conservation Reserve Program (CRP) grasslands located in two different land cover areas (rangeland and cropland) in northeastern Colorado in 2013-2014 and analyzed for pesticides (in 2014 bees were also collected from wheat fields). Habitat quality and quantity are metrics currently being used to better understand the ecosystem services CRP fields provide for native pollinators. However, there is limited information on the effect of pesticide exposure to native bees as they forage in these areas. Bees were collected bi-monthly from May to September at each land cover type. The bees were analyzed for over 100 current-use pesticides, including neonicotinoid insecticides and fungicides (two groups of pesticides shown to be toxic to pollinators). Results of 25 composite samples from 2013 show that most of the pesticide detections in bees occurred in samples collected from the rangeland sites. The compounds detected in more than one sample include the neonicotinoid insecticides (clothianidin, imidacloprid, thiamethoxam), the insecticide fipronil and one of its degradates (fipronil desulfinyl), and the fungicide fluxapyroxad (a new fungicide first registered in 2012). The 2014 bees have yet to be quantified and further analysis will be conducted to compare pesticide exposure to nearby land uses and bee foraging areas. Information from this data set will help us better understand the exposure and accumulation of agricultural pesticides to native pollinators in an attempt to define potential effects at the population level, particularly on CRP lands where conservation efforts to promote pollinator health are being implemented.

AGRO 216

Risk assessment for imperiled butterflies exposed to a mosquito control pesticide on a national wildlife refuge

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Pesticide application for mosquito control threatens conservation of threatened and endangered species. In 2014, the Florida leafwing and Bartram's hairstreak butterflies were federally listed as endangered under the Endangered Species Act. Both taxa existed on the National Key Deer Wildlife Refuge where pesticides have been used to control adult mosquitoes. Laboratory and field studies, along with risk modeling, were conducted to assist resource managers responsible for conservation of these butterfly taxa. Acute exposure studies were conducted to estimate lethality and sublethal response (ChE activity). LD50 values (dose to the thorax) for 3 adult butterfly taxa ranged from 2.0 – 7.6 µg/g, while values in the literature ranged from 0.0012 – 30.1 µg/g. In addition, toxicity from wing-only exposure was evaluated following literature reports of lethality following wing-only exposure. Toxicity declined as the distance between the thorax and dose location on the wings increased. In addition to the laboratory studies, response of adult and larval butterflies after aerial naled applications was measured and compared to naled deposition onto pesticide samplers and to adult mosquito response during 4 field trials. Near complete mortality and greater than 90% ChE inhibition was observed for adult and larval butterflies in areas targeted by the sprays. Response of butterflies at locations adjacent to the targeted area indicated naled drift into the nontarget area at levels potentially hazardous (mortality and ChE inhibition) to nontarget butterflies. Adult butterfly exposure, based naled residues on the samplers, was compared to a species sensitivity distribution to estimate the likelihood of mortality following aerial naled applications. Risk likelihood for adult butterflies was high in areas targeted by aerial naled applications under existing field trial conditions. Results of this study indicate that it may not be possible to conserve threatened and endangered butterflies existing within areas targeted by aerial naled applications for adult mosquito control.

AGRO 217

Aquatic modeling to estimate pesticide exposure to threatened and endangered species

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The National Marine Fisheries Service, U.S. Department of Agriculture, U.S. Environmental Protection Agency, and U.S. Fish and Wildlife Service have been developing an approach to estimate pesticide exposure to threatened and endangered species for national scale assessments of pesticide registrations. This approach is based on recommendations from the National Research Council (NRC) Committee on Ecological Risk Assessment. This paper will outline procedures developed by the interagency workgroup for aquatic exposure assessment portion of Step 2 of the analysis, determining the likelihood of adverse effects to listed species at the individual scale. Topics covered will include a description of the fate and transport models to be used, model parameterization to estimate exposure in generic aquatic habitats with physical characteristics corresponding to habitat volumes and flow rates used by listed species, incorporation of geospatial data sets to derive exposure estimates for listed species that vary temporally and spatially, and procedures to account for uncertainty in the exposure estimates for different generic aquatic habitats.

AGRO 218

Aquatic endangered species assessment of chlorpyrifos: I. Overview and risk characterization in Step 1

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This presentation describes Step 1 of an aquatic endangered species assessment of the organophosphate insecticide chlorpyrifos that employs the "interim approach" being applied in registration review by the US Environmental

Protection Agency and the Services (US Fish and Wildlife Service, US National Marine Fisheries Service). Step 1 focuses on co-occurrence, the spatial overlap between Federally listed species range (species locations and designated Critical Habitat) and the Action Area (locations of chlorpyrifos use plus off-site areas where chlorpyrifos may be present at levels that may impact Federally listed aquatic species). Spatial delineation of the Action Area requires defining the chlorpyrifos use footprint as well as comparing predicted chlorpyrifos concentrations with metrics of direct and indirect effects – a screening-level ecological risk assessment (SLERA). In the SLERA, estimated exposure concentrations (EECs) in water bodies within or adjacent to the chlorpyrifos use footprint are predicted using EPA's Surface Water Concentration Calculator (SWCC) configured for relevant aquatic habitats representing static, flowing, and marine water bodies in each of the 18 large watersheds (designated by 2-digit Hydrologic Unit Codes, or HUC2s) that comprise the continental United States. For many groups of aquatic taxa, EECs in the SLERA are below the effects metrics; for these taxa, a "No Effect" determination can be made without the need for further analysis. For taxa whose effect metrics are exceeded by EECs in the SLERA, the extent of downstream transport and off-site chlorpyrifos drift where effects metrics are exceeded is calculated and added to the use footprint to determine the spatial extent of the Action Area. Listed species ranges are then overlaid on the Action Area to identify areas of co-occurrence where chlorpyrifos use "May Affect" the listed species, which are then further evaluated in Step 2 of the assessment.

AGRO 219

Aquatic endangered species assessment of chlorpyrifos: 2. Screening level exposure modeling, action area definition, and co-occurrence

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This presentation describes the screening level exposure modeling, action area definition, and co-occurrence analysis conducted as part of Step 1 of an aquatic endangered species assessment of the organophosphate insecticide chlorpyrifos that employs the "interim approach" for such assessments being tested by the US Environmental Protection Agency and the Services (US Fish and Wildlife Service, US National Marine Fisheries Service) in registration review. Screening-level aquatic EECs from EPA's SWCC tool are used to determine which species can be removed from further consideration in the refined assessment because conservative estimates of exposure do not exceed the relevant effects metrics. In Step 1, each species is assigned to the highest vulnerability aquatic habitat bin that could provide habitat for that species. Next, appropriate PRZM standard crop scenarios are chosen for each crop group and each HUC2 watershed, then associated with a species if it and the crop group occur within the same HUC2 watershed. For large HUC2 regions, multiple weather stations may be used to ensure the worst case weather is captured. The screening level modeling takes into account existing label mitigations, including buffers for spray drift. The spatial delineation of the Action Area is determined by expanding the chlorpyrifos use site footprint associated with each crop group by an off-site transport zone due to both spray drift and runoff downstream from use sites. The spray drift expansion zone is determined based on appropriate spray drift curves and the aquatic screening level effects metrics, unique for each crop group. A downstream dilution approach is conducted at the NHDPlus catchment level using crop group specific EECs assigned to relevant spatial areas of the use footprint. A crop group specific co-occurrence analysis is applied to determine which species may be impacted by each chlorpyrifos use pattern in each HUC2 watershed area.

AGRO 220

Using targeted monitoring to evaluate mitigation strategies that reduce pesticide loading to streams

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The recent National Academy of Science (NAS) recommendations for evaluating pesticide impacts on endangered species isolates the usability of ambient surface water monitoring data to a qualitative rather than quantitative role in the evaluation process. In 2014, WSDA staff met with NMFS biologists to develop a targeted monitoring approach that may result in improved utility and accuracy of mitigation. This first study evaluates reduced pesticide loading following aerial applications of pesticide to berry fields where woody riparian vegetation, approximately 6-10 meters in width, is maintained along adjacent waterbodies. Of the three pesticide a.i.'s currently applied by air for control of *Drosophila suzukii* in blueberries and raspberries in Whatcom County, Washington, the organophosphate insecticide malathion was selected for this study based on its pesticide consultation history and importance to the berry industry. Data was collected from ten individual application events across several study locations. Timed surface water samples were collected immediately before, during, and after aerial applications on adjacent fields. Depositional samplers were deployed within four transects adjacent to treated fields and collected after each malathion application. Study sites with riparian vegetation will be compared to control sites with no riparian vegetation in an attempt to evaluate the effectiveness of said vegetation in reducing pesticide loading to streams. The intent of the study is to better define potential mitigation measures that could continue to allow pesticide use and limit impacts on ESA listed species.

AGRO 221

National endangered species assessment for malathion: Case study

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An endangered species ecological risk assessment is currently underway for malathion, an organophosphate pesticide, and one of the three case study organophosphate pesticides (malathion, chlorpyrifos, diazinon) being evaluated by the Environmental Protection Agency (EPA). The case studies were initiated after the release of the National Academy of Sciences panel report and subsequent interim guidance provided by the Federal Family on conducting endangered species assessments. Malathion is an interesting case study for the endangered species process due to the large number of use patterns, relative to the other OP test chemicals. In this presentation, we present our problem formulation, Step 1 analyses, and our Step 2 analysis plan for aquatic and terrestrial species potentially exposed to malathion. Our Step 1 analyses began with a screening-level ecological risk assessment (SLERA) to identify listed taxonomic groups potentially at risk of direct or indirect (e.g., effects to habitat or prey availability) effects in treated areas and adjacent off-site transport zones. Subsequently, a co-occurrence analysis, refined crop footprints, and proximity analysis were used to develop the refined malathion action area. In Step 2 refined ecological risk assessment methods are employed to characterize risk for listed species and their critical habitats for those listed species that screen through from Step 1. Details on the approaches applied in Step 1 and Step 2 will be presented.

AGRO 222

Ecological risk assessment for Pacific salmon exposed to dimethoate in California

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A probabilistic risk assessment of the potential direct and indirect effects of acute dimethoate exposure to salmon populations of concern was conducted for three evolutionarily significant units (ESUs) of Pacific salmon in California. These ESUs were the Sacramento River Winter-Run Chinook, the California Central Valley Spring-Run Chinook, and the California Central Valley Steelhead. Refined acute exposures were estimated using the Soil and Water Assessment Tool (SWAT), a river basin scale model developed to quantify the impact of land management practices in large, complex watersheds. Both direct effects (i.e., inhibition of brain acetylcholinesterase activity) and indirect effects (i.e., altered availability of aquatic invertebrate prey) were assessed. Following refinements of exposure estimates to reflect realistic flow volumes, risk to salmon and their aquatic invertebrate prey items was determined to be *de minimis*. Therefore, acute exposure to dimethoate is not expected to have direct or indirect adverse effects on Pacific salmon in these three ESUs.

AGRO 223

Protecting endangered species from pesticides with stakeholder solutions

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Pesticide use has the potential to harm endangered species. Proposed federal protection measures would have prohibited pesticide use in habitat areas and buffer zones and consequently alarmed and alienated key stakeholders. Federal agencies have recently affirmed policies encouraging more stakeholder input, earlier in the regulatory process. California engaged diverse stakeholders using interest-based conflict resolution techniques. Voluntary protective strategies emerged that were practical, scientifically defensible, and credible among the regulated community. Delivering these solutions through an Internet-accessible database allowed users to obtain protection strategies customized to their location, proximity to endangered species habitats, and pesticide use preferences. State and federal fish and wildlife agencies endorsed the strategies, and county pesticide regulators appreciated practical alternative protective measures. Since most habitats are on private lands, voluntary compliance by landowners of practical measures encourages a cycle of greater protection for habitats and recovery of species.

AGRO 224

Stereoselective biotransformation of β-blockers and antidepressants in the aquatic environment

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Determination of the fate of individual enantiomers of chiral pharmaceuticals in the aquatic environment is critical in environmental risk assessment because enantiomers typically have different toxicological activities. The aim of this study was to establish if biodegradation in aquatic environment of β-blockers and antidepressants is predominantly stereoselective. Under laboratory conditions, the stereoselectivity of biotransformation of atenolol and fluoxetine was investigated in river water and wastewater effluents from a local municipal wastewater treatment plant (WWTP). In the river water microcosm test, biodegradation of R-atenolol was more pronounced than S-atenolol. Over a 10-day period, the enantiomeric fraction changed from 0.50 to 0.42. In contrast, the rate of biodegradation of S-fluoxetine was higher than R-fluoxetine, and the EF increased from 0.50 to 0.80. To determine whether enantiomers undergo chiral inversion, enantiopure standards were used in microcosm experiments. No chiral inversion was observed in river water. These results suggest that biodegradation of atenolol and fluoxetine in river water is stereoselective. Additional results on stereoselective biodegradation of atenolol and fluoxetine in wastewater will be discussed at the meeting.

AGRO 225

Transport of tetracycline antibiotics under field conditions

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Spreading livestock manure on soils is a common agricultural practice as a waste disposal method and fertilizer source. However, selection of resistant bacteria from the land application of antibiotic-contaminated manure might pose a significant human health concern in the United States and globally. Tetracyclines are the most utilized antibiotics in the livestock industry and account for more than 40% of the antibiotics utilized in the food animal industry in the U.S. Studies have reported the occurrence of Tetracyclines in agricultural fields fertilized with Tetracycline-contaminated manure and can accumulate in the soil with repeatedly application; yet little research has evaluated the fate and transport of Tetracyclines under field conditions. The objective of this study is to utilize a physical and chemical non-equilibrium model to quantitatively represent the transport of Tetracycline antibiotics under field conditions. The Two-Region and Two-Site models has shown reasonable agreement (r^2 = 91% to 99 %) to column transport experiments of Chlortetracycline (CTC) under laboratory conditions (no transformation or degradation). Under field conditions, transformation and dilution affect differently the overall selection pressure against or in favor of resistance. Tetracyclines sorb strongly to soils, yet are still bioavailable to microorganisms suggesting a potential scenario for resistance to develop; even at extremely low concentrations resistance can be selected (< 20 ng/ml). Therefore, is essential to understand the fate and transport of Tetracycline antibiotics on soil under natural conditions to subsequently address the question whether the usage of

Tetracyclines in the animal production industry is a significant contributor of resistance selection.

AGRO 226

Reconnaissance study of agricultural emerging contaminants (AECs) in the South Fork watershed of the Iowa River using polar organic chemical integrative samplers (POCIS)

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In the past 5 – 10 years, passive sampler technology has been developed for water quality monitoring, and the polar organic chemical integrative sampler (POCIS) is becoming one of the most commonly used devices. POCIS are manufactured to sample hydrophilic compounds such as antibiotics. In this reconnaissance study, we are using the POCIS to monitor agricultural emerging contaminants (AECs) in the South Fork watershed (SFW) of the Iowa River. AECs, are any agricultural naturally occurring or synthetic compound, or any microorganism detected in the environment, which is not routinely monitored, and has the potential to cause a known or perceived health risk to humans or the environment. The AECs of concern are tylosin, and sulfamethazine. Tylosin and sulfamethazine are antibiotics used in swine production. Atrazine was used as a frame of reference, due to its ubiquitous nature in the SFW. The SFW is an agricultural watershed, encompassing approximately 78,000 ha (193,000 acres). It's mainly dominated by corn and soybean production with an extensive amount of confined swine feeding operations. Located on the eastern edge of the Des Moines lobe, the landscape consists of poorly drained soils, resulting in 80% of the SFW in tile drainage. Previous monitoring studies in the South Fork watershed have resulted in non-detects or low detection of tylosin and sulfamethazine. POCIS have the ability to improve detection limits of chemicals that are ordinarily hard to detect with current sampling techniques. Three in stream locations (IATC-323, IABC-350, and IASF-450) and two tile drain (IATC-241 & IATC-242) were used in the study. Bi-monthly POCIS (n=2), grab samples (n=2), and sediment samples were taken at each sampling site. Grab samples and sediment samples were processed using solid phase extraction (SPE). Each POCIS was processed by removing the HLB sorbent from its housing followed by solvent extraction. All elutes were analyzed for tylosin A, sulfamethazine, and atrazine using an ABSciex 5500 QTrap with an Agilent 1260 Infinity LC. We developed and used laboratory calibrated sampling rates (Rs) for tylosin, sulfamethazine, and atrazine. This evaluation of the POCIS could provide an accurate picture of AEC concentrations in the SFW.

AGRO 227

Determination of antibiotics, estrogenic hormones, and UV filters in water, sediment, and crayfish from an urban watershed

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Antibiotics, estrogens, and UV filters have been widely used in medicine, agriculture, and personal care products for many years; consequently, these compounds have been detected in different environmental compartments. The appearance of these emerging contaminants in the environment has drawn increased attention due to potential impacts on human and ecological health. In urban settings with leaking wastewater infrastructure, these concerns are amplified by the constant input of emerging contaminants to sensitive ecosystems. Macroinvertebrates, like crayfish, might be useful bioindicators of ecological exposure to these emerging contaminants; however, studies on the uptake and toxicological effects of these contaminants in macroinvertebrates are limited. The objective of this study was to measure 34 antibiotics, three estrogens, and four UV filters in water, sediment, and crayfish (*i.e.*, *Cambarus bartonii* and *Orconectes virilis*) collected from urban watersheds in Baltimore, Maryland. Target compounds were analyzed using online solid-phase extraction liquid chromatography electrospray ionization tandem mass spectrometry. Partitioning of antibiotics, estrogens, and UV filters between the water, sediment, and biota phases was determined. Three antibiotics, namely ofloxacin, clarithromycin, and sulfamethoxazole, were detected in surface water from select locations at concentrations up to 1300 ng/L. Two UV filters (*i.e.*, benzophenone-3 and octocrylene) were present at all locations. Estrone (0.5-15 ng/L) was the only estrogenic hormone detected in the surface water samples. As UV filters and estrogenic hormones exhibit a high log D, these molecules were also identified in the sediment and crayfish samples. These findings confirm that the freshwater macroinvertebrates are exposed to, and accumulate, emerging contaminants; furthermore, given their prevalence throughout the United States, crayfish may serve as a model organism for ecotoxicological studies aimed at understanding ecosystem changes influenced by antibiotics and endocrine disrupting chemicals.

AGRO 228

Effect of Irrigation Water Quality on Antibiotic Persistence in Soil

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Antibiotics are being introduced into agricultural soils at trace quantities by irrigation with treated wastewater and concentrated animal feeding operation (CAFO) lagoon effluent. The cointroduction of antibiotics and the complex water matrix may have an impact on the fate of these chemical contaminants in soil by their interactions with dissolved organic matter, particulate organic matter, or nutrients in the irrigation water. An Illinois silt loam agricultural soil was hydrated to 50% of water holding capacity with one of 7 treatment waters that contained antibiotics, to create an initial soil concentration of 250 µg/kg for each compound. Periodically, for a total of 35 d, triplicate soil subsamples were serially extracted with sonication by acetone:methanol (1:1). Antibiotic residues in extracts were prepared by solid phase extraction (SPE) for analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). Treatments explored the relationships between antibiotic persistence in soil when introduced through pure lagoon effluent, sterilized lagoon effluent, sterilized lagoon effluent filtered to remove particulate organic matter, pure deionized water, and deionized water amended with nitrogen, phosphorus, or potassium. Decay kinetics of each compound were assessed for each treatment and statistically compared.

AGRO 229

Rapid screening of metabolism potential of pharmaceutical and personal care products (PPCPs) in plants using plant cell cultures

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With the increasing use of treated wastewater and biosolids in agriculture, residues of pharmaceutical and personal care products (PPCPs) in these reuse resources may contaminate food produce via plant uptake, constituting a route for human exposure. The final levels of PPCPs in plants depend on not only the uptake, but also metabolism within plants. However, at present little information is available on the metabolism of PPCPs in plants. In this study, the metabolism potential of 18 PPCPs was evaluated using a carrot cell culture. Ten PPCPs were found to be recalcitrant in the culture during a 90-h incubation, including trimethoprim, atenolol, acetaminophen, carbamazepine, DEET, diazepam, caffeine, meprobamate, primidone, and dilantin. Triclocarban was mostly adsorbed to the cell tissues and not metabolized in the culture. The other 7 PPCPs, i.e., atorvastatin, triclosan, sulfamethoxazole, diclofenac, ibuprofen, gemfibrozil, and naproxen, displayed rapid metabolism, with only 0.4-47.3% remaining in the culture after 96 h. After hydrolysis with glucosidases, a fraction (1.28-20.6%) of naproxen, diclofenac, ibuprofen, or gemfibrozil was found to be in the glucose-conjugated form. Six transformation intermediates were further identified for carbamazepine, four of which were reported for the first time in plants. Results from this study showed that cell cultures may be a useful tool for rapid and inexpensive screening of the metabolism capacity of PPCPs in higher plants and for exploring metabolic pathways.

AGRO 230

Influence of soil texture on the uptake of antibiotics in wastewater irrigated lettuce

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Increasing demands on water for irrigation necessitate the use of alternative water sources, such as recycled agricultural or municipal wastewater, which can contain traces of organic compounds including antibiotics. In this study, we evaluate the influence of soil texture on antibiotic uptake in wastewater-irrigated lettuce. Replicated experiments were conducted with lettuce grown in a greenhouse using sub-irrigation with water containing three antibiotics: lincomycin, sulfamethoxazole, and oxytetracycline. After six weeks, lettuce was harvested and samples of soil and lettuce were collected. Soil samples were taken from the top and bottom 2 cm of the soil profile. The remaining lettuce flats were then irrigated with uncontaminated water and harvested 5 and 10 days later. Direct plant toxicity, demonstrated by lower plant yield and discoloration, was observed in lettuce grown in soil with a high sand content. Mobility of the compounds within the soil and uptake in the plants was influenced by both soil texture and physicochemical properties of the antibiotics. Uptake of the antibiotics resulted in concentrations as high as 68 ng/g lincomycin in lettuce leaves. Concentrations decreased at ten days after antibiotic exposure which may indicate a reduction in the bioavailable fraction in the soil or degradation within plant leaves. Best management practices for the use of recycled wastewater in food crops should consider uptake of trace contaminants in irrigation and use an alternate water source prior to harvest

AGRO 232

Transformation of organoarsenicals in water using the UV and UV-H_2O_2 systems

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Roxarsone (ROX) and nitarsone (NIT) are used as additives in animal feeding operations and have been detected in animal manure, agricultural retention ponds, and adjacent surface waters. This work investigates treatment of organoarsenicals using UV-based treatment processes, namely UV irradiation at 253.7 nm and the UV-H_2O_2 advanced oxidation process. The apparent molar absorptivity was mapped for ROX and NIT across pH and wavelength. For UV irradiation at 253.7 nm, the fluence-based pseudo-first order rate constant (k'_p) and effective quantum yield (Φ) for ROX were $8.10–29.7\times10^{-5}$ cm^2/mJ and $2.34–8.37\times10^{-3}$ mol/Einstein, respectively; the corresponding constants were slightly lower for NIT. The observed rate constants are higher during advanced oxidation (*e.g.*, $k'_{p,ROX}$ = $3.92(\pm0.19)–217(\pm48)\times10^{-4}$ cm^2/mJ). Second order rate constants for organoarsenical transformation by hydroxyl radicals were determined to be $3.40(\pm0.45)\times10^9$ and $8.28(\pm0.49)\times10^8$ $M^{-1}s^{-1}$ for ROX and NIT, respectively. Solution pH and nitrate concentration did not significantly impact ROX transformation during advanced oxidation; however, bicarbonate and dissolved organic matter (sourced from chicken litter to represent agricultural wastewater/runoff) reduced ROX transformation through hydroxyl radical scavenging. Inorganic arsenic was the predominant transformation product of ROX during UV-H_2O_2 treatment; however, a mass balance approach did indicate that intermediate organo-arsenic compounds were formed.

AGRO 233

Colorimetric microtiter plate receptor-binding assay for the detection of freshwater and marine neurotoxins targeting the nicotinic acetylcholine receptors

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Anatoxin-a and homoanatoxin-a, produced by cyanobacteria, are agonists of nicotinic acetylcholine receptors (nAChRs). Pinnatoxins, spirolides, and gymnodimines, produced by dinoflagellates, are antagonists of nAChRs. In this study we describe the development and validation of a competitive colorimetric, high throughput functional assay based on the mechanism of action of freshwater and marine toxins against nAChRs. *Torpedo* electrocyte membranes (rich in muscle-type nAChR) were immobilized and stabilized on the surface of 96-well microtiter plates. Biotinylated α-bungarotoxin (the tracer) and streptavidin-horseradish peroxidase (the detector) enabled the detection and quantitation of anatoxin-a in surface waters and cyclic imine toxins in shellfish extracts that were obtained from different locations across the US. The method compares favourably to LC/MS/MS and provides accurate results for anatoxin-a and cyclic imine toxins monitoring. Study of common constituents at the concentrations normally found in drinking and environmental waters, as well as the tolerance to pH, salt, solvents, organic and inorganic compounds did not significantly affect toxin detection. The assay allowed the simultaneous analysis of up to 25 samples within 3.5 hours and it is well suited for on-site or laboratory monitoring of low levels of toxins in drinking, surface, and ground water as well as in shellfish extracts.

AGRO 234

Development and application of a salivary antibody 6-plex immunoassay to determine human exposure to environmental pathogens

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This study describes the development and application of a multiplex immunoassay using the Luminex xMAP™ platform to simultaneously measure specific antibodies to six common waterborne pathogens and evaluate its performance in a pilot study of saliva collected from beachgoers. Antigens from *Campylobacter jejuni*, *Helicobacter pylori*, *Toxoplasma gondii*, hepatitis A virus (HAV) and two genotypes of noroviruses (GI.1, GII.4) were coupled to Luminex carboxylated microspheres and used to measure salivary IgG responses from beachgoers in Puerto Rico. More than 5,500 saliva samples from over 2,000 study participants were prospectively collected upon visiting a beach, then two weeks and 6 weeks post-beach visit. Preliminary data show salivary antibody responses were log-normally distributed for all pathogen targets; antibodies to the noroviruses were most commonly detected, followed by *H. pylori*, HAV, *T. gondii* and *C. jejuni*. A cut-off that differentiates positivity from negativity was determined by calculating the mean plus 3 standard deviations of the control uncoupled microspheres as previously described. Prevalence of exposure to each organism in the sample population was calculated in the baseline samples and incident infections were estimated from immunoconversions (4-fold increase in specific antibody responses) in prospectively collected saliva samples. Further research may investigate associations between these antibody responses and self-reported health outcomes or swimming exposures. The Luminex multiplex salivary immunoassay may facilitate rapid, low cost, and non-invasive surveillance of waterborne infections as indicators of exposure in large epidemiological studies.

AGRO 235

Immunoassays for environmental contaminants using single domain heavy chain antibodies (VHH)

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Single domain heavy chain antibodies are a unique IgG type found in camelids and sharks. The binding domain lacks a light chain and therefore is smaller and less complex than common IgG making it more amenable to cloning techniques. The engineered antibodies, known as VHHs, have affinities to their antigens that are similar to the corresponding IgG. The VHHs have additional advantages such as thermal stability, stability toward chaotrophic agents, improved water solubility and ease of genetic manipulation. These characteristics plus the ability of the smaller antibody to penetrate membranes have made VHH a viable agent in clinical diagnostics and therapeutics. However, there have been few reports of the development of VHH for small molecules, in particular environmental contaminants. We have developed alpaca-derived VHH for flame retardants tetrabromobisphenol A (TBBPA) and polybrominated diphenyl ether congener 47 (BDE-47). Panning strategies for isolating practical VHHs included the use of heterologous selection antigens, early introduction of solvents, competitive elution with specific analyte, increased stringency of washing and post adsorption to decrease background binding. These strategies led to selection of VHH that were comparable in sensitivity and possessed similar cross reactivity patterns to the existing polyclonal and monoclonal assays. The versatility of the VHH was demonstrated by their use in classical enzyme immunoassay as well as in a microfluidic biosensor driven by a smart phone and an impedance biosensor. We also demonstrated the ease of genetic manipulation by fusing the VHH to a common reporter enzyme, alkaline phosphatase, for a one-step assay for TBBPA. Recombinant VHHs have proven to be an excellent alternative to existing antibody technology and should prove valuable for the next generation of immunoassay.

AGRO 236

Recombinant antibodies that distinguish between methylated and non-methylated derivatives of phenanthrene, a major polycyclic aromatic hydrocarbon present in crude oil

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Polycyclic aromatic hydrocarbons (PAHs) are a toxic and persistent class of environmental contaminants. Many unalkylated PAHs enter the environment as a result of incomplete combustion and are thus referred to as ‘pyrogenic’. In the crude oil, however, alkylated ‘petrogenic’ PAHs often predominate and methylated forms of phenanthrene are a major component of many crude oil samples. The ultimate goal of this research is to develop antibody tools to both identify petrogenic PAHs derived from oil spills and monitor their migration, accumulation and remediation. A recombinant scFv antibody library (~6x10^6 individual scFvs) was prepared from mice immunized with methylated phenanthrene-protein conjugates. Two selection systems, phage and yeast display, were used sequentially to select rare clones from this library that could bind to soluble phenanthrenes and/or methylated (alkylated) phenanthrenes. After three rounds of phage selection, selected scFv fragments were cloned into a yeast display vector to generate a yeast mini-library. Yeast display coupled with fluorescence-activated cell sorting (FACS) was used to analyze and select scFv pools that bound to soluble phenanthrene and/or methylated phenanthrenes. Finally, analysis of single cells isolated from these pooled samples allowed us to select three specific monoclonal scFvs, each with unique amino acid sequences and binding specificities as shown in the data from competitive ELISA. Antibody **A** had very little ability to differentiate among methylated versus unmethylated phananthrenes, while the **B** and **C** antibodies showed preferential binding to the 4-methyl and 2-methlyphenanthrenes, respectively. Studies are underway to isolate additional antibodies from this library and to further characterize the binding properties of all isolated scFvs. Supported by the NIEHS (1U19ES020677 and 1U19ES020676) and the National Science Foundation (OISE-1253272)

AGRO 237

Nanobody based immunoassay for soluble epoxide hydrolase detection using polyHRP for signal enhancement: The rediscovery of polyHRP?

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Soluble epoxide hydrolase (sEH) was shown to be a potential pharmacological target for treating hypertension, vascular inflammation, COPD, cancer, pain and multiple cardiovascular related diseases. We developed a single domain antibody (sdAb, nanobody or VHH) based immunoassay for sEH detection. We then improved its sensitivity significantly by using polymeric horseradish peroxidase (PolyHRP). Ten alpaca nanobodies highly selective for native human sEH were isolated from a phage display VHH library using magnetic beads. These nanobodies can be produced rapidly and cheaply in bacterial cultures, while also maintaining excellent selectivity for the target. The classical sandwich format consisting of the polyclonal anti-sEH (capture Ab), VHH (detection Ab) and the HRP labeled anti-HA tag (tracer) showed a marginal sensitivity (0.0016 OD�mL/ng) in low abundance tissues. However, the introduction of the PolyHRP as the tracer demonstrated a 130.6 fold increase in the sensitivity (0.2090 OD�mL/ng), indicating the powerful potential of the PolyHRP as a label. The enhanced sEH immunoassay using the PolyHRP was further evaluated in terms of selectivity against other epoxide hydrolases and detection of the target protein in tissue homogenates, including 6 human tissues and 10 B-lymphocyte samples. Comparison with the enzyme activity based assay and the Western-blot for sEH detection reveals good correlation of the immunoassay. The work demonstrates the promise of nanobodies for sEH detection specifically and pharmacologically and toxicologically important proteins generally and shows the value of rediscovering the PolyHRP for enhancing sensitivity after a long silence of several decades in the literature.

AGRO 238

Development of a proteomic-based technique for evaluation of natural removal of contaminants from groundwater

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The Department of Defense has over 26,000 contaminated groundwater sites with over 25% of the remedies-in-place using enhanced in situ bioremediation (ISB) and over 50% of remedies using monitored natural attenuation (MNA) as a polishing step. As the DoD transitions from costly active remediation to passive MNA, ISB is typically a requirement for a long-term monitoring of a wide range of chemical, geochemical, and microbial parameters to clearly prove that natural attenuation is occurring and/or progressing as expected. One such group of contaminants of major concern is chlorinated volatile organic contaminants (CVOCs). As part of the monitoring process, typically real-time PCR (qPCR) is used for the quantification of relevant microorganisms and/or genes involved in the degradation of CVOCs. However, measurement of the gene products (i.e., proteins) of interest would provide the advantage over qPCR measurements of CVOCs attenuation at a functional level. Such an advantage would likely be realized since gene copy numbers and transcript levels do not always correlate with protein levels.

While immunoassays and/or activity-based assays would offer a simple measurement of reductase protein levels or activity levels, such assays are not available for these settings due to a variety of challenges, such as: matrix interference, lack of antibodies that target reductive dehalogenases, large number of species that can degrade CVOCs (e.g., variation in protein sequence), and lack of facile methods to extract active proteins from groundwater. To this end, we have developed a targeted proteomic technique that enables identification and quantification of reductive dehalogenases from ground water samples. The approach was enabled by population analysis of contaminated groundwater samples (16S rDNA sequence analysis) followed by a bottom-up targeted proteomic analysis to identify peptides of interest. Following identification and verification of proteins of interest, a multiple-reaction monitoring (MRM) assay is under development. For future monitoring efforts, data from these analyses could be used to develop a greater understanding of natural attenuation or *in situ* bioremediation at sites contaminated with CVOCs, which may in turn inform decision makers on remediation actions. While here we demonstrated the technique for CVOC monitoring, the method is applicable to removal of other contaminants (e.g., pesticides, etc.) where immunoassays are not readily available.

AGRO 239

Effects of chlorpyrifos and TCP on human kidney cells using toxicity testing and proteomics

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An Adverse Outcome Pathway (AOP) is a conceptual framework to apply molecular pathway-based data for use in risk assessment and regulatory decision support. The development of AOPs requires data on the effects of chemicals on biological processes (i.e., molecular initiating events, key intermediate toxicity events, and proteomic changes) to link exposure to effects. Here, we describe the application of morphological, biochemical and proteomics analysis on human cell cultures to support AOP development. HEK293 (ATCC, CRL-1573) cells were cultured in DMEM supplemented with FBS and exposed to chlorpyrifos at 10, 30, 60, and 90 µg/mL, or 3,5,6-trichloro-2-pyridinol (TCP) at 50, 100, 150, and 200 µg/mL. After 24 h of exposure, the cells were removed from the culture plates, stained with trypan blue, counted and tested for cytotoxicity. Flow cytometry on cells stained with Annexin V and propidium iodide was also performed to determine apoptosis. Overall, the cytotoxic effect was in a dose and time dependent manner with the effect becoming more obvious with an increase in time and concentration. Using a proteomic approach, over 1000 peptides were identified and assigned to 372 proteins using high-resolution mass spectrometry. Data evaluation uncovered groups of proteins that consistently appeared to be up-regulated or down-regulated in response to the toxicity of the compounds used. We found that a group of 25 proteins was detected after the cells were exposed to either chlorpyrifos or TCP, including cDNA FLJ54290, ADP-ribosylation factor 3 and Serpinei mRNA binding proteins. A total of 19 proteins were detected after exposure to TCP alone whereas three proteins were detected after exposure to just chlorpyrifos. The addition of proteomic analysis to the study of exposure to TCP on human embryonic kidney cells provided a distinct correlation among cytotoxicity, apoptosis, cytokine expression, and the recovery of damaged cells leading to the discovery of putative biomarkers of exposure that still need to be confirmed and validated.

AGRO 240

Development and testing of genetically modified crop products throughout their life cycle

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Genetically modified crops have been the most rapidly adopted modern agricultural technology. These products are designed to address agricultural issues or needs of the growers. The development of a GM crop encompasses trait identification, gene isolation, plant cell transformation, plant regeneration, efficacy evaluation, commercial event identification, safety evaluation, and finally commercial authorization. This is a lengthy, complex, and resource-intensive process. Crops produced through biotechnology are the most highly studies food or food component consumed. Careful examination of the gene's performance and impact on the plant's performance and composition are just part of the assessment process that is undertaken on the path to market. Before commercialization, these products are shown to be as safe as conventional crops with respect to feed, food, and the environment. The global process and the various analytical tests that must accompany the product during the course of development, throughout its market life, and beyond are described.

AGRO 241

GPCR targets for new arthropod vector insecticides: Dopamine receptors

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New mode-of-action chemistries are needed for control of arthropod vectors of infectious disease as resistance to currently used commercial insecticides is a continuing problem. G protein-coupled receptors (GPCRs) represent promising targets for insecticide discovery because of their critical role in the neurological processes of arthropods. The Purdue Invertebrate Receptor Group (PIRG) is a collaborative effort designed to pursue insecticide discovery around these receptors. To date, multiple arthropod vector genomes have been mined by our group to identify suitable candidate GPCR targets. Initial efforts have focused on D_1-like dopamine receptors (DARs) from the yellow fever mosquito, *Aedes aegypti,* the malaria mosquito, *Anopheles gambiae,* the northern house mosquito, *Culex quinquefasciatus,* and the Lyme disease tick, *Ixodes scapularis.* Pharmacological profiles in response to biogenic amines and a panel of DAR antagonists and agonists were evaluated against these receptors expressed in reporter cell lines. The arthropod D_1-like DAR, DOP2, from these four vectors exhibits pharmacological properties distinct from human D_1-like DARs. High-throughput chemical library screening was employed to identify small molecule antagonists to *Ae. aegypti Aa*DOP2 and *I. scapularis Is*DOP2 targets. The most potent antagonists were further characterized via *in vitro* assays, and molecules with high selectivity (up to 140-fold) for the invertebrate target versus the human DAR, hD1, were identified. These compounds caused significant mortality of *Ae. aegypti* and *C. quinquefasciatus* larvae and *Ae. aegypti* adults *in vivo*. Our results demonstrate the promise of this strategy for identification of unique insecticidal leads that are effective against key vector species.

AGRO 242

Identification of immunogenic tick saliva proteins secreted into the host during 24-48 hours after attachment

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Chemical acaricide application is the predominant mechanism of tick control worldwide. However, the issues of resistance development and environmental pollution have shifted the focus to developing new, more efficient, and environmentally friendly strategies. One of these possible alternatives is vaccination against ticks, which is supported by evidence that some hosts can develop immunity to tick infestation. Our research goal is to identify immunogenic saliva proteins that ticks inject into the host during the first 24-48 hours after attachment, which represent valuable targets for anti-tick vaccine development. Our study was focused on *Amblyomma americanum* and *Ixodes scapularis* ticks species, because of their abundance and medical importance in the United States. We used rabbit antibodies to 24-48 hour-fed tick saliva proteins for screening phage display cDNA expression libraries, prepared from whole female ticks fed for 24 or 72 hours. After four rounds of biopanning, immune-sensitive cDNA libraries were subjected to next generation sequencing, *de novo* assembly, and bioinformatic analysis. A total of 895 and 182 contigs were assembled from *A. americanum* and *I. scapularis* biopanned libraries, respectively. Based on BLAST analysis and observed matches in GenBank, contigs were provisionally identified and classified as proteases, protease inhibitors, ligand binding proteins, enzymes, ribosomal proteins, proteins of miscellaneous function, and orphan immunogenic tick saliva proteins that are conserved in ticks but not in mammals. Data in this study provide the foundation the tick feeding proteins exposed to the host during the first 24-48 hours after attachment. Immunization targeting immunogenic proteins that are involved in tick feeding in the first 24-48 hours after attachment might impede the feeding process before the majority of tick borne pathogens can be transmitted.

AGRO 243

G-protein-coupled receptor/PKA signaling pathway in insecticide resistance in the mosquito, *Culex quinquefasciatus*

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G-protein-coupled receptors (GPCRs) are involved in signal transmission, which play pivotal physiological roles in insects, and have been proposed as a regulatory factor in mosquito insecticide resistance. Characterizing GPCRs and their regulatory pathway in insecticide resistance is fundamental for understanding the development of insecticide resistance in mosquito. Our current study has found that the expression pattern of a GPCR gene was significantly overexpressed in resistant mosquitoes and this overexpression was increased in mosquitoes following permethrin selection. Functional study through double-stranded RNA-mediated interference (RNAi) revealed that knockdown of GPCR gene in resistant mosquitoes resulted in increased susceptibility to permethrin, simultaneously causing the decreased gene expression of a protein kinase A gene (PKA, the downstream effector of GPCR pathways) and 4 resistance-related cytochrome P450 genes. The involvement of the PKA gene in permethrin resistance through the GPCR regulatory pathway by regulation of P450 gene expression was further characterized. Our study suggested an important role of the GPCR/PKA-mediated regulatory pathway governing P450 gene expression and P450-mediated resistance in *Culex* mosquitoes.

AGRO 244

Sabadilla vs. pyrethroids: A comparison study of toxicity and characterization of insecticidal modes of action

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Insecticide resistance is an increasing problem in many insects that vector disease. Currently, the pyrethroid class of insecticides is the only approved compound class for insect treated bednets for the control of mosquitoes that vector disease. Target-site resistance due to point mutations occurring in the voltage-gated sodium channels has been attributed to the reduction in efficacy of standard use pyrethroid insecticides on the market. Long-lasting insecticidal bednets incorporating the use of piperonyl butoxide as a synergist to suppress metabolism-based resistance and increase the efficacy of the pyrethroid to both susceptible and resistant mosquitoes have been implemented as a further pest management strategy. Yet, the insecticide resistance problem is ever present, and the need for novel target site resistance breaking mechanisms is even more imperative. To contest the increasing frequency of multiple resistance mechanisms in mosquitoes, the goal of this research is to examine the use of established sabadilla alkaloids as alternative chemistries to mitigate pyrethroid resistance in mosquitoes that vector disease. The objective of this study is to estimate the acute toxicities of standard-use pyrethroid mosquitocides and experimental sabadilla alkaloids to insecticide-susceptible and -resistant *Aedes aegypti* mosquitoes. This information will not only improve chemical control strategies for mosquitoes, but will guide the discovery and design of safe and selective mosquitocides in addition to the development of resistance-breaking chemistries for vector mosquito management.

AGRO 245

Novel roles of DSC1 and interactions of DSC1 with para in determining the sensitivity of pyrethroids and DDT

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Voltage-gated sodium channels (i.e., Na_v channels) are critical for electrical signaling in the nervous system and are the primary targets of the insecticides DDT and pyrethroids. In *Drosophila melanogaster*, besides the canonical Na_v channel, Para (also called $DmNa_v$), there is a sodium channel-like cation channel called DSC1 (*Drosophila* sodium channel 1). Temperature-sensitive paralytic mutations in $DmNa_v$ ($para^{ts}$) confer resistance to DDT/*pyrethroids*, whereas DSC1 knockout flies exhibit enhanced sensitivity to pyrethroids. To further define the roles and interaction of $DmNa_v$ and DSC1 channels in pyrethroid neurotoxicology, we generated a $DmNa_v$/*DSC1* double mutant line by introducing a $para^{ts1}$ allele (carrying the I265N mutation) into a *DSC1* knockout line. We confirmed that the I265N mutation in a $DmNa_v$ variant reduced the sensitivity to two pyrethroids, permethrin and deltamethrin in sodium channels expressed in *Xenopus* oocytes. Computer modeling predicts that the I265N mutation confers pyrethroid resistance by allosterically altering the second pyrethroid receptor site on the $DmNa_v$ channel. Furthermore, we found that I265N-mediated pyrethroid resistance in $para^{ts1}$ mutant flies was almost completely abolished in $para^{ts1}/DSC1^{-/-}$ double mutant flies. Unexpectedly, however, the *DSC1* knockout flies were more resistant to DDT, compared to the control flies (w^{1118A}), and the $para^{ts1}/DSC1^{-/-}$ double mutant flies were even more resistant to DDT compared to the DSC1 knockout or $para^{ts1}$ mutant. Heterologous expression of DSC1 in the presence of DDT demonstrated that DDT interacts with DSC1. Furthermore, a mutation in DSC1 (D1924N) negated the effect of DDT on DSC1 under voltage-clamp conditions. These findings revealed distinct roles of the $DmNa_v$ and DSC1 channels in the neurotoxicology of pyrethroids and DDT and implicate the exciting possibility of using DSC1 channel blockers or modifiers in the management of pyrethroid resistance.

AGRO 246

Activity of voltage-gated potassium channel blockers and their potential as new type of insecticide to control disease vector mosquitoes

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In the current study, we evaluated the effects of N-((1-phenylcyclopentyl)methyl)benzamide derivatives for toxicity to mosquitoes and for potency of inhibition of delayed-rectifier potassium currents by electrophysiological methods, including whole-cell patch clamp and muscle depolarization methods. For whole-cell patch clamp experiments, we tested 17 compounds on a HEK 293 cell line expressing the malaria mosquito *Anopheles gambiae* Kv2.1 channel, and a homologous channel in SH-SY5Y cells, a human-derived neuroblastoma cell line. All the compounds showed potential to affect potassium currents. Most of their half inhibition concentrations on the amplitude of potassium currents were at the micromolar level. The IC_{50} values of the most potent compound (2S-65465) was 217 nM on HEK 293 cells, and 194 nM on Sy5y cells. From muscle depolarization assays, we also found that depolarized muscle of yellow fever mosquito, *Aedes* aegypti, larvae with an EC_{50} value of 3.9 µM. Toxicity assays on the malaria mosquito, *Anopheles gambiae*, were done using WHO paper assays, as well as topical assays against adult females. In the WHO paper assay (1 mg/ml), after 24 hours observation, 6 of 17 compounds showed ability to kill mosquitoes. Again, 2S-65465 showed the highest toxicity with 82% mortality, and in topical assay, its LD_{50} was 230 ng/mosquito. The results suggest that N-((1-phenylcyclopentyl)methyl)benzamide derivatives were effective blockers of delayed-rectifier potassium current, and some are potential leads for novel insecticidal compounds.

AGRO 247

2015 Kenneth A. Spencer Award address: A career in crop protection discovery

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Following graduate studies in synthetic organic chemistry, I joined DuPont in 1979 to pursue a career in crop protection discovery. Sales of the agrochemical business at that time were less than 2-3% that of corporate but there was significant growth with each new decade. I and other chemists were hired at that time to work on a newly discovered class of potent acetolactate synthase (ALS) inhibiting herbicides called sulfonylureas. The discoverer, George Levitt, was a great mentor to many of us starting our careers. I was encouraged to explore for new areas of biological activity and the eighties were an exciting time for the agrochemical research community as far as new product discoveries. However, I soon discovered how challenging finding new chemistry opportunities could be. In the mid-eighties I did have success in discovering a novel class of triazolopyrimidine herbicides only to find that long soil residual properties precluded commercial development. Fortunately in the mid-nineties, I was part of team involved in the discovery of the powdery mildew fungicide proquinazid where photostability issues around early analogs were successfully addressed for commercial advancement. A number of other novel herbicide and fungicide programs were undertaken with some candidates undergoing extensive worldwide field testing. One herbicide candidate was selected as a commercialization candidate in the late nineties but subsequently abandoned. It was not until the early 2000s that I had opportunity to work in one of the most exciting research areas of my career, a new mode-of-action class of potent insecticides called anthranilic diamides. Our effort on this chemistry gave rise to two exciting products: chlorantraniliprole and cyantraniliprole that provide excellent levels of broad-spectrum insect control. Having worked in all three disciplines (herbicides, fungicides and insecticides) over four decades, highlights from my discovery career will be presented with some valuable lessons learned along the way.

AGRO 248

Mesoionic insecticides: A novel class of insecticides that inhibit rather than activate nicotinic acetylcholine receptors

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A novel class of mesoionic compounds has been discovered with exceptional insecticidal activity on a range of Hemiptera and Lepidoptera. These compounds have been found to have a potent desensitizing effect on the nicotinic acetylcholine receptor which correlates to their insecticidal potency. The discovery, synthesis, biological activity, structure-activity relationship and mode of action will be discussed.

AGRO 249

Synthesis and SAR studies of insecticidal pyridazin-3-yl amides, hydrazides, hydrazines, and hydrazones

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Current global crop losses due to pest damage are estimated to be between 10 – 16 %. Dow AgroSciences is part of the world wide R & D effort to develop novel fungicides, insecticides and herbicides to overcome the on-going development of resistance to current crop protection products. The synergy resulting from having these three research areas 'under one roof' has resulted in the discovery of insecticidal activity in a series of compounds initially intended as herbicides. This presentation will outline how efforts to find biosteric replacements for a synthetically challenging motif led to the discovery of pyridyl pyridazines with activity against cotton aphid and green peach aphid. Development of synthetic routes and a description of structure-activity relationships (SAR) will be discussed.

AGRO 250

Synthesis and insecticidal activity of *N*-(5-aryl-1,3,4-thiadiazol-2-yl)amides

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While investigating a series of insecticidal *N*-(6-arylpyridazin-3yl)amides, the isosteric replacement of a pyridazine ring with a 1,3,4-thiadiazole ring led to a new class of insecticides with activity against a variety of sap feeding pests. Optimization of both the aryl ring and the amide substituent of the new *N*-(5-aryl-1,3,4-thiadiazol-2-yl)amides has revealed several structure-activity relationship trends. The synthesis and biological activity of relevant molecules will be discussed.

AGRO 251

Novel class of heterocyclic sulfonamides for the control of soil nematode

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The discovery and development of new pesticides that are highly effective against target pests, work by new modes of action, and meet societal demands for safety to humans and the environment are essential in the defense of crops. Soil dwelling nematodes are among pests responsible for significant crop damage and high yield loss in agricultural production. Furthermore, most current nematicidal products are under severe regulatory pressure due to a range of toxicological and environmental issues. This presentation will describe the discovery and properties of a new class of heterocyclic sulfonamides with broad spectrum nematode control

AGRO 252

Total synthesis of indole alkaloids

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This presentation will describe recent total syntheses of indole alkaloids completed by our laboratory, in addition to related advances in synthetic methodology.

AGRO 253

How can product usage inform pesticide exposure assessments? Examples of the use of AgroTrak® and CA Pesticide Use Reporting data

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As part of a national analysis, data on recent pyrethroid usage from GfK Kynetec AgroTrak® and the CA Pesticide Use Reporting (PUR) were accessed to provide additional context for national pesticide risk assessments. This provided information on the amount (pounds and acres), location, application method and crops for which individual pyrethroid active ingredients have been applied over a recent four-year period (2009-2012). According to AgroTrak®, pyrethroids are applied to over 50 different crops annually, and therefore usage data, together with preliminary Tier II modeling, helped focus higher-tier exposure modeling on those crop uses that are most potentially significant. AgroTrak® and PUR data were used to understand the crops most often treated with any pyrethroids, the fraction of crop area treated for individual crops or crop groups, the percentage of applications made aerially as well as the relative market share of each of the individual active ingredients. These factors were expressed in terms of either their national or regional geographic distributions. These data were of great value for refining potential exposure estimates and their associated uncertainties. This presentation will illustrate how these data were extracted and examine the implications of some of the uncertainties underlying the information. This study provides very clear examples of how usage data can be used to help focus and enhance exposure and risk assessments on individual active ingredients as well as for groups of pesticides.

AGRO 254

Use of monitoring data, toxicity identification evaluations, and usage information in the ecological risk assessment of pyrethroid insecticides

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For the synthetic pyrethroids, there are a wide variety of agricultural and outdoor urban uses which could result in exposure to non-target organisms. These insecticides have been recognized as highly toxic to non-target organisms residing in the water column and the benthos. At the USEPA, the Registration Review of the pyrethroids, pyrethrins and synergists is ongoing, and the ecological risk assessments are scheduled to be published in fiscal year 2016. The EPA has traditionally relied solely on modeling, using the Tier II aquatic models, PRZM/EXAMS (Pesticide Root Zone Model/EXposure Analysis Modeling System) and more recently the SWCC (Surface Water Concentration Calculator), to obtain estimated exposure concentrations in surface and pore waters. The Pyrethroids Working Group (PWG) provided monitoring data in whole (unfiltered) water, filtered water, suspended sediment and bed sediment samples from a number of open literature studies and other sources. The monitoring data are primarily from California but include locations in many other states. Pyrethroids were detected in all types of samples with more frequent detections in suspended and bed sediment samples. Additionally, toxicity identification evaluation (TIE) techniques have been developed for the pyrethroid insecticides. TIE manipulations have pinpointed the cause of toxicity of water and sediment samples as pyrethroids in many cases. For the agricultural uses of pyrethroids and pyrethrins, the Agency has available usage information. In this presentation, modeled and monitored concentrations and predicted toxicity with a comparison to TIE will be explored and compared with the emphasis on heavy agricultural crop use patterns.

AGRO 255

Evaluation of time-dependent sorption of pesticide in soil using parameters generated from lab data

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The U.S. Environmental Protection Agency (USEPA) released the Pesticide Root Zone Model for Groundwater (PRZM-GW) in 2012 to estimate potential groundwater concentrations following agricultural applications of crop protection products. PRZM-GW assumes that soil sorption is linear and constant over time. However, crop protection products often demonstrate soil sorption characteristics are non-linear and increase over time (both characteristics which can reduce mobility potential in soil significantly). The objective of the study is to present model-data comparisons and to demonstrate that the use of Freundlich-type non-linear sorption and time-dependent sorption processes in leaching modeling is necessary to better describe compound movement. Comparisons support the conclusion that predicted groundwater concentrations are more appropriate and realistic when modeling includes these processes. However, this presentation will not just show how PRZM can be calibrated to field studies. It will show how you can use the data generated from lab studies to compute the values needed in the PRZM model for non-linear sorption and time-dependent sorption. There will be a 4-step comparison which shows PRZM-GW with linear sorption, Non-linear sorption (lab generated values), non-linear sorption + time dependent sorption (lab generated values), and NLS-TDS (calibrated values). It will show that using the lab generated NLS/TDS parameters will generate groundwater concentrations that are still conservative. Additionally, we plan to show a sensitivity analysis of NLS/TDS parameters on GW concentrations over a range of DT50 values and Kocs. This outcome highlights the need for refinement options in the current regulatory groundwater modeling which does not include non-linear and time-dependent sorption behavior.

AGRO 256

Incomplete pesticide models for soil and water: A fate and transport - chemical kinetics disconnect

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Hydrology engineers have created about 40 pesticide fate and transport hydrology models for pesticides in soils. One of them is PRZM.gw which is used by the Pest Management regulatory Agency and US EPA together under NAFTA. PRZM.gw has a good description of water transport. Instead of a realistic description of chemical mechanisms it uses k_D and persistence half lives as constants. A dozen predictive spreadsheet models have recently been created for the kinetics and mechanisms of pesticides in soil and water. Experimentally determined stoichiometry produced realistic descriptions of the chemical kinetics and mechanisms for these models. They do not account however, for the hydrology transport of pesticides through soils. A preliminary attempt to harmonize the two types of models has revealed a mismatch of experimental conditions and chemistry descriptions. k_D and reaction half lives are found experimentally to not be constants. They are also influenced by the water to soil ratio. An early conclusion is that comprehensive models will have to be created if the existing two types cannot be harmonized.

AGRO 257

Comparison of residential pyrethroid exposure predictions based on EPA Tier 2 standard scenarios and SWMM/AGRO scenarios based on residential use survey data

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The approach followed by the US EPA for prediction of aquatic pesticide exposure resulting from residential uses of pesticides in screening level risk assessments is to apply SWCC Tier 2 standard scenarios. There are currently two residential standard scenarios used for this purpose, one located in Texas designed originally for an endangered species assessment for the Barton Springs Salamander (ResidentialBSS) and the second designed to address exposure for the California Red-Legged Frog (CAresidentialRLF). Both scenarios include a simple pervious (turf) component and an single impervious component that are combined to estimate total pesticide loads entering a receiving water body. Recent work on modeling exposure from residential pesticide applications in California has resulted in the development of a new conceptual model and set of residential scenarios using the Stormwater Management Model (SWMM) and the AGRO-2014 model. The key element of this new scenario is the ability to treat multiple "use areas" around each home (e.g. lawns, patios, driveways.) at different rates and frequencies, and to assign variable runoff characteristics to different surfaces, resulting in a better Tier 2 representation of residential pesticide applications and washoff. This allows data from residential use surveys and field studies to provide better screening EECs that reflect the varying behaviors of chemicals on different surfaces. The model has been validated with monitoring data from a high intensity residential development in California. The scenarios developed are derived from residential use survey data that covers seven different geographical regions across the United States. In this presentation, we will compare the two conceptual models, compare the model predictions of aquatic EECs, and evaluate the predictions of pesticide loads from both models against measurements from a California urban drainage system. The new SWMM/AGRO approach is appropriate for use in both screening level and refined risk assessments.

AGRO 258

Use of soil fumigant exposure assessment system (SOFEA) outside of California

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Soil fumigants are widely used in agriculture. Inhalation exposure to fumigants may occur post-application as the result of off-gassing. Regulatory agencies have established buffer zones for fumigants to protect bystanders in proximity to fields. However, there is also a potential for lower level, longer duration exposures to residents that live in agricultural areas with significant soil fumigant use, even if they are not in close proximity to an application. The Soil Fumigant Exposure Assessment System (SOFEA) is an air dispersion modeling tool that is capable of simulating fumigant applications across an airshed and outputting concentrations at different averaging times needed for risk assessment. It has been used by state authorities in California for the regulation of the soil fumigant 1,3-dichloropropene. California has an extensive pesticide use database that provides detailed data on the timing and location of pesticide applications. This database has been used to parameterize SOFEA for California applications. No other state has such a system. This paper will demonstrate a methodology to apply SOFEA outside of California. Crop locations will be simulated using the United States Department of Agriculture (USDA) Cropland Data Layer (CDL), which is a Geographical Information System (GIS) database of crop locations at 30 m resolution and developed by remote sensing. Combined with data on total county-level pesticide use, fumigant application locations can be simulated using the CDL data and applied in SOFEA. An example application will be provided for demonstration.

AGRO 259

Factors to consider when developing screening level and more refined estimates of potential human and aquatic ecological exposures and risks resulting from chemical releases in household wastewater

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As part of the risk assessment process for pesticides with indoor uses, US EPA considers potential concentrations in surface waters arising from chemical releases in household wastewater. The "down-the-drain" module of E-FAST (2014) (Exposure and Fate Assessment Screening Tool) is used to develop preliminary estimated concentrations. This presentation examines the assumptions inherent in that assessment and their implications for different classes of chemistry. In addition, the presentation considers the principal factors which should be examined when this screening tool indicates that further evaluation and refinements are necessary to complete a risk assessment. A comparison of modeled exposures with data coming from Publicly Owned Treatment Works (POTWs) monitoring programs and bench scale and pilot processing studies will be presented. POTWs (also known as wastewater/sewage treatment plants) are typically owned by local government agencies and process vast volumes of household wastewater before they are reintroduced to US waters. A recent study monitored effluent from 32 California POTWs that together treat >40% of California's wastewater for eight pyrethroids (bifenthrin cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin, and permethrin) in influent, effluent and biosolids. Together with bench scale processing studies, the real-world data show that POTW effluent pyrethroid concentrations are greatly reduced by wastewater processing. Additionally, these data allow the corresponding screening level exposure estimates to be interpreted and placed into context and also provide strong indications on which additional factors should be considered in more refined models to address this regulatory need. Examples of possible higher tier modeling approaches (e.g. iSTREEM) will be discussed.

AGRO 260

Using data to improve the efficiency of tiered assessment of pesticide exposure in groundwater

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The susceptibility of groundwater to contamination by a specific pesticide can vary greatly spatially according to the pesticide usage, agronomic, and weather patterns at a site. Consequently, exposure modeling of high-risk sites can predict high pesticide concentrations that don't occur unless a combination of vulnerability factors all co-occur. Conservative assumptions for pesticide behavior (relating to mobility and persistence in the topsoil, vadose zone, and aquifer) in early screening model runs can further increase the pesticide exposure estimates. Tier 1 modeling values can be much higher than observed concentrations when the full combination of assumptions regarding site variables and pesticide behavior do not occur in the field. There is also a risk of underestimation of exposure from monitoring data which are rarely robust enough to represent the full range of exposure potential. Furthermore, in drinking water exposure evaluations model estimates for highly vulnerable areas are only relevant to human exposure assessments when drinking water wells utilize the vulnerable ground water in these areas. This paper discusses refinements in modeling tailored to pesticide-specific use patterns, site-specific and depth-specific sorption and degradation pesticide properties, and the degree of co-location of drinking water wells and vulnerable aquifers and pesticide use areas. Strategies for the improvement of model input selection and use of monitoring data to inform tiered modeling are presented. The goal is verifiable relaxation of the conservatism of model estimates earlier in the tiered exposure evaluation process thereby reducing the need for resource-intensive refinements in risk assessments.

AGRO 261

Nutrient runoff from agricultural watersheds in southeast Indiana (USA) and development of the watershed conservation regime

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From 2011 through 2014, ten streams in southeast Indiana were routinely sampled across all hydrologic conditions to examine the effectiveness of agricultural best management practices (BMPs) to reduce nutrient loading to streams. The watersheds were 60% or more cultivated crops. No effect of the practices could be detected in the nutrient data, however the data revealed characteristics about the runoff that could improve the design of effective agricultural BMPs. Turbidity ranged from <5 NTU to >1000 NTU across hydrologic extremes and there was a strong correlation between turbidity and total phosphorus. On average, nitrate-N was 97% of dissolved inorganic nitrogen across all flow conditions and maximum nitrate-N concentrations were >15 mg/L. The input of nitrate and phosphorus occurred under different hydrologic conditions and by different mechanisms, suggesting multiple BMPs are required to reduce loading of both nutrients. Controlling soil erosion could be effective at reducing phosphorus inputs, but the mobility of the nitrate ion presents challenges for BMP designs. In this light, the study suggested a new concept for designing and assessing conservation practices within watersheds: the conservation regime. The watershed conservation regime is akin to an ecological disturbance regime, with each BMP assessed in terms of its magnitude, extent, frequency/duration, and potential interaction with other practices. Summed across all BMPs within a watershed, the conservation regime provides a comprehensive assessment of the effect of multiple BMPs, including the potential for synergistic or antagonistic interactions among practices. Matching the spatial and temporal extent of BMPs relative to nutrient sources areas and timing of nutrient loss is a critical, but often neglected, aspect of efforts to reduce nutrient loss from watersheds. The concept of a conservation regime addresses this and other problems that have historically hindered programs to reduce nutrient loss from agricultural watersheds in the midwestern U.S. and elsewhere.

AGRO 262

Can changes in conservation reduce nitrogen export from agricultural watersheds?

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Excess fertilizer nutrients entering Midwestern agricultural streams degrade both local and downstream water quality, resulting in algal blooms and subsequent hypoxic “dead zones” far from the nutrient source. We are quantifying the benefits of watershed-scale conservation practices that may reduce nutrient runoff to adjacent freshwaters. Specifically, research is lacking on whether the watershed-scale planting of winter cover crops (e.g., annual ryegrass) after corn/soybean harvest can reduce export of excess fertilizer nutrients from agricultural fields to subsurface tile drains and ultimately inputs to adjacent streams. After a pre-treatment year of data collection, in August 2013, we began planting cover crops on >70% of croppable land (>1600 acres) in a small sub-watershed located in the Tippecanoe River Basin in Indiana. Both before and after cover crop planting, we collected water samples every 14d from 25 tile drains and 10 longitudinally-distributed sites along 8km of stream to quantify the effects of cover crops on tile drain and stream export of nitrate-N. We found that tile drain concentrations were spatially-variable, highlighting the importance of field-specific agricultural management practices (e.g., extent and timing of fertilizer application). Nevertheless, the planting of cover crops decreased average tile nitrate-N concentrations by 36% in Winter/Spring, resulting in a seasonal 45% reduction in N-flux from subsurface tiles compared to pre-cover crop planting. In addition, annual watershed nitrate-N export decreased by 31%, translating to an additional 10,500kg of N retained annually on the landscape for crop uptake. After this first year of watershed-scale planting, winter cover crops significantly reduced nutrient export from agricultural fields, showing promise for improved water quality in agricultural landscapes. Additionally, successful outcomes highlighted through watershed-scale demonstration projects may facilitate widespread adoption, making them powerful agents of change for advancing conservation success.

AGRO 263

Reducing nutrient movement in manure-treated, tile-drained fields

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Loss of nutrients from cropped soil represents an economic loss to producers and a threat to environmental quality. In this study, we monitored water, nutrient, and sediment in tile drainage from agricultural fields treated with manure in western Minnesota. Phosphorus results will be presented here. In this multi-year study, we measured flow-weighted soluble reactive phosphorus (SRP) concentrations in tile drainage year-round. We compared phosphorus transport in fields 3 years before and after conversion from open tile inlets (in which ponded water is drained directly into the subsurface tile system) to buried inlets (in which ponded water must infiltrate through a gravel bed before reaching the subsurface tile system). Median annual SRP concentrations were reduced by 35% using gravel inlets (0.064 vs 0.099 mg/L). In 5 of the 6 years, SRP load in tile drainage was greater during snowmelt than during non-snowmelt periods. Median SRP concentrations during snowmelt were 6 to 10 times greater than for the non-snowmelt period. These results demonstrate that in these agricultural landscapes, replacing open inlets with buried inlets can reduce phosphorus export by tile drainage.

AGRO 264

Slow-release, nonpolluting, cost-effective fertilizer system

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Presented here is a polymer system designed to release fertilizers over a wide range of time that consists of a few weeks to several years, a system that obviates problems associated with fertilizer runoff pollution and fertilizer evaporation. This product (fertilizer system) is produced by co-extruding recovered thermoplastics such as polyethylene, polypropylene, polyesters or polyamides with chemical nutrients commonly used as fertilizers and then cutting the extruded product into pellets. Some of the unique properties of this product are the simplicity of its application, its slow release capabilities and the biodegradation of the thermoplastic. The pellets are placed in the soil with the seeds and by designed biodegradation of the thermoplastic, release the chemical nutrient. Since the product is placed underground there is very little nutrient (fertilizer) loss resulting from fertilizer runoff or evaporation This fertilizer system could significantly reduce the pollution of rivers, lakes, oceans and the atmosphere resulting fromfertilizer runoff and evaporation. Fertilizer runoff has been estimated to be 10 to 50% or more depending upon when and how much rainfall there is after the fertilizer is applied. Scientific studies have showed that 30 to 70% of surface applied urea can be lost by evaporation the elimination of fertilizer loss due to fertilizer runoff and evaporation gives the farmer the option of using less fertilizer to obtain current crop yields. Since the farmer can use less fertilizer and there is no additional fertilizer application cost, the cost of using this product can be less than the cost of conventional fertilizer use. The use of this product results in biodegradation of the thermoplastic; therefore, the thermoplastic will never end up in a landfill. The current cost of slow-release fertilizers is 3 to 5 times the cost of regular fertilizers, this limits it's use in high-value crops and field crops such as corn, wheat, soy beans, etc. The projected cost of this fertilizer system is 1.25 to 1.50 times the cost of regular fertilizer.

AGRO 265

Limus, a novel urease inhibitor for agriculture: Enhanced effect of two thiophosphoric triamides

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Nitrogen fertilization of crops is essential for yield and quality. However, of approximately 100 million tons of nitrogen fertilizer produced globally each year, less than 50% of the applied nitrogen is taken up by crops. Part of the nitrogen not taken up by crops is lost to the environment where it can have negative impacts on air and water quality, biodiversity, and climate, in addition to resulting in financial losses for farmers. Urea is by far the most important commercial fertilizer worldwide, comprising around 50% of all nitrogen fertilizers. Urea fertilizers have advantages such as a high nitrogen concentration, safety of storage and application, and low shipping costs, but these fertilizers can also be a source of gaseous nitrogen losses as ammonia (NH3). The potential for urea hydrolysis at the soil surface makes urea particularly inefficient under many circumstances. Depending on soil and weather conditions, more than 50% of the nitrogen fertilized as urea may be lost as gaseous NH3. To delay the urea hydrolysis at the soil surface and thus drastically reduce nitrogen losses as NH3, BASF developed the novel urease inhibitor Limus®. Limus® comprises two triamides N-(n-butyl) thiophosphoric triamide and N-(n-propyl) thiophosphoric triamide that together are more effective at reducing ammonia emissions than a single inhibitor. This makes Limus® a highly efficient urease inhibitor for agricultural to prevent nitrogen losses. This has been proven extensively in both lab and field trials. The new formulation also provides better storage stability on treated urea. The higher nitrogen use efficiency from using urea plus Limus® results in economic benefits for farmers, and the environmental benefits of Limus® help improve sustainability of food production.

AGRO 266

Discovery, mode of action and development of nitrapyrin as a nitrification inhibitor

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For over 60 years, management of crop nitrogen inputs has been a top priority for growers and scientists. As early as the 1950's, it was realized that if nitrogen inputs could be better utilized, a more efficient cropping system could be achieved with better yields. In more recent times, the needs to feed an ever increasing global population while protecting the environment has been a very active area of biology and chemical research. Nitrification inhibitors have proven to be an effective tool to maximize crop production, maximize nutrient use efficiency, and reduce environmental issues such as leaching and nitrous oxide emissions. Nitrapyrin, 2-chloro-6-(trichloromethyl) pyridine, has been used for over 40 years as a commercialized nitrification inhibitor. In this presentation, a background of its discovery, its mode of action, its current uses, key breakthroughs in formulation science to enhance utility and future global development will be covered.

AGRO 267

Formulation innovations for nitrapyrin nitrification inhibitor for use with multiple fertilizer types

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Nitrapyrin was first discovered in the early 1950's and was first sold as a nitrification inhibitor in the United States in 1974 under the trade name N-Serve®, an oil-soluble formulation of nitrapyrin. N-Serve nitrogen stabilizer was primarily used in corn production with soil injected anhydrous ammonia fertilizer, because both are volatile. Nitrapyrin inhibits Nitrosomonas and related nitrifying soil bacteria thereby maintaining a greater amount of nitrogen fertilizer in the NH4+ form longer into the growing season. Delaying the oxidative conversion of NH_4^+ to NO_3^- has direct agronomic benefits to the grower by maintaining more nitrogen in the crop root zone and thus optimizing the yield potential of the crop. Nitrapyrin also has direct environmental benefits by reducing both nitrate leaching into ground and surface water while reducing nitrous oxide emissions into the atmosphere. These benefits have been observed over the past 40 years through university testing and documented in over 200 publications reporting effects of nitrapyrin on soil nitrogen.

In 2009, Dow AgroSciences introduced a novel aqueous based capsule-suspension formulation of nitrapyrin, sold under the trade name of Instinct® in the US corn market. Instinct can be used with ammoniacal fertilizer compositions and manure to bring the same nitrification inhibition that N-Serve delivers with anhydrous ammonia. The unique capsule-suspension formulation protects nitrapyrin from volatility and allows surface stability for up to ten days prior to being incorporated through mechanical means, precipitation or irrigation. Dow AgroSciences now actively sells the nitrapyrin CS formulation across the globe in various crops to aid growers in their nitrogen management programs. This paper outlines these formulation advancements and the value they have demonstrated for growers

AGRO 268

Management and mitigation of nitrates from nitrogen fertilizers in California

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The environmental fate of nitrogen from fertilizers is unlike other synthetic chemicals like pesticides used in agricultural operations. Nitrogen can lead to the production of nitrates, through the microbial cycle, which have public health impacts if contaminated groundwater is used as a drinking water sources. Therefore, effective management and use of nitrogen fertilizer is critical including establishing crop-based user-friendly nitrogen fertilizer use rate information for growers. California has implemented regulations designed to address nitrate reduction in aquifers from nitrogen fertilizers. There is a concerted effort to also study and evaluate methodologies and management practices that limit the movement of nitrogen past the root zone. However, many challenges exist from both management practice and scientific research aspects. This abstract will discuss existing efforts to address nitrogen management in California from an agronomic perspective and the challenges in managing and mitigating nitrates in consideration of the state's diverse specialty crop production sector. Also discussed will be the research and education gaps that have been identified and completed based on sound scientific research. Existing tools developed for growers show the complexity of managing nitrogen and nitrates in non-point systems. New technologies can be used to ensure regulatory certainty.

AGRO 269

Next generation sample preparation materials for selective matrix removal

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Sample preparation methods for complex samples often provide a choice between a laborious cleanup that gives a clean sample and a minimal cleanup that allows large amounts of coextracted matrix into the final sample. For a laboratory doing contaminant analysis, this often means choosing between a method that will take numerous technical steps and additional time and money, or a method that will introduce unwanted contamination to the sample and analytical system. The latter is especially problematic for samples high in lipid content; causing chromatographic interferences, matrix effects, carry over, and overall poor data quality. A new matrix removal material solves this dilemma by providing remarkable cleanliness for complex QuEChERS extracts, without additional steps, cost, or hardware. Data will be presented to demonstrate the high level of cleanliness, accuracy, and precision that can be achieved with multi-analyte, multi-class contaminant analysis applications on LC and GC. The high performance and selectivity of this material make it an attractive option for those working with the most challenging matrices and applications, especially where lipid removal is required.

AGRO 270

Recent dioxin survey and results in meat and poultry

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USDA's Food Safety and Inspection Service (FSIS) is the food safety agency responsible for maintaining the safety of meat, poultry, and egg products. In partnership with the Agricultural Research Service, the Food and Drug Administration and the Environmental Protection Agency (EPA), FSIS has conducted surveys on a five-year cycle to analyze dioxin-like compounds (DLCs) in animal fats to identify sources of DLCs in the food supply. The Agency completed the fourth survey in 2012–2013. The last survey of the same magnitude, duration, statistical design, and analytical methodology was conducted in 2007–2008.

In the 2012–2013 dioxin survey, the data were compared to past results to determine temporal trends. Thus far, median TEQ values for these slaughter classes appear to have decreased during the past five years — ~9.5% in beef; 31% in pork; 33% in chicken; and 35% in turkey. The results were used to calculate consumer exposure estimates to DLC based on consumption rates of beef, pork, and poultry. The highest exposure is from non-lean beef at 0.098 pg TEQ/kg-day. However, these values are well below the oral reference dose (RfD), released by the EPA, of 0.7 pg/kg-day. From the mid-1990s to 2008, DLC levels in beef, pork, and poultry have either declined or remained constant. In the 2007–2008 dioxin survey, 17 toxic PCDD and PCDFs and 4 PCBs were analyzed for in 510 beef, market hog, young turkey, and young chicken samples.

The 2012–2013 survey used calculations based on the RfD to determine dioxin trigger levels (2 pg TEQ/g beef fat and 4 pg TEQ/g poultry and pork fat) that would initiate on-farm investigations to identify sources of DLCs. Currently, nine investigations are underway, including seven beef samples, one pork sample and one chicken sample.

AGRO 271

Ambient ionization of T-2 and HT-2 toxin from food and feed matrices utilizing direct analysis in realtime (DART) coupled to mass spectrometry

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Direct analysis in real time (DART) ionization coupled to mass spectrometry (MS) was used for the rapid quantitative analysis of T-2 toxin, and the related HT-2 toxin, extracted from corn. Sample preparation procedures and instrument parameters were optimized to obtain sensitive and accurate determination of the toxins. The lowest calibration levels (LCL) were 50 µg/kg for T-2 toxin and 300 µg/kg for HT-2 toxin. Quantitative analysis was performed with the use of matrix-matched standards employing the ^{13}C-labeled internal standard for T-2 toxin. DART-MS of corn extracts spiked with T-2 toxin gave a linear response over the range of 50–1000 µg/kg. With the isotope dilution technique, good recoveries (99-110%) and repeatabilities (RSD 7.4–11.6%) were obtained at T-2 toxin spiking levels of 100 and 1000 µg/kg. Adaptability of the developed method was demonstrated by analysis of T-2 and HT-2 toxins from an oat flour QC material and field oat samples. The applicability of DART-MS masked forms of the toxins was evaluated by analysis of the glucosyl forms of the toxins. The results further indicate the potential for application of ambient ionization mass spectrometry to provide sensitive, convenient quantitation of mycotoxins from a variety of grain and grain-related materials.

AGRO 272

Survey of glyphosate residues in honey, corn, and soy products

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Food consumption is an important pathway of human exposure to pesticides and other chemical contaminants. Studies have shown that exposure to contaminants in food could pose a public health risk. Contaminants can enter the food supply in various ways including direct pesticide application to food crops, indirect application through the air (drift), through the soil (from previous growing seasons), through the water supply (run-off), or through food processing (cross-contamination from shared processing equipment). The use of glyphosate in agriculture has increased significantly with the introduction of transgenic crops such as Roundup-Ready® soybeans and corn, which enable farmers to directly apply low cost broad spectrum herbicide products to their fields without harming crops. In the US, glyphosate is currently the most widely used herbicide, with 180-185 millions pounds applied in the agricultural sector during 2007, 5-8 million pounds used in homes and gardens, and 13-15 million pounds used in industrial, commercial and governmental weed control applications. The dramatic increase in the use of glyphosate in agriculture and landscape maintenance is occurring not only in the US, but throughout the world. This high level of use has led to concerns about its effects on humans and the environment. Samples of honey, pancake and corn syrup, soy sauce, soy milk, and tofu, purchased in the Philadelphia, US metropolitan area were analyzed for glyphosate residue using ELISA. Fifty-nine percent of the honey samples had glyphosate concentrations above the method LOQ (15 ppb), with a concentration range between 17-163 ppb and a mean of 64 ppb. Eleven of the tested honey samples were organic, forty-five percent, contained glyphosate concentrations above the method LOQ, with a range of 26-93 ppb and a mean of 50 ppb. Of the fifty-eight non-organic honey samples, sixty-two percent, contained glyphosate concentrations above the method LOQ, with a range of 17-163 ppb and a mean of 66 ppb. In addition to comparison of production method (organic vs. conventional), the honey results were evaluated according to pollen source and by country of origin, grouped by GMO usage (prohibited, limited, or permitted). Glyphosate concentrations above the method LOQ (75 ppb) were also found in ten of the twenty-eight soy sauce samples evaluated, with a concentration range between 88-564 ppb and a mean of 242 ppb; all organic soy sauce samples tested were below the method LOQ.

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Halogenated flame retardants in baby food from the United States and from China and the estimated dietary intake by infants

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Polybrominated biphenyl ethers (PBDEs), Dechlorane plus (DP), and the replacements of PBDEs, including Firemaster 550 (mainly 2-ethylhexyl-2,3,4,5-tetrabromobenzoate and *bis*(2-ethylhexyl)-tetrabromophthalate), pentabromobenzene (PBBZ), pentabromoethyl benzene (PBEB), hexabromobenzene (HBB), and decabromodiphenylethane (DBDPE) have been used as flame retardants in various commercial and household products. They are ubiquitous in the environment and are accumulating in biota. Infants are exposed to flame retardants via many pathways, including dietary intakes. Although human breast milk is the major food of infants, more and more mothers stop breast feeding early, and many infants are fed ready-to-use baby food, including infant formula; cereals; and vegetable, fruit, and meat purees. Not much is known about the flame retardant concentrations in these baby foods. Three categories of baby food (formula, cereal, and puree) were bought from United States and Chinese stores in 2013 and analyzed for PBDEs and related flame retardants. The primary goal of this project was to investigate whether there were differences in the levels of flame retardants between these two nations' baby foods. The median concentrations of total PBDEs (sum of BDE-17, 28, 47, 49, 99, 100, 153, 183, and 209) were 21 and 36 pg/g fresh weight for the Unites States and Chinese baby foods, respectively. Among non-PBDE flame retardants, HBB, DP, and DBDPE were frequently detected (22-57%) with median concentrations of 1.6, 8.7, and 17 pg/g fresh weight for United States samples, and 1.3, 13, and 21 pg/g fresh weight for Chinese samples. In general, the flame retardant concentrations in the United States and Chinese samples were not statistically different, but very high DP concentrations were observed in one Chinese formula sample (4000 pg/g) and in one United States cereal sample (430 pg/g), possibly suggesting contamination of the raw materials or contamination during production of these two samples. A comparison of median estimated daily dietary intake rates of BDE-47, 99, and 153 with existing reference doses for neurodevelopmental toxicity and other existing criteria suggested no concerns for the intake of these baby foods.

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Arsenic speciation in high matrix food products: Striving for a complete mass balance

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In recent years, major efforts have been devoted to developing methods for arsenic speciation as the toxicity and biological effects of arsenic are known to be species dependent. Therefore, reliable measurements pertaining to individual arsenic compounds are required to accurately determine the ecological impact and health risks associated with arsenic exposure. Although sensitive and robust analytical methodologies (*i.e.* LC-ICP-MS) have been developed for arsenic speciation, the lack of certified reference materials (CRMs) with certified mass fraction values for individual arsenic species has been a major limiting factor in method development and validation for arsenic speciation analyses. Here we detail our efforts in an attempt to certify individual arsenic species in several candidate CRMs (kelp, apple juice, seafood, etc.), giving special attention to developing a quantitative extraction method and surveying various chemistries for speciation analysis in order to pursue a complete mass balance.

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Analysis of two classes of persistant organic pollutants in edible oil samples

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Persistant organic pollutants (POPs) are lipophilic, and can collect in the fatty tissues of living organisms. For this reason, they bioaccumulate through the food chain and can be found in edible oils and oil-containing foods. Testing oily samples for POPs can be problematic due to the high background created by the fats present. Common extraction techniques such as liquid-liquid extraction use nonpolar solvents, which result in large amounts of fatty matrix being

coextracted with the analytes. As a result, clean up using large solid phase extraction (SPE) cartridges of silica or alumina is often required for these types of samples prior to chromatographic analysis. This work will present an extraction method for PCBs and PAHs from edible oil samples using a new dual-layer SPE cartridge. Specifically, the method will be applied to the extraction of polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) from soybean oil, and PAHs from butter. For soybean oil, undiluted sample was weighed directly onto the cartridge, and elution of the analytes performed in a single step using acetonitrile. The extracts were concentrated and analyzed directly by GC/ECD for PCBs or HPLC/FLD for PAHs. PAHs were extracted from butter using a modified version of this method that included a secondary cleanup with a small silica gel SPE cartridge. Analysis was then performed by GC/MS-SIM. Details of the methods will be outlined, and data will be presented showing recovery and reproducibility of spiked replicates.

AGRO 276

Challenges of measuring pyrethroid adsorption coefficients in sediments using automated solid phase micro extraction (SPME) techniques

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In order to predict any potential ecological effects of highly hydrophobic organic chemicals (HOCs) or to interpret the results of sediment toxicity studies, it is critical to understand the bioavailable fraction of the HOC present in a sediment water system. Equilibrium partitioning (EqP) theory indicates that this requires measuring adsorption coefficients (Koc) estimated on the basis of the bioavailable (i.e. free) fraction of chemical in solution rather than classical batch-equilibrium Koc study data (1:100 ratio of sediment:water for HOCs) based on total chemical in the aqueous phase. An effective approach for this employs Solid Phase Micro Extraction (SPME) techniques and data have been published for pyrethroid insecticides using this approach. However, in the course of a study conducted on nine pyrethroid molecules in two sediments using automated SPME equipment, significant practical problems were experienced due to the inability to prevent rapidly decreasing concentrations in the standard solutions used to calibrate free chemical SPME fiber responses due to vessel wall adsorption. This presentation documents efforts to conquer this problem and presents an effective but non-ideal solution based on staggered calibration sample preparation. The presentation will also compare the resulting SPME-derived and "classical" pyrethroid Koc coefficients measured for the nine active ingredients in two sediments. The bioavailable Koc coefficients approach theoretical values predicted from carefully measured slow-stir Kow coefficients and, as expected, are typically 1.8 to 7 times greater than the corresponding coefficients based on total aqueous concentrations. The presentation will also discuss the generation of Kocs measured under the conditions of regulatory sediment ecotoxicity studies (1:2 sediment:water ratio) and associated Kdoc coefficients. These new data are important for assessing regulatory ecotoxicological thresholds for pyrethroids and the associated techniques will be applicable to other classes of HOC.

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Modeling compound loss from passive sampler sorbents

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A model was developed to quantify volatile losses of compounds from a passive sampler's sorbent layer. Volatile loss could occur between the time of retrieval and processing of the passive sampler or in intertidal environments where the passive sampler could be exposed from the surface water during low tides. The model focused on initial losses when external mass transfer resistances control evaporative losses. The results suggest that thicker sorbent layers should be used and the samplers should be processed rapidly onsite or kept at low temperatures after retrieval enroute to an off-site facility for processing to ensure retention of more volatile compounds. For example, to retain 90% of naphthalene for a 24 hour exposure to ambient air (20°C) a PDMS sorbent thickness of 1.4 cm would have to be used. The model developed can also be applied to other passive sampling sorbents to estimate initial desorption rates and sampling times necessary to achieve a specific level of compound retention. The model suggests that passive samplers routinely used to monitor hydrophobic organic compounds may not provide quantitative measurement of naphthalenes or other volatile compounds without special efforts to reduce losses of these compounds.

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Investigating soot-water partition coefficients of organic compounds using frontal chromatography and polyparameter linear free energy relationship

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Although black carbon only counts for about 9% of organic carbon in sediment, it dominates the sorption of many hydrophobic organic compounds and greatly reduces their bioavailability. In this study, we measured isotherms of 19 organic compounds with diverse functional groups (for example, ketone, ether, phenol, amine, chlorinated) on a well characterized model black carbon (NIST diesel soot) by frontal chromatography. Based on these soot-water partition coefficients at different sorbate activities, a sorbate-concentration dependent polyparameter linear free energy relationship was found: $log_{10} K_{BC,\,soot}$ (L/kg) = (4.39±0.12) V + (-3.33±0.13) B + (-0.35±0.02) $log_{10}\, a \times E$ +(-2.13±0.11) (N=95, R^2=0.94, SE=0.20), where V is McGowan's characteristic volume for the sorbate; B is the hydrogen basicity; E is the excess molar refraction and a is sorbate's activity in water (saturated = 1). This relationship shows that molecular size of sorbate is the predominant factor forcing its partition into soot, while its basicity (electron donating capacity) discourages this process. Also, sorbate's polarizability increased its adsorption, especially at lower chemical activities. Using this relationship, soot-water partition coefficients of other organic compounds of interest (e.g., atrazine) at various concentrations may be estimated.

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Attenuating historically contaminated sediments by black carbon amendments: Effects of sediment types and contact time

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Historical contaminated sediments from recalcitrant organic chemicals is always challenging for remediation strategies. The effectiveness of conventional dredging is questionable. In the current study, black carbon was used as sediment amendment for immobilizing legacy hydrophobic compounds. The remediation efficiency was evaluated through the measurement of bioavailability of those compounds, which was estimated by the isotope dilution method (IDM). Different activated carbon amendment rates, sediment types, and sorbent contact time were evaluated for their effects on the carbon remediation efficiency. Activated carbon was an efficient sorbent to reduce the bioavailability of the legacy hydrophobic organic contaminants in a marine Superfund site sediment on Palos Verdes Shelf (PVS), CA. In addition, contact time study on freshly spiked ocean sediment suggested presence of natural attenuation of contamination with time in the sediment with and without activated carbon amendment. The effectiveness of activated carbon amendment on remediation and its efficiency increase with contact time may help understand the risk management of the contaminated sediment sites and lead the remediation practices.

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Equilibrium sampling of hydrophobic organic contaminants in sediment

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Hydrophobic organic contaminants (HOCs) reaching the aquatic environment are largely stored in sediments. The risk of contaminated sediments is challenging to assess since traditional exhaustive extraction methods yield total HOC concentrations, whereas freely dissolved concentrations () govern diffusive uptake and partitioning. Equilibrium sampling can deliver , e.g. by using glass jars with µm-thin silicone coatings on the inner walls for *ex situ* equilibration [1] and a device housing a number of silicone-coated fibers for *in situ* equilibration [2]. In both cases, parallel sampling with varying silicone thicknesses can be applied to confirm valid equilibrium sampling [1,2]. The measured equilibrium concentrations in silicone (C_{Sil}) can be compared to C_{Sil} from silicone equilibrated with other media such as biota [3]. Moreover, C_{sil} can be divided by silicone/water partition ratios to yield . Concentrations in lipid at thermodynamic equilibrium with sediment () can be calculated via lipid/silicone partition ratios () [4], which has been done in studies with limnic, river and marine sediments [1,3-6]. The data can then be compared to lipid-normalized concentrations in aquatic organisms or regulatory thresholds. Finally, chemical activities (a) can be calculated [7]. This overview lecture shows that equilibrium sampling has proven to be a straightforward, precise and sensitive approach to determine C_{free}, a and of HOCs, and that it can provide the basis for thermodynamic assessments of polluted sediments.

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AGRO 281

Development and application of freshwater sediment-toxicity benchmarks for currently used pesticides

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Several currently used pesticides are being monitored in streambed sediments by the U.S. Geological Survey (USGS) in regional studies. To assess potential for adverse effects on benthic organisms, two types of freshwater sediment benchmarks for pesticides were developed by using either spiked-sediment toxicity data from the literature and from available databases, or equilibrium-partitioning-based values for compounds without spiked-sediment toxicity data. Benchmarks were established to define pesticide concentrations above which there is a high probability of adverse effects on benthic organisms (Likely Effect Benchmark, or LEB), and below which adverse effects are not expected (Threshold Effect Benchmark, or TEB). LEBs and TEBs were determined for 123 pesticide compounds. When sediments contain mixtures of pesticides, LEB-quotients (environmental concentrations divided by the LEBs) can be summed for all detected pesticides to produce a Sediment-Pesticide Toxicity Index (Sediment-PTI) for the mixture, assuming an additive model. In an example application, data for pesticides measured in bed sediments collected from about 200 streams in USGS studies were evaluated by using the new sediment benchmarks, and the results were compared to measured toxicity from whole-sediment toxicity tests conducted with the amphipod *Hyalella azteca* (28-day exposures) and the midge *Chironomus dilutus* (10-day exposures). Amphipod survival, dry weight, and biomass were significantly and inversely related to the Sediment-PTI, whereas midge survival, ash-free dry weight, and biomass showed no relationship. The LEBs for currently used pesticides were rarely exceeded (2% of samples), but these samples all were toxic to amphipods. About 20% of sites with pesticide concentrations below all TEBs also were toxic to amphipods, indicating that other factors besides the pesticides measured may adversely affect benthic organisms in these samples. Ongoing studies will apply these sediment benchmarks to further investigate pesticide bioavailability and mixtures in sediments and their influence on toxicity to benthic organisms.

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Comparing bioavailability neasurement methods

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Hydrophobic or non-polar compounds are preferentially sorbed to particulate and dissolved organic matter in sediment, necessitating the consideration of bioavailability in risk assessment. There are a wide variety of bioavailability measurement methods, with some (e.g., SPMD, PED, SPME) aiming to detect the freely dissolved concentration C_{free} while others (e.g., mild solvent extraction, Tenax desorption, cyclodextrin extraction) to quantify the reversibly desorption fraction. A challenge for practitioners is which method to use, or if different methods produce the same results. We have compared different methods in aquatic toxicity assays as well as in simulated remediation treatments. In this presentation, we will discuss the similarity and differences among the methods in their working principles, evaluate advantages and limitations of individual methods, and highlight procedures in assuring the correct use of these methods in sediment quality assessment.

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New interpretations of the results of HOC monitoring studies and sediment ecotoxicity studies for HOCs based on refined adsorption coefficients

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Interpreting and applying data from ecotoxicity tests to ecological risk assessment or from ambient water monitoring studies requires appropriate quantification of potential test organism exposures to bioavailable (free) toxicant concentrations. This is challenging for highly hydrophobic organic compounds (HOCs) due to their exceptionally strong adsorption to sediments which renders large fractions non-bioavailable in natural systems. HOC monitoring data are often reported simply as measured concentrations without accounting for this bound fraction. Quantifying bioavailable benthic exposures in HOC sediment ecotoxicity tests is also practically very difficult due to low pore-water volumes and low free concentrations. In the absence of direct measurements, equilibrium partitioning theory (EqP) can predict pore water concentrations based on organic carbon (OC)-normalized sediment concentrations using partition coefficients (K_{oc}). However, for highly hydrophobic compounds, the only appropriate Kocs to use would be those based on free aqueous phase concentrations (rather than totals of bound and free chemical). Recent research by the Pyrethroid Working Group (PWG) using Solid Phase Micro-Extraction (SPME) technology has measured bioavailable Koc and Kdoc coefficients for pyrethroids under a number of conditions. The SPME approach improved upon traditional Liquid-Liquid Extraction (LLE) methods since it estimated the desired bioavailable fraction in "true" solution in pore water; $K_{OC\text{-}SPME}$ coefficients were systematically higher than corresponding $K_{OC\text{-}LLE}$ values. Additionally, coefficients measured under standard batch equilibrium guideline study conditions with 1:100 sediment:water ratios were markedly higher than those generated to reflect sediment ecotoxicity studies (1:2 sediment:water ratios). This presentation will explain how these more refined Koc coefficients may be used to refine pore water estimates for sediment ecotoxicity studies and how ultimately these refinements improve risk characterization. In the same way, these biologically relevant adsorption coefficients, in conjunction with measured suspended solids and DOC measurements, should be used to interpret monitoring studies in HOCs and develop regulatory thresholds.

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Anaerobic abiotic reduction of dichloroacetamide safeners in Fe(II)-amended, heterogeneous minerals systems

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Safeners are added to herbicide formulations to improve selectivity by protecting crop plants from toxic effects of the active herbicide. Safeners vary widely in terms of structure and are co-formulated with a specific class of herbicide; for example, dichloroacetamide safeners (the subject of the current work) are typically paired with chloroacetamide herbicides. Dichloroacetamide safeners are structurally similar to chloroacetamide herbicides that have been detected in natural waters. The environmental fate of safeners is poorly understood; evidence suggests that as dichloroacetamide safeners transform, they can become more biologically active. Manganese oxides are important redox-active species that frequently co-occur with iron oxides as secondary soil minerals. Dichloroacetamide safeners have been shown to transform via reductive dechlorination in anaerobic abiotic systems with iron oxy-hydroxide minerals, but were not monitored in the presence of manganese oxide. This study examines the reductive dechlorination of the dichloroacetamide safeners benoxacor, furilazole, and dichlormid in Fe(II)-amended, anaerobic abiotic mineral suspensions. Batch reactors examined the transformation rate of the dichloroacetamide to monochlorinated products. Reactors were sampled over time and analyzed via gas chromatography (GC). The safener dichlormid did not transform over a 6-hour sampling period. Benoxacor and furilazole both transformed by a pseudo-first-order reaction in the slurries containing Fe(III) oxide, Mn(IV) oxide, and $FeCl_2$. The presence of Mn(IV) oxide had an appreciable effect on the transformation rate of benoxacor and furilazole, decreasing the reaction rate with increased Mn(IV) oxide. A critical value of 1 was determined for the ratio of Fe(II) to Mn(IV) oxide. Below the critical value, safener transformation decreased as the ratio decreased. Above the critical value, furilazole transformation occurred at a constant rate and benoxacor transformation rate increased as the ratio increased. This study is the first to examine the transformation of furilazole and highlights the importance of accounting for mixed-mineral effects in agrochemical transformations.

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Risk mitigation strategies of DDT and dieldrin residues in historical orchard soils

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Repeated applications of organochlorine pesticides in historical orchards have resulted in contaminated soils that require remediation to minimize risk to local ecosystems. Most physical and chemical remediation techniques involve removing or processing the contaminated soil and are costly and time consuming. Economical approaches are needed that can reduce risks effectively while meeting the requirements of state and federal regulations. The critical factors to be considered when evaluating the risks posed by of organochlorine pesticides are bioavailability, bioaccessibility, and bioaccumulation. We conducted microecosystem studies to determine the effects of several soil amendments with the potential to reduce bioaccumulation of DDT and dieldrin residues in soil invertebrates like earthworms (*Lumbricus terrestris L.*) and the subsequent biomagnification or reduced bioavailability of these compounds in the terrestrial food chain. The same amended soils were also tested with the addition of various plant covers and compared to an unamended control soil with plant cover. The use of low-cost organic compost was found to be effective at lowering the bioavailability of organochlorine pesticides in soils thereby protecting sensitive receptor species and mitigating movement of these residues into the food chain. Results of this work can be used in the decision-making process for natural resource and regulatory managers who are seeking lower cost remediation solutions to mitigate ecological risks at orchard sites.

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Comparison of two approaches to modeling ground water exposure with EPA's PRZM-GW model

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Currently, EPA uses the PRZM-GW (Pesticide Root Zone Model for Groundwater) model to derive estimated drinking water concentrations (EDWCs) in groundwater as part of the human dietary risk assessment. When the residues of toxicological concern consist of parent and one or more degradates, simplifying assumptions must be used to run PRZM-GW since the model is currently designed to only model the fate and transport to groundwater of one chemical at a time. When appropriate, EPA's Office of Pesticide Programs uses a simplified modeling approach which treats the total toxic residues as a single compound based on the combination of fate data for the residues of concern. In this presentation, an example of this approach is provided for groundwater exposure estimation using PRZM-GW. A limitation of this approach is that the toxicity of all the residues of concern are considered equivalent. Another approach is to separately model the parent and degradates of concern with PRZM-GW (similar to the Residue Summation approach used by OPP for kinetic analysis of environmental fate studies) and combine the estimated concentrations from each model run. Both approaches have been used with the PRZM-GW model for estimating groundwater exposure concentrations for pesticide residues of concern. The results show the importance of careful consideration of input parameter selection in using the model for estimation of total exposure to multiple toxic products arising from application of a single pesticide.

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Inclusion of biphasic kinetics and non-linear sorption to refine estimated regulatory groundwater concentrations of pesticides

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Bi-phasic degradation and non-linear sorption of pesticides are often observed in laboratory environmental fate and field dissipation studies. Non-first order behavior is captured in the NAFTA regulatory modelling kinetics tool as a bi-exponential (double first order in parallel (DFOP)) model. The NAFTA kinetics guidance recommends the slow phase half-life as the representative input for regulatory models. This conservative strategy does not reflect realistic pesticide behavior in soil and leads to over-prediction of groundwater concentrations. Incorporation of half-life values from both (slow and fast) phases, use of mass conservation principles, and non-linear sorption kinetics are proposed to represent the complete bi-phasic degradation profile and concentration-dependent sorption behavior for groundwater concentration predictions. Model results using the modified approach show significant refinement compared to the current screening level approach.

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Antifeedant and antifungal activity of nanobiopesticide synthesized by Eucalyptus plant extract

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Rapid increasing in world population required increase agriculture production to fulfill the requirement of growing world which would be 9.1 billion by 2050. It is estimated that more than 25-40 % of crop losses due to pest and pathogens worldwide. Synthetic pesticides is widely using for the control of pest and diseases. The role of synthetic pesticide in pest management is major concern for environmental safety. As we know residues of synthetic pesticide shows negative impact on environment and living beings. The uncontrolled uses of synthetic pesticides are increasing resistance in the pest along with environmental toxicity. The most important challenge to achieve production targets should be safe and in sustainable manner. So there is need for development of new pesticide which can provide sustainable and ecofriendly greener solution of pest problem. Intelligent use of chemicals on the nano scale level can be a suitable solution for this problem, which provides delivery of correct quantity of nutrients and pesticides that promote productivity and ensure environmental safety and high efficiency. In the present study we developed iron nanoparticle using Eucalyptus plant extract. These bio-synthesized nanoparticles applied as antifungal and anti-insecticidal agents. Bio synthesized Iron nanoparticles showed antifungal activity against *Fusarium oxysporum*, *Rhizoctonia solani* and *botrytis cinerea*. The antifungal activity increased from 33-80% at increasing amount of nanoparticle solution. Similarly, Antifeedant assay measured against Diamond back moth (*Plutella xylostella*) and nano-bio-pesticide showed 61.6% antifeedant activity.

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Larvicidal activity of prenylated stilbene analogs

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In our continuing effort aimed at looking for novel biologically active stilbenes, 33 stilbene analogs were synthesized. The stilbenes were tested for larvicidal activity against *Aedes aegypti*, the primary vector of dengue fever, yellow fever, and other diseases. Two analogs, (*E*)-4-(3,5-dimethoxystyryl)-2-(3-methylbut-2-en-1-yl)phenol (**1**) and (*Z*)-4-(3,5-dimethoxystyryl)-2-(3-methylbut-2-en-1-yl)phenol (**2**) demonstrated strong larvicidal activity. Data suggest that a prenyl group directly attached to the ring enhances activity against *A. aegypti* larvae.

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Effect of lanthanum on yield and components of soybean seedlings under supplementary UV-B radiation stress

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The effect of spraying lanthanum among different concentration (30 $mg \cdot L^{-1}$、90 $mg \cdot L^{-1}$ and 150 $mg \cdot L^{-1}$) on yield and components of Soybean Seedlings in soybean early flowering stage exposed to high level of UV-B radiation and low level of UV-B radiation(simulated ultraviolet radiation increased by 12.80% - T2 and 4.33% - T1 compared with natural light of Harbin in heilongjiang province) was studied by using soybean Dongnong 42 with planting in the pot. The results showed that: 1 Compared with that of the control, supplementary low level of UV-B radiation caused 13.43% increase in yield and components of Soybean Seedlings; While, supplementary high level of UV-B radiation caused 24.60% decrease in yield and components of Soybean Seedlings. 2. The seed number per plant, seed weight per plant, 100-seeds weight and yield of soybean were increased with the decreasing of La concentration under the supplementary low level of UV-B radiation stress. In addition, spraying $30mg \bullet L^{-1} LaCl_3$ made the maximum value with 61.30 seeds per plant, 15.83 grams per plant, 27.48 grams per 100-seeds and 516.91 $kg \bullet ha^{-1}$, which were increased 5.15%, 21.39%, 15.35% and 21.39% respectively, compared with that of the control. The seed number per plant, seed weight per plant and yield of soybean were smaller than that of the control. While, 100-seeds weight were increased 13.37% compared with that of the control under the supplementary high level of UV-B radiation stress. In addition, spraying $30mg \bullet L^{-1} LaCl_3$ made the maximum value with the decrease of 18.03%, 9.03% and 9.03% respectively in those three indexes compared with that of the control. The results show that proper increase of low level of UV-B radiation could increase the yield of soybean, while high level of UV-B radiation stress produce unfavorable effects on soybean yield and yield components. Spraying rare earth lanthanum in optimum fertilization concentration at flowering period can alleviate the adverse effects and even increase the yield, in which 30 $mg \cdot L^{-1}$ is the best dosage. This study provides reference for alleviating the negative impact on soybean seedlings under Supplementary UV-B radiation stress and improving the yield of soybean through spraying the best appropriate concentration of rare earth lanthanum.

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Effect of nano silicon preparation on the nutrient content of rice plant aerial parts

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Dynamic changes of TN,TP and TK's content in the leaves,stem-sheath and spike of rice during tillering stage,booting stage and maturation stage were studied with the rice "DongNong 427"in the Northeast Agricultural University Gardening test station potted condition in Harbin of Heilongjiang Province,by spraying two kinds of nano silicon fertilizer solution(Si50,Si60) during tillering stage and booting stage.The main results were as follows:Spraying two kinds of nano silicon fertilizer solution(Si50,Si60)can increase rice plants absorb N,P,K.They can significantly improve the content of TN,TK in the rice spike,leaves,and the content of TP in the spike,leaves,stem-sheath,but Si60 is better than Si50.This study provides a reference for efficient and reasonable utilization of nano silicon preparations.

AGRO 292

Effect of nano silicon fertilizer on rice yield and component factors

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The effects of nano silicon fertilizer on rice yield and component factors were studied with the rice "longjing 27" in nenjiang of Heilongjiang Province in China, by spraying two kinds of nano silicon fertilizer solution (Si50, Si60) during the booting stage. The main results were as follows: Compared with blank, spraying two kinds of nano silicon fertilizer solution during the booting stage can increase rice yield, and improve the value of rice yield component factors. The highest yield is the treatment of Si60 , increasing the yield by 20.51%（$P>0.5$） with blank; Spraying Si50,seed setting rate and number per panicle will achieve maximum by 94.75% and 98 ears, both increased 1.60%（$P<0.5$） and 1.38%（$P>0.5$） respectively; Spraying Si60 have largest influence on other production factors, thousand seed weight is 24.86 g, ear length is 17.43cm, number per panicle is 99.They increased 1.00%（$P>0.5$）, 10.71%（$P<0.5$）and 20.57%（$P>0.5$） respectively. This study provides a reference for efficient and reasonable utilization of nano silicon fertilizer to increase rice yield.

AGRO 293

Renewable syntheses of agrochemicals and pharmaceuticals from biomass-derived platform chemical 5-(chloromethyl)furfural (CMF)

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Currently, more than 95% chemicals are sourced from petroleum. Structurally complex specialty chemicals (e.g. agrochemicals, pharmaceuticals) are typically synthesized from petroleum-derived platform chemicals (e.g. aromatics, olefins, and alcohols). However, the availability of petroleum is limited. On the contrary, biomass is a renewable, carbon-neutral, and environmental-friendly resource that has attracted serious interest as a commercially viable alternative to petroleum for energy, materials and chemicals. Recently, a high-yielding synthesis of 5-(chloromethyl)furfural (CMF) has been demonstrated by acidic hydrolysis of cellulosic wastes (e.g. newspaper, cotton, corncobs) within a biphasic reactor. CMF retains, at least partially, the structural features of the highly functionalized sugars. In the course of developing CMF as a biomass-derived platform chemical, a series of value-added products have been synthesized. In terms of agrochemicals, the natural herbicide δ-aminolevulinic acid was prepared from CMF in three steps involving azide substitution, photooxidation and catalytic hydrogenation with an overall 68% yield. CMF was also converted into prothrin, a biodegradable insecticide, along with some analogues, in five to six steps with overall yields between 50-70%. With respect to pharmaceuticals, the blockbuster antiulcer drug ranitidine was synthesized efficiently from CMF in four steps with an overall 68% yield. Furan fatty acids, naturally occurring antioxidants, can also be prepared from CMF in seven steps featuring a Suzuki coupling and a Wittig olefination with an overall 50% yield. These described syntheses suggest that CMF could serve as a renewable resource to conveniently provide a variety of valuable pharmaceuticals and agrochemicals.

AGRO 294

Development of a high-throughput screening system for the detection of PaOA₁ octopamine receptor antagonists and agonists from *Periplaneta americana*

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Octopamine is a biogenic amine that has been implicated in the regulation of numerous physiological roles, such as reproduction, learning-and-memory, and nerve stimulation, to name a few. Previous studies have demonstrated that octopamine has a very limited role in mammalian systems, and thus octopamine receptors represent a promising target for the development of safer insecticides. Compounds identified to act upon these receptors would thus have little to no effect in mammalian and other non-target organisms. To-date, we have developed a line of Chinese Hamster Ovary cells (CHO) that are stably expressing an α-adrenergic-like octopamine receptor that has been isolated from the American cockroach (*Periplaneta americana*). Functionality of this receptor was screened using a calcium liberation detection assay using Fluo-4 NW dye (Invitrogen Tech.) Functional screening determined that octopamine is the preferred ligand with an EC50 of 41nMol; tyramine, a closely related biogenic amine, binds with a lower affinity with an EC50 of 674nM. Using multiple concentrations of terpenoids commonly found in plant essential oils, we have identified viable screening concentrations for the identification of agonists and antagonists at this receptor. These compounds represent novel leads for the development of future insecticides that modulate the activity of the octopamine receptor in arthropods.

AGRO 295

Development of passive samplers for measuring bioavailability of pesticides in contaminated water with performance reference compound calibration

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Long-term monitoring of contaminants in surface water systems is of global environmental concern. Pyrethroids and fipronil are widely used in urban environments, leading to their occurrence in urban surface waterways. The determination of freely dissolved concentration (C_{free}) is therefore essential for estimating the bioavailability of pesticides.

A range of passive samplers have been successfully used for measuring C_{free}. For *in situ* field applications, thin-film samplers are considered to be more suitable compared with injector-type or fiber-SPME. In this study, different types of thin films were tested for their absorption capacity of 8 pyrethroids, 4 fipronil compounds, diazinon, and chlorpyrifos in water. The polyurethane (PU) film displayed the most effective enrichment for both pyrethroids and fipronils, thus, it was selected as the passive sampler material in further experiments.

The absorption kinetics of pesticides for PU films were then evaluated in stimulated surface water. Diazinon, chlorpyrifos, and fipronils were quickly reached an apparent equilibrium within 2 d. In the case of pyrethroids, the concentrations on the film did not reach equilibrium until 43 d. To circumvent the long equilibrium time requirement, two stable isotope labeled compounds, deuterated d_5-bifenthrin and phenoxy-$^{13}C_6$-*cis*-permethrin, were preloaded on the PU film as performance reference compounds (PRCs) before use. The desorption of PRCs was compared with absorption of pyrethroids under mixing conditions. The sum of desorbed fraction of the labeled pyrethroids and absorption fraction of each target pyrethroid varied from 0.8 to 1.2 (Figure 1), suggesting that the desorption of labeled PRCs was isotropic to absorption of their native counterparts. The PRC calibration approach therefore may be used to monitor both fipronil compounds and pyrethroids under non-equilibrium conditions with flexible sampling time.

AGRO 296

Modeling the vibrational spectroscopy of amorphous carbonaceous materials using DFT

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Hydrothermal chars (hydrochars) are carbonaceous materials derived from the thermal decomposition of biomaterials in aqueous solutions under pressure, and these hydrothermal char's applications include catalysis, energy storage, and water purification. Despite promising applications and on-going effort, the structure of hydro chars remain incompletely characterized. Infrared and Raman spectroscopy can provide more details about the functional groups present, the degree of aromaticity, and structure of hydro char. In particular, vibrational spectroscopy contains a wealth of structural information that has not been fully utilized due to the complexity of the spectra and difficulty in making definitive assignments. In this work, we have used DFT to simulate the vibrational spectra of model compounds and hydrochar model structures to guide assignment of the vibrational spectra features. Specifically, we have modeled the Raman and infrared spectra of polyaromatic hydrocarbons and functionalized aromatic compounds. We can then view the individual vibrational modes of these model compounds to get a better understanding of how complex molecular structure is represented in Raman and IR spectra. Specific emphasis has been placed on understanding the molecular significance of the G and D bands, graphite and defect assignments based on spectroscopy of low-defect graphitic materials, for the poorly ordered and defect-rich hydrothermal char. In simple polyaromatics the wavenumber position of these bands was found to decrease with increase in the number of aromatic rings. The relative intensities of the characteristic peaks in the D and G band bands were found to fluctuate based on the number of rings, and the symmetry of the molecule. That is, whether the molecule consisted of rings arranged in the shape of simple polygons such as pyrene, or merely as a straight chain of rings as in tetracene. For example as shown below, molecules consisting of polygonal structures had a decrease in the wavenumber location of their D band, an A1G ring breathing mode. Similar trends have been discovered for more complex aromatic compounds such as arylfurans and aromatic carboxylic acids.

AGRO 297

Prospecting of oil and deoiled cakes of Jatropha curcas L. and Pongamia pinnata L. for pesticidal activity

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Biodiesel has emerged as a major alternative to petro-diesel. Inadvertent use of chemicals for pest control has posed serious threat to our environment & alternatives for chemical insecticides are being screened. Oil and deoiled cakes of Jatropha curcas L. and Pongamia pinnata L. were evaluated for the bioactivity against P. xylostella, S.litura and Helicoverpa armigera. The oil was extracted using hexane as solvent (JA,PA) seed cake left after oil extraction was extracted with methanol (JB,PB) and also seed were extracted directly with methanol (JC,PC). All the extract were then evaluated for bioactivity against P. xylostella , S.litura using anti-feedancy bioassay and Helicoverpa armigera using diet mixing bioassay. It was found that 5% Jatropha extract showed the highest mortality rate against Helicoverpa armigera and among the two oil Pongamia oil 5% (90.60%) was more active than Jatropha oil 5% (56%). Very mild antifeedancy was observed against S.litura. The highest percent feeding inhibition of S. litura obtained in Pongamia methanol PC (47.59), Jatropha methanol JC (47.31) while comparable activity was found Jatropha hexane JA (41.62), Pongamia methanol from seed cake PB (40.13) while low antifeedant activity was found in Jatropha methanol after hexane JB (20.00), Pongamia hexane (10.08). The highest feeding inhibition of P. xylostella was observed obtained in Pongamia methanol extract obtained from seed cake PB (97.05) and Pongamia methanol PC (91.14). Good feeding inhibition was also obtained with Jatropha methanol from seed cake JB(73.78). Lowest feeding inhibition is found in PA(47.85), JE(56.96), JC(60.0), JA(64.25), PE(64.26). The methanol extract of Jatropha and Pongamia were formulated capable of application at field level, which is based on the solubility of extract in different solvents.

AGRO 298

Flunixin urine residues in culled dairy cows and its relevance to food safety and environmental concerns

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Flunixin is a US-FDA approved non-steroidal anti-inflammatory agent; it is prominent due to violative meat residues detected by the US-FSIS in dairy cows. The effects of route of administration (2.2 mg/kg) and endotoxin challenge on flunixin elimination and residues were investigated. High urinary flunixin concentrations (> 100,000 ng/mL) were measured in 30%, 70%, and 90% of cows on dosing days 1, 2, and 3, respectively (2 h post-dose). The cow with the highest 96-h withdrawal urinary flunixin level had violative milk and liver flunixin residues; the cow having the second highest urinary flunixin had violative milk residues. At the 96-h withdrawal period, flunixin concentrations were > LOQ in 45% of plasma and in 100% of urine samples. Urinary flunixin concentrations were not significantly altered by dosing route or endotoxin, but the endotoxin treated cows had higher flunixin/5OH-flunixin ratios. These data suggest urine could be an alternative to predict violative flunixin residues.

AGRO 299

Measurement of pyrethroids and their environmental degradates in fruits and vegetables using a modification of the quick easy cheap effective rugged safe (QuEChERS) method

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Pyrethroid insecticides are used extensively in agriculture and they, as well as their environmental degradates, may remain as residues on food products such as fruits and vegetables. Since pyrethroid degradates can be identical to the urinary markers used in human biomonitoring of pyrethroid exposure, it is important to understand the contribution of these degradates when studying sources of human pyrethroid exposure.

We modified a widely used pesticide extraction and clean-up method (QuEChERS) to measure several commonly used pyrethroids (*cis*/*trans*-permethrin, cypermethrin, deltamethrin, esfenvalerate, bifenthrin, cyfluthrin, and cyhalothrin) and their degradates (3-PBA, *cis*/*trans*-DCCA, 4-F-3-PBA, DBCA, and MPA) in selected fruits and vegetables. We determined extraction efficiencies from: tomatoes, oranges (whole, peeled, peel only), grapes, apples, bananas (peeled, peel only), onions, lettuce, green peppers, carrots and broccoli. For a subset of these items (apples, grapes, tomatoes, lettuce and banana peel), we also established limits of detection and quantitation (LOD/LOQ).

Each sample was homogenized then fortified with pyrethroids and degradates. 15 g sub-samples were extracted with acetonitrile, then salted and partitioned with NaCl and $MgSO_4$. The extract was divided and further cleaned using graphitized carbon black (pyrethroids) or C18 (degradates). Sample analysis was via liquid chromatography/tandem mass spectrometry (LC/MS/MS).

Considering the mean recoveries each of the 14 analytes in all 13 matrices: 41% of the recoveries were ≥ 90%, 73% were ≥ 80%, and 94% were ≥ 70%. All LOD's were less than 100 ng/kg, except 3-PBA (132 ng/kg, tomato), MPA (129 ng/kg, tomato), and *trans*-permethrin (141 ng/kg, banana peel).

We applied the method to samples (subset of 5) collected weekly for four weeks from local supermarkets. At least one pyrethroid was present in measureable concentrations in all matrices except banana peels. In contrast the only metabolites detected were *cis*/*trans*-DCCA, in one lettuce sample.

AGRO 300

Effect of pH and surfactants in stereoselective fate of beta-blockers in wastewater

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Although polar micropollutants are considered less likely to sorb to organic matter, several studies showed the occurrence of beta-blockers in sludge at concentrations ranging from ng/l to µg/L. In California, 75% of biosolids and an increasing amount of reclaimed water from wastewater treatment plants (WWTP) are used in agriculture, posing environmental and human health risks. Establishing stereoselective fate in wastewater is critical for improving risk assessment for beta-blockers. In this study, we collected effluent and mixed liquor from a local municipality WWTP. Stereoselectivity in biodegradation was monitored through a wastewater microcosm experiment. There was no significant change in enantiomeric fraction (EF) of salbutamol after 6 days of incubation. However, the EF of pindolol increased from 0.50 to 0.53, but acebutolol decreased from 0.51 to 0.41. Effect of surfactants and pH on sorption behavior of the beta-blockers in sludge was determined by following EPA's test guidelines. Increasing pH from 6.50 to 8.49 resulted in an increase in the amount sorbed on the sludge. Adding Triton X-100, a nonionic surfactant increased the sorption of the beta-blockers. However, sodium dodecyl sulfate, an anionic surfactant resulted in a decrease in adsorption, and the decrease was more pronounced for acebutalol. Thus, presence of nonionic surfactants in WWTP may result in an increase in sorption of basic pharmaceuticals to sludge. The effect of pH and surfactants on biodegradation of salbutamol, pindolol and acebutalol will be reported at the meeting.

AGRO 301

Studies toward understanding the SAR around the sulfoximine moiety of the sap-feeding insecticide Isoclast™

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Isoclast™ active represents the first product from a novel class of insect control agents based around a sulfoximine chemical scaffold. It exhibits broad spectrum control of many sap feeding insect pests, including aphids, whiteflies, hoppers, and *Lygus*. The discovery of Isoclast stemmed from a novel scaffold-based approach toward identifying bioactive molecules. Lead optimization efforts to understand the structure-activity relationships (SAR) and improve potency led to significant variation of the substituents flanking each side of the sulfoximine moiety. Synthetic routes were established which enabled diversification at both the carbon atom linking the sulfoximine group to the pyridine ring (R1,R2), as well as the terminal position of the molecule (R3). This talk will discuss the synthesis and insecticidal activity of these novel analogs, as well as key findings from exploring this portion of the SAR.

AGRO 302

Developing a scalable process to Isoclast™ - a new crop protection agent

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Isoclast^{TM}, a new class of sulfoximine insecticide discovered by Dow AgroSciences, controls a broad spectrum of sucking pests while offering a unique mode of action for superior aphid control on a variety of major crops. This talk will present development efforts toward a scalable strategy to Isoclast™ active. The technology involves a *de novo* approach to a fully functionalized pyridine sulfide intermediate using enamine-mediated cyclization followed by two inexpensive and efficient oxidations to establish the sulfoximine moiety.

AGRO 303

Agrochemical process research: Searching for the holistic solution

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Using examples from Process research and the industrial production of active ingredients some of the thinking beyond the development of an agrochemical technical process will be discussed. Emphasis will be placed on issues which really drive the technical feasibility of large scale AI production at a cost that is applicable for an agrochemical.

AGRO 304

Process research of DAS-Hb1, a 6-alkylpicolinate broadleaf herbicide

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Picolinic acid herbicides have been an active area of commercial interest for decades. Recently, a 6-alkyl-5-fluoropicolinate, DAS-Hb1, was identified as a novel broadleaf herbicide. The initial synthesis of DAS-Hb1 was accomplished by an early stage Discovery route, which utilized an electrophilic fluorination reaction using F-TEDA, a Stille cross-coupling reaction, and a Pd-catalyzed hydrogenation reaction. The Dow AgroSciences Process Chemistry team investigated several synthetic strategies to eliminate costly and/or toxic reagents, minimize precious metal-catalyzed reactions, and ultimately develop a cost effective process route. An overview of the route scoping efforts will be presented and key advances will be highlighted.

AGRO 305

Learning from Mother Nature: Natural products as a source of ideas and inspiration for agrochemcials

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Natural products (NPs) have long been used as pesticides and have broadly served as a source of inspiration for many commercial synthetic organic fungicides, herbicides and insecticides that are in the market today. In light of the continuing need for new tools to address an ever changing array of fungal, weed and insect pests, NPs continue to be a source of models and templates for the development of new pest control agents. Interestingly, an examination of the literature suggests that NP models exist for many of the pest control agents that were discovered by other means, suggesting that had circumstances been different, these NPs could have served as inspiration for the discovery of a great many more of today's pest control agents. With an emphasis on insecticides, an attempt will be made to answer questions regarding the existence of NP models for existing pesticides, and using the spinosyns as a reference point, what is needed for the discovery of new NPs and NP models for pest control agents.

AGRO 306

Discovery of naphthalene isoxazoline insecticides

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A series of naphthalene isoxazolines have been designed as insecticides for crop protection. We will present the chemical synthesis, biological activity and structure-activity relationships. The naphthalene isoxazoline derivatives shown here have excellent insecticidal activity on insects, such as Diamondback Moth, Fall Armyworm, Potato Leafhopper etc. at 2ppm. We also unambiguously determined that only the S configuration of the naphthalene isoxazoline molecule is the biologically active form by single X-Ray structural analysis.

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AGRO 307

Aryl heterocyclic amines (AHA) insecticides

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Fipronil is a broad-spectrum insecticide belonging to the phenylpyrazole chemical family that disrupts the insect central nervous system by blocking the passage of chloride ions through the GABA receptor and glutamate-gated chloride (GluCl) channels, components of the central nervous system. Specificity of fipronil on insects may come from a better efficacy on the GABA receptor, but also due to the non-existence of GluCl channels in mammals. Thus, GABA-gated chloride channel inhibition is a highly desirable mode of action targeting a chewing insect product goal. This presentation will detail our efforts in this area dealing with closely related pyrazolo-pyrimidine analogs, eventually morphing into a project we termed the Aryl Heterocyclic Amines (AHA's). The similarities and differentation factors vs. Fipronil, insect spectrum, bioactivity, field results and bioavailability of the AHA's will be discussed.

AGRO 308

Streamlining refined aquatic exposure estimation for agricultural uses by understanding the significance and limitations of standard Tier II assumptions

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This presentation focuses on potential aquatic ecological exposure following off-target transport to receiving waters due to drift, erosion, and runoff. USEPA Tier II modeling involves running standard models with standard crop/field scenarios to predict exposure concentrations in water, pore water, and sediment. One modeling refinement retains the maximum use rates and numbers of applications while modifying application methods (e.g. ground vs. aerial) and application sequence scheduling to reflect real-world agronomic practices. More refined modeling should also incorporate exposure reductions due to label-required mitigation practices such as droplet size and drift as well as vegetative filter strips. Additionally, the effect of providing increased realism with respect to refined sediment dynamics using the AGRO model and the resulting effects on estimated environmental concentrations (EECs) (especially for hydrophobic AIs) will be discussed. Another important standard assumption relates to how well the selected soil/climate scenario reflects the actual distribution of crop-soil co-occurrence across the USA. Two example key crops reflecting drift- or erosion-driven scenarios for pyrethroids demonstrate the effects of each of these refinements on 90th-centile (EECs) as well as the distributions of concentrations across 30 years. These simple refinements also provide the background for more detailed assessments of the sources of uncertainty potentially impacting aquatic exposure assessments. The conclusion is that careful attention needs to be paid to ensure standard modeling is based on reasonable worst case (i.e. protective) but nevertheless agronomically realistic input assumptions and that it also reflects current label mitigations. Using these realistic inputs as the starting point for further refinement allows uncertainty analyses to help quantify the degree of confidence associated with standard modeling outputs.

AGRO 309

Test version of a spatial aquatic model (SAM) to estimate spatial and temporal pesticide exposures in water

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The U.S. EPA Office of Pesticide Programs (OPP) is developing a spatial aquatic model (SAM) to address the need for more spatial and temporal contexts for pesticide aquatic exposure assessments. The model provides an efficient way to estimate how often, how long, and where possible adverse impacts may overlap with populations at risk. Spatially and temporally-explicit datasets of land use, soil, weather, and hydrology are integrated with optimized fate and transport model routines to develop the Ohio River basin test version of SAM. Model inputs for soil-land cover-weather combinations and meteorological updates have been automated. Users will be able to access the model from a webpage hosting OPP models, and model inputs and outputs will be available and stored (temporarily) on a central cloud. This presentation will describe the conceptual model used for SAM, identify key spatial datasets and model routines, and provide preliminary evaluations of model outputs.

AGRO 310

Drinking water exposure assessment for chlorpyrifos in North America: Overview and conclusions

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Chlorpyrifos is widely used to control insect pests in a variety of agricultural and non-agricultural use sites. The potential exposure to chlorpyrifos and chlorpyrifos-oxon in drinking water as a result of its use is dependent on physical-chemical and environmental fate properties, weather, watershed vulnerability, agronomic practices (*e.g.*, application date and method) and drinking water treatment. A national screening level drinking water assessment was completed and a conceptual model was developed for conducting a screening level assessment for community drinking water systems. This model reflects the development of the drinking water intake percent cropped area (PCA)/percent use area (PUA) adjustment factors and demonstrates that exposure to chlorpyrifos and chlorpyrifos-oxon is expected to be highly localized. However, the incorporation of spatial refinements introduces uncertainties **stemming from the available** resolution of weather and usage data, land cover, and drinking water treatment methods that **present difficult challenges to conducting a** watershed by watershed analysis for risk assessment purposes.

AGRO 311

Higher tier aquatic exposure assessment for imidacloprid

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Regulatory exposure models are mostly deterministic models which predict exposure concentrations considering certain use patterns, weather, and soil conditions. A standard assessment using the deterministic approach for imidacloprid, a widely used insecticide, is not capable of characterizing the exposure concentrations of imidacloprid reflective of the spatial and temporal variability in relevant agronomic and environmental conditions.. This paper describes a higher tier ecological aquatic exposure assessment of imidacloprid performed using a probabilistic modeling approach. The assessment considered probability distributions of critical environmental and agronomic factors spatially or temporally, including application date, weather and soil conditions, drift fraction, percent cropped area, and the mitigation measures such as runoff buffer zones on the label. The results demonstrated that the probabilistic modeling approach is able to refine the screening level exposure concentrations to reflect more realistic exposure conditions.

AGRO 312

Higher tiered aquatic exposure assessment of a recently developed pesticide under realistic agricultural production practices improves understanding of environmental fate

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Concerns regarding the persistence and potential accumulation of the metabolite in sediment were raised based on results of laboratory measurements in an anaerobic aquatic metabolism study. A higher tier aquatic exposure assessment was carried out over three years in two agricultural small watersheds to investigate the fate of a recently developed pesticide and its primary metabolite. Applications of the product were made by the growers to the watershed draining to the pond(s) at each location every year during the study period. Monitoring was performed under real world usage, meaning growers rotated crops and applied product as they would normally, rather than prescribe maximum label usage. The sampling locations, represented potential "worst case" environments, included farm pond(s) receiving drift and runoff, intermittent streams, and perennial streams fed by the farm ponds. Composite water and sediment samples (top two inches) were collected monthly from each sampling location. The parent and metabolite were measured in water, pore water and sediment extracts. Results indicate that seasonal variations due to cropping practices, cover crops, and significant rainfall events leading to runoff were the predominant factors governing transport of residues into surface waters. The co-occurrence of parent and its primary metabolite in samples suggests that the metabolite was forming in the field via soil metabolism and/or soil photolysis, and then transported to the water/sediment by runoff events. While the anaerobic aquatic metabolism lab study showed significant formation of the metabolite in the sediment study, the laboratory study was conducted under highly reducing conditions, which are not likely to exist in typical agricultural watersheds that have higher oxidative conditions, especially in intermittent or perennial streams. No environmentally relevant accumulation was found in any environmental compartment and residues were well below concentrations predicted by tier one conservative models.

AGRO 313

Improved modeling approach to evaluate pesticide product for impacts to surface waters in California

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California Department of Pesticide Regulation (CDPR)'s Surface Water Protection Program (SWPP) is developing a more consistent and transparent modeling approach for the evaluation of pesticide products submitted for registration in California. Currently we are working on multiple projects (http://www.cdpr.ca.gov/docs/emon/surfwtr/review.htm) to help evolve and improve the model. Recent development includes advance tools for 1) urban pesticide uses, 2) data needs and modeling approach for evaluating pesticide degradate, 3) effects to marine/estuarine organisms, and 4) modeling scenarios for California-centric receiving water body. For urban evaluation, USEPA's tier-2 models and modeling scenario for "San Francisco impervious surface" are generally accepted in the CDPR development, while the landscape description and simulation design are significantly improved to represent reasonable worst-case conditions of pesticide aquatic exposure in California urban areas. Degradate evaluation is started with an initial screening with potential data request for degradate toxicity tests, and followed by pesticide toxicity index approach for assessing total toxic residues of parent-degradates mixture to aquatic organism. A methodology is also developed to identify pesticide products with relatively high potentials to enter estuaries and the ocean. The method is designed as a standard procedure for determining the appropriate toxicity endpoint in evaluating pesticide products. Modeling scenarios for California receiving water body are under development (http://www.cdpr.ca.gov/docs/emon/pubs/protocol.htm). The products will include the model selection and water body dimensions representative to California field conditions, which are anticipated to replace the USEPA scenario for standard farm pond for the pesticide evaluation and risk assessments.

AGRO 314

Integrating modeling and monitoring for pesticie aquatic exposure assessment

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Current pesticide regulatory exposure assessments are conducted with computer models biased toward worst-case

scenarios of use and environmental conditions. Although there is wide existence of pesticide aquatic monitoring programs by industry, government and academic organizations, measured data have been used less in the regulatory assessment process largely due to variability in sampling designs (frequencies, timing) and lack of context in exposure conditions. In this work, we present a framework to connect monitoring data and modeling techniques to characterize realistic potential of exposure based on recently developed approaches including principle component analysis (PCA), bias factor (BF), and kriging methods. A decision tree is proposed, beginning with PCA for watershed vulnerability, modeling informed by BF, and daily data imputation by watershed covariates. Examples of applying these approaches are provided to demonstrate their usefulness for watershed scale assessments.

AGRO 315

Microbial dechlorination of PCBs—it's not just for sediments any more

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Polychlorinated biphenyls (PCBs) are persistent, toxic, and bioaccumulative pollutants, and their environmental impacts are widespread. One of the very few ways that PCBs are degraded in natural environments is via dechlorination by anaerobic bacteria, which use the PCB molecule as an electron acceptor. Until recently, dechlorination of PCBs had only been observed in contaminated sediments, most famously those of the Upper Hudson River. Recently, however, examination of congener patterns in several US waterways has suggested that microbial dechlorination of PCBs occurs in landfills, sewers (especially combined sewers), and contaminated groundwater. This presentation will present evidence for the dechlorination of PCBs occurring in the Delaware River Basin, the New York/New Jersey Harbor, and the Portland, Oregon Harbor superfund site. In all three locations, measurement of 209 PCB congeners by EPA method 1668 has provided large data sets that we have mined using factor analysis (specifically Positive Matrix Factorization or PMF). This approach suggests that PCB congeners that are products of the dechlorination process constitute 19% of total point-source PCB loads to the Delaware River, and 22% of all of the PCBs found in the water column of the Portland Harbor. The spatial patterns suggest that dechlorination occurs in sewers, landfills, and groundwater in the Delaware River Basin, but primarily and perhaps exclusively in the groundwater at the Portland Harbor superfund site.

AGRO 316

Non-chlorinated dibenzo-*p*-dioxin daughter product detected in sediment microcosms from two contaminated sites originally amended with 1,2,3,4-tetrachlorodibenzo-*p*-dioxin

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Contamination of soils, sediments and groundwater with toxic chlorinated contaminants is a serious problem worldwide. A major goal in remediation of environmental media contaminated with chlorinated chemicals is removal of all chlorines from the organic molecule through reductive dechlorination to allow further biodegradation to occur. Although this has been observed for chlorinated solvents, it is less commonly observed for aromatic compounds.

Sediment samples from two geographically separate sites originally contaminated with environmental polychlorinated dibenzo-*p*-dioxins (PCDDs), were placed in serum bottles with anaerobic minimal medium. The sediments were amended with 1,2,3,4-tetrachlorodibenxo-*p*-dioxin (1,2,3,4-TCDD) as a model congener to study methods for enhancing reductive dechlorination. Triplicate microcosms were amended with electron donor (organic acids), and two treatments were amended with soluble alternate halogenated compounds intended to enhance dioxin dechlorinators (tetrachlorobenezene or trichloroacetophenone). After prolonged incubation (years) abundant 2-monochlorobibenzo-*p*-dioixn was detected in all treatments and lesser amounts of non-chlorinated dibenzo-p-dioxin (up to 9.8 mol% of the original parent compound) were detected in some treatments.

Production of non-chlorinated dibenzo-*p*-dioxin from 1,2,3,4-TCDD, even after long incubation times, raises the possibility that reductive dechlorination could result in complete removal of chlorines from the PCDDs.

AGRO 317

In situ pilot studies evaluating the efficacy of bioaugmentation for treatment of PCB-impacted sediments

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In situ treatment with granular activated carbon (GAC) has been used successfully to sequester aromatic POPs such as PCBs in sediments effectively minimizing their interaction with the biological food chain. The objective of this research is to develop and test the efficacy of a bioamended form of GAC embedded with microorganisms to sequester PCBs from the food chain and concurrently dechlorinate and degrade weathered PCBs in sediments. Two pilot studies were initiated to demonstrate and validate this environmentally sustainable technology at PCB-impacted field sites. Treatability studies in sediment mesocosms demonstrated PCB levels were reduced by up to 80 % after treatment by bioaugmentation. Effects of different quantities and types of inocula on total PCBs, congener distribution and bioavailability were determined for optimal field application. Among the challenges for the pilot field studies were development of methods for production level scale-up of the microorganisms without residual POPs, production of SediMite modified as a carrier for the bioamendments, development of a system to introduce active PCB transforming microorganisms into SediMite pellets during dispersal of the pellets at the site, and maintaining viability of the anaerobes and aerobes during the deployment process. Methodology, challenges associated with deployment at a sewage treatment pond and a wetlands tributary and preliminary results for the field demonstration studies will be discussed. The results of the pilot studies will access the feasibility of using bioremediation for full-scale treatment of PCB-impacted sites. *In situ* treatment by bioaugmentation where applicable would have a significantly reduced environmental impact compared with dredging by reducing the health risks associated with sediment disruption, reducing overall energy use, effectively negating the requirement for extensive waste management and substantial habitat restoration.

AGRO 318

Biofilm enhanced bioremediation of polychlorinated biphenyls in soil and sediment

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Removal of polychlorinated biphenyls (PCBs) from contaminated sediments is a priority because of degrading bacteria. Commonly applied remedies (dredging/capping) are associated with challenges due to issues such as resuspension of contaminants and destruction of the surround environment. Bioremediation using *in situ* microbial degradation of PCBs represents a significant improvement. However, previous attempts have failed because of PCB stability, low bioavailability and low abundance/activity of PCB-degrading organisms. Co-localizing PCB-degrading microbes onto surfaces of adsorbents in high density biofilms their toxic and carcinogenic properties and utilizing them as inoculum delivery system provides a novel PCB bioremediation approach. In this study, biofilm covered activated carbon (AC) particles were applied for enhancement of PCB dechlorination in sediments.

Biofilms of anaerobic *Dehalobium chlorocoercia* DF1, enrichments from wastewater and aerobic *Burkholderia xenovorans* strain LB400 were formed on AC. Mature biofilms were inoculated into PCB contaminated sediment mesocosms. PCB concentrations were determined by GC. Molecular techniques included: DNA extraction, q- PCR with specific 16S rDNA primers, identification by DHPLC and Illumina sequencing. Microscopic analyses included: DAPI, PNA-FISH, SEM and CLSM.

Biofilm formation on AC was observed for all organisms via staining/microscopy. Biofilm inoculation into mesocosms increased the no. 2-fold and PCB degradation was enhanced (biofilms: 31% vs. planktonic inoculum: 6%) over 200 days. The bacterial diversity of PCB dechlorinating and overall bacterial populations showed significant changes simultaneously with bioremediation. The application of biofilm covered AC particles enhanced the PCB degradation likely due to PCB adsorption onto AC ensuring direct contact and electron transfer between the biofilm and adsorbed PCBs. This two-phased approach provides an efficient and cost-effective method for delivering microorganisms for on-site bioaugmentation.

AGRO 319

Investigating anaerobic dechlorination of organochlorine pesticides

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Hexachlorocyclohexane (γ-HCH; Lindane) was a widely used organochlorine insecticide until about 1997. Because of slow rates of degradation, it still persists in many subsurface environments, particularly in certain agricultural soils, and near industrial facilities where it was manufactured, posing a health hazard owing to toxicity and probably carcinogenicity. It is well established that γ-HCH can degraded aerobically but it is also known to be anaerobically reductively dechlorinated to chlorobenzene and benzene. Chlordecone ($C_{10}Cl_{10}O$) is a much more toxic and highly recalcitrant organochlorine insecticide formerly used on banana plantations in the Caribbean. In this study, we investigated anaerobic microbial dechlorination of γ-HCH and chlordecone. We compared this dechlorination to the well-established dechlorination of chlorinated solvents such as trichloroethene in microcosms established from Caribbean soil.

Anaerobic dechlorination of γ-HCH and TCE was readily observed in soil microcosms. γ-HCH dechlorination resulted in the production of chlorobenzene and benzene at a ratio of about 3:1. The challenge now is to stimulate further degradation of chlorobenzene and benzene under anaerobic conditions, which can be achieved by inoculation with competent chlorobenzene- and benzene- degrading cultures. The analytical challenges faced with chlordecone analyses are significant yet we have observed partial dechlorination of this substrate using LC/MS/MS. Pyrotag analyses of the 16S rRNA gene has revealed presence and possible enrichment of several putative organohalide-respiring bacteria in the soil of active microcosms and in subsequent transfer cultures, including *Dehalobacter*, *Dehalococcoides, Clostridium* and *Geobacter*. The implications are that in the anaerobic conditions typical of organic rich soil, reductive dechlorination of a wide range of halogenated organics can occur naturally, slowly detoxifying the soil. The process can be accelerated through biostimulation and bioaugmentation and the knowledge of the microbes responsible provides a means to monitor the process. The advantages and challenges of such remediation approaches will be presented.

AGRO 320

Ecology and evolution of aerobic bacteria that utilize vinyl chloride as a carbon and energy source

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Vinyl chloride (VC) is a known human carcinogen and common groundwater pollutant. VC can enter groundwater directly via inadvertent spills of VC monomer, but is more commonly generated by anaerobic transformation of chloroethenes and chloroethanes. Subsurface VC biodegradation occurs under either anaerobic or aerobic conditions. A prevalent mechanism for anaerobic VC biodegradation is reductive dechlorination to ethene by various *Dehalococcoides* sp. There are also aerobic bacteria that can fortuitously oxidize (i.e. co-metabolize) VC in the presence of oxygen and a growth supporting compound (e.g. methane). A potentially important group of VC-oxidizers is the ethene-oxidizing bacteria (also called "etheneotrophs"). These obligate aerobes can co-metabolize VC in the presence of ethene, and under the appropriate conditions can also utilize VC as a sole source of carbon and energy.

Functional genes involved in VC and ethene biodegradation by etheneotrophs include *etnC*, which encodes the alkene monooxygenase (AkMO) alpha subunit. AKMO initiates the initial enzymatic attack on VC. The second enzyme in the pathway is epoxyalkane:coenzyme M transferase, encoded by *etnE*. In recent efforts, we have developed a quantitative, real-time PCR (qPCR) method that targets these key functional genes and their transcripts. We are currently applying these methods to study the abundance and activity of VC-oxidizing bacteria at contaminated sites. Laboratory VC adaptation experiments with an ethene-assimilating isolate, *Mycobacterium* sp. strain JS623, has revealed that missense mutations in *etnE* facilitate transition from cometabolic to growth-coupled VC oxidation. Recently we have investigated *etnE* diversity in environmental samples and employed metagenomics, RT-qPCR, and stable isotope probing to study the transition to growth-coupled VC oxidation in an aerobic mixed culture derived from a contaminated site in Alaska. Continued research in both the laboratory and in the field is expected to shed new light on the ecology and evolution VC-oxidizing etheneotrophs in contaminated groundwater environments.

AGRO 321

Using factor analysis to find evidence of microbial degradation in the subsurface at a historically contaminated site

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We recently applied a sophisticated statistical tool to elucidate evidence of microbial degradation in an existing database of concentrations of subsurface contaminants measured at a historically contaminated site in New Jersey. Statistical analysis via Positive Matrix Factorization (PMF) was applied to a dataset of concentrations of halogenated organic compounds and their degradation products measured in the groundwater between 1990 and 2013. The database also contains nutrient concentrations and ancillary parameters such as pH and dissolved oxygen, which assisted in interpreting PMF results. Furthermore, temporal and spatial variations of individual degradation profile patterns were examined. Analysis various datasets indicates degradation via dechlorination in the subsurface.

PMF analysis of the chlorinated benzene dataset reveals a degradation factor containing both monochlorobenzene and benzene, which is correlated positively with nitrite and negatively with sulfate and sulfide. This may indicate at high nitrite levels, the reduction of monochlorobenzene to benzene and/or the degradation of benzene may be inhibited. In addition, it is known that the reduction of monochlorobenzene to benzene and/or the degradation of benzene can occur under sulfate reducing conditions. Analysis of the chlorinated ethene dataset reveals two dechlorination factors, representing different stages of dechlorination. Nearly all of the vinyl chloride and *cis*-dichloroethene and most of the *trans*-dichloroethene reside in the partial dechlorination factor, which is positively correlated with sulfate. The factor representing advanced dechlorination contains all of the ethene and ethane and is correlated positively with alkalinity, ferrous iron, and methane and is correlated negatively with sulfate. This research has demonstrated that PMF is a useful tool for investigating large data sets on contaminant concentrations in the subsurface and can provide evidence of natural attenuation.

AGRO 322

Recent developments in sample preparation and GC-MS/MS analysis of environmental contaminants and pesticides in food samples

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Residues of hazardous contaminants in food present a concern in food safety programs. Simple and sensitive analytical methods are needed to monitor the residues and ensure that the food is safe for consumption.
A fast, high throughput analytical method for simultaneous determination of >200 diverse contaminants was developed and evaluated for fish samples. The contaminant list included environmental pollutants: polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated biphenyl ethers (PBDEs), novel emerging flame retardants and diverse pesticides.
The sample preparation was based on QuEChERS (quick, easy, cheap, effective, rugged, and safe) technique with acetonitrile extraction and dispersive solid-phase extraction (d-SPE). This sample preparation approach was further streamlined by performing d-SPE clean-up in filter-vials, thus eliminating a need for an additional centrifugation step, and reducing sample preparation time. A single analyst can prepare 12 pre-homogenized samples in one hour, and the calculated sample preparation cost is ≈$3 per sample using bulk materials. The sample preparation was originally developed for fish and seafood (shrimp), and is being evaluated for meat samples.
Low pressure vacuum outlet gas chromatography tandem triple quadrupole mass spectrometry (LPGC-MS/MS) allows for simultaneous determination of >200 chemical contaminants plus internal standards in one chromatographic run of 10 min. Recoveries of 70-120% were achieved for the majority of analytes with relative standard deviations under 20% (n=5) even at a low spiking level (1-5 ng/g), making the method applicable for analysis at environmentally relevant concentrations.
To minimize sample size, and reduce amounts of organic solvents needed for extraction, variables affecting QuEChERS-based extraction yields of incurred contaminants in fish samples were recently investigated. Our results showed that 2 g subsamples were adequate for analysis of the incurred contaminants.Smaller subsample size often translates into faster, easier, and less wasteful methods, provided that the subsample adequately represents the original sample.

AGRO 323

Synthesis, spectral characterization, biological activity, and soil:water fate of brominated 17β-estradiol isomers

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Estrogens are eliminated from nearly all animals in significant quantities, and when released into the environment they can act as endocrine disrupting compounds, particularly to aquatic organisms. Tracing the movement of estrogens from animal waste to impacted waters is complicated by historical estrogen deposits in soils, as well as their uncontrolled introduction by wildlife. Tracer studies can be conveniently performed with radiochemicals; however, these are not always suitable for field-scale research. Stable, brominated analogs of estrogens may prove a fruitful method to study the movement of theses endocrine disrupting compounds in the field. Therefore, the aim of this project was to synthesize brominated 17β-estradiol analogs, to spectrally characterize the products formed, conduct bioassays evaluating the estrogenicity of synthetic products, and to utilize one of the isomers synthesized in a field-scale tracer study. Mild bromination of 17β-estradiol was accomplished with molecular bromine, and various experimental conditions were tested to evaluate the effects on product yield and regioselectivity of bromination. Full characterization of products by ^{1}H- and ^{13}C-NMR, as well as electron impact-gas chromatography/mass spectrometry were completed. The biological activity of each brominated 17β-estradiol isomer was evaluated in the E-Screen assay, measuring cell proliferation in response to exposure to estrogenic compounds in non-transfected MCF-7 BOS cells. The soil:water fate and transport of two of the monobrominated 17β-estradiol isomers will be reported from laboratory batch sorption experiments using radiolabeled analogs relative to parent 17β-estradiol. Bromination reactions were also applied to estrone, testosterone , androstendione, progesterone, cortisol, and cholesterol in order to elucidate the bromination reaction mechanism.

AGRO 324

Pesticide multiresidue analysis in straw roughage using the QuEChERS approach and HPLC/MS/MS

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The use of pesticides in food and feed bring unintended and adverse impacts to the health of both humans and animals. Roughage is an important part of the food chain of which humans and animals sit. As the main feed resources of feeding herbivores, roughage includes hay, straw, abortive shell and parts of the leaves, etc. Straw roughage can fill rumen, stimulate the rumen wall to ensure the normal digestion, to provide energy and improve the butter-fat percentage. This feed is very vital for livestock in farming areas, which has many advantages such as extensive sources, high output, variety and cheap. While the field trials for pesticide registration we have finished indicated that pesticide residue in straw and shell matrixes are quite high and it could pose health risks for animal products. However, there has been no report on the pesticide multi-residue analysis in straw roughage to date. In this article, a multi-residue method using the QuEChERS Approach combined with liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) was established and validated for rapid determination of 69 pesticide residues at different levels in 3 representative straw roughage (wheat straw, rice straw and rice hulls). During method development, the extracting method and several different sorbents (C18, PSA, GCB and MWCNT) for d-SPE cleanup were investigated and compared with respect to the recoveries and precision. In the quantitative analysis, the recoveries ranged from 60 to 120 % at the level of 1−100 μg/kg and consistent relative standard deviations ＜30 % （n＝5） were achieved for most of the target analytes (77% of the pesticides in wheat straw, 84% of the pesticides in rice straw and 80% of the pesticides in rice hulls). While the recoveries of some analytes were lower in all three matrixes due to their chemical structure, such as pymetrozine, mepiquat chloride and nicosulfuron, and some of the other pesticides response were very low in mass spectrometry, for instance, trifluralin, dinotefuran and oxadiargyl. The LOQs for diverse pesticides ranged from 1 to 10 μg/kg. In qualitative detection, retention times and ion-ratios of matrix-matched calibration solutions kept consistent with standard calibration solutions generally. The developed QuEChERS method achieved satisfactory separation, sensitivity, linearity, and repeatability for most pesticides and the preparation method is fast, easy and high throughput.

AGRO 325

Target and non-target screening for emerging environmental contaminants using high resolution and accurate mass LC-MS/MS

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There is an increasing demand for analytical techniques and methods combining targeted identification and quantitation with retrospective and non-target data analysis. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC-MS/MS run.

A QuEChERS extraction procedure was used to extract residues and contaminants from food samples. Extracts were subsequently analyzed by LC-MS/MS using a SCIEX TripleTOF® system operated in information dependent acquisition (IDA) mode. IDA combines a TOF-MS survey scan with the automatic acquisition of TOF-MS/MS to collect molecular ion and fragment ion information of every compound present in the sample.

MS and MS/MS data was explored to confidently identify and accurately quantify targeted chemicals based on retention time, accurate mass, isotope pattern and MS/MS library searching. EU proficiency test samples were analyzed to verify method performance. 43 out of 44 pesticides were identified correctly. One false negative was caused by low QuEChERS recovery. No false positive results were reported due to use of MS/MS for structural identification.
In addition, sample-control-comparison was successfully used to find unexpected contaminants. Identification was based on accurate mass MS and MS/MS information, including empirical formula finding, ChemSpider searching, and automatic MS/MS fragment ion interpretation. This challenging data processing workflow was automated and allows easy result review and reporting in the latest version of MasterView™ software.

AGRO 326

Sample preparation and cleanup for multiresidue analysis of foodstuffs and environmental samples: Simple SPE strategies for complex matrices

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Among the most important challenges in multi-residue analysis is the need to identify and quantify hundreds of compounds in each sample, preferably in a single analysis. With so many compounds with myriad chemical properties traditional methods of sample preparation and cleanup may not be appropriate. Multiresidue extraction procedures such as Quechers methods are useful for initial preparation of many foodstuffs and related samples but the analyst usually must perform some sort of cleanup prior to introduction of the sample to the GC-MS or LC-MS instrument. In this presentation we will discuss sample cleanup using SPE in dispersive and pass-through modes as well as the more traditional retain, wash and elute mode. The degree and complexity of the cleanup required is not only dictated by the complexity of the sample but also by the sensitivity and selectivity of the chromatographic system used for analysis. Among the analytical applications discussed will be multi-residue pesticides, persistent environmental pollutants and multi-residue mycotoxins. Samples prepared from relevant matrices are analyzed using both moderate and high sensitivity instruments with cleanup procedures optimized for each level of instrument sensitivity. Of highest priority is to keep the required cleanup strategy as simple as possible.

AGRO 327

Analysis of perfluoroalkyl substances in food, drinking water, and indoor dust from New York State and the assessment of human exposure

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Perfluoroalkyl substances (PFASs) have been used as surfactants and surface protectors in many consumer products. PFASs have been reported to elicit numerous adverse health effects in humans. Americans have the highest levels of PFASs in their bodies in comparison with populations from other countries. Little is known on the sources and pathways of human exposure to PFASs. In the present study, twelve PFASs were analyzed in 139 food and beverage samples, 23 drinking water samples, and 23 indoor dust samples collected from New York State, by using isotopic dilution high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Among 139 food samples, 121 (87%) samples contained at least one of the PFASs. Among the 12 PFASs analyzed, PFOS was detected at the highest frequency, at 72%, followed by PFOA (55%). In 23 drinking water samples, PFOA concentrations ranged from <LOQ to 17.4 pg/mL; PFNA concentrations ranged from <LOQ to 38.4 pg/mL. In 23 indoor dust samples, total PFAS concentrations ranged from 59.3 to 9074 ng/g (mean ±SD :1441 ± 2302 ng/g). The calculated median human exposure doses in the present study were 0.65 ng/kg bw/day for PFOS, and 0.57 ng/kg bw/day for PFOA, which were similar to the values derived from serum biomonitoring data for the U.S. general population. Based on the concentrations measured for drinking water, foodstuffs, and indoor dust collected from New York State, the relative contributions from each source to the total PFAS exposures were derived. Food consumption is the major source for both PFOS and PFOA exposure, followed by indoor dust ingestion. Meat and poultry are the major contributors for PFOS exposure, while drinking water is the major contributor for PFOA exposure.

AGRO 328

Shoot-and-Dilute gas chromatography-mass spectrometry: Polycyclic aromatic hydrocarbons screening in food using streamlined sample preparation and alternative carrier gases

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Food contamination with toxic polycyclic aromatic hydrocarbons (PAHs) occurs by exposure to environmental contamination and during some food preparation processes. Methods explored here employed streamlined sample preparation and Shoot-and-Dilute gas chromatography-mass spectrometry (GC-MS). While classic sample extraction methods such as Soxhlet and Pressurized Fluid Extraction (PFE) yield excellent quantitative results for PAHs, they are resource intensive and relatively slow. A highly modified QuEChERS extraction was paired with a simple silica solid-phase extraction (SPE) cleanup. This sample preparation method is much less resource intensive and provided satisfactory recovery of all PAHs tested.

Shoot-and-Dilute GC-MS/MS methods used split injection which can alleviates matrix related issues occurring at the inlet and column. There are notorious problems associated with splitless injection of dirty samples, most notably for PAHs is drastic response changes. This can occur very quickly with real samples, especially without exhaustive sample cleanup. An easy way to reduce inlet and column maintenance is to use split injection when possible. Increased flow through the inlet during split injection minimizes poor response for involatile compounds (e.g. PAHs, PCBs, dioxins and furans) and maintains acceptable data quality longer. Shoot-and-Dilute GC-MS/MS and splitless injection GC-TOFMS methods were used to screen for EU 15+1 PAHs and compared with splitless injection GC-MS/MS for food samples (e.g. tea).

Hydrogen and nitrogen carrier gases were explored as alternatives to helium which is a finite resource, more expensive and becoming harder to obtain. Hydrogen, which is easily generated, is more efficient and faster than helium. Nitrogen can be very efficient as a carrier gas, but is very slow compared to hydrogen and helium, increasing analysis time. A "translated" method will deliver essentially the same separation as hydrogen, but with a slower analysis time. However, the analysis time with nitrogen can be shortened by scaling column dimensions producing a very similar chromatogram.

AGRO 329

Solvent free emulsifier blend for solvent free EC formulations

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The use of solvents in today's pesticide formulations is becoming an issue due to environmental concerns. AkzoNobel is committed to delivering Eco Premium Solutions to design more sustainable products for customers and the planet. Pesticide emulsifiable concentrate (EC) formulations contain a mixture of emulsifiers, active ingredients, and solvents. A solvent-free EC can eliminate the need for a solvent if the active ingredient is a liquid with low viscosity. Historically products claiming to be solvent-free may not be truly solvent free because the surfactants used to formulate the ECs typically contain a solvent used to aid in processing and handling. Typical emulsifier blends for EC pesticide formulations are a mixture of an anionic surfactant, a nonionic surfactant, and EO-PO block copolymer. The anionic surfactant generally is a salt (Ca or Amine) of dodecyl benzene sulfonate (DDBS). If the anionic surfactant is a Ca-DDBS or amine-DDBS, the viscosity is very high if no solvent is used. Therefore, the current Ca-DDBS and amine-DDBS mixtures in the market always contain various solvents. Typical solvents include 2-ethylhexyl alcohol (high odor), isopropyl alcohol (flammable), butanol (flammable), ethanol (flammable), aromatic solvents (flammable/high odor) and glycols. The amount of solvent in the product varies depending on many factors but can account for almost 40 - 50% of the weight. All of these solvents serve only one function – to make the DDBS salts less viscous for processing and provide little to no benefit to the emulsification. In the work that will be presented we have formulated solvent free DDBS salts (Ca and amine) and it is used in a truly solvent free liquid emulsifier package (Sponto EC-430 SF) that can be used to make stable ECs such as 2,4-D ester.

AGRO 330

Design and development of a novel green solvent: An unsaturated alkyl amide as a surfactant-solvent hybrid

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Over the past two decades, the crop protection industry has sought to reduce or eliminate the use of naphtha-based solvents in liquid formulations and replace them with new bio-based solvents. This global trend, initially driven by increasingly stringent regulations on carcinogens, regulatory actions on certain solvents classes, and the need for environmentally safer formulations has led to the development of numerous green solvents, which have mostly been based on esters. Beyond the obvious advantages of lower toxicity and environmental impact, bio-based solvents can, in some cases, offer better performance over traditional solvents. However, a recent challenge in the crop protection industry has been formulating with increasingly complex active ingredients that have large molecular weights, sensitive functional groups, and more chiral centers. As a result, new green solvents with even greater performance benefits are needed to solubilize these complex actives that are entering the market. In this presentation, we report the development of a novel solvent that combines the effectiveness of a low HLB non-ionic surfactant with the strength of a powerful solvent. This metathesis-derived compound contains amide and olefin functionalities and is synthesized from a renewable feedstock. Additionally, it is non-flammable and has a low vapor pressure, a mild, low odor and a low pour point. With these advantages, combined with a Kauri-butanol value over

1000, this solvent is able to solubilize a wide range of actives to give stable, safer formulations.

AGRO 331

Soybean oil as a “green” carrier for agrochemical formulations

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Benefits of soy-based agrochemical formulations as alternatives to petroleum-based are well-recognized (i.e., renewable, biodegradable, environmentally benign). Soy-based adjuvants have seen some recent success, where approximately 20% of all adjuvants used in crop protection are soy-based. However, soybean oil as a carrier for agrochemical formulations has been slower to develop due in part to technical hurdles, including poor performance as a solvent and difficulties to emulsify. Recent advances in oil dispersion (OD) formulation technology show promise for using soybean oil as a carrier. One of the main technical challenges is solubility of chemical compounds in soybean oil. Low solubility is favorable for OD formulations, whereas higher solubility is useful for emulsifiable concentrate (EC) formulations, possibly with a co-solvent. A study that explores the feasibility of using soybean oil as a carrier for OD formulations and as a solvent for EC formulations is presented. Compatibility of several chemical compounds (e.g., glyphosate, 2,4-D) was investigated for solubility and chemical stability in soybean oil. Based on the compatibility, the feasibility for developing an OD formulation was explored in terms of active ingredient loading, ease of milling, and evaluation of suitable dispersants, emulsifiers, and suspending or thickening agents. Further evaluation of active ingredient loading and suitable soy-based emulsifier systems for EC formulations was also investigated.

AGRO 332

Use of yeast stress-induced proteins to affect the function of surfactants and their application in agricultural formulations

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It is known in the literature that surfactants have long been used to affect the structure and function of proteins in certain applications. However, the use of proteins to affect the function of surfactants has not been explored. This work will show that small proteins (< 30 kDa) derived from yeast stress-induced systems improve the performance of a broad range of surfactants via formation of a protein-surfactant complex (PSC) and have applications in several agrochemical adjuvant applications. Proteins produced by stress induced yeast reduced the crtical micelle concentration (CMC) of example systems of nonionic, anionic, cationic, and amphoteric surfactants. Additionally, the dynamic surface tension (DST) were reduced with the presence of the proteins. Wetting and penetration of water containing PSCs into tomato and cabbage leaves was observed to be faster and more complete than water with surfactant alone. Also, the wetting and penetration of dry soil by water containing PSC was compared with commercial soil wetting agents. The end result of moisture retention is presented in a turf application.

AGRO 333

Understanding the applicability of in-vitro assays for assessing eye irritation and skin sensitization potential to support crop protection formulation development

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The development of new crop protection formulations increasingly requires consideration of acute toxicity as a design parameter. The use of non-animal toxicity screening, together with novel formulation technologies, increases the level of confidence in delivering formulations without critical acute toxicity effects (eg. eye irritation and skin sensitization) which could potentially limit the ‘registrability’ of the product in certain geographies.

The performance of two *in vitro* cytotoxicity-based eye irritation prediction assays Neutral Red Release (NRR) and EpiOcular™ (EO) as well as the KeratinoSens™ assay for prediction of skin sensitization were evaluated on a range of agrochemical formulation types for which *in vivo* animal data were already available to correlate. The eye irritation *in vitro* assays were implemented in a tiered approach to over 70 agrochemical formulations and exhibited greater than 80% overall accuracy while the KeratinoSens™ assay correctly classified 17 of 18 active ingredients (AI) and their formulations.

An overview of these results is presented with intent to build a reliable toolbox of animal-free methods for screening acute toxicity potential. Ultimately, these tests could be incorporated into an overall weight-of-evidence assessment approach aimed to minimize or even replace the use of animals needed for the actual registration of agrochemical formulations.

AGRO 334

Chlorpyrifos formulations and leachability studies

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Large numbers of case studies have been reported for residues of chlorpyrifos in water bodies above MRL values in India. There is an extensive use of chlorpyrifos not only in crops for control of various pests being broad spectrum, but it is also used widely for termite control after the ban of Aldrin (highly toxic) in India which was used as a homicide by some farmers.
It is generally recommended at the rate of 1 Lm^{-2} at 20% EC formulation but its usage is higher than its recommended dose by pest controllers and also by ignorant farmers. A study was carried out to know the leachability of various chlorpyrifos formulations to know the best suitable formulation for effective usage, avoiding contamination of water resources through leaching.
Chlorpyrifos emulsifiable concentration (EC), wettable powders (WP), liquid concentrate (LC), wettable granules (WG) and micro encapsulation (ME) formulations were studied in lysimeters in neutral soil to know the persistence and leachability.

For leachate samples, maximum leachability was found in EC (102.147 µg mL^{-1}) and minimum in ME formulation (29.712 µg mL^{-1}) of chlorpyrifos in one year. Leaching in water samples was found to be in increasing order of ME<WG<WP<LC<EC. The result indicated that safety of ME and WG formulation was high as compared to the other formulations of chlorpyrifos from leaching point of view. However, there are other reports of high residue in crop produce of ME and WG formulations of chlorpyrifos (Alberto et al. 2011).
Therefore, while recommending ME and WG formulations as in edible crop produce, studies would be required for residue analysis in local crops. ME formulation of chlorpyrifos can be safely used in turfs, buildings, crevices, foundations etc. due to less leachability in view of not being an agricultural commodity.

AGRO 335

Lead generation: Revving up the engine of discovery

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Since the 1800's, crop protection products have been critical for enabling farmers to feed the global population. 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 lead chemistries with novel modes of action. While crop protection industries have been successful in meeting some of these demands, the challenge is far from over. Many lead generation strategies across the industry have been employed to increase the probability of success. At Dow AgroSciences, we have established a portfolio of approaches to accelerate lead generation. These approaches, along with few case studies, will be discussed.

AGRO 336

Fluorine chemistry at Bayer: Enabling new products

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The talk will give an overview of several aspects of fluorine chemistry at Bayer which explores the incorporation of fluorine into different structural motifs to be found in modern agrochemicals. The challenges associated with achieving technical feasibility for the large scale production of an agrochemical will be a uniting theme.

AGRO 337

Design and synthesis of pyridine and pyrimidine derivatives as insecticides

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A series of pyridine or pyrimidine derivatives shown below demonstrate excellent insecticidal activity on Hemiptera insects, such as green peach aphid, cotton melon aphid, corn planthopper. We will give the details of chemical synthesis, biological activity and structure-activity relationships during this presentation.

References:
Xu, M. PCT Int. Appl. WO 2012/087630. **2012**; *Chem. Abstr.* 2012*, 157*, 151634.

AGRO 338

Pro-insecticidal approach toward increasing *in planta* activity

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In the pursuit of new agrochemicals, many new lead generation hits show initial promise against isolated targets, but fail to demonstrate the desired biological effects *in planta*. Recently, while exploring thiourea analogs (compound 1) as potential new insecticides against sucking pests, it was found that this area of chemistry showed potent biological activity against aphids and whiteflies in early screening. However, the activity did not translate to more stringent field-simulated testing conditions. This presentation will discuss the design and synthesis of a series of more lipophilic "pro-insecticide" analogs. Specifically, the effect of protecting groups on transport through leaf tissue, metabolism *in planta* on a variety of crops, and ultimately the effect on insecticial activity translation will be discussed.

AGRO 339

Molecular modeling of inhibition of fatty acid biosynthesis by post-emergent herbicides

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Acetyl-CoA carboxylase has been a useful and important target of aryloxypropionic acid derivatives and the substituted cyclohexanediones, used to control annual and perennial grasses. These classes of herbicides act by blocking fatty acid biosynthesis in grasses and other monocots while leaving broadleaf crop plants (dicots) unaffected in treated fields. Inhibition takes place by blocking fatty acid biosynthesis at the transcarboxylation step in the transformation of acetyl Co-A into malonyl Co-A by the enzyme acetyl Co-A carboxylase in monocots. Development of herbicide resistance is a problem, which can be addressed by modeling and understanding of the protein domain and binding mechanism by which the herbicide acts. Known crystal structures from yeast ACCase show that both the aryloxypropionic and cyclohexanedione derivatives bind near the interface between two domains of this multidomain protein in slightly different zones and with different residues. Although ACCase crystal structures in plant ACCase are not available, sequences in yeast ACC are highly conserved in plant ACCase. In this study homology modeling followed by molecular dynamics simulation and has been used to transform the available ACCase structures into those for wheat, corn, and weed grasses. A series of aryloxypropionic acid and cyclohexanedione congeners have been docked into the transcarboxylation domain of these ACCases and scored. Binding affinities in the docked poses were computed and compared using ab initio and density functional methods. This work emphasizes the utility of molecular modeling as a tool for discovery in pesticide chemistry.

AGRO 340

Challenges encountered in the structure elucidation of metabolites

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

test material itself is extensively metabolized. More often than not, the structure elucidation of metabolites becomes even more complicated due to complexity of bound residues. The industry standards for the extent of characterization and identification of bound residues will be discussed.

The analytical tools which are used to identify metabolites are constantly advancing and it becomes imperative to use the state of the art technique to facilitate identification of metabolites. Normally, identification of metabolites is carried out by co-chromatographic retention time comparison with known reference compounds using two dissimilar chromatographic systems. However, unequivocal identification of unknown metabolites poses significant challenges especially for more polar metabolites and conjugates. The identification of polar metabolites and conjugates becomes increasingly difficult when they are relatively not retained on a reversed phase HPLC column. It requires a significant amount of HPLC method development to purify and isolate the more polar metabolites and conjugates

Depending on the purity of the isolated polar metabolite or conjugate, the identification by LC/MS/MS or NMR as appropriate may pose additional challenges. Identification by NMR spectroscopy is very rare in metabolism studies as we are dealing with nanogram to microgram quantities. The industry standards for characterization and identification of metabolites will be presented.

AGRO 341

Trace level metabolite identification using high resolution mass spectrometry coupled to low flow separations

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The development and registration of new agricultural chemicals requires the identification of a variety of trace level unknown environmental metabolites. Within regulatory studies, trace level metabolites are generated in a variety of plant, animal, and environmental systems. In Discovery, the translation of in-vitro activity to field activity often requires an understanding of metabolism of potential lead compounds in the host species, target species, as well as soil and other environments. These unknowns are generally present at ultra-trace (low pg) levels, requiring that their initial identification be performed using high resolution/accurate mass LC/MS. We will describe our use of high resolution mass spectrometry combined with micro and nano-LC separations to separate and detect these trace level metabolites. These approaches, combined with the application of controlled isotopic labelleling, can provide a rapid and effective approach for metabolite identification.

Metabolites were generated in soil, plant cell culture, and whole plant systems. These samples were analyzed using UHPLC separations on several LC/MS systems including: 1) a Sciex 5600 (QqTOF), 2) a Thermo Q Exactive (QO), and 3) a Thermo Fusion (QOLIT) instrument. These were configured for conventional UPLC, microLC, as well as nanoLC separations. In conventional LC, radiolabeled metabolites were tracked using online (RAM) detection, and ESI ionization combined with accurate MS and MS/MS data acquisition was performed. When required, the fractionated LC effluent was collected and assayed to localize fractions containing the peaks of interest. These fractions were then concentrated and analyzed using 1) nano infustion on an Advion Nanomate, 2) microLC on an Eksigent Ekspert MicroLC 200, or 3) 2-D nanoLC on an Eksigent NanoLC Ultra operating in trap and elute mode. Data was collected in a combination of MS, MS/MS, and MS^n modes at resolutions up to 450,000 (FWHM) depending on the experimental demands. These methods were employed in the identification of xenobiotic metabolites produced in both plant and soil systems.

AGRO 342

Identification of Indaziflam metabolites in the rat

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Indaziflam is an alkylazine herbicide for non-selective pre-emergent and early post-emergent control of grass and dicot species in trees, nuts, vines, industrial vegetative management, and turf. As part of the development program for this compound, metabolism studies in the rat were performed where a variety of oxidative metabolites were identified in excreta and tissues. The structures of those metabolites were determined using a combination of chromatographic, mass-spectral, and nuclear magnetic resonance techniques. The methodology used to determine the regio- and stereochemical locations of metabolic oxidation will be presented.

AGRO 343

Fractionation and characterization of bound and unextractable pesticide residues in plants

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Bound pesticide residues are basically those residues remaining in plants after exhaustive solvent extraction. These residues have been detected to incorporate, conjugate, and/or encapsulate in the protein, starch and/or lignin/cellulose constituents of plant tissue. Determination of the nature and quantity of bound pesticide residues in plants has been a challenging problem for researchers. Fractionation methods in soybean and wheat matrices are discussed to establish the bound residue into natural cellular components.

AGRO 344

Fishing for unknown metabolites of nonradio labeled molecules “cold compounds” in samples of biological, environmental, and complex origins using high resolution time of flight mass spectrometry and METABOLYNX™

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Fishing for metabolites of non-radio labeled molecules in variety of samples using HPLC-MSTOF will be discussed. For many decades industries have relied on radio labeling “Tagging” of their lead compounds prior to initiating long term metabolism and environmental fate (E-Fate) studies. Tagged molecules accurately account for compound’s mass-balance in a particular media, therefore from the regulatory standpoint the entire metabolic profile or degradation and biotransformation pathway are clearly demonstrated leaving no ambiguity if there were leftover potential toxic metabolites or degradation compounds unstudied.

The Carbon-14 (C^{14}) radiolabel has been the most common technique to tag molecules. Depending on the chemistry and complexity of C-14 synthesis, making a C^{14} molecule can potentially be a lengthy process. Therefore, in the interest of time and at the inception of discovery and at the compound selection criteria stage, and for cost considerations, industries rely on accurate mass LC-MS technologies to gather preliminary data on biotransformation pathways and rate of metabolism to choose a candidate with which to move forward.

The HPLC-MS-TOF in conjunction with powerful software "METABOLYNX™" utilized to fish out new metabolic by-products. This software is designed to facilitate metabolism scientists in discovering metabolites from samples without the need for radiolabel tagging. The accurate mass technology provides the confidence needed to resolve a legitimate metabolite from matrix-co-extractives while METABOLYNX™ tool directs a metabolism chemist on potential metabolic products based on its built-in various metabolic pathways which have been derived and designed from academic and extensively-published sources. In addition, in the case with halogenated molecules as an added bonus, the METABOLYNX™ employs an additional built-in technique called "Isotopic Cluster Analysis-The Strip Program," which provides a way of removing unwanted background and noise from a data file that will be discussed.

AGRO 345

Transformation of [^{14}C]Fluensulfone into lactose in the lactating goat

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Characterization of metabolites transformed into natural products is an important yet challenging aspect of metabolism studies. In a lactating goat metabolism study conducted with two radiolabeled forms of the nematicide fluensulfone, methods were required to characterize radiolabel association with natural products. HILIC HPLC and normal-phase TLC were used in conjunction with phenylhydrazine derivatization for analysis of radiolabel; using glucose and lactose as saccharide standards. Fluensulfone was found to be extensively metabolized with no parent compound detected, and the radiolabel being incorporated into natural products; including the disaccharide lactose. HILIC chromatography and derivatization proved to be useful tools for characterization of ^{14}C-residues from test substances whose metabolism involves biosynthetic pathways.

AGRO 346

Corrinoid quantity and quality determine reductive dechlorination rates and extents

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Hydrogen-driven reductive dechlorination is the only energy-conserving pathway in obligate organohalide-respiring *Dehalococcoides mccartyi* (*Dhc*). The reductive dehalogenase (RDase) enzyme systems that serve as terminal oxidoreductases require a corrinoid cofactor for catalytic activity. Remarkably, *Dhc* strains lack the ability for *de novo* cobamide biosynthesis and strictly depend on corrinoid scavenging. *Dhc* strain BAV1 cultures that received 25 µg vitamin B_{12} L^{-1} dechlorinated 1,2-dichloroethene (cDCE) to ethene, but incomplete dechlorination to vinyl chloride (VC) occurred in cultures amended with 1 µg vitamin B_{12} L^{-1}. Using *Dhc* strain BAV1 and strain GT carrying the cDCE/VC reductive dehalogenase genes *bvcA* and *vcrA*, respectively, the effects of cobamides with different lower bases on dechlorination rates and extents were explored. Amendment of 5′,6′-dimethylbenzimidazolyl-cobamide (DMB-Cba) to *Dhc* strain BAV1 and strain GT supported cDCE-to-ethene reductive dechlorination at rates of 100 and 70 µM Cl^- released day^{-1}, respectively. The dechlorination rates decreased up to 5-fold with cobamides carrying 5′-methylbenzimidazole (MeBen), 5′-methoxybenzimidazole (MeOBen), or benzimidazole (Ben) as the lower base. Strain-specific responses were observed in cultures supplied with MeOBen-Cba or Ben-Cba, and only strain BAV1 harboring the BvcA VC RDase, but not strain GT expressing the VcrA VC RDase, produced ethene. Amendment with DMB restored the VC-to-ethene-dechlorinating phenotype in strain GT. These findings reveal that cobamide quantity and quality (i.e., the type of lower base) affect *Dhc* reductive dechlorination rates and extents (i.e., ethene formation) and thus impact bioremediation.

AGRO 347

Reductive dechlorination of dichlorobenzene isomers and monochlorobenzene by *Dehalobacter* spp

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Chloroaromatic compounds, including chlorobenzenes (CBs), are major industrial and agricultural pollutants. We enriched and purified three cultures of *Dehalobacter* (Dhb) from sediments, each on a different dichlorobenzene (DCB) isomer. All strains used H_2 as an electron donor and dechlorinated their respective DCBs to monochlorobenzene (MCB). Culture 12DCB1 used 1,2-DCB and other singly-flanked CB congeners. Culture 13DCB1 used both 1,2-DCB and 1,3-DCB, as well as other congeners with single-flanked or meta chlorines, including 1,3,5-trichlorobenzene, produced from hexachlorobenzene by *Dehalococcoidia* that use doubly-flanked chlorines. Culture 14DCB1 used 1,4-DCB and other congeners with para chlorines. In contrast to the DCB utilizers, we have been unable to greatly purify a Dhb culture that dechlorinates MCB to benzene, but we have obtained a mixed methanogenic and dechlorinating culture that uses butyrate as an electron donor.
We used Illumina HiSeq 2x100 technology to determine draft genome sequences of the three DCB-utilizing Dhb cultures. Their genomes range from 2.88-3.06 Mb and contain 28-40 reductive dehalogenase (RDase) genes as well as genes for sporulation, motility, and chemotaxis. We used blue native polyacrylamide gel electrophoresis (BN-PAGE) and shotgun proteomics to identify the RDases involved in DCB dechlorination in each strain. Surprisingly, only a single RDase was found in each case, and the RDases were close homologues, with 12DcbA and 13DcbA sharing 95% amino acid identity and 12DcbA and 14DcbA showing 90% identity. More recently, we used Illumina MiSeq 2x300 technology to obtain a metagenome sequence from the butyrate-MCB culture. Fourteen contigs ranging from 24-924 kb were identified as coming from Dhb, totaling 2.9 Mb, essentially complete. Also identified in the metagenome were contigs from acetotrophic and hydrogenotrophic methanogens, and the butyrate oxidizing *Syntrophomonas*, all organisms expected in a butyrate utilizing methanogenic consortium. BN-Page identified an MCB RDase with 96% identity with 12DcbA. Thus, relatively small sequence changes led to changes in substrate specificity among the four enzymes. These RDases and their genes are potential biomarkers for chlorobenzene dechlorination in the environment. Using recently developed heterologous expression systems and guided by recent RDase crystal structures, it will be interesting to explore the effects of sequence variation on substrate specificity.

AGRO 348

Role of the genus *Dehalogenimonas* in anaerobic chlorinated alkane dehalogenation: Polychlorinated ethanes and propanes

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Both species of the bacterial genus *Dehalogenimonas* that have received formal taxonomic descriptions to date (i.e., *D. alkenigignens* and *D. lykanthroporepellens*) possess the unique metabolic trait of growing via organohalide respiration. Under anaerobic conditions, both species are able to utilize environmentally-important polychlorinated ethanes (e.g., 1,2-dichloroethane and 1,1.2-trichloroethane) and propanes (e.g., 1,2-dichloropropane and 1,2,3-trichloropropane) as electron acceptors for growth. A growing body of evidence suggests that these novel metabolic abilities allow both species to play an important role in *in-situ* contaminant transformation. The recent isolation and phenotypic characterization of a novel strain that clusters within the genus *Dehalogenimonas* based on 16S rRNA gene sequencing but with a lineage distinct from previously described species provides enhanced understanding of the diversity of this genus. Like previously described species of the genus, the novel *Dehalogenimonas* strain utilizes several polychlorinated alkanes including 1,2-dichloropropane and 1,2,3-trichloropropane as electron acceptors. The determination of a draft genome sequence for the strain and comparison with reference genomes provides insights into the genetic diversity of this organohalide-respiring clade.

AGRO 349

Microbiology, biochemistry, and genomics of the transformation of halogenated aromatics by *Dehalococcoides* strains

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Halogenated aromatic compounds encompass many of the legacy persistent organic pollutants but also emerging contaminants including pesticides, disinfectants, flame retardants and pharmaceuticals. Strains of the bacterial genus *Dehalococcoides* can use such halogenated aromatics as terminal electron acceptor for energy conservation and growth ("Organohalide respiration"). *Dehalococcoides mccartyi* strain CBDB1 is a model organism for which the microbiology is well described, the genome is sequenced, expression profiles with various electron acceptors are known, a chlorobenzene reductive dehalogenase has been identified by native gel electrophoresis and mass spectrometry and the major lines of the anabolism are described. The presentation gives an overview of the research with strain CBDB1 and highlights current research on the debromination of brominated flame retardants, the biochemical description of the reductive dehalogenase complex, and model-based prediction of dehalogenation pathways.

AGRO 350

Characterization of the activities of *cis*-3-chloroacrylic acid dehalogenase homologues: Analysis and implications

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The *cis*- and *trans*-3-chloroacrylic acid dehalogenases (*cis*-CaaD and CaaD) catalyze the cofactor-independent hydrolytic dehalogenation of the *cis*- and *trans*-isomers of 3-chloroacrylic acid to yield malonate semialdehyde and HCl. They are found in bacterial pathways that are partially responsible for the rapid degradation of the isomeric mixture of 1,3-dichloropropene, which has extensive agricultural use as a nematicide. Kinetic, mechanistic, and structural studies identified the key components of the catalytic machinery for both enzymes. Characterization of recently identified *cis*-CaaD homologues provides new insights into the mechanism, specificity, and reactions of *cis*-CaaD and CaaD.

AGRO 351

Complexity of spray drift research: Knowing where to look for trends that are out of the ordinary

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Spray drift research has become central to understand when, where and how pesticides should be applied. The easiest way to mitigate spray drift is through droplet size management (i.e. selection of low drift nozzles, formulations and adjuvants). Laser diffraction is commonly used to measure droplet size, but this approach has limitations. While laser diffraction measurements are high throughput, it does not account for droplet velocity or other external factors, but can give some indication of what the drift potential may be knowing that larger droplets have a greater propensity to deposit in the targeted area. A series of experiments were conducted at the Pesticide Application Technology Laboratory at the University of Nebraska-Lincoln's West Central Research and Extension Center in North Platte, NE. The studies show that there are complex relationships that must be consider to provide applicators the best recommendations on spray drift mitigation. For example, certain nozzle types, such as the TT nozzles from TeeJet, are not compatible with certain spray adjuvants and formulations, such as emulsion based products. Knowing where to look for these complex interactions is important so that as accurate of recommendations can be made. Additionally, initial studies indicate that even if two sprayers are producing similar droplet sizes and making applications the same way, there may be differences in the drift potential due to droplet velocity or other application parameters.

AGRO 352

Comparison of multiple sampling methods for evaluation of off field airborne chemical movement

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Three active ingredients (dicamba, 2,4-D, and imazethapyr) with different physio-chemical properties were sprayed with different spray nozzles in the field. After spray solutions dried, several different samplers were used to determine if residues could be detected. The use of large orifice air induction nozzles producing relatively coarse sprays greatly reduced measurable residues compared to smaller orifice

conventional nozzles which produce finer sprays with smaller droplets. Results from this study indicate that atomization rather than physical-chemical properties determined the quantity and duration of residues measured. Additionally, the PUF sampler technique which is commonly used by many researchers recently, proved to be the least desirable sampling technique based on residue trend and analytical ease of use.

AGRO 353

Wind-controlled approach for spray drift testing

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Off-target movement of spray drift remains a concern. While "zero drift" is acknowledged as an impossible standard to meet, more scientific research on spray drift effects and reduction are needed. Spray drift studies in the field are challenging, expensive, and time consuming, due to continuously changing wind patterns and the need to "hurry up and wait" for suitable wind conditions to spray and collect downwind samples. To overcome this challenge, a full-scale, weather-independent spray testing facility is used. The spray testing facility allows spray drift testing in a wind-controlled environment, effectively reducing the time and costs typically associated with field studies. The spray testing facility is approximately 150 ft long with a 20 ft by 20 ft cross section, and can support low-speed and high-speed wind tunnel conditions. An approach for conducting wind-controlled spray drift testing is presented, and includes both spray droplet size characterization at the nozzle source and downwind sediment and airborne drift sample collection. Different drift reduction technologies, formulation parameters, spray and wind conditions can be efficiently tested to collect a robust, defensible data set to support further risk assessments of products.

AGRO 354

Recommendations for uniformity in spray drift field studies

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The October 2014 launch of USEPA's Drift Reduction Technology program provides registrants with an opportunity to gain quantitative credit in the risk assessment by demonstrating reduced drift from modern spray nozzle design. Registrants can take advantage of this credit by generating drift data for specific active ingredients and formulations using different nozzle technologies. These data can then be used to reduce spray setbacks (or buffers) on product labels for both off-target organisms and endangered species. With different companies and scientists generating these data (as opposed to a single entity such as the Spray Drift Task Force) there is a need to establish uniform methods for generating and presenting these data. These data will help develop an eventual model or other statistical representation of the results that will allow omitting the expensive field studies in the assessment of drift technologies. In this future, droplet spectra from wind tunnels can be entered into the model to generate reliable results that reflect the reality of the field. Guidelines from EPA, ISO and ASABE for general study design currently exist. Consideration of equipment, procedures, and data reduction for consistent methodologies will be discussed.

AGRO 355

Probability of multiple applications having the same wind speed and key meteorological parameters and the resulting impact on pesticide loadings and exposure

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U.S. EPA aquatic exposure modeling includes a 5% drift loading for aerial application in their standard Tier II modeling. If a drift buffer is included on the label, the USEPA uses the AgDRIFT® aerial Tier I default values (wind speed of 10 mph, temperature is 86°F, and relative humidity is 50%) to calculate the drift on the standard pond. For a single application, that approach is a feasible worst-case hypothesis. However, the likelihood of these conditions co-occurring for each one of a series of multiple applications (which are common on pyrethroid insecticide labels) decreases rapidly as the number of applications within a cropping season increases. This presentation examines the potential for aerial drift when the actual hourly wind speed, temperature, and humidity are taken into account on the day of application. Thirty years of wind speed will be analyzed for various U.S. EPA standard scenario weather files over cropping seasons. Comparisons of the drift loadings and associated aquatic EECs in the standard pond will be provided to show the difference between using standard U.S. EPA spray drift defaults and using the spray drift loads based on real-world measured wind data. The results show that the magnitude of the effect is dependent upon location and numbers of applications but that the default drift loading assumptions can exaggerate 90th percentile EECs up to 2 fold compared to using the real-world weather data. The presentation will also examine the accompanying probabilities of multiple applications having similar wind directions.

AGRO 356

Emulsion-based drift control: Influence of interfacial properties

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Observations of reduced driftable fines for emulsion-containing agricultural spray solutions have led to proposed sheet break-up mechanisms that are dependent on the interfacial properties of the spray solution, including the oil-water and water-air interfaces. To further probe the variation in fine droplet creation as a function of interfacial properties, a Design of Experiments (DOE) was constructed with four oils, three surfactants, three oil-to-surfactant ratios, and two emulsion (or total oil) loadings. This resulted in an intentionally wide range of measured colloidal and interfacial properties, including dynamic and equilibrium interfacial tension of the oil-water and water-air interfaces, zeta potential, spreading coefficients, and emulsion particle size. Aqueous dilutions of each of the formulations were also sprayed to quantify the amount driftable fines, which are defined here as the volume percent of droplets less than 150 µm in diameter. It was found that the DOE parameters contributed significantly to all measured responses, including driftable fines. The relationships found between the measured interfacial properties and observed driftable fines results including the capacity for these physical property measurements to reliably predict fine droplet production in emulsion-based systems will be presented.

AGRO 357

Software visualization and automation for making sense of the ever increasing amounts of mass spectral data

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Modern mass spectrometers produce a wealth of analytical information capable of identifying targeted and unknown compounds in complex mixtures. As the amount of data generated increases, the importance of software for interpreting the results becomes even more important. Software is particularly critical for understanding large amounts of data, by improving visualization of data, automation of processing, and performing the actual storage / retrieval of this data.
Visualization is important for understanding data. It is much easier to grasp the meaning of large amounts of data when it is displayed graphically and interactively, than when it is simply a table of numbers. Examples from both screening and quantitation software will be shown that demonstrate the importance of interactivity and visualization for understanding data.
Automation of processing is another area where software plays a critical role. Existing solutions include the ability to automatically identify unknown compounds through library searching and formula finding. Where we, as a community, go next is very exciting. Today's results files are increasingly open formats (XML) or convertible to other formats. Simple, command line driven, tools exist for processing data and generating reports. Combining open formats with simple tools could empower a variety of custom workflows.
Underlying visualization and automation, is the data itself. The amount of data, the number of compounds (known or unknown at analysis time) and the speed at which answers can be mined from it, all become important. Data independent acquisition (SWATH) ensures that MS/MS for all possible compounds is collected, but it does increase the amount of the data being collected. As the amount of data being collected increases, the speed of extracting chromatograms and MS/MS becomes a critical step.
Visualization, automation, and speed of processing are critical factors for analysing and understanding the wealth of data produced by modern mass spectrometry analysis.

AGRO 358

Impurity characterisation of the fungicide flutriafol using liquid chromatography and time of flight MS detection to aid pesticide product registration

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The identification and quantification of impurities plays a critical role at all stages of both the pesticide development and manufacturing processes, and are essential requirements for the registration of crop protection products. The structure of impurities present at or greater than 0.1% must be identified to ensure that the overall safety of the formulated product is well understood. During the development process, different routes of synthesis may be evaluated, each producing its own distinct impurity profile. It is critical that these synthesis routes are thoroughly investigated with an aim to identify relevant impurities alerted by the threshold criteria. In this study, technical grade flutriafol was analysed by LC/ToF-MS/UV and relationships between the minor unknown components and the flutriafol were identified.
Technical grade flutriafol was used to prepare a stock solution of 1 mg/mL in methanol prior to analysis. An LC method was developed that consisted of a 2.1 x 100 mm C18 column (1.6 µm particle size) and a mobile phase gradient that utilised 0.1% formic acid in water and acetonitrile. Positive ion electrospray ionization (ESI+) MS was performed on a high-resolution QToF monitoring a mass range covering 50-950 Da. An orthogonal detection method, photo diode array (PDA), was acquired simultaneously from 210-400 nm and used to calculate the chromatographic peak area % concentrations.
Peak area % contributions exceeding the chromatographic peak area threshold greater than 0.1% as determined by PDA detection (220 nm) were flagged for characterisation using Q-ToF MS. Two components were tentatively identified as isomers of the active ingredient flutriafol, a third unknown component was also observed. The unknown component was found to have structural features that were common to the flutriafol active ingredient. Based on the elemental composition of the fragments isotopic fit to theoretical elemental compositions, knowledge of the synthetic route and the structure of the active ingredient, a structure for the unknown component having *m/z* 314.1303 was tentatively proposed. Based on the results of this study, a process for pesticide impurity profiling for pesticide development is proposed.

AGRO 359

Applying tensor decomposition model for high-dimensional toxicogenomics data analysis and interpretation

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High-throughput techniques in toxicogenomics provide a cost-effective way in studying the vast and ever-increasing number of toxicants, but generate a large amount of high-dimensional data in the meantime. These data often feature the simultaneous measurements of numerous genes' expression, as well as complicated relationships among multiple experimental factors such as genes, time points, and toxicant concentrations. Compared to the dimensions of the data, the experimental sample size is usually limited to draw confident conclusions using analytic methods designed for low-dimensional data. Tensor decomposition is a type of unsupervised learning method that separates high-dimensional data into principal components, each representing a stereotypical pattern of variation in the data. As the effect of the major sources of variation is decoupled through these components and filtered-out from the background noise, this improves our ability of extracting possible causal relationships. In this work we applied PARAFAC (parallel factor analysis), a specific tensor decomposition model to our three-dimensional (gene × time × concentration) gene expression time series data. We found that we could uncover several plausible toxicity mechanisms of action by decomposing the original high-dimensional data into several components and investigating them individually. The results of PARAFAC also helped us quantify the dose-response relationship, namely the relationship between the toxicological responses and toxicant concentration for each possible toxicity mechanism. Our results indicated that tensor decomposition is an invaluable tool for toxicogenomics studies with complex design and can become an integral part of the data analysis workflow.

AGRO 360

Computer systems validation and e-data

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Scientists are generating an increasing amount of electronic data (e-data) used to support regulatory submissions. Regulatory requirements in the GLP arena to this point have allowed for differing levels of interpretation as to which standard or guidance to follow for CSV within the industry. The increasing volume of e-data, advancing technology and the variation in interpretation of requirements are causing scientists to rapidly transition from the paradigm all "raw data is paper" to raw data is the "initial capture" whether in paper or electronic form. In an effort to ensure data integrity during the collection, processing, and storage of e-data, we have implemented a risk management based Computer Systems Validation (CSV) strategy as a means to ensure necessary testing has been performed on computer systems. We have developed strategies to validate and maintain our computer systems to ensure integrity of the data throughout the entire data life cycle. The objective is to ensure standardization through creation of:

- A sustainable CSV life cycle process including system assessments, validation strategies, change management, and retirement processes.
- A sustainable e-data life cycle strategy including acquisition/analysis standardization, backup, archival, searchability, and retrievability.

The ultimate goal is to establish an environment where the capabilities exist to:
Perform all tasks from initiation to summarization electronically (inventory management, protocol/report generation, data collection, analysis, and maintenance)

- Seamlessly share data with global partners (internal/external)
- Transmit Electronic Study Files to regulatory bodies for review/audit

AGRO 361

Straightforward, unified approach to tracking compound progression, analysis, and work-requests

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Keeping track of the status of substances (compounds, formulations, etc.) from initial synthesis through all levels of analysis and testing is a challenge in any laboratory setting. It is critical to know what work has been requested and whether it has been completed. What compounds have been made? Which have been analyzed? Which have progressed through each assay, and do the results justify testing them further through the battery of analyses and assays? This becomes even more complex to track when monitoring compound or assay data from multiple sources. Fully integrated software tools allow users to monitor the lifecycle of the entire workflow and request process; from requesting and tracking the experiments for synthesis of chemical substances to analysis and multiple stages of testing with a single search, and quickly drill into details for individual syntheses, analyses, or assays.

AGRO 362

Allotrope framework: An innovative collaboration to improve data interchange, increase research efficiency, and realize the full value of your data

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Allotrope Foundation is building a sustainable solution that addresses the complexities and gaps across the analytical data lifecycle created by the diverse array of proprietary file formats, and incomplete, inaccurate or inconsistent metadata. We can't easily share or compare data across laboratories due to the incompatible file formats. We can't understand or interpret data later because the context is incomplete, inconsistent, or spread across multiple applications or systems. The Allotrope Framework is comprised of: vendor neutral, extensible file format to store data & metadata built on existing standards; Metadata definitions & repository to provide accurate, complete & consistent contextual metadata; and Class libraries (software toolkit) that instrument & software vendors can use to embed the standards into their software. The Framework will enable capturing the complete context of the analytical workflow, in the same vendor neutral file that contains instrument and processed data and results, described in an industry standard controlled vocabulary.

Allotrope Foundation is an international consortium founded by pharmaceutical and biopharmaceutical companies in 2012 to develop a Framework for transforming the management of analytical data throughout its lifecycle. A series of workshops and other outreach in 2014 have created dialog in other related industries that utilize analytical chemistry, including Agrochemical, leading to the conclusion that the same underlying problems are common to the use of analytical chemistry and that the benefits of a solution would be equally shared.

In this presentation we will share details on the strategy and approach of Allotrope Foundation, as well an update on progress towards the goal of a first release of production quality Framework in 2015, including examples of efforts at Foundation members in 2015 to integrate the Framework in their informatics infrastructure. Tangible progress has been made, and the project is on target to deliver its value.

AGRO 363

Assessment of human biomonitoring data in a public health risk context: Utility of biomonitoring equivalents

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Measurements of environmental chemicals in air, water, or other media can be compared to health-based screening values to identify chemical exposures that may be of concern, or to identify chemicals for which a wide margin of safety appears to be present. Interpretation of biomonitoring data for environmental compounds is hampered by a lack of similar screening criteria applicable to measurements of chemicals in biological media such as blood or urine. Such screening criteria would ideally be based upon data from robust epidemiological studies that evaluate a comprehensive set of health endpoints in relationship to measured levels of chemicals in biological media. However, development of such epidemiologically-based screening values is a resource- and time-intensive effort. As an interim effort, the development of Biomonitoring Equivalents (BEs) has been proposed (Hays et al. 2007). A Biomonitoring Equivalent (BE) is defined as the concentration of chemical in a biological medium (blood, urine, or other medium) that would be predicted to result from exposure at an existing health-based screening criterion under the conditions of exposure consistent with that criterion. This presentation will highlight chemical-specific BEs that have been developed and how they can be used in helping to put human biomonitoring data (including pesticides) into a public health risk context.

AGRO 364

Monitoring trends in exposure to contemporary insecticides in the US population

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Insecticides represent a significant portion of the pesticides used in commercial and residential settings in the United States. Interest in assessing the extent of human exposure to these pesticides exists because of their widespread use, their potential adverse health effects, and because this class of chemicals is constantly changing to adapt to insect resistance. In 1990, pyrethroids, organophosphates and carbamates dominated the insecticide market. Today, neonicotinoid and fipronil insecticides are quickly beginning to dominate the market. We have improved and validated an analytical method to measure trace levels of select metabolites of organophosphate and pyrethroid insecticides in urine. In addition to being selective and sensitive for these chemicals, the method is flexible to allow adding metabolites of new insecticides as they become relevant. We have applied this analytical method to the analysis of thousands of samples from different biomonitoring studies including the National Health and Nutrition Examination Survey (NHANES). NHANES provides useful data to examine temporal trends of metabolite concentrations of commonly used insecticides and to evaluate whether trends vary by demographic variables in the United States. We have also observed trends in the concentration of the metabolites similar to the trends in usage of the insecticide. These findings stress the usefulness of biomonitoring as a tool to assess the extent of human exposure to commonly used insecticides and to track the public health effectiveness of legislative actions to reduce exposure to pesticides.

AGRO 365

Protein adducts in dried blood spots as exposure biomarkers in epidemiological research

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While chronic disease and cancers are caused by both genetic and environmental factors, it has been estimated that a majority of disease risk is due to differences in environments. To investigate the role of the environment in disease etiologies, untargeted approaches are needed that can compare chemical profiles across groups in an unbiased fashion. Candidate biomarkers identified in such studies can then be targeted in population-based studies using simple blood collection methods, such as dried blood spot (DBS) sampling. While it is currently not possible to measure all chemicals in the human body in single experiments, important classes of reactive chemicals can be targeted. Here we report a novel approach using protein adduct (addition product) profiles as molecular fingerprints of environmental exposures, called "adductomics". Because protein adducts persist in the blood for the life span of the protein, adducts represent an integration of exposures occurring over weeks to months (e.g., mean residence times are 28 days and 63 days for human serum albumin (HSA) and hemoglobin, respectively). Consequently, protein adducts serve as ideal biomarkers for measuring exposures to environmental chemicals when chronic exposure scenarios are of greatest interest. Once discordant adducts are identified in discovery-based experiments, we then apply targeted protein adduct methods to dried blood spots (DBS), which can be collected using a simple and minimally-invasive finger prick. To achieve unparalleled analytical sensitivity using extremely small volumes of blood, adducts are quantified using a state-of-the-art Agilent 6490 QQQ mass spectrometer with a nano-chip HPLC interface. This analytical platform permits biomarker detection down to the zemptomolar concentration (10^{-21} mol/L) using micro-liter blood volumes that can be obtained from simple and minimally-invasive finger stick blood smaples.

AGRO 366

Organochlorine pesticides in follicular fluid of women undergoing assisted reproductive technologies

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Human are exposed to pesticides due to occupational, dietary and environmental exposures. Some pesticides may interrupt the normal functions of the endocrine system. Female infertility rates have increased significantly since the 1980s. This study was to investigate possible adverse effects of organochlorine pesticide (OCP) exposure on female fertility. Follicular fluid samples were collected from 127 Chinese woman patients (aged 20-35) who underwent assisted reproductive technologies and had no records indicating occupational exposure to OCPs. Among the 17 OCPs analyzed, methoxychlor dominated and accounted for 13% of the total OCPs with a mean concentration of 168 ± 34 ng/g lipid weight, followed by heptachlor-epoxide, hexachlorocyclohexanes, endrin and dichlorodiphenyltrichloroethanes. The levels of OCPs in the follicular fluid samples in the present study were greater than those reported from developed or industrialized countries. The concentrations of OCPs in follicular fluid samples positively correlated to the patients' age.

AGRO 367

Biomonitoring of pyrethroid exposure in Thai farmers and consumers by immunoassay

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Pyrethroids account for a significant portion of the worldwide pesticide sales market, just behind the organophosphates. Because of its high use and potential for human toxicity, it was selected as a target for the national biomonitoring program (NHANES) as well as California's biomonitoring program. 3-Phenoxybenzoic acid, a metabolite of a number of commonly used pyrethroids is used as the monitoring species in urine. We developed an immunoassay for 3-PBA, providing a rapid and cost effective tool for exposure monitoring. The method was validated using spiked samples against an LC-time of flight instrument. Most recently we have used the assay to compare exposure between farmers and consumers in an agricultural area near Chiang Mai, Thailand. Urine and blood samples were collected from 100 farmers and 100 consumers, with an equal portion of males and females. Samples were hydrolyzed to release bound species or products of secondary metabolism, extracted and cleaned up using solid phase extraction. Results showed that the detection rate for 3-PBA was higher in the plasma of

farmers, but the concentrations found were similar in farmers and consumers. On the other hand, the detection rate in urine was similar, but the concentration range found for the farmer group was much higher. In one of the first reported comparisons of 3-PBA concentrations between plasma and urine samples, no correlation was found. This is likely because transient exposures are more likely to be detected in urine, while plasma values represent more long term exposures due to the longer half-life of bound species. The cost of utilizing the immunoassay was about ¼ the cost of an LC method.

AGRO 368

Development of *Helisoma Trivolvis* pond snails as biological passive samplers for the biomonitoring of an agricultural fungicide in wetlands

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Estimating environmental exposure of current-use pesticides (e.g. fungicides) is difficult because most have short water half-lives; making pulsed doses the most relevant exposure scenario. Moreover, many hydrophobic pesticides will partition to suspended material reducing bioavailability. Therefore, typical sampling regimes using infrequently gathered water samples do not provide good measures of exposure. Alternatively, passive samplers that integratively collect available fractions of pesticides from water could be used; however, they need to be deployed and recollected, requiring a planned sampling design and multiple trips to sites. Non-target organisms residing in imbedded cropland wetlands have the potential to be exposed to fungicides through spray drift or accidental direct spraying. Our hypothesis is that some aquatic organisms may accumulate fungicides and maintain residues for longer periods than in surface waters; thus, field-collected organisms could serve as passive samplers based on tissue residues. *Helisoma trivolvis* pond snails were evaluated for their use as biomonitoring tools to predict water concentrations of pyraclostrobin, a strobilurin fungicide ingredient frequently applied to corn surrounding depressional wetlands. Dose-response exposures indicate that *H. trivolvis* is tolerant of pyraclostrobin with a median lethal concentration of 441μg/L (95% confidence intervals of 359-555μg/L). This concentration is greater than environmentally relevant concentrations of pyraclostrobin (86μg/L) assuming direct spraying into shallow wetlands (16cm deep) and full water incorporation using Headline AMP. Following constant pyraclostrobin exposure at concentrations below acutely toxic doses, snails reach equilibrium concentrations within 96hr and have a bioconcentration factor of 170mL/g. Further, depuration studies suggest multiphasic elimination (R^2=0.925) with "fast" and "slow" release compartments with residue half-lives of 11.6hr and 190hr, respectively. As such, fungicide body residues can be measured in snails long after water concentrations fall below detection limits. With high fungicide tolerance, rapid accumulation and long depuration, *H. trivolvis* pond snails may be a viable biomonitoring tool for pyraclostrobin.

AGRO 369

Organohalide respiration in *Sulfurospirillum multivorans*: Structure and function of the tetrachloroethene reductive dehalogenase

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The epsilonproteobacterium *Sulfurospirillum multivorans* was isolated in the mid 1990's from activated sludge. The organism is able to grow under anoxic conditions with chlorinated or brominated ethenes as energy substrates. Tetrachloroethene (PCE) is converted via trichloroethene (TCE) to *cis*-1,2-dichloroethene. The reductive dechlorination of PCE is coupled to energy conservation via a chemiosmotic mechanism (organohalide respiration) in a widely uncharacterized respiratory chain. The key enzyme of this metabolic pathway is the PCE reductive dehalogenase (PceA), a corrinoid-containing iron-sulfur protein [1]. In general, reductive dehalogenases display no sequence homologies to other corrinoid-containing proteins, the structures of which are known. The corrinoid cofactor of PceA is the unusual norpseudo-B_{12} [2], the composition of which differs from vitamin B_{12}. From the necessity of low potential electron donors for the PCE reductive dechlorination the involvement of a superreduced [Co^I] was deduced. The [Co^I] is proposed to function as the reactive species initially attacking the organohalide. This presentation will summarize the actual knowledge about the composition and topology of the PCE respiratory chain in *S. multivorans* and the PceA structure and function.

The PceA enzyme forms a homodimer, which is bound to the outer face of the bacterial cell membrane. Proteome analyses of *S. multivorans* cells cultivated with either PCE or fumarate as terminal electron acceptor allowed for the identification of respiratory chain components specifically required for PCE respiration. Recently, the 3D-structure of the PceA enzyme was solved [3]. In crystals of PceA the norpseudo-B_{12} is bound in its *base off* conformation via hydrogen bridge linkages to the polypeptide chain. The halogenated substrate enters the active site via a defined substrate channel. From the topology of the two [4Fe-4S] clusters a sequential electron transfer to the norpseudo-B_{12} can be concluded. The PceA crystals were found to bind TCE in the active site of the enzyme. The positioning of the substrate in the active site points towards a dissociative electron transfer from [Co^I] to TCE rather than a nucleophilic attack. This result supported earlier findings, which indicated that a substrate radical intermediate might be involved.

[1] Neumann et al. (1996) J Biol Chem 271: 16515-16519
[2] Kräutler et al. (2003) Helv Chim Acta 86: 3698-3716
[3] Bommer et al. (2014) Science 346: 455-458

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Degradation of halogenated alkaloids by the catalytic hemoglobin dehaloperoxidase from *Amphitrite ornata*

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Dehaloperoxidase (DHP), the hemoglobin of the terebellid polychaete *Amphitrite ornata,* is an ancestral multifunctional system that possesses a multitude of activities (peroxidase, peroxygenase, oxidase, and oxygenase), each of which is of sufficient catalytic activity as to degrade a broad range of halogenated aromatic toxins found in marine environments. The two known isoforms of DHP, A and B, have been shown to oxidize trihalophenols to dihaloquinones in an oxidative

dehalogenation reaction that utilizes hydrogen peroxide as the oxidant, and mechanistic and isotopic labeling studies will be presented that confirm a peroxidase-based mechanism for this transformation. More recently, we have shown that DHP possesses both peroxygenase and oxidase activities using a new class of substrate, specifically haloindoles. Using 5-Br-indole as a representative substrate, the major monooxygenated degradation products were found to be 5-Br-2-oxindole and 5-Br-3-oxindolenine. Isotope ^{18}O-labeling studies confirmed that the oxygen atom incorporated was derived exclusively from $H_2^{18}O_2$, indicative of a previously unreported peroxygenase activity for DHP. Reactivity with 5-Br-3-oxindole in the absence of H_2O_2 also yielded 5,5′-Br_2-indigo above the expected reaction stoichiometry under aerobic conditions, and O_2-concentration studies demonstrated dioxygen consumption. Non-enzymatic and anaerobic controls both confirmed the requirements for DHP and molecular oxygen in the catalytic generation of 5,5′-Br_2-indigo, and together suggest a newly identified oxidase activity for DHP. Additional studies detailing our investigations into halopyrrole degradation by DHP will also be presented.

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Challenges and new approaches to the defluorination of fluorinated aromatic compounds

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Fluorinated organic compounds, including fluorinated arenes, are becoming ever more abundant in agrochemicals, pharmaceuticals and other chemical applications. Their rise will almost certainly bring with it environmental contamination of natural waters. At the present time, there are very few options for remediating sites contaminated with fluorinated arenes. This presentation will focus on the challenges associated with aromatic defluorination reactions, including thermodynamics and possible kinetic pathways. An overview of successful approaches will also be presented.

AGRO 372

Reductive dehalogenation is endogenous in vertebrates and other animals

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Industry is not the only source of halogenated compounds in our bodies! Vertebrates generate iodinated tyrosine derivatives during biosynthesis of the thyroid hormones, 3,3',5,5'-tetraiodothyronine (thyroxine, T4) and 3,3',5-triiodothyronine (T3). Bromo- and chlorotyrosine are also produced from stimulation of our immune system. All of these compounds are subject to reductive dehalogenation in vivo, a process unusual for aerobic organisms. Thyroid hormones are deiodinated by a selenocysteine-containing enzyme that belongs to the thioredoxin structural superfamily. In contrast, the tyrosine derivatives are dehalogenated by a flavoprotein from the nitro-FMN reductase structural superfamily. This enzyme entitled iodotyrosine deiodinase (IYD) represents the best opportunity to describe the unique role of the redox cofactor flavin in a reductive dehalogenation process that supports dechlorination, debromination as well as deiodination. The selectivity of these processes are based on substrate-induced organization of the active site and concurrent activation of the flavin. Single electron transfer of the flavin is only observed in the presence of substrates and their analogues. Key residues of an active site lid are critical for substrate recognition and were used to identify potential IYD homologues throughout the tree of life. Subsequent expression of representative genes confirms IYD activity exists throughout all Metazoans tested to date. Even some bacteria contain IYD. Consequently, a wide range of aerobic organisms have the ability to dehalogenate haloaromatic compounds by a reductive process in the absence of metal-containing cofactors.

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Reductive dehalogenation of perchloroethene and trichloroethene in chemostat reactors and a continuous flow column

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The goal of in-situ bioremediation of perchloroethene (PCE) or trichlorethene (TCE) is to achieve complete dehalogenation to ethene as a non-toxic endproduct. Results will be presented from laboratory studies where the Evanite Culture (EV), that is highly enriched in *Dehalococcides mccartyi,* was grown under steady-state conditions in chemostat reactors and in a continuous flow column. The degree of dehalogenation was monitored along with other important chemical parameters. Molecular methods were applied to track the microbial communities that developed. In the continuous flow column study, the EV culture was bioaugmented to aquifer material from the Hanford, DOE site. When excess amounts of fermentable substrates were added, PCE was mainly transformed to ethene and SO_4^{2-} was completely reduced. Formate was found to be a more effective than lactate or propionate in maintaining effective dehalogenation. Formate maintained higher aqueous H_2 concentrations and less Fe^{2+} was present in the column effluent. Clone library analysis indicated great numbers of geobacter were present when lactate or propionate were added. The electron donors were directed to the following processes: iron reduction (47%), sulfate reduction (18%), acetate production (12%), and PCE transformation (8%). EV culture was grown in chemostats for over 5 years on TCE and formate. When excess formate was added, stable operation was achieved and TCE was transformed mainly to ethene. Dehalogenation, acetate and biomass production represented ~ 60 %, 25%, and 12% of the electron donor utilization, respectively. With limited formate addition TCE was transformed incompletely to VC (50%) and ethene (50%). Dehalogenation and biomass production represented 85% and 14% of the electron donor utilization, respectively. *cis*-DCE and VC transformation rates were greatly reduced along with strains *Dehalococcoides mccartyi* capable of growth on *cis*-DCE and VC. The column and chemostats studies illustrate the importance of the electron donor in maintaining effective dehalogenation conditions.

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Degradation of triclosan and triclocarban and formation of degradation products in activated sludge using benchtop bioreactors

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Triclosan (TCS) and triclocarban (TCC) are chlorinated antimicrobials commonly found in consumer personal care products. Extensive use of products containing these compounds has led to their presence and detection in the wastewater treatment (WWT) process, where they are only partially removed leading to their eventual release to the environment - presenting toxicological, endocrine disruption, and microbial resistance issues. Additionally, of growing concern are the formation of TCS and TCC degradation products and their environmental fate. Notably, under aerobic conditions, TCS can be biologically degraded to the more persistent and lipophilic methyltriclosan (MeTCS). This study aims illustrate TCS and TCC removal potential as well as identify the formation of different degradation products. Degradation tests were performed utilizing benchtop bioreactors inoculated with mixed liquor from a large municipal WWT plant to simulate activated sludge treatment. Reactors were run, in duplicate, at temperatures of 21°C and 30°C with dissolved oxygen and pH concentrations of 4 mg/L and 7.0, respectively. Reactors were covered with aluminum foil to prevent photolysis. Reactors were run for 122 hours to simulate extended hydraulic retention time; no additional mixed liquor was added during the studies. Samples were collected from the reactor at predetermined time points and analyzed quantitatively for TCC, TCS, and MeTCS. Concentrations of TCS at time zero were approximately 3 to 3.5 times higher than TCC. Fifty percent (50%) removal of both TCC and TCS occurred after approximately 24 to 30 hours, at both temperatures. MeTCS increased during reactor runs, with concentrations being directly related to TCS. Qualitative scans of three TCC and three additional TCS degradation products are currently being performed to determine any presence/formation during experiments. Preliminary results indicate the presence of the TCS degradation product 2,4-dichlorophenol and the TCC metabolite 3-chloroaniline. Finally, all samples were analyzed for solids, COD, and microbial activity.

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Glufosinate – spray quality effects with tank mixes and nozzle selections

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The introduction of 2,4-D and dicamba tolerant crops offers growers many more options for weed control systems, particularly controlling glyphosate resistance weeds. As a potential common tank mix partners for these weed management systems, the glufosinate use pattern is expected to change significantly following the approval of the auxin-type of herbicide tolerant crops. With ever increasing concerns on spray drift and weed resistance, EPA reguires registrant to include languages on pesticide labels as to what spray quality must be used to be in compliance with the label. These recommendations focus on mitigating drift on non-target species by increasing the spray droplet sizes. However, glufosinate is a contact herbicide and its efficacy relys on plant absorption primarily through their leaves and other green parts. Coverage is essential for the performance of glufosinate. The efficacy may decrease as spray droplet size increases. With the potential tank mix practices of glufosinate with certain groups of systemic herbicides, a series of studies have been conducted to understand how the same recommendations on nozzle selection and spray settings may influence efficacy. The study findings will be shared in this presentation.

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Confirmation of the drift reduction performance of Enlist Duo* Herbicide applied with various spray nozzle designs

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The drift reduction performance of Enlist Duo Herbicide, a proprietary formulation of 2,4-D choline salt and glyphosate, has been exhibited in a variety of wind tunnel and field drift deposition experiments. Based upon these initial experiments, air induction-type spray nozzles have been shown to provide a balanced mix of drift reduction and desired herbicide efficacy. To expand the number of nozzles available for use with the product, an extensive set of wind tunnel testing and comparative modeling was performed. A designed experiment with 36 spray nozzles was executed in the University of Nebraska-Lincoln wind tunnel facility, spanning a range of nozzle designs and operating pressures. Operating within their rated pressure ranges, 32 of the 36 tested nozzles yielded % fines measurements equal to or less than the field-tested standard nozzle TeeJet AIXR11004 operated at 40 psi. The % fines results were found to be directly related to the operating pressure for a given nozzle, and thus maximum pressures that would meet the percent driftable fines specification were defined for each nozzle. Additional verification of the results was carried out by comparative modeling of near-field deposition with the AGDISP model. The modeling confirmed the conclusions of the experimentally-derived percent fines results.

[*]Enlist Duo is a trademark of Dow AgroSciences LLC

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Influence of droplet size, application pressure, and adjuvants on the retention of dicamba spray droplets on leaves

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Off-target movement of growth regulator herbicides can cause severe injury to susceptible plants. Apart from not spraying on windy days, making herbicide applications using nozzles that produce large droplets is the preferred method to reducing herbicide drift. Although large droplets maintain a higher velocity and are more likely to reach the leaf surface in windy conditions, their ability to remain on the leaf surface is not well understood. This study was conducted to evaluate how droplet size, adjuvants, and pressure impact spray retention on a leaf surface. Common lambsquarters and soybean plants were grown inside a greenhouse located at the Pesticide Application Technology Laboratory, West Central Research and Extension Center, University of Nebraska-Lincoln in North Platte, NE. Three nozzles (XR, AIXR, and TTI) were evaluated at 138, 259, and 379 kPa. Dicamba (0.14 kg ae ha^{-1}) was applied alone and with a non-ionic surfactant (NIS), crop oil (COC), methylated seed oil (MSO), silicone, or drift reduction adjuvant (DRA) and contained a tracer dye. Dicamba spray retention when applied using the XR nozzle, which produced the smallest spray droplets, was 1.75 times greater than when applied with the TTI nozzle which had the largest spray droplets. Applying dicamba with MSO resulted in spray retention on leaf surfaces nearly four times the amount achieved when applying dicamba without an adjuvant. The lowest application pressure (138 kPa) had more than 10% more dicamba spray retention compared to the higher

pressures 259 and 379 kPa. By understanding the impacts of these application parameters on dicamba spray droplet retention, applicators can select application parameters, equipment, and adjuvants that will maximize the amount of dicamba spray retained on the target leaf surface while minimizing dicamba spray drift.

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Exposure and risk assessment for spray drift deposition of isoxaflutole on non-target plants

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The risk of isoxaflutole to non-target plants after spray application on soybean was assessed in a tiered and integrated approach. The assessment started with default assumptions using drift deposition values from the standard EPA model AgDRIFT. Further refinement was achieved by considering individual drift curves for coarse nozzles based on the label language using the CLA RegDISP tool and considering drift data from Canadian PMRA. Eventually the most realistic drift exposure figures were generated by conducting formulation based spray drift trials in the field with different nozzles and wind speed conditions. The field experiments were complemented by wind tunnel tests on a larger variety of nozzles and operating conditions and subsequent AgDISP modeling of drift curves to evaluate the scope and validity of the field generated drift data for other non-tested nozzles. About 8-fold reductions in required buffer distances to non-target plants could be accomplished with this tiered approach while keeping within EPA's risk assessment paradigm.

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Novel formulation technology for reducing pesticide drift

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Dow AgroSciences has developed a new drift reduction technology (DRT) that significantly reduces the fraction of driftable fines in ground applications of pesticides. The technology can be built into a pesticide formulation or utilized as a standalone technology that can be tank mixed with many pesticide products. Spray chamber, wind tunnel and field drift trials have shown ≥ 50% drift reduction with conventional flat fan and hollow cone nozzles. Additionally, the technology has shown similar drift reductions with low-drift air induction flat fan and hollow cone nozzles. The DRT technology has demonstrated that it works with a broad range of herbicides, insecticides, and fungicides and formulation types. Efficacy trials have shown no reduction in efficacy when the DRT technology is used in standard or air induction nozzles. The new DRT technology will offer growers and pesticide applicators greater flexibility to reduce pesticide drift when choosing modes of action across all pesticides and crop types.

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Beyond AgDRIFT – Analysis of expanded ground sprayer deposition data

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In the mid-1990s, the Spray Drift Task Force (SDTF) carried out an extensive program of field experiments to characterize off-site deposition of pesticides from spray drift. The SDTF effort concentrated upon aerial application equipment and carried out only a limited number of trials with ground-based sprayers. The ground trial results were analyzed to produce a set of ground sprayer deposition curves, which were incorporated into the AgDrift simulation shell for regulatory exposure assessments. Because of the small number of trials, the curves were very general in nature and thus could not reflect the influence of environmental conditions or nozzles producing large droplets. To account for the improved spray drift performance of more contemporary application technology, the CropLife America Spray Drift Issues Management Team commissioned the analysis of additional deposition data sets generated by Agriculture and Agri-Food Canada (AAFC) in 2000, 2004 and 2011. Detailed environmental conditions and application equipment settings were compiled, where available, from the both the SDTF and AAFC data and analyzed with multiple regression techniques. Based upon the regression analysis, the relative impact of the most important conditions and settings on spray deposition will be presented. From these results, a set of generalized ground deposition curves will be proposed, reflecting the importance of environmental conditions as well as improvements in spray application technology.

AGRO 381

Connecting spray particle size to biology for pesticide applications

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The application of pesticides is central to efficacy of the greater than 900 million pounds of pesticides used each year in the U.S. However, it is often one of the most over looked or disregarded aspects of the process replaced by considerations of pest size or stage, density, product rate and many other things. Spray particle size is one of the most important aspects of pesticide delivery systems for both efficacy and off-target movement. Studies conducted at the Pesticide Application Technology Laboratory within the University of Nebraska-Lincoln's West Central Research and Extension Center in North Platte, NE incorporated biological indicator crops to try to correlate droplet size to downwind deposition, flux and injury. The studies indicate that droplet size is a good indicator of drift potential but that biological response as well as efficacy of a pesticide application are much more complicated. The studies conducted show that there is a correlation between droplet size and deposition, but the correlation of deposition is much closer to injury than droplet size is to injury of susceptible crops. Further investigation is needed to understand how to maximize pesticide applications for both drift and efficacy.