Poisonous Plant Research (PPR)

Volume 1 Article 3

12-17-2018

Proceedings for the 10th International Symposium on Poisonous Plants (ISOPP).

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The 10th International Symposium on Poisonous Plants (ISOPP) was held on September 16-20, 2018 at the Red Lion Conference Center in St. George, Utah, USA. The meeting was truly international with 55 attendees from across the globe. The attendees were a diverse mix of research scientists, academicians, students, veterinarians, private industry representatives, extension agents and government regulators. Dr. Joseph Betz, Acting Director of the Office of Dietary Supplements at the National Institutes of Health, was the plenary speaker for the symposium, wherein he spoke regarding the safety of botanical supplements. There were six sessions of oral presentations including sessions on Global Perspectives on Poisonous Plants, Natural Toxins and the Systems They Affect, Emerging Poisonous Plant Problems, Diagnostics, and Advances in Research. Two posters sessions provided a great opportunity for interaction and discussion. The highlight of the meeting was an evening banquet whereupon Drs. Anthony Knight, Kip Panter, Steven Colegate, and Franklin Riet-Correa were honored with the ISOPP Lifetime Achievement Award. These four individuals dedicated their scientific careers to studying poisonous plants. They were also key players in many of the previous ISOPPs. There was also time for networking and socializing during a bus tour of Southern Utah / Northern Arizona to observe the natural plant communities in these areas as well as during a Dutch oven dessert social one evening. We are already looking forward to the next ISOPP, which is going to be held at the veterinary school campus in Lyon, France in July of 2021 or 2022. Stay tuned for further developments. We hope to see you all there.

10th International Symposium on Poisonous Plants (ISOPP 10)

Red Lion Hotel

September 16-20, 2018

St. George, Utah, USA

10th International Symposium on Poisonous Plants

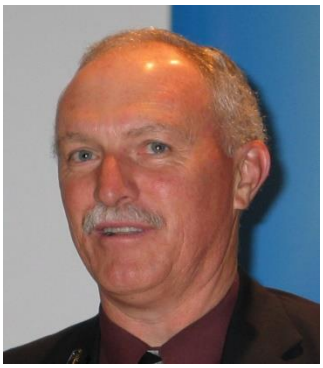
Program and Abstracts

Chairpersons:	**Dr. Daniel Cook and Dr. Jim Pfister**	**2018, St. George, Utah, USA**
Past Chairpersons:	**Dr. Mengli Zhao**	**2013, ISOPP9, Hohhot, China**
	Dr. Franklin Riet-Correa	**2009, ISOPP8, João Pessoa, Brazil**
	Dr. Kip Panter and Dr. Jim Pfister	**2005, ISOPP7, Logan, Utah, USA**
	Dr. Tom Acamovic	**2001, ISOPP6, Glasgow, Scotland, UK**
	Dr. Tam Garland	**1997, ISOPP5, San Angelo, Texas, USA**
	Dr. Steven Colegate and Dr. Peter Dorling	**1993, ISOPP4, Fremantle, Australia**
	Dr. Lynn James	**1989, ISOPP3, Logan, Utah, USA**
	Dr. Alan Seawright and Dr. Ross McKenzie	**1984, Joint U.S./Australian Symposium, Brisbane, Australia**
	Dr. Alan Seawright, Dr. Selwyn Everest, and Dr. Lynn James	**1977, Joint U.S./Australian Symposium on Poisonous Plants, Logan, Utah, USA**

Awards and Dedications:

ISOPP Lifetime Achievement Awards

Dr. Kip Panter, Logan, Utah, USA



Dr. Kip Panter recently retired after a long and distinguished career as a research scientist at the USDA-ARS Poisonous Plant Research Laboratory (PPRL), Logan, Utah; Dr. Panter also served as Research Leader from 2007 to 2016. Dr. Panter completed a BS degree in Animal Science (1975) and MS degree in Reproductive Physiology (1978) at Utah State University (USU). He completed a PhD in Toxicology (1983) from the University of Illinois. Dr. Panter also worked at the PPRL as an animal caretaker and technician during his undergraduate and graduate programs at USU before becoming a research scientist in 1983. Dr. Panter's research career focused on the effects of poisonous plants and natural toxins on reproduction in livestock, including embryonic and fetal growth and development. Dr. Panter's pioneering research into birth defects in livestock propelled him to the forefront of the teratology field. Dr. Panter developed a Spanish goat model to determine the mechanism of action of lupine-induced "crooked calf syndrome" in cattle, including evaluating the toxicity of individual alkaloids. Further, his innovative collaboration with the Lahey Medical Center has led to the biomedical application of the goat model to improve treatment of cleft palate in children. Dr. Panter's vision and in utero surgical skills with goats have led to discoveries in reproductive toxicology that one day may eliminate numerous surgeries for children born with a cleft palate and its concomitant facial disfigurement. In addition to his work on cleft palate origins and repair, Dr. Panter has characterized the reproductive dysfunction in livestock caused by locoweeds in sheep, goats, and cattle. Dr. Panter has also evaluated the toxicity of individual alkaloids from larkspur and determined structure activity relationships using a mouse model. Dr. Panter contributed his expertise in other research endeavors such as selenium toxicity, and pine needle and snakeweed-induced abortions in cattle. Dr. Panter has also contributed greatly to the ISOPP meetings, as he was the ISOPP Chair in 2005 in Logan, UT, and provided critical assistance in organizing several other ISOPP meetings. Dr. Panter's contributions to toxic plant research have been of great benefit to the livestock industry and to teratology research in livestock and humans. It is with great pleasure that the ISOPP community recognizes Dr. Kip Panter with the Lifetime Achievement award in 2018 at the 10th ISOPP meeting held in St. George, Utah, USA. We express our appreciation for his lifetime efforts in the area of poisonous plant research with this award. (Jim Pfister)

Dr. Franklin Riet-Correa, Colonia, Uruguay

Dr. Franklin Riet-Correa was born and raised in Rocha, Uruguay, the son of a veterinarian. He graduated in 1968 in veterinary medicine from the Universidad de la República in Montevideo. After a faculty appointment, and then founding the first state diagnostic laboratory in Uruguay, he spent 1973 to 1974 in Australia as a visiting scientist with CSIRO. In 1977 he became a visiting professor of Veterinary Pathology at the Federal University of Pelotas (UFPEL), in southern Brazil. In Pelotas, he created the Regional Diagnostic laboratory, and served as director from 1978 to 2002. He also held an appointment as a researcher at the Research Veterinary Laboratories Miguel C. Rubino in Uruguay between 1998 and 2007. He obtained an MS degree in Animal Health in 1982 at the Federal University of Pelotas, and PhD in Experimental and Comparative Pathology at the University of São Paulo (USP) in 1999, working with Dr. Silvana Lima Gorniak. In 2002, he moved to the Federal University of Paraíba (later the Federal University of Campina Grande) in Veterinary Medicine working at the agricultural branch campus in Patos, Paraíba state in northeastern Brazil. He helped to establish and became the coordinator of the Graduate Program in Veterinary Medicine at the Federal University of Campina Grande. He has been a longstanding member of the Advisory Committee of Veterinary Medicine of the CNPq (Brazilian Council for Scientific and Technological Development). He has worked, tirelessly and persistently, often times under difficult circumstances, for nearly 50 years in diagnostic pathology and plant-caused intoxications in livestock. Franklin has been supported in his research efforts by his spouse and fellow faculty member in Patos, Dr. Rosane M. T. Medieros. While working in northeastern Brazil, he obtained millions of dollars in federal and state funding as the coordinator of the National Institute of Science and Technology (INCT) project entitled "Control of Plant Intoxications." Franklin has brought great energy to the study of toxic plants, mentoring countless graduate and undergraduate students, and assisting and supporting research programs at various universities across Brazil, from the Amazon region in the north, to west-central Brazil, and into southern Brazil. He organized and chaired the 1st and 2nd Latin American Symposium on Toxic Plants. In 2014 he returned to Uruguay to coordinate The Animal Platform on Animal Health, a new program created by the National Institute of Agricultural Research (INIA) to improve animal health in Uruguay. Franklin continues to work for INIA in Colonia, Uruguay. During a long and illustrious career, he has authored or co-authored more than 400 peer reviewed manuscripts, 4 books, and advised nearly 80 graduate students. It is with great pleasure that the ISOPP community recognizes Dr. Franklin Riet-Correa with the Lifetime Achievement award in 2018 at the 10th ISOPP meeting held in St. George, Utah, USA. His lifetime contributions to the study of poisonous plants cannot be measured, as Dr. Riet-Correa's influence will be felt throughout Latin America for generations to come through the efforts of his students and others whom he has assisted and advised. (Jim Pfister)

Dr. Steven Colegate, Ocean Grove, Victoria, Australia

Dr. Steven M. Colegate is awarded the ISOPP Lifetime Achievement Award in recognition of his contributions in the area of the chemistry of poisonous plants and his leadership and mentoring of the international community interested in toxic plants. Dr. Colegate received his B.Sc. (Chemistry) and his Ph.D. (Organic Chemistry) degrees from the University of Western Australia. He joined the Department of Toxicology and Pharmacology in the School of Veterinary Science at Murdoch University Western Australia in 1978 and was principal research chemist working on a number of plant-induced poisoning problems from the Western Australia region. In 1996 Dr. Colegate moved eastward across Australia to join the Plant Toxins unit of CSIRO Animal Health in Geelong, Victoria Australia in which he served as Senior Research Scientist and then later in 2000 became Principal Research Scientist and Group Leader for the Plants Toxins Unit. With CSIRO, research continued on plant associated poisonings in livestock of national and international interest. Dr. Colegate retired from CSIRO in 2008 and shortly thereafter was invited to come to the Poisonous Plant Research Laboratory in the United States as a Visiting Scientist enabling Dr. Colegate to continue his research interest in the chemistry of pyrrolizidine alkaloids. Dr. Colegate's research has focused on the chemistry of a broad range of plant-associated toxins, not only of regional Australian interest but of great international interest also. Some highlighted research includes his early work on Swainsona species that lead to the isolation and identification of the toxic polyhydroxy alkaloid known as swainsonine, a toxin now of international interest and found associated with locoweeds in the US and China and the Ipomoea and related species of South America. His seminal work in the identification of swainsonine was critical in moving forward research on locoweeds in the US and leading to an expansion of interest in structurally related polyhydroxy alkaloids internationally. At CSIRO, Dr. Colegate worked on numerous aspects of annual ryegrass toxicity including analytical methods and isolation of corynetoxins, the toxicity of corynetoxins, and research on a vaccine to protect livestock from annual ryegrass toxicity. A major emphasis of Dr. Colegate’s research throughout his career has been focused on the pyrrolizidine alkaloids. This has included the development and application of analytical methods for the detection of pyrrolizidine alkaloids in plants and related food and feeds for both animals and humans. His association with the Poisonous Plant Research Laboratory and extending to that of the international research community (ISOPP) has continued in numerous ways, both in collaborative research and also as advisor, mentor, and friend, to many of the scientists working on poisonous plants. He has been a key participant of the ISOPP meetings and co-organizer of ISOPP 4 held in Fremantle, Western Australia in 1993. These associations have proven over time as a most valuable contribution to the research community and to the livestock industry. It is with great pleasure that the ISOPP community recognizes Dr. Steven M. Colegate by the awarding of the Lifetime Achievement award in 2018 at the 10th ISOPP meeting held in St. George, Utah, USA for the outstanding contributions for 40 years dedicated to research on the chemistry of poisonous plants. (Dale Gardner)

Dr. Anthony Knight, Tucson, Arizona, USA

Dr. Knight was born on a ranch in Kenya where he developed a passion and love for animals, livestock production, and working with livestock producers. This led him to study veterinary medicine at the University of Nairobi in Kenya where he graduated in 1968. While in school, he was encouraged by several visiting Colorado State University faculty to apply to the CSU graduate program. He applied and was awarded the Rockefeller Scholarship and subsequently moved to Fort Collins, Colorado. Veterinary students and graduate school colleagues that knew him during his early studies described Tony as an intelligent, soft spoken, skinny boy with an "interesting" African/English accent. He exceled at CSU and in 1970 he received a master's degree in Veterinary Science. After going home and working for a year at the University of Nairobi, he decided to return to Fort Collins and joined the CSU faculty in 1971, which he made home for over 40 years. Tony is board certified by the College of Veterinary Internal Medicine, has served as department head of Clinical Sciences at CSU for 18 years, and has taught and mentored thousands of veterinary students, interns, and residents. At CSU, Dr. Knight quickly became the local authority and soon a world expert on toxic plants and toxin-related diseases. Upon retiring in 2012 from CSU, he moved to Tucson, Arizona where, in addition to gardening, he continues to actively advise and interact with researchers at the University of Arizona and throughout the world. Dr. Knight is a prolific writer and he is the "go to" author of books, papers, and chapters concerning poisonous plants that affect livestock and pet animals. He is a talented photographer and skilled plant taxonomist as seen in the figures and illustrations of his noted book "A Guide to Plant Poisoning of Animals in North America." He has always been very generous in sharing his photographs and subsequently he has contributed impeccable figures to other authors, including PPRL personnel, for various publications and books. Perhaps Dr. Knight's biggest legacy lies in the education and teaching of his students. Dr. Knight and his colleagues developed the CSU veterinary toxicology courses which included toxic plant lectures that are still used by CSU and many other veterinary schools. His students unanimously love him as an instructor and as a group they are amongst the very best veterinary graduates in their understanding of toxic plants and the diseases they cause. It is with great pleasure that the ISOPP community recognizes Dr. Anthony Knight with the Lifetime Achievement award in 2018 at the 10th ISOPP meeting held in St. George, Utah, USA. It is an honor to recognize Dr. Knight for his accomplishments in poisonous plant research. (Bryan Stegelmeier)

SESSION 1. GLOBAL PERSPECTIVES ON POISONOUS PLANTS Session Chair: Dr. Tam Garland	
Invited Lecture: Safety of botanical dietary supplements	Joseph Betz
Induction of resistance in cattle to poisoning by monofluoroacetate from plants using non-toxic salt	Benito Soto-Blanco
Non-toxicological factors affecting cattle responses to poisonous plants	Ben Green
EURL for mycotoxins & plant toxins in food and feed	Monique de Nijs
Ferulenol exposure of calves in Corsica (France)	Gilbert Gault
Ergot as a potential emerging disease in Pacific Northwest, USA	A. Morrie Craig
Photosensitization diseases of animals: classification and a weight-of-evidence approach to primary causes	Mark Collett
Restoration of rangelands degraded with annual grasses that also contain poisonous plants	Clint Stonecipher
SESSION 2. NATURAL TOXINS & THE SYSTEMS THEY AFFECT Session Chair: Dr. Ben Green	
A functional metabolomics analysis of lolitrem B and its biosynthetic intermediates in murine brain	Jane C. Quinn
'Go Slow' myopathy in dogs: a secondary plant toxicity?	Hayley Hunt
Diterpene acids in Gutierrizia sarothrea and G. micorocephala and implications for cattle abortions	Dale Gardner
The ophthalmic lesions of locoweed poisoning in livestock, wildlife, and rodents.	Bryan Stegelmeier
Pyrrolizidine alkaloids – natural toxins risk in Queensland honey	Natasha Hungerford
Potential toxicity of pomegranates to cattle	Elizabeth Read
Toxicity and anticancer activity of aloperine derived from Sophora alopecuroides L.	Mingning Qiu
Eupatorium adenophorum induces toxicological effect on mice liver by activating pyroptosis via GSDMD/IL-1? pathway	Yanchun Hu
POSTER SESSION #1 Advances in Research	
A novel analytical method for simultaneous quantification of the carcinogens Ptaquiloside, Caudatoside and Ptesculentoside	Vaidotas Kisielius
The disaster and toxicant mechanisms and prevention and control of locoweed in Western China	Baoyu Zhao
The progress of distribution, harm and exploitation of Ligularia in northwestern Sichuan pastoral area	Xi Liu
Swainsonine in select North and South American Astragalus species	Daniel Cook
The study of metabolites from fermentation culture of Undifilum oxytropis	Hao Lu
Cytotoxicity of Terminalia ferdinandiana extracts in intestinal and hepatic cancer cell lines	Saleha Akter
Earwax: innovative method to detect fluoroacetate intoxication in cattle	Paulo Cunha

Grazing of *Delphinium occidentale* (duncecap larkspur) by susceptible and resistant cattle	Jim Pfister
Herbicide control of death camas	Clint Stonecipher
Hazard cause, distribution, damage and control of poisonous weeds in natural grassland of China	Baoyu Zhao
Idiopathic enzootic calcinosis in a goat herd in New Zealand	Mark Collett
Ptaquiloside and pterosin B levels in four Brazilian ferns	Benito Soto-Blanco
Liquid chromatography coupled to quadrupole time-of-flight mass spectrometry assay for quantification of protodioscin	Benito Soto-Blanco
SESSION 3. EMERGING POISONOUS PLANT PROBLEMS AND DIAGNOSTICS Session Chair: Dr. Patricia Talcott	
The clinical and histologic changes of Salvia reflexa intoxication in cattle, goats and mice	Bryan Stegelmeier
Diterpenoids from Salvia reflexa associated with hepatotoxicity in cattle	Dale Gardner
Endophyte infected perennial ryegrass (Lolium perenne) effects on pregnant camels	Linda Blythe
First giant fennel (Ferula sp.) intoxication in continental France	Denis Grancher
Mass mortality of eastern grey kangaroos associated with Panicum gilvum related hepatogenous photosensitisation	Jane C. Quinn
Accumulation and depletion of the toxic amino acid indospicine in calves fed Indigofera spicata	Mary Fletcher
Risk assessment of indospicine residues in bovine muscle and liver from north-west Australia	Gabriele Netzel
ProMED-Mail and toxic plants	Tam Garland
POSTER SESSION #2 Emerging Poisonous Plant Problems and Diagnostics	
North American hard yellow liver disease: an old problem readdressed	Bryan Stegelmeier
Effect-based screening of water leachable compounds of invasive plants employing in vitro bioassays	Bettina Gro Soerensen
Pyrrolizidine alkaloids in blue heliotrope (Heliotropium amplexicaule) in Australia	Natasha Hungerford
Hypoglycin A exposure of horses with atypical myopathy	Gilbert Gault
Relative accumulation of indospicine and its deamino-metabolites in camels fed Indigofera spicata	Gabriele Netzel
Spontaneous photosensitization by Brachiaria ruziziensis in sheep	Benito Soto-Blanco
Toxic effects of saponins from Cestrum axillare leaves in goats	Benito Soto-Blanco
Characterisation and bioactivity of secondary metabolites of Amaranthus retroflexus L. implicated in livestock poisoning	Jane C. Quinn
Regulation of swainsonine production and gene expression in fungal symbionts	Ramanujam Nadathur

Acute neurological signs and deaths in rams associated with horse chestnut (Aesculus indica) ingestion	Kathleen Parton
Clinical aspects of the experimental poisoning in cattle by the pods of Stryphnodendron obovatum	Paulo Cunha
Cardiac changes of cattle experimentally poisoned by Palicourea marcgravii and prevented with acetamide	Fabrício Carrião dos Santos
SESSION 4. DIAGNOSTICS Session Chair: Dr. Barry Pittman	
Invited Lecture: Arizona Livestock Incident Response Team (ALIRT)	Peter Mundschenk
Diagnosis of dehydropyrrolizidine alkaloid (DHPA) poisoning	Bryan Stegelmeier
The search for biomarkers of facial eczema following a sporidesmin challenge in dairy cows	Zoe Matthews
Diagnosis and surveillance of Brassica-associated liver disease (BALD) in cows	Mark Collett
Evaluation of noninvasive specimens to determine livestock exposure to teratogenic Lupine spp.	Stephen Lee
SESSION 5 – Continued - DIAGNOSTICS Session Chair: Dr. Barry Pittman	
Towards understanding the aetiology of acute bovine liver disease in Australia	Elizabeth Read
Diagnosis of selenium poisoning in livestock	Zane Davis
Development of a PCR-based method for detection of Delphinium species in poisoned cattle	Daniel Cook
SESSION 6 – Continued- ADVANCES IN RESEARCH Session Chair: Dr. Dale Gardner	
Ipomoea asarifolia and Ipomoea muelleri: investigation of tremorgenic indole diterpenes	Stephen Lee
Oral toxicity of progoitrin-derived nitriles in rats and rabbits	Zoe Matthews
Quantitation of Phalaris alkaloids throughout a growing season in Victoria, Australia	Elizabeth Read
Is tremetone really the toxin in white snakeroot and rayless goldenrod that is responsible for causing milk sickness?	Zane Davis
Asphodel exposure of sheep in Algeria	Gilbert Gault
Metabolic profiling of cytotoxic steroidal saponins in five Australian Panicum species	Yuchi Chen
Investigating photosensitisation in livestock resulting from ingestion of the pasture legume *Biserrula pelecinus L.*	Jane C. Quinn
The effect of drying temperature on the tremorgenicity of Ipomoea asarifolia in mice	Kevin Welch

Safety of botanical dietary supplements

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In 1994, the U.S. Food, Drug, and Cosmetic Act was amended to create a special category of foods called dietary supplements. Permitted ingredients in this category include raw and processed botanicals. The perception that natural substances are inherently safer and more healthful than synthetic survives despite the experiences of Socrates and the Borgias. Natural toxins can occur in botanical ingredients or products constitutively or as contaminants and can originate in eukaryotic plants (phytotoxins), fungi (mycotoxins), algae (phycotoxins) and prokaryotic microorganisms (cyanotoxins). In general, adverse events associated with botanicals may be placed into one of several categories: misidentification, deliberate adulteration, misuse, and inherent toxicity, including those caused by herb/drug interactions. Enhanced pharmacovigilance has demonstrated that serious adverse events associated with dietary supplements do occur, and with increased reporting, the number of cases has risen. Of particular concern are hepatoxicities associated with dietary supplements reported to NIH's Drug-Induced Liver Injury Network (DILIN). Potential sources of hepatotoxic agents in botanical products include phytochemicals that occur naturally in the plant, mycotoxins in raw materials, co-harvested weeds, misidentified plant materials, and materials that have been subjected to economically motivated adulteration. Several products with ingredients not known to contain previously reported toxins have been associated with hepatotoxicity, including those that contain green tea extracts, kava, and black cohosh. In addition to concerns raised about acute toxicities of supplement products, not all adverse health effects manifest in a timeframe that permits immediate assignment of causality, and delayed increases in morbidity and mortality following exposure are seldom reported.

Induction of resistance in cattle to poisoning by monofluoroacetate from plants using non-toxic salt

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Monofluoroacetate (MFA) is considered one of the most toxic substances known, and it is found naturally in several plant species responsible for causing sudden death syndrome in ruminants. Due to the hyperacute evolution of the poisoning and the impossibility of effective treatment, the induction of animal resistance might be the best tool to control MFA poisoning in ruminants. The objective of this study was to promote resistance in cattle to the toxic effects of MFA through its degradation by the ruminal microbiota after administration of sodium trifluoroacetate (TFA). Ten calves were used, distributed into two groups: control group (N=3) and treated group (N=7). The animals from the treated group received TFA, while those in the control group received water, both for 28 consecutive days. The animals were submitted to daily clinical evaluation and weekly blood biochemical determination, in order to identify any sign of poisoning. After 28 days of administration of TFA or water, application of the leaves of *Palicourea marcgravii* was performed to determine the occurrence of resistance. The administration of TFA was not responsible for any clinical or biochemical changes in blood. The administration of *P. marcgravii* induced clinical changes in the control group of cattle, but no alteration was observed in the animals of the treated group. Thus, the administration of TFA to cattle induces resistance to MFA poisoning.

Non-toxicological factors affecting cattle responses to poisonous plants

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Cattle respond to poisonous plants as individuals, and these responses can be affected by factors such as cattle breed, age, and sex. For example, when Angus steers are orally dosed with a standardized dose of *Delphinium barbeyi* as yearlings, and again at two years, there are significant differences between the walking times 24 hours after dosing of the steers as yearlings and at two years of age (16.0 ± 5.3 minutes as yearlings, 40 minutes as two year olds, $P = 0.0015$). The response to larkspur also appears to be sex-dependent. When yearling Angus heifers, steers, and bulls receive a standardized dose of D. barbeyi, the cumulative walk times for the yearlings 24 hours after dosing were 3.0 ± 1.2, 16.6 ± 1.9, and 15.5 ± 2.4 minutes for 30 heifers, 61 steers, and 33 bulls, respectively ($P < 0.0001$, Kruskal-Wallis test). Further research is needed to better understand the mechanism(s) underlying the age and sex-dependent changes affecting cattle responses to toxic plants and to exploit knowledge of the mechanisms to reduce cattle poisonings. Finally, whenever possible, yearling Angus heifers and steers should be kept from grazing larkspur-infested rangelands as a simple management tool to avoid catastrophic losses to a poisonous plant common in western North America.

Ferulenol exposure of calves in Corsica (France)

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Giant fennel (*Ferula* sp. *L*) is a tall plant of the Apiaceae family that grows all around the mediterranean sea. In Corsica most of the Giant fennel belongs to the genus *Ferula* and the species *communis*. All parts of the plant contain 2 major prenylated coumarins, ferulenol and ferprenin, that are efficient inhibitors of the vitamin K recycling enzyme VKOR. The deadly haemorraghic syndrome that occurs after consumption of the plant concerns all mammals. In France most clinical cases were described in Corsica because of the free ranging of livestock. Only 1 clinical case was described in continental France. In order to study the ferulenol exposure of cattle in Corsica we sampled blood from different animals (27 females, 9 males) of a cow-calf herd with free access to giant fennel and with clinical cases of ferulosis and 4 samples of milk. The herd consisted of pure breed animals (1 Aubrac bull and 10 Corse cows) and crossbreed animals (1 bull and 15 heifers Aubrac X Corse). 9 calves (weaned and unweaned) were present. Each year the Aubrac purebreed bull was intoxicated and treated with vitamin K1. Some crossbreed heifers were also poisoned but seemed to be more resistant and have never been treated.

5 animals were newborn calves (1-3 weeks old), 4 were post weaning calves (3-6 months old) and 27 adults (> 1 year old). Adults and postweaning calves were grazing and fed a local hay. Blood and milk samples were tested for ferulenol and dicoumarol with LC-MS-MS according to the method developed by Fourel et al. LOQ was respectively 1.0 µg/l for ferulenol and 0.5 µg/l for dicoumarol. Adult and postweaning calves were positive to dicoumarol (respectively med 4.4 and 1.2 ng/ml). Most samples showed positive results to ferulenol (adult 26/27 positive, med = 8.9 ng/ml, postweaning calves 4/4, med = 2.8 ng/ml and unweaned calves 4/5, med = 1.1 ng/ml). The milk samples (3/4) contained ferulenol (med 2.1 ng/ml) and didn't contain dicoumarol. No reported intoxication in unweaned calves is consistent with the low level of ferulenol. There was no significant difference in ferulenol blood concentration between the pure breed Aubrac bull and the crossbreed heifers. Giant fennel eating cows excrete ferulenol in milk and expose the suckling calves. There is a trend of correlation between the blood level of ferulenol of the cow, the consentration in its milk and in the blood of its calf. This trend must be studied further.

Dicoumarol is usually found in moldy sweet clover (*Melilotus* sp.). In the grazing areas we could observe fresh and not moldy sweet clover.

Giant fennel is so frequent in Corsica and the animals are almost all in free ranging that we wonder if there is an ability of induced resistance in local cattle. Moreover we wonder now if this intoxication might be a health problem for people eating liver sausages (traditional habit), meat or cheese.

Ergot as a potential emerging disease in Pacific Northwest

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Ergot causes disease due to a fungus, *Claviceps purpurea*, which produces ergopeptine alkaloids. These alkaloids cause vasoconstriction, reproductive diseases, and aglactia. The Pacific Northwest produces 70% of the world's cool season grasses. When harvested, 30% of the harvest ends up as seed screenings which often have usable amount of protein and are made into pellets for animal feed. In the past, at the OSU Endophyte Service Laboratory, *Claviceps purpurea* was seen about once a month. These past three years, ergot positive samples were seen three or more times per week. Ergot is an emerging disease in the Northwest. We are finding that ergot is not only produced by *Claviceps purpurea*, but also *Claviceps humidiphila*. This latter *Claviceps* produces ergot alkaloids with a unique alkaloid profile. The banning of grass field burning 20 years ago, most likely explains the higher incidence of the soil fungus that we are seeing in feed samples. To meet this demand, new protocols for sample extraction and chromatographic determination have been developed. Oregon has the second largest dairy in the United States with 30,000 milking head at the Three Mile Canyon Dairy. Ergot has been found in hay samples from the 57,000 acres surrounding this dairy which provides part of the TMR ration. Limited studies on milk production document that when ergot is in the feed, dairy production reduced from 87 pounds/day to 64 pounds/day. The increase in ergot in both pellets and hay has a large potential impact for dairy operations.

Photosensitisation diseases of animals: classification and a weight-of-evidence approach to primary causes

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Clare's (1952) classification system for photosensitisation diseases (Types I, II, III and Idiopathic) has endured many years of use despite some confusion regarding his secondary, or Type III, category, as well as the more recent discovery of two mechanisms (Types I and II) of phototoxicity.

Therefore, to reduce confusion in terminology, I propose that Clare's four groups be known as *primary* (or direct), *secondary* (indirect or hepatogenous), *endogenous* (aberrant haeme pigment synthesis), and *idiopathic*. The use of the word *type* is then reserved for the mechanisms of phototoxicity.

Many plant species are incriminated in the idiopathic category. Most of these are likely to be primary; however, the weight-of-evidence (WOE) for all but a few is sparse. With respect to plants (and certain mycotoxins and insects) implicated in primary photosensitisation outbreaks, McKenzie's "toxicity confidence rankings" (*Australia's Poisonous Plants, Fungi and Cyanobacteria*, 2012) has been adapted to "phototoxic agent confidence rankings". Thus, plants, mycotoxins or animals can be categorised regarding *phototoxicity* (definite; some evidence; suspected; or phototoxin isolated but no field cases known) and *WOE* (field cases; experimental feeding produces photosensitisation; phototoxin isolated; phototoxin produces photosensitisation experimentally; and/or correlation of the action spectrum/chromatogram in blood or skin with the absorption spectrum/chromatogram of the phototoxin). As a result, confidence rankings ranging from 0 to 5 can be allocated. From available literature, only a few plant species can be ranked as 5 (definite phototoxicity with maximum WOE).

Restoration of rangelands degraded with annual grasses that also contain poisonous plants

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Invasive annual grasses are replacing native vegetation on rangelands throughout the western U.S. Medusahead (*Taeniatherum caput-medusae*) has altered the natural succession of vegetation on the Channeled Scablands of eastern Washington and reduced forage options for livestock. Research conducted by the Poisonous Plant Research Lab has shown that cattle start to consume velvet lupine (*Lupinus leucophyllus*) as medusahead and other annual grasses mature and decrease in palatability and nutrient content. Velvet lupine, in the Channeled Scablands, is teratogenic to cattle when consumed between 40 and 100 days of gestation, resulting in a condition known as crooked calf syndrome. Revegetation is necessary to restore these degraded rangelands to a healthy perennial grassland and thus increase forage value and abundance, providing an alternative forage source to reduce cattle consumption of velvet lupine.

A functional metabolomics analysis of lolitrem B and its biosynthetic intermediates in the murine brain

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The neuroactive mycotoxin lolitrem B causes a neurological syndrome in grazing livestock resulting in hyperexcitability, muscle tremors, ataxia and, in severe cases, clonic seizures and death. Lolitrem B is the endpoint in a biosynthetic pathway of indole diterpenoid toxins present in fodder yet the neuroactive status of it's pathways intermediates remains undefined. To define the effects of lolitrem B and it's pathway intermediates terpendoles B, C and E in the brain, a functional metabolomic study was undertaken in which cordination and tremor were quantified and metabolomic profiling undertaken to determine quantification anad relative abundance of both toxin and key neurotransmitters in various brain regions. Marked differences were observed in the duration of tremor and coordination between pathway members, with some showing protracted effects and others none at all. Quantification of lolitrem B using LCMS/MS QQQ identified presence of Lolitrem B in liver and kidney, cerebral cortex, thalamus and brain stem but not in cerebellum. Metabolomic profiling by LCMS/MS-QToF of brain isolated from intoxicated animals using showed significnat variation in targetted neurotransmitter and amino acid profiles over time. This study demonstrates for the first time bioaccumulation of lolitrem B in the brain, with absence of detectable levels of toxin in the cerebellum, as determining a dynamic catecholenergic response over time. This data is indicates that the indole diterpenoid toxins induce alterations in catecholamine pathways in the brain as well identifying pathway intermediates with non-tremorgenic profiles. This study identifies a functional metabolomic approach for physiological profiling of neurotoxic agents in the brain.

'Go Slow' myopathy in dogs: a secondary plant toxicity?

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'Go Slow' myopathy (GSM) is an acquired myopathy in dogs in New Zealand. The disease is characterised by a sudden onset of trembling, exercise intolerance, weakness and/or collapse, and typical clinical pathology findings include increased activities of creatine kinase and aspartate aminotransferase in serum. Microscopic lesions in the skeletal muscle of affected dogs include muscle degeneration in the absence of significant inflammation, and electron microscopic changes are consistent with a toxic mitochondrial myopathy. Cardiac muscle is not affected. Recent research has shown that the disease is associated with the consumption of wild pork in specific geographical regions of New Zealand, and combined with the pathological findings, GSM is considered most consistent with a secondary plant toxicity. Interestingly, the disease shares many features of *Ageratina altissima* (white snakeroot) and *Isocoma pluriflora* (rayless goldenrod) poisonings in animals and people, but these plant species are not naturalised in New Zealand. To further investigate the mechanism and cause of GSM, untargeted liquid chromatography-mass spectrometry was performed on aqueous and lipid extracts of liver samples from affected dogs, and results were suggestive of a defect in mitochondrial fatty acid oxidation. Several of the significant mass spectrometric features identified when comparing affected dogs (n=15) and control dogs (n=24) could be consistent with plant-derived alkaloids, including convoline and several other tropane alkaloids. Further work is required to identify the definitive cause of this myopathy.

Diterpene acids in *Gutierrizia sarothrea* and *G. micorocephala* and implications for cattle abortions

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Broom snakeweed (*Gutierrezia sarothare*) and threadleaf snakeweed (*G. microcephala*) are perennial plants found on western US rangelands. If eaten, the plants have been reported to be toxic to cattle, sheep and goats and may in cattle cause premature parturition (abortions) in late term animals. The toxic components are not known, but some propose that the diterpene acids may be both toxic and abortifacient similar to those found in Ponderosa pine. In order to better understand the diterpene acids that occur in *G. sarothare* and *G. microcephala*, plants were collected from Colorado, Oklahoma, New Mexico, Texas, Arizona and Utah, identified by classical taxonomy and then analyzed by a general GC-MS procedures to determine individual chemotypes. The GC-MS fingerprints were diverse showing 15 possible different chemotypes. Four major chemotypes accounted for 73% of the samples and from which the major diterpenes acids were determined by extraction, preparative chromatography and characterization by NMR, MS, IR and UV spectroscopy. The diterpene acids were found to be a mix of furano, lactone, di-acid and labdane type acids. Several of the diterpene acids appear to be new compounds and most have not been previously described as from *G. sarothare* or *G. microcephala*. Chemotype BSW-2 is of particular interest because it was found to contain a compound identified as labd-7-en15,18-dioic acid, which is structurally similar to isocupressic acid and agathic acids which are known to cause abortions in cattle.

The ophthalmic lesions of locoweed poisoning in livestock, wildlife and rodents

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Livestock and wildlife are poisoned when they continuously eat swainsonine-containing plants. Poisoning in cattle is characterized by reluctance to move and dull appearing eyes that producers have used to identify poisoned animals that should be removed from contaminated areas to avoid permanent damage. Original reports of locoweed-induced ophthalmic disease described cytoplasmic vacuolation of inner nuclear neurons and epithelial cells of the ciliary body. The originally described tissues were compared with the ophthalmic lesions of acute poisoning in sheep, goats, cattle, horses, mule deer, hamsters, rats, and guinea pigs that had clinical and histologic neurologic disease. Fundascopic studies of these poisoned horses were uniformly normal. Histologically the eyes from these animals had none or minimal vacuolation of inner nuclear neurons. These same animals had massive Purkinje cell vacuolation and loss. In animals with extended intoxication (animals repeatedly dosed continuously) all neurons including minimal vacuolation of neurons in the retina were identified. However, there was extensive vacuolation of larcrimal glands even in mildly poisoned animals. Clinically poisoned animals often had “dry eye” (reduced tear production using Schirmer tear test). Reduced tear production correlated with lacrimal gland vacuolation. Poisoned animals were all visual. The “dull eyes” described in locoed cattle is probably due to altered tear film and dry eyes. These findings suggest that although eyes of poisoning animal have altered gross appearance, the ophthalmic lesions of poisoning are less useful as indicators of poisoning or prognosis as they occur with severe disease when poisoning is obvious.

Pyrrolizidine alkaloids – natural toxins risk in Queensland honey

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Pyrrolizidine alkaloids are widely distributed natural toxins and their consumption has been connected with acute and chronic liver damage, and even death, in livestock and humans. There are more than 600 pyrrolizidine alkaloids, with the 1,2-unsaturated pyrrolizidine alkaloids being potent carcinogens. Their presence in food concerns food regulators, and Food Standards Australia New Zealand (FSANZ) established a provisional tolerable daily intake for these alkaloids of 1 μg/kg.BW/day.

Pyrrolizidine alkaloids and their *N*-oxides have been identified from >6,000 species across the Asteraceae, Leguminosae and Boraginaceae families. Internationally it has been reported that such toxins can be found in honey due to transfer by bees of pollen/nectar from certain flowers, particularly *Heliotropium*, *Crotalaria*, *Echium* and *Senecio* species.

In this survey, honey samples sourced from markets and shops in Queensland, Australia, were analysed by UHPLC-MS/MS for 20-30 common pyrrolizidine alkaloids. Correlations between the occurrence of pyrrolizidine alkaloids and the botanical/ geographical origin of the honey are essential as pyrrolizidine alkaloid contamination at up to 4000 μg/kg has been detected. In this study, the predominant alkaloids detected were isomers lycopsamine, indicine and intermedine, displaying identical MS/MS spectra. Separation of these isomers by UHPLC has enabled comparisons of the relative proportions present in honey to alkaloids in suspect source plants. Overall plant pyrrolizidine alkaloid profiles will be compared to those found in honey samples to help identify the most important plants responsible for honey contamination. The native Australian vines of *Parsonsia* spp. will be discussed as a likely a contributor to high levels of lycopsamine.

Potential toxicity of pomegranates to cattle

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Pomegranates (*Punica granatum*) have been extensively researched for their health benefits for humans, presumably because of the high tannin content present primarily at punicalagin. Similarly, it has been suggested that feeding pomegranate pieces or extracts to cattle may provide similar benefits, thus improving livestock production. However, in June 2013, a farmer in Victoria, Australia fed pomegranates to their cattle as a feed alternative. Over the following 9 days, 9 cattle became ill and died. Furthermore, punicalagin was previously implicated in liver toxicity of sheep and cattle in Queensland, Australia. This project aimed to determine if punicalagin or any other potentially toxic metabolites were present in the pomegranates that contributed to the deaths of the cattle.

Pomegranate pieces were collected from this farm and extracted with aqueous methanol. The crude extract was fractionated over a polyamide column and the resulting seven fractions were analysed using LCMS and NMR spectroscopy. The punicalagin content of each fraction was determined and then fractions were tested for cytotoxicity against bovine kidney (MDBK) cells. Punicalagin and the fractions that contain significant amount of punicalagin were found to have a moderate cytotoxic effect. However, a fraction that contained no punicalagin was also moderately cytotoxic. Additional fractionation and LCMS analysis alongside cytotoxicity assays identified gallic acid as a potentially toxic metabolite. A discussion of the implications of this research will be provided.

Toxicity and anticancer activity of aloperine derived from *Sophora alopecuroides* L

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Sophora alopecuroides L, a toxic traditional Chinese herb, is a dominant plant population distributed in arid and semi-arid region in Northwest China. Quinolizidine alkaloids were identified as the active components in *Sophora alopecuroides* L. To investigate the anticancer activity of the alkaloids derived from *Sophora alopecuroides* L, we evaluated inhibitory effect of the alkaloids on ovarian and bladder cancer cells. Aloperine was found to play the strongest effect on cancer cells. Bladder cancer cells were indicated more sensitive to Aloperine. Cell proliferation, cell cycle, apoptosis, metastasis, adhesion and autophagy were measured to determine the anticancer effect of Aloperine. We have found that Aloperine could suppress proliferation and adhesion, induce cell cycle arrest, apoptosis and autophagy in bladder cancer cells, whereas didn't affect metastasis. We analyzed the anticancer mechanism of Aloperine in bladder cancer cells by RNA-seq and found that Aloperine might function through Lysosome, Inflammatory mediator regulation of TRP channels and Glycosaminoglycan biosynthesis pathways. Moreover, we assessed acute and subacute toxicity of Aloperine to BALB/c mice after intraperitoneal injection. The acute toxicity study revealed the LD_{50} dose of Aloperine was 176.243 mg/kg in mice. The sub-acute toxicity study indicated certain toxicity in liver and kidney whereas the damage was recovered to a degree after withdrawal of Aloperine. No obvious injury was detected in other organs. These results suggest the potential of Aloperine derived from *Sophora alopecuroides* L to be developed as an anti-bladder cancer drug and a recovery time is necessary when Aloperine is used for experimental and clinical researches.

Eupatorium adenophorum induces toxicological effect on mice liver by activating pyroptosis via GSDMD/IL-1? Pathway

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It has been reported that *E. adenophorum* causes hepatotocicity of animals. The purpose of this study was to present the molecular mechanism of hepatotoxicity induced by *E. adenophorum* in mice. 40 of female 8-week-old mice with similar body weight were randomly divided into 4 groups. Group A, B and C were fed with 10% (20 g/kg BW,), 20% (40 g/kg BW) and 30% (60 g/kg BW) level of *E. adenophorum* dry power for 6 weeks, respectively. Mice administrated with nutrient balanced feed were used as control. The results showed that the mice fed in group C presented a markedly decreasing bodyweight ($p < 0.05$) and appeared obvious toxicological effect. The alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase in group C had significant increase compared with control group ($p < 0.01$). Histological observation showed that necrosis accompanying inflammatory reaction in liver could be induced by high dose of *E. adenophorum* (Group C). *E. adenophorum* triggered the activation of IL-1? in dose-dependent, which were measured by ELISA, western blotting and immunohistochemistry. *E. adenophorum* could induce hepatocytes pyroptosis in dose-dependent manner, which could confirmed by the increasing of annexin V and propidium iodide double-positive hepatocytes and the released of lactate dehydrogenase. In addition, the Gasdermin-D (GSDMD) which is an executor of pyroptosis, is also activated in Group C. In conclusion, the definite toxicity of *E. adenophorum* in diets revealed to a great threat to mice by inducing damage on liver by activating pyroptosis.

A novel analytical method for simultaneous quantification of the carcinogens ptaquiloside, caudatoside and ptesculentoside

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Pteridium sp. (Bracken fern) are worldwide abundant species of vascular plants containing the carcinogenic glycoside Ptaquiloside. Ptaquiloside is reported to cause cancer in ruminant animals ingesting the ferns. The chemical structure of Ptaquiloside makes it highly water soluble and mobile in soils and sediments. Previous studies have demonstrated significant leaching of Ptaquiloside to water bodies receiving water from the surface of the plants after precipitation. Ptaquiloside can occur in drinking water resources and pose oncological threat to people. Besides, compounds similar to Ptaquiloside (Ptesculentoside and Caudatoside) have recently been detected in different *Pteridium* species. We have isolated the abovementioned compounds, investigated their physical-chemical properties and developed techniques for their simultaneous quantification. The HPLC-MS quantification method is developed towards its versatility (simultaneous analysis in a single run), fast speed (up to 5 min. per sample analysis) for high-throughput screening of numerous water samples and aiming at a low limit of detection for the analytes (1 ng/l). The analytes are separated in an Agilent InfinityLab Poroshell 120, 2.7 um EC-C18, 3.0x50 mm HPLC column with an Agilent 1260 Infinity reversed-phase HPLC system. This project is part of the network investigating natural toxins in aquatic environments (www.natoxaq.ku.dk) funded by European Union’s Horizon 2020 research and innovation programme.

The disaster and toxicant mechanisms and prevention and control of locoweed in western China

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Locoweeds, which belong to the genera *Oxytropis* and *Astragalus*, are an important species of poisonous plants and mainly distributed in 9 provinces of natural grassland in western China, such as Tibet, Qinghai, Inner Mongolia, Xinjiang, etc. These regions account for more than 60% and 70% of the country's land area and natural grassland area respectively, and it is major pastoral areas frontier minority areas, source of national rivers and water towers, and ecological barriers in China, so its strategic position is extremely important. Since the 1970s, the amount of locoweed has increased year by year in natural grasslands of western China, and the area has spread from 7.60×10^6pa to 2.15×10^7pa. Locoweeds have become the most serious poisonous grass that threatens the Chinese grassland livestock husbandry production. For that reason, many scientists have carried out the joint research of multi-disciplinary, multi-sectoral and multi-provincial regions through 33 years, and collected 23,484 samples distributed in 8,432 sampling sites of 213 counties, and ascertained locoweed species and distribution pattern, and explored locoweed disasters and poisoning mechanisms, and presented some new concept on prevention and control of locoweed, for example, "Five Benefits and Two Harmfulness", "Three Changes Three Uses" and "Three-five" technical system. Meanwhile, series of drugs were developed and extended in some serious locoweed poisoning areas in Western China, and obtained and obvious control effects. In a word, these comprehensive measures will be applied as a fundamental approach on the green prevention and control of locoweed in China in future.

Preliminary studies on poisonous plants, endophytes and metabolites of *Ligularia*

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In recent years, with the development of super-resistant bacteria as a disease that is difficult to overcome, the search for highly effective and broad-spectrum antibacterial active substances from natural plants has become a hotspot and an important way to create new antibacterial drugs. A large number of research results show that the antibacterial mechanism of natural plants is different from antibiotics, and it is not easy to produce drug resistance, which greatly reduces the environmental problems caused by drug residues.

Endophytic fungi is a new microbial resource to be developed. Not only can beneficial endophytic fungi be isolated from traditional medicinal plants, but also other plants that grow in some special environments have also found a large number of endophytic fungi that produce active metabolites. Endophytic fungi increase crop nitrogen fixation, biological control and other aspects. It plays an important role. And its metabolites have some special effects, such as the use of antibiotics.

The poisonous plants of *Ligularia* are dominant poisonous plants on the grasslands of Ganzi Prefecture, Sichuan Province, China, especially the *Ligularia ulmoides*. It only reached 304,666 hectares in Litang County and Seda County, seriously affecting the growth of high-quality forage grass in the pastoral areas and herdsmen's grazing. In order to prevent the poisonous plants of the genus *Ligularia*, except for a large amount of man-powered excavation, some herbicides are applied to accumulate pesticides in grassland soil and water sources, resulting in excessive pesticide residues in livestock products, and even human and livestock poisoning, constraining the local green ecological livestock husbandry. Industry development. Therefore, we conducted a series of studies on the metabolites of the plant and its endogenous bacteria. The results of the study showed that the plant extracts and endophytic metabolites have broad-spectrum antibacterial activity. Research results show that there is no relevant research in China and other countries.

Swainsonine in select North and South American *Astragalus* species

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The indolizidine alkaloid swainsonine is found in a number of plant species worldwide, and causes severe toxicosis in livestock grazing these plants, leading to a chronic wasting condition characterized by weight loss, depression, altered behavior, decreased libido, infertility, and death. Swainsonine is produced by a vertically transmitted fungal endophyte, *Alternaria* Section Undifilum species. Swainsonine has been detected in several North and South American *Astragalus* species of which some are part of four taxonomic sections, the Densifolii, Diphysi, Inflati, and Trichopodi. These sections belong to two larger groups representing several morphologically related species, the Pacific Piptolobi and the small flowered Piptolobi. We hypothesized that there may be a common morphological and/or phylogenetic relationship for swainsonine-containing species. The objective of this study was to screen the remaining species for swainsonine in sections Densifolii, Diphysi, Inflati, and Trichopodi previously not known to contain swainsonine. To broaden the scope further, species within the remaining sections of the Pacific Piptolobi and the small flowered Piptolobi were screened for swainsonine. Furthermore, several South American species were screened, including a subset that are phylogenetically related to the above mentioned North American species. Swainsonine was detected in several species previously not reported to contain swainsonine. A strong correlation was observed between the occurrence of swainsonine and species that share common morphological features and genetic relatedness. A systematic examination for swainsonine in these species provides important information on the toxic risk of these species and would be a valuable reference for land managers.

Cytotoxicity of *Terminalia ferdinandiana* extracts in intestinal and hepatic cancer cell lines

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Plant foods are considered non-toxic and safe due to their natural origin and traditional use in the diet. However, studies involving the efficacy and safety of some plant foods have indicated that frequent consumption over a prolonged period of time may produce toxic effects possibly due to undesirable interactions between some phytochemicals and other dietary molecules, contamination with heavy metals or pesticides, and/or adulteration. With the current emphasis on the safety of plant food, it is important to explore the potential toxicity of plants utilized in the food industry. Therefore, the aim of this study was to assess the potential risk of polyphenol-rich extracts of *Terminalia ferdinandiana* fruits and seeds by determining their cytotoxicity in undifferentiated and differentiated Caco-2 (enterocytes), HT29-MTX-E12 (goblet) and Hep G2 (liver) cancer cell lines. Nine different concentrations ranging from 33 to 200,000 µg/ml were investigated using the Cyquant NF Cell proliferation assay to detect viable cells. Changes to cell viability in response to the extracts produced IC50 values ranging from 4415 to 12,878 µg/ml. Comparatively, the IC50 values for standard ellagic acid varied from 1055 to 2243 µg/ml. Overall, when compared to standard ellagic acid, higher concentrations of fruit and seed extracts were required to inhibit the proliferation of the different cancer cells. Results reported here provide valuable information regarding the safe use of *T. ferdinandiana* fruits and seeds in commercial food products.

Earwax: A clue to discover fluoroacetate intoxication in cattle

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Palicourea marcgravii is one of the most important plants related to sudden death syndrome in cattle in Brazil and the toxic principle is monofluoroacetic acid (MFA). An innovative method was developed to detect fluoroacetate poisoning in cattle by headspace/gas chromatographic analysis of earwax samples of intoxicated cattle. For the experiment, earwax samples were collected from 7 calves, males, aged 6 and 8 months included in the study randomly divided into two groups (T1 and T2), consisting of 4 and 3 calves, respectively. The animals were experimentally intoxicated with *P. marcgravii* at a dose of 1,8 g/kg by forced ingestion of the dried and ground plant, 3 h after oral administration of single doses of acetamide of 1,0 g/kg (group T1) and 2,0 g/kg (group T2). The animals presented clinical signs evident of intoxication, however discrete or of short duration. Earwax samples were collected 30 days after the intoxication, using a metal curette, transferred in eppendorf tubes, immediately stored in a freezer and analyzed within 7 days of freezing. The levels of MFA encountered in groups (T1) receiving a lower dose of acetamide were higher than group (T2) receiving a higher dose of acetamide. In spite of that, the same dose of MFA that was administer indicating the amount of MFA detected in earwax is inversely proportional to the dose of acetamide which is the antidote. Thus, earwax analysis represents a successful approach for detection and monitoring of fluoroacetate poisoning.

Grazing of *Delphinium occidentale* (duncecap larkspur) by susceptible and resistant Angus cattle

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Delphinium spp. (larkspurs) often fatally poison grazing cattle. Angus cattle differing in susceptibility to larkspur poisoning were used to assess selection of larkspur (*Delphinium occidentale*) while grazing. During July 2015, 2016, and 2017, diet selection of 12 cattle (6 susceptible, 6 resistant) was determined for 2 to 3 weeks. During 2015 resistant steers ate 6% of their diets as total larkspur compared to 7% ($P > 0.34$) for susceptible steers. During 2016 there was a treatment x d interaction ($P = 0.02$) for total larkspur consumption as resistant steers consumed more larkspur on 5 trial d than did susceptible steers. Overall, resistant steers ate 6% of their diets as larkspur compared to 3% for susceptible steers. During 2017 there was a treatment x d interaction ($P = 0.03$) for total larkspur consumption as resistant heifers consumed more larkspur on 2 trial d than did susceptible heifers. Overall, during 2017 resistant heifers ate 7% of their diets as larkspur compared to 3% for susceptible heifers. The average serum concentration of methyllycaconitine in severely intoxicated, susceptible animals was approximately 700 ng/mL compared to approximately 1,000 ng/mL for severely intoxicated, resistant animals. The only fatalities (2) were in susceptible animals. The diet selection and comparative responses of resistant and susceptible animals on rangeland appears to validate the phenotyping done in the laboratory, but must be verified in a controlled setting. Selecting resistant cattle to graze larkspur-infested rangelands may reduce losses; however, further research is required to develop genetic biomarkers to identify such animals.

Herbicide control of death camas

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Death camas (*Toxicoscordion* spp.; syn. *Zigadenus* spp.) is a bulbous perennial that grows on foothill ranges in the western U.S. and is one of the first plant species to emerge in the spring. The lack of alternative forage at this time can result in livestock consuming death camas which in turn can lead to death loss. Research on herbicide control of death camas is limited with most control studies conducted decades earlier. Previous work only identifies 2,4-D as a possible control agent. Six herbicides (aminopyralid, 2,4-D, chlorsulfuron, imazapic, quinclorac, and 2,4-D + triclopyr) were applied at two different stages of plant development to determine control of death camas. Imazapic, 2,4-D, and 2,4-D + triclopyr controlled death camas when applied at early vegetative growth and after flower pod development, thus providing producers and land managers with alternative herbicides to control death camas.

Hazard cause, distribution, damage and control of various species of poisonous weeds in natural grassland of China

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The grassland of China is an important resource for livestock production, which even is the largest ecological barrier and the constructive foundation of ecological civilization. Also, it has some other significant functions, such as maintaining diversity of animal and plant species, guaranteeing the national ecosystem, prairie security and food safety and protecting the environment of human beings and animals. However, grassland already have been degenerating by global climate change, drought, overgrazing and irrational utilization, which led to the shortage of pasture and spread of poisonous weeds. Poisonous weeds which cause mounts of livestock death and disorder ecological structure of plant population are called “grassland killer”, seriously restricting the sustainable development of husbandry. This review summarizes the current research about poisonous weeds in grassland of China, such as the cause of disaster, characteristics of occurrence, distribution of species, status of damage and trend of prevention and control. We also give some suggestions and countermeasures of prevention and control for natural grassland of China, which has certain value for administering poisonous weeds scientifically.

Idiopathic enzootic calcinosis in a goat herd in New Zealand

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Findings in 25 out of 40 Toggenburg goats from one property necropsied over a period of 27 years included calcification of the endocardium, aorta, small arteries and lungs. We hypothesise that this syndrome is hypervitaminosis-D, or enzootic calcinosis. Enzootic calcinosis caused by the dietary intake of certain plants that contain elevated concentrations of vitamin D or vitamin D-analogues has been reported in some countries, but not New Zealand.

Exogenous sources of vitamin D include dietary supplements, certain rodenticides, and known calcinogenic plants, such as *Trisetum flavescens*, *Solanum glaucophyllum* and *Cestrum diurnum* among others (these contain metabolites or glycosides of vitamin D3), as well as *S. esuriale* and *Nierembergia veitchii* (for which the vitamin D-like substance remains unidentified). None of these plants are known to occur on the farm, and no supplements nor rodenticides were accessible to goats.

Serum 25-hydroxyvitamin D2, D3, total calcium and phosphorus (n=15 goats) were measured two-monthly for 12 months and compared to control goats (n=22). The calcinosis flock showed higher serum vitamin D2, but not D3.

Vitamin D2 is synthesised in the cell membranes of fungi (including yeasts) and its presence in plants such as *Medicago sativa*, *Lolium perenne*, and hay, is attributed to endophytes and/or fungal contamination. Goats browse more widely than other species and reported sources of vitamin D2, including mushrooms, lichens, hay, and *Pinus radiata* pollen, are present on the farm. In addition, the goats ingest willow, poplar, cedar bark, and large quantities of *Solanum nigrum*. Investigations are continuing.

Ptaquiloside and pterosin B levels in four Brazilian ferns

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Ptaquiloside is a carcinogenic compound present in several fern species and is responsible for poisoning in cattle. It can be transferred into milk potentially causing tumors in humans. Pterosin B is an end-product of ptaquiloside breakdown and also occurs naturally in some ferns. This study aimed to measure the concentrations of ptaquiloside and pterosin B in four fern species: *Pteridium arachnoideum*, *Dicranoptris flexuosa*, *Gleichenella pectinata* and *Sticherus lanuginosus*. The measurements of both compounds were performed by HPLC-UV using pterosin B as analytical standard. Pterosin B was isolated from *P. arachnoideum* sprouts and LC-QTOF/MS was used for confirming the identity of chemical. Fronds (n=28) and sprouts (n=29) of *P. arachnoideum* were collected from two states: Minas Gerais (southeastern Brazil) and Rio Grande do Sul (south Brazil). Furthermore, fronds of *D. flexuosa*, *G. pectinata* and *S. lanuginosus* were collected in Minas Gerais state. Ptaquiloside and pterosin B levels in *P. arachnoideum* were 2.631±0.471 mg/g and 0.805±0.153 mg/g in fronds and 16.136±3.881 mg/g and 7.305±2.642 mg/g in sprouts. Ptaquiloside levels did not significantly differ between plants collected in the two states. Ptaquiloside and pterosin B levels were 2.466±0.173 mg/g and 1.572±0.166 mg/g in *S. lanuginosus*, and 0.892±0.215 mg/g and 0.466±0.083 mg/g in *D. flexuosa*. On the other hand, *G. pectinata* showed no detectable levels of both ptaquiloside and pterosin B. In conclusion, Brazilian *P. arachnoideum* showed high concentrations of ptaquiloside, and they did not vary between locations. Furthermore, ferns of species other than *Pteridium* also contain ptaquiloside that can cause poisoning.

Liquid chromatography coupled to quadrupole time-of-flight mass spectrometry assay for quantification of protodioscin in *Brachiaria* grasses

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The reported work aimed to develop and validate a method to detect and quantify protodioscin in *Brachiaria* grasses using ultraperformance liquid chromatography (UPLC) coupled to high-resolution quadrupole time-of-flight mass spectrometry. Samples were extracted by acetonitrile–water 50:50 v/v mixture and ultrasonication. The mobile phase consisted of 5 mM ammonium acetate in water–methanol and sacetonitrile containing 0.1% formic acid. The parameters used to validate the method for determining protodioscin comprised determination of the selectivity, ionization suppression/enhancement (matrix effect), linearity of the calibration curve, the limit of detection (LOD), the lower limit of quantitation (LLOQ), and the precision and accuracy of the method. The LLOQ of protodioscin was determined as 0.1 µg mL^{-1}, and the LOD was 0.03 µg mL^{-1}. The developed method was applied for determining protodioscin levels in *B. decumbens* collected from three pastures where sheep showed signs of photosensitization. The obtained values ranged from 0.71% to 1.12%. Thus, the developed method for determining protodioscin in *Brachiaria* grasses by LC coupled to high-resolution quadrupole time-of-flight mass spectrometry showed high accuracy, precision, and sensitivity.

The clinical and histologic changes of *Salvia reflexa* intoxication in cattle, goats and mice

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Salvia reflexa or mint weed was first reported to be toxic in Australian livestock in 1940. Poisoned animals were described as having "twitching muscles" followed by recumbence and death. Various toxins were proposed and original reports suggested nitrites were the cause though this was never confirmed nor was the pathology thoroughly described. Recently *S. reflexa* has been identified as the cause of cattle poisoning in the western United States. *Salvia* contaminated hay initally produced clinical disease in cattle with clinical signs and biochemical changes were indicative of acute liver failure. Histologically affected animals had massive centrilobular hepatic necrosis characterized by hepatocellular swelling, necrosis and lysis resulting in collapse of hepatic cords and hemorrhage. Clinically sublethally poisoned cows were biopsied and found to have a progression of lesions ranging from hepatocellular necrosis to mixed inflammation with fibrosis and periportal fibrosis and biliary proliferation. These sublethally poisoned animals were averted to eating additional *S. reflexa* and would sort through contaminated feed to avoid it. The disease with similar clinical progression, serum biochemically changes and hepatic necrosis was reproduced expermentally in cattle, goats and mice using both ground *S. reflexa* and varios extracts of both the plants and contaminated hay. The chemistry and toxins associated with this disease have identified as salviarin and other related furanoditerpenes. These findings indicate that North American *S. reflexa* contains potent hepatoxins. More work is needed to determine the distribution and concentrations of the toxins in other plant populations and other *Salvia* species.

Diterpenoids from *Salvia refl*exa associated with hepatotoxicity in cattle

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Several diterpenoids were isolated as part of a bioassay guided fractionation of weedy hay via a mouse hepatotoxic bioassay. The diterpenoids isolated included those compounds identified as salvarin, salvianduline D, and rhyacophiline. A new diterpenoid, 7-hydroxyrhyacophiline, was isolated and the structure elucidated by 1D and 2D NMR. The purified diterpenoids were tested in a mouse bioassay and salvarin, salvianduline D and 7-hydroxyrhyacophiline were found to induce sever hepatic nacreous within 48 hours after single oral dosage (~500 mg/kg). At similar dosage levels rhyacophiline was found not to induce the hepatotoxic effects. The identified diterpenoids are known to be found among the different *Salvia* species which led to the identification of dried plant parts (stem and flower pods) of *Salvia reflexa* found among bales of hay. A reexamination of the hay field location found a significant population of *S. reflexa* along the hay field edges and irrigation ditch banks.

Endophyte infected perennial ryegrass (*Lolium perenne*) effects on pregnant camels

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Perennial ryegrass (*Lolium perenne*) infected with endophyte *Neotyphodium lolii* contains toxic alkaloids with lolitrem B, most commonly measured to diagnose "ryegrass staggers". Another ergot alkaloid, ergovaline, is concurrently produced by the endophyte at approximately one-third the level of the lolitrem B. It causes vasoconstriction and reproductive problems in cattle. This study measured the effects of varying levels of lolitrem B and ergovaline in straw fed over 42 days to mid to late term pregnant camels. 18 pregnant camels were divided into 4 groups and fed either perennial ryegrass straw containing lolitrem B at less than 100ppb (group A, negative control), or 1000 ppb (Group B) or 1500ppb (Group C) or 2300ppb (Group D, as a positive control). Camels were videotaped initially and weekly during the trial for detection of "ryegrass staggers". After 4 days on the high dose, one camel in late gestation aborted, and the high dose (Group D) was discontinued. By the end of the 42 days on the B ration, 3 of the 6 camels delivered weak calves with death of 2 in four days and agalactia evident in all 3 affected camels. One of these camels also had ryegrass staggers. In the C group, 3 camels developed ryegrass staggers. In addition, one of the three had an abortion at 10 months, one had a weak, low birth weight calf that died 3 days post-natal, and one had agalactia. No problems occurred in the A group. Dilution of straw containing greater than 500ppb lolitrem B is recommended.

First giant fennel *(Ferula* sp.) intoxication in continental France

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Giant fennel (*Ferula* sp. L) is a tall plant of the Apiaceae family that grows all around the mediterranean sea. All parts of the plant contain 2 major prenylated coumarins, ferulenol and ferprenin, that are efficient inhibitors of the vitamin K recycling enzyme VKOR. The deadly haemorraghic syndrome that occurs after consumption of the plant concerns all mammals. It was described only in Corsica and involved *Ferula communis* var. *communis*. In continental France three species of Giant fennel were described: *Ferula communis communis* (from the Rhône river to the italian border, in Corsica), *Ferula communis microcarpa* close to the spanish border at the west and *Ferula glauca* between the two previous areas. F.microcarpa and F.glauca were traditionally considered as non toxic chemotypes.

In 2016 deadly clinical cases were observed for the first time in sheep in continental France. 200 local breed ewes were grazing an area to clear the scrub and maintain firebreaks. The animals had access to this area during winter (from January to March). The first day the shepherd found 3 dead ewes. The same day 17 more ewes died. The day after 15 more sheep died. The only observed symptoms were prostration, weakness and pale mucosae. Spontaneous hemorrhage without epistaxis was present. Necropsy findings were hemorraghe in lungs and hepoperitoneum.

There was Giant fennel overall in that area that was determined to host only *Ferula communis* (L) var. *microcarpa* (CAUWET-MARC). A previous study showed that the levels of ferulenol in *Ferula communis* var. *microcarpa* were significantly lower than those of *Ferula communis* var. *communis* (respectively 13.7-849 µg/g DM (min-max) vs 1.19-8331 µg/g DM (min-max)). Two samples of Giant fennel leaves were taken and their ferulenol and ferprenin levels were determined. The levels were consistent with the previous study : average dry matter (N=2) was 17.5 % and ferulenol level was respectively 81 µg/g and 119 µg/g of dry matter of leaves. No ferprenin could be detected. Blood and liver samples from 2 sheep were analysed for ferulenol content by LC/MS-MS. The levels in blood and fresh liver were respectively 9.7 and 41 ng/ml and 411 and 519 ng/g. Though the difference in ferulenol levels between both varieties the risk is present and the toxicity of giant fennel *var. microcarpa* is evident when the animals are farmed in free ranging during winter without added fodder. This first continental description of Giant fennel intoxication in sheep points out the relationship between the weather conditions and the grazing practices.

Mass mortality of Eastern Grey Kangaroos (*Macropus giganteus*) associated with hepatogenous photosensitisation subsequent to ingestion of *Panicum gilvum*

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The impact of introduced toxic plant species, to which evolutionarily naïve native mammals have little tolerance, is poorly documented but is an emerging issue in wildlife population health. Blindness associated with photosensitivity, as well as abnormal behaviours including shade seeking in domestic buildings and straying onto roads and roadside verges resulted in subsequent mass mortalities of Eastern Grey Kangaroos (*Macropus giganteus*), which occurred in the Wagga Wagga region of New South Wales in April 2014. Necropsy of affected cases revealed lesions indicative of dermal and corneal photosensitivity. In particular, animals presented with marked corneal oedema with a ventral to dorsal progression indicative of progressive stages of the disease. Dermal and ocular lesions were associated with cholangiohepatopathy of varying severity and chronicity and hyperbilirubinaemia. This syndrome was suspected to be a plant toxicosis resulting in hepatogenous photosensitisation. The presence of acicular clefts in hepatic portal regions suggested saponin toxicity.

An analysis of pasture proportion and species indicated a high proportion a *Panicum* grass initially identified as *Panicum gilvum*. *P. gilvum* has been reported to contain steroidal saponins that have been implicated in crystalline hepatopathy and cholestasis resulting in photosensitivity in livestock. UPLC/QToF mass spectrometry indicated that steroidal saponins similar to diosgenin were found in the leaves, stems and inflorescence of *P. gilvum. Trace metabolites were also evaluated in livers of affected kangaroos and are as yet unidentified.* Subsequent outbreaks have occurred since 2013, highlighting the importance of understanding how invasive weeds species can adversely impact populations of native mammals.

Accumulation and depletion of the toxic amino acid indospicine in calves fed creeping indigo (*Indigofera spicata*)

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Consumption of pasture plants of *Indigofera* spp by grazing animals over a prolonged period is reported to cause both liver damage and reproductive losses, with the responsible toxin indospicine (an arginine analogue) also forming persistent tissue residues. In this study, separate accumulation and depletion feeding experiments were undertaken in calves fed *Indigofera spicata* (3 mg indospicine/kg bodyweight per day) to determine the accretion and elimination of indospicine from various tissues in these animals. In the accumulation trial, indospicine concentrations increased throughout the 42-day feeding period reaching 15 μg/mL in plasma, 19 μg/g in liver and 33 μg/g in muscle. In the depletion trial, calves were fed *I. spicata* for 35 days, after which the plant was removed from the diet. The rate of elimination was relatively slow and estimates of half-life were 31, 25 and 20 days for muscle, liver and plasma, respectively. Indospicine concentrations measured in bovine tissues in this trial are comparable with those in horsemeat and camel meat reported to cause fatal hepatotoxicity in dogs, albeit a species known to be particularly susceptible to this toxin. The persistence of indospicine residues in bovine tissues and the widespread distribution of *Indigofera* species (such as Birdsville indigo – *I. linnaei*) in tropical and sub-tropical grazing lands warrant further investigation, as indospicine has been established as causing reproductive losses, and likely contributes to calf losses in these regions.

Risk assessment of indospicine residues in bovine muscle and liver from north-west Australia

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Indospicine is a natural toxin found only in *Indigofera* plant species, including species prevalent in northern grazing regions of Western Australia. These legumes are palatable to cattle and indospicine residues derived from these plants accumulate in cattle tissue. Indospicine-contaminated horse and camel meat have caused hepatotoxicosis and mortalities in dogs after dietary exposure. Since the risk for human consumption is not known, a survey study was undertaken in Western Australia to assess the indospicine levels in samples collected from abattoirs over 4 seasons in 2015-2017 and to predict the likelihood of significant residues being present. Muscle and corresponding liver samples from 776 cattle originating from Kimberley/Pilbara regions were collected at abattoirs across Spring and Autumn seasons. The indospicine concentrations ranged from below detection to 3.63 mg/kg, with residue prevalence being generally greater in Autumn, than in Spring collections. @Risk best fit probability distributions showed ninety-fifth percentile (P95) indospicine concentrations of 0.54 mg/kg for muscle and 0.77 mg/kg for liver in this 2015-2017 period. When considered with average Australian daily meat consumption data, the estimated consumer exposure from this P95 muscle was 0.31 mg indospicine/kg bw/day, which compares favourably with our calculated provisional tolerable daily intake (PTDI) of 1.3 mg indospicine/kg bw/day. Canine exposure is also of concern with active working dog exposure calculated to exceed this PTDI by a factor of 25 based on P95 indospicine concentrations of 0.54 mg/kg in muscle. Even allowing for the conservative nature of the PTDI calculation, this canine exposure risk is of potential concern.

ProMED-mail and toxic plants

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There are a number of accessible data bases for searching and perhaps identifying toxic plants. Search engines have greatly increases what we can find, especially on the internet but there is also much misinformation on some sites. However, where is the data base recording the specifics of those poisoning? How many people or animals were involved? Was this an inadvertent poisoning or was the situation perhaps to collect on insurance somewhere?

ProMED-mail, a global, moderated, reporting system may fill the niche. This is the **Pro**gram for **M**onitoring **E**merging **D**iseases. It is an archived and searchable system. This reporting system is more than infectious disease, it includes emerging situations, toxicities and chemical warfare agents. It involves human beings, animals, and plants of economic concern.

The program is free to your email when you subscribe. While it does request some information, it is for contacting the member who may be in an area where there is an outbreak/toxicity and ProMED-mail is looking for first hand reporting of the situation. ProMED-mail does not sell, publish or otherwise release your information.

Website: http://www.promedmail.org. ProMED-mail is a program of the International Society for Infectious Diseases. http://www.isid.org

North American hard yellow liver disease: an old problem readdressed

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Hard yellow liver disease or fatty cirrhosis, first described in 1931 in western Texas, is reported to periodically affect cattle, sheep, goats, pronghorn antelope (*Antilocapra americana*) and whitetail deer (*Odocoileus virginianus texanus*). Described as chronic liver disease with progressive deterioration of condition, icterus and liver failure. At slaughter the liver is often yellow and many have multiple focal firm, hard, often gritty foci scattered throughout the liver with most near the capsule and margin surface. Many lesions have been misidentified as carcinomas identified at slaughter that later is microscopically identified as hard yellow liver disease. Early studies included feeding studies using potential toxic plants, climate and forage studies, infectious disease surveys and mycotoxin analyses have not reproduced this syndrome. So after nearly a century of work, hard yellow liver disease continues to affect animals and impair animal health and production. Recently with continued occurrence, we have collected tissues, blocks and slides from affected animals. Additional studies were done evaluating tissues for plant toxin metabolites or fragments of nucleic acid from potential pathogens. No plant toxin metabolites or pathogens were detected. Extensive review of reports and histologic studies of archived samples show that hard yellow liver disease is variable with a spectrum of pathology. This suggests that this syndrome is probably a collection of different diseases of both toxic and immune mediated causes. The objective of this presentation is to incite discussion and further collaboration to understand and solve this problem.

Effect-based screening of water leachable compounds of invasive plants employing *in vitro* bioassays

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Invasive plants have by definition an increasing distribution and often occur in high abundance in both the natural and agricultural environment. For some, we know of apparent toxic effects, such as the phototoxicity of *Heracleum mantegazzianum* by skin contact, and allelopathic effects of *Fallopia japonica*. They are however overall under-investigated in terms of toxicity to animals, humans and ecosystems. In the present study, we employed an array of *in vitro* cellular reporter gene assays addressing sublethal effects such as endocrine disruption, mutagenicity and adaptive stress response (oxidative stress, inflammatory response and DNA repair). Plants were collected in the environment, and extractions made by shaking with mineral water after homogenization. Solid-phase extraction was applied for the enrichment of samples prior to dosing, and fractionation for separation of cytotoxicity and specific effects. Species investigated include *Impatiens glandulifera*, *Solidago canadensis*, *Heracleum mantegazzianum* and *Fallopia japonica*. Results TBA. This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No. 722493 (NaToxAq). www.NaToxAq.ku.dk

Pyrrolizidine alkaloids in Blue Heliotrope (*Heliotropium amplexicaule*) in Australia

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Pyrrolizidine alkaloids (PAs) are secondary metabolites present in 3% of all flowering plants, which are spread across the globe, with each plant having a characteristic PA profile. PAs have been reported to have hepatotoxic, and their inadvertent consumption has been associated with acute and chronic liver damage (and even death) in wildlife, livestock and humans. These alkaloids are potent carcinogens and their presence in food commodities as even low level contaminants is of concern to food regulators. Bees can transfer PAs from pollen and nectar into honey, raising food safety concerns and therefore the presence of PAs in honey is monitored and regulated. Blue heliotrope (*Heliotropium amplexicaule*) is native to South America and is an introduced flowering plant in Australia that has been reported to produce PAs. Previous studies identified the retronecine monoester indicine as its major alkaloid. The presence of additional pyrrolizidine alkaloids has been reported in this plant but no structures have been elucidated for these compounds. This study aimed to isolate and identify these unknown PAs via NMR and LCMS and thus provide a comprehensive PA profile for blue heliotrope. This profile allowed for comparison with PAs found in commercial honeys as to determine if *H. amplexicaule* was a major source of contamination in Australian honey. These results will be discussed.

Hypoglycin A exposure of horses with atypical myopathy

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Hypoglycin A (HGA) and its lower homologue alpha-methylenecyclopropyl glycine (MCPG) are contained in a lot of unripe edible fruits of the Sapindaceae family like ackee, longan, rambutan, litchi and cause a severe hypoglyceamic encephalopathy in human. HGA is also present in sycamore maple seeds and leaves and is responsible for atypical myopathy (AM) in horses. The toxicity is due to the metabolism of HGA into methylenecyclopropyl-acetic acid (MCPA) whose ester MCPA-CoA disrupts the ß-oxydation of lipids and gluconeogenesis. To evaluate the exposure of horses between 2000 and 2014 HGA was measured in blood samples of 39 horses (26 horses with a single blood sampling and 13 with serial blood sampling) according to the method developped by Carlier et al. (UHPLC-HRMS/MS). Two seeds of sycamore maple were tested for HGA. Among the 26 single blood samples HGA couldn't be detected in 9 samples. Considering that the first sample of the serial sampling could correspond to the single sample the median level of HGA in serum was 575.9 ng/ml (N=30 ; min : 51.7 ;max : 1323.6) at the first sampling. The group with serial blood sampling (N=13 ; from 2 to 7 samplings between 1 to 64 hours after the first sampling) shows a decrease in the HGA concentration but 65% of the initial level was remaining after 24 hours (N=5). The HGA content of the seeds was 120 and 740 µg/g.

Relative accumulation of indospicine and its deamino-metabolites in camels fed *Indigofera spicata*

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Indospicine is a natural toxin which is found in *Indigofera* plant species and accumulates in tissues of livestock grazing these plants. It is known to cause both primary and secondary hepatotoxicosis, including the fatal poisoning of domestic dogs in Perth from the consumption of indospicine-contaminated camel meat in 2009.

In-vitro experiments have demonstrated that camel digesta fluid has the capacity to metabolise indospicine but such metabolism is in competition with absorption of indospicine from the digestive system. Six camels (2-4 years, 270-390kg) fed *Indigofera spicata* (337ug indospicine/kg.bw/day) for 32 days at which time 3 camels were euthanized. The remaining camels were monitored for further 100 days after cessation of this diet.

In a retrospective investigation relative levels of indospicine metabolites in plasma and tissue samples collected during both accumulation and depletion stages of this trial have been examined. Indospicine as well as the metabolites 2-aminopimelamic acid and 2-aminopimelic acid were analysed by UHPLC-MS. Low levels of indospicine were still present in plasma after 100 days. In contrast, the intermediate metabolite 2-aminopimelamic acid could be detected at very low levels (<LOQ) in almost all plasma samples, whereas 2-aminopimelic acid could not be detected. 2-aminopimelamic acid could be found in all tissues except muscle, whereas 2-aminopimelic acid was only found in pancreas and kidney tissue. It is concluded that metabolites formed during gastrointestinal digestion are metabolised/excreted much more readily than indospicine itself, and that increasing the gut microbial capacity to degrade indospicine presents an opportunity to prevent formation of persistent residues.

Spontaneous photosensitization by *Brachiaria ruziziensis* in sheep

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Brachiaria grasses are responsible for hepatogenic photosensitization, mainly *Brachiaria decumbens*, *B. brizantha* and *B. humidicola*. On the other hand, there just two reports of photosensitization by *Brachiaria ruziziensis* in Brazil. The present study aims to describe clinical and pathological changes in an outbreak of hepatogenic photosensitization caused by *B. ruziziensis* in sheep. In addition, samples of young and old leaves (n=4 for each type) were collected for 8 weeks for quantification of the steroidal saponin protodioscin and spores of *Pithomyces chartarum*. Sixteen of 43 crossbred Santa Inês sheep fed *B. ruziziensis* grass showed signs of photosensitization. Clinical signs included jaundice, apathy, dehydration and photosensitization, characterized by facial edema and cutaneous scars, especially in the ears. Six sheep died, and pathological examination was performed in one of them. Gross findings were generalized jaundice, crustal lesions on the face and especially on the ears, the kidneys and liver were firm and greenish. Histologically, the liver had diffuse infiltrate of foamy macrophages, occasional multinucleated cells, and megalocytosis. The lesions in skin included epidermal and dermal necrosis. Mean protodioscin levels were 0.065% (n=32) in young leaves and 0.020% (n=32) in old leaves. Mean number of *P. chartarum* spores were 480 $x10^3$ spores/g (n=32). Thus, it is likely that the photosensitization by *B. ruziziensis* described herein was caused mainly by sporidesmin produced by the fungus.

Toxic effects of saponins from *Cestrum axillare* leaves in goats

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Cestrum axillare (*C. laevigatum*) causes spontaneous acute poisoning in cattle, goats and sheep. The main pathological finding is centrilobular hepatic necrosis. The toxic principle was not yet definitively proven, but some authors attribute the toxicity of the plant to the presence of saponins gitogenin and digitogenin. The objective of this work was to determine whether saponins are the compounds responsible for the hepatotoxic effects produced by *C. axillare* leaves. The effects of the administration of the leaves were compared with those produced by the saponins isolated from the leaves in goats. Six goats were randomly assigned to three experimental groups that received [1] *C. axillare* leaves, [2] saponins extract from leaves or [3] water (control group). The presence of gitogenin and digitogenin in saponins extract was confirmed by LC/MS. For goats receiving the dry leaves the administered dose of plant was 10 g/kg for one animal and 5 g/kg for the other one. Saponins extract was administered at a dose equivalent to 20 g/kg repeated after 24 hours. The dry leaves administered at a dose of 10 g/kg to a goat produced severe clinical signs of poisoning and centrilobular hepatic necrosis. At the dose of 5 g/kg of dry leaves, no clinical sign was observed, but hepatic necrosis was found; 15 days after the last administration, the hepatic parenchyma was regenerated. The administration of extracts of saponins to goats did not produce significant toxic effects, proving that these compounds are not responsible for poisoning.

Characterisation and bioactivity of secondary metabolites of *Amaranthus retroflexus* L. implicated in livestock poisoning

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Amaranthus retroflexus, originating from South America is an introduced weed in many parts of the world, including Australia. Ingestion of the plant material has been associated with acute renal failure in a number of livestock species including cattle, sheep and horse. While previous reports indicated that its foliage contains high levels of nitrogen, the characterization of the chemical composition of *A. retroflexus* foliage has not been performed fully, with differentiation of constituents in plant parts such as the leaf, stem, inflorescence and root. We report here the composition of the secondary metabolites of *A. retroflexus* foliage and the results of bioassay-guided cytotoxicity assessment of *A. retroflexus* extracts in an attempt to isolate and identify the associated toxins. As determined by UPLC-MS QTOF, all tissues (leaves, flowers, stems and roots) of *A. retroflexus* possessed an abundance of nitrogen-containing compounds, the primary constituents of which had apparent molecular formulae of $C_8H_{18}NO_2$ and $C_9H_{20}NO_2$, potentially the methyl and ethyl esters, respectively, of 4-oxo-1-butanaminium, a structural relative of choline. Cytotoxicity, as measured by MTT assay against the murine fibroblast cell line NIH3T3, was associated with a single compound in the extracts of *A. retroflexus* leaves, which we have tentatively identified as a compound with a high degree of similarity to the modified peptide N-acetyl-L-α-aspartyl-L-alanyl-L-α-aspartyl-L-α-glutamyl-O-(carboxymethyl)-L-tyrosyl-L-leucinamide, a mammalian metabolite known to play a role in cell signalling. Confirmation of the bioactivity of the putative toxin by comparisons with an authentic standard is required to ascertain nephrotoxic activity associated with livestock poisoning.

Regulation of swainsonine production and gene expression in fungal symbionts

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Locoweed consumption by animals has caused toxicosis problems in the western USA and China. Locoweeds contain fungal-produced swainsonine, a phytotoxin that causes locoism. Genome analyses of the biosynthesis pathway of swainsonine in endophyte-infected locoweed revealed a consensus region of orthologous gene clusters containing a multifunctional swnK gene. To determine the relationship between the swnK region and the toxicity of fungi, four fungal species from the USA and China were analyzed for gene expression. The endophyte *Alternaria* section *Undifilum* sp. was isolated from the locoweeds *Astragalus strictus* and *Oxytropis ochrocephalia*, and the pathogens *A. bornmuellerii* and *Slafractonia leguminicola* were grown on PDA. Oligonucleotide primers were designed from three domains in the swnK region, Ketide Synthase (KS), A (adenylylation) domain, AT (Acyl Transferase). Total RNA was isolated from 21-day old cultures and cDNA was generated. Quantitative PCR data revealed that the RNA expression from the KS region was highest for the *Alternaria* sp. isolated from *Oxytropis* and *Astragalus* species, while lower for *Slafractonia* and negligible for *A. bornmuellerii*. Gene expression data from the A and AT regions will be presented. Quantification of swainsonine levels in laboratory-grown cultures using high-performance liquid chromatography (HPLC) was performed. Our findings suggest a correlation between KS mRNA and swainsonine levels in endophytes. Detailed understanding of factors influencing swainsonine levels will improve methods to predict toxicity.

Acute neurological signs and deaths in rams associated with horse chestnut (*Aesculus indica)* ingestion

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A New Zealand Gisborne based veterinary practitioner investigated the acute death of four rams and examined five other rams with neurological signs in a mob of 15 Suffolk cross and Polled Dorset in June 2016 (early winter in Southern Hemisphere). The rams were being rotationally grazed on flat ryegrass pasture. A week prior to the discovery of four dead rams, the rams were moved to a paddock with access to horse chestnut trees *Aesculus indica*. Mildly affected rams would crouch and tremble when approached. Severely affected rams displayed neurological signs such as intention tremors and a high-stepping gait. Terminally the rams became laterally recumbent, salivated profusely, paddled and displayed dyspnoea. Over a 36-hour period 12 of the 15 rams died. On post mortem examination, the rumen contained numerous horse chestnuts. History, clinical examination, post mortem and blood sampling results excluded other potential differential diagnoses. Internationally, horse chestnuts are reported to be poisonous to ruminants [Castell *et al* 1992], however, there are no reported cases in New Zealand. All species in the genus *Aesculus* contain a saponin-class toxin called aesculin (6,7-dihydroxycoumarin 6-glucoside). Toxic levels of aesculin may fluctuate throughout the year. The young leaves and flowers are usually considered the most toxic parts, but the bark and seeds (nuts) also contain aesculin.

Clinical aspects of the experimental poisoning in cattle by the pods of *Stryphnodendron obovatum*

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The barbatimão (*Stryphnodendron obovatum* Benth) can cause abortions, digestive disorders and photosensitization in cattle. The objective was to report clinical aspects of the experimental poisoning in Nellore cattle by the pods of *Stryphnodendron obovatum*. Six Nellore calves received doses of 5g/kg for 10 consecutive days and after they were followed clinically for another 60 days. The six animals (A1, A3, A4, A5, A6, A8) showed apathy, anorexia, muscle tremors, hypersalivation and tympanism. The clinical signs of apathy and anorexia began around the fifth day after intoxication and worsened until the intoxication was over. Bovines A3, A5 and A6 had diarrhea, while in calves A1, A4 and A8 dry faeces were observed. Cattle A1, A4 and A8 showed intense muscle tremors and sialorrhoea was noticed in animals A3, A5 and A6 from the fifth to the tenth day. At the end of the intoxication, cattle A3 and A8 had ruminal hypomotility, tachycardia and tachypnea, and seven days after the end of the intoxication protocol, these calves died. The four surviving animals showed signs of apathy, reduced appetite and muscle tremors up to 50 days after intoxication, and recovery at 60 days. Thus, the photosensitization reported in the literature could not be reproduced. The experimental protocol performed induced mainly digestive disorders and could even lead to death.

Cardiac changes of cattle experimentally poisoned by *Palicourea marcgravii* and prevented with acetamide

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Acetamide (C_2H_5NO) has been tested to prevent intoxication by sodium monofluoroacetate (MFA), a toxic principle present in *Palicourea marcgravii*, the main toxic plant that causes sudden death in cattle in Brazil. The objective was to evaluate the heart rate (HR) and the serum creatine phosphokinase MB (CK-MB) concentrations of 12 male calves between 6 and 8 months of age, experimentally poisoned with 1.8g/kg *Palicourea marcgravii*, after 3 hours of administration of an acetamide dose of 1.0g/kg (T1) and 2.0g/kg (T2). Evaluations occurred at the time of administration of acetamide (reference value) and 6, 12, 18 and 24 hours after intoxication. All animals presented clinical signs regardless of the treatments, with the death of 1 animal of T1 and 2 animals of T2. The mean values of T1 (HR 65.6±6.0, CK-MB 148.5±80.1) and T2 (HR 62.8±15.0, CK-MB 129.3±43.3) were not different ($p>0.05$). Although cases of bradycardia (T1 0/6, T2 3/6) occurred, the occurrence of tachycardia (T1 4/6, T2 4/6) was more frequent, predominantly present in the dead animals, regardless of the treatment. CK-MB, an enzyme indicative of heart attack, did not show large variations in elevation (T1 1/6, T2 1/6) and decrease (T1 0/6, T2 3/6). The results suggest that the intoxication promoted cardiac alterations, mainly related to the cardiac rhythm and may be associated to the cardiotoxic effect of the MFA. Despite the presentation of symptomatology, acetamide avoided the death of 75% of the animals intoxicated by *Palicourea marcgravii*.

Arizona Livestock Incident Response Team (ALIRT)

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The Arizona Livestock Incident Response Team is a based on private practitioners, extension specialists, Arizona Diagnostic Lab, UA cooperative extension specialists in plants, water and range management. It is the mission of the ALIRT to respond to suspicious livestock deaths, massive livestock disease outbreaks, or potential threats such as Agro or Bioterrorism. This is done by consistent sample collection as well as covering costs for advanced diagnostics if needed. Through a complete work up as well as plant and range evaluation the producer gets a better idea of what management changes can be implemented to prevent the problem in the future. This presentation will go over the structure of a response and also how the ALIRT training can prepare the team for other responses.

Diagnosis of dehydropyrrolizidine alkaloid (DHPA) poisoning

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Dehydropyrrolizidine (DHPA) containing plants are found throughout the world and human, livestock and wildlife exposure is common. Poisoning is much less frequent as it is dose, alkaloid, duration, and dose accumulation dependent. Identifying poisoned animals is dependent on exposure, clinical signs and biochemistry, disease and pathologic lesions, and chemical detection of metabolites. A definitive diagnosis is dependent on a consensus of all these indicators as false positive and negative results are common and often confuse the diagnosis. This paradigm will be compared to select toxic plants and the diagnostic path required for their identification.

The search for biomarkers of facial eczema following a sporidesmin challenge in dairy cows

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Facial eczema (FE), caused by the mycotoxin sporidesmin, is a disease of ruminants that is of major economic importance to New Zealand. Because secondary photosensitisation following liver damage is the most important clinical sign, there can be serious animal welfare implications.

Currently it is difficult to diagnose subclinically affected animals and those in the early stages of the disease. The goal of this project was to apply modern analytical techniques to attempt to identify biomarkers of early FE in lactating dairy cows following the administration of a single oral dose (0.24 mg/kg) of sporidesmin. Serum was analysed using ^{1}H NMR, UPLC/ESI-MS and UPLC tandem MS/MS. Milk yields were compared between groups before and after treatment. Multivariate analyses and time series statistics were performed.

Serum metabolites differed between dosed cows before and after treatment, and samples from cows that developed clinical signs could be distinguished from all others. Prominent metabolites detected by ^{1}H NMR were a mixture of glycoproteins, carboxylic acids, ketone bodies, acetates, lipids and lipoproteins. Molecular ions detected by MS in the clinical cows were taurine- and glycine-conjugated bile acids. The milk yield of all dosed cows, including those that showed no clinical signs nor liver enzyme activity elevations (non-responders) started to decrease on Day 1, and reached a nadir (decrease of 25 - 100%) by Day 7.

It appears that the overall effects of sporidesmin on milk production in New Zealand are hugely underestimated. Further work to establish the significance of the change in metabolites is needed.

Diagnosis and surveillance of *Brassica*-associated liver disease (BALD) in cows

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"Opportunistic" research into photosensitization and liver disease in cows grazing bulb turnips (*Brassica rapa*) began when the odd affected cow was examined during late summer/fall, normally a warm and dry period, of 2002 at Massey University, Palmerston North. At this time of the year, sporidesmin toxicity (facial eczema), is the most prevalent cause of photosensitization and highly elevated serum liver enzyme (e.g. γ-glutamyl transferase, GGT) activities in cattle, sheep and alpacas. However, fast-growing turnip and rape (*B. napus* ssp. *biennis*) crops that provide high quality, readily digestible feed are valued as grazing during this time of the year in many parts of NZ. This research culminated in the publication in 2014 of, firstly, a description of this bovine disease and, secondly, an hypothesis that the liver damage in affected cows is caused by nitrile derivatives of one or more dominant glucosinolates in these brassicas. Shortly after this information became available, a widely publicised outbreak of liver disease and photosensitization in dairy cows grazing swedes/rutabaga (*B. napus* ssp. *napobrassica*) occurred during an unusually mild winter and early spring that promoted excessive growth and retention of swede leaf in Southland and Otago in NZ. The company that sells the majority of swede products in NZ undertook to fund research to better understand risk factors for brassica-associated liver disease (BALD).

Evaluation of noninvasive specimens to determine livestock exposure to teratogenic lupine spp.

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The livestock industry in the western United States loses over $500 million annually from death losses and abortions due to poisonous plants. This may be underestimated because poisonous plant-induced death losses often go undiagnosed due to a lack of appropriate or available biological specimens for analysis. Recommendations have been made to assist in collection and preparation of tissue specimens and gut contents for diagnosis of plant poisonings. However, earwax, hair, and other noninvasive specimens have been largely neglected as potential specimens for determining livestock consumption of poisonous plants. Earwax and hair from livestock in controlled dosing studies and livestock grazing lupine-infested ranges were analyzed for toxic/teratogenic lupine alkaloids by high-performance liquid chromatography-high resolution mass spectrometry (HPLC–HRMS). These noninvasive specimens may prove to be valuable tools in the assessment of livestock exposed to toxic and teratogenic lupines.

Towards understanding the aetiology of acute bovine liver disease in Australia

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Acute Bovine Liver Disease has been recognised for decades as a threat to cattle in the South East of Australia, mainly occurring in parts of Victoria and Tasmania. Its sporadic occurrence has hindered efforts to identify and control the cause even though it is associated with rough dog's tail (RDT), a grass that is usually found in 'toxic' paddocks as old, dry forage. Investigation into fungal species found on RDT at the time of harvesting has identified a few species of *Pyrenophora (*formerly *Drechslera)* as possible culprits. However, the toxin that causes ABLD and its origins are currently unconfirmed.

To help solve this, different types of pasture grass were collected during and ≥ 7 days before or after an outbreak of ABLD and analysed using LCMS/MS. No metabolites were detected exclusively in the outbreak group, but many metabolites differed significantly in their abundance between the two groups. The top 10 of those metabolites were analysed further to provide putatively identification for some from the MS^2 data. In addition, LCMS/MS and NMR profiles of liver and urine from healthy and sick cattle were compared. 19 metabolites in liver and 31 metabolites in urine were found exclusively in samples from affected cattle. 4 of those metabolites were common to both liver and urine samples. Putative molecular formulas were determined from MS^2 spectra for identification purposes. A discussion about the link between fungal species, pasture, and animal disease will be provided.

Diagnosis of selenium poisoning in livestock

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Selenium is an essential element, however it has a very narrow window between ingested amounts that cause deficiency and toxicosis. Selenosis can be either acute or chronic and commonly involves one of three types of exposure history: 1, grazing animals ingest forages containing elevated selenium concentrations subsequent to having grown on seleniferous soils; 2, toxicity from environmental contamination of water, reclaimed soils from mining, or fly ash; or 3, accidental overdoses by supplement injection or misformulation of feed selenium supplements. Toxic selenium doses vary depending upon the species, age, selenium status, and chemical form of Se ingested. Recent research has helped to better understand why and when animals consume seleniferous forages. Clinical signs of poisoning and tissue concentrations can often differ and there is not always a clear diagnosis depending upon clinical signs. Even though cases of selenium toxicosis are not as common as deficiencies they commonly occur and cause extensive financial losses on the livestock industry. Selenium toxicity has been extensively researched; however, their remains a significant amount of knowledge that needs to be discovered in order to fully understand conditions of exposure and both the beneficial roles and negative impacts of selenium.

Development of a PCR-based method for detection of *Delphinium* species in poisoned cattle

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Toxic plants such as *Delphinium* spp. (larkspur) are a significant cause of livestock losses in western North America. Correctly determining the causative agent responsible for the death of an animal, whether by disease, poisonous plant, or other means, is critical in developing strategies to prevent future losses. The objective of this study was to develop an alternative diagnostic tool to microscopy and analytical chemistry to determine whether a particular poisonous plant was ingested. Polymerase chain reaction (PCR) is a tool that may allow detection of the genetic material from a specific plant within a complex matrix such as rumen contents. A pair of oligonucleotide primers specific to *Delphinium* spp. was developed; using these primers, a PCR product was detected in samples from an *in vivo*, *in vitro*, and *in vivo/in vitro* coupled digestion of *Delphinium occidentale*. Lastly, larkspur was detected in a matrix of ruminal material where the amount of larkspur was far less than what one would expect to find in the rumen contents of a poisoned animal. The PCR-based technique holds promise to diagnose larkspur and perhaps other toxic plant-caused losses.

Ipomoea asarifolia and *Ipomoea muelleri*: investigation of tremorgenic indole diterpenes

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Ipomoea asarifolia and *Ipomoea muelleri* have been associated with a tremorgenic syndrome in livestock in Brazil and Australia, respectively. *I. asarifolia* and *I. muelleri* were investigated by high-performance liquid chromatography–high-resolution mass spectrometry (HPLC–HRMS) and high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) for indole diterpene composition. Indole diterpenes were detected in both species. Terpendole K and the three new indole diterpenes were isolated. The structures of the new indole diterpenes were determined by NMR spectroscopy and given the names of 11-hydroxy-12,13-epoxyterpendole K, 6,7-dehydroterpendole A, 6,7-dehyro-11-hydroxy-12,13-epoxy-terpendole A. The tremorgenic activity of terpendole K and the new indole diterpenes were evaluated in a mouse model.

Oral toxicity of progoitrin-derived nitriles in rats and rabbits

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Liver disease and secondary photosensitisation in cattle grazing brassica forage crops, especially turnips (*B. rapa*), was described in 2014. Later in the same year in New Zealand, there was a large, unprecedented outbreak of liver disease, photosensitisation, loss of production, and mortality in dairy cows grazing swedes (rutabaga, *B. napus* ssp. *Napobrassica*). The cause of this brassica-associated liver disease (BALD) is unknown, but it has been hypothesised that glucosinolate-derived nitriles could be to blame.

Each brassica variety contains 20 or more different glucosinolate (GSL) compounds in varying ratios and concentrations. We hypothesised that the conditions in the rumen of hungry, pregnant or lactating cows favour the formation of daughter nitriles/epithionitriles from ingested GSLs. Progoitrin has been identified as the dominant GSL in swedes, turnips and forage rape (*B. napus* spp. *biennis*). For progoitrin, the secondary compounds of interest are 3-hydroxy-4-pentenenitrile (crambene) and the epithionitrile, ?-hydroxy-thiiranepropanenitrile).

The serum biochemical and pathological responses to single or multiple consecutive daily oral doses of either crambene, the epithionitrile, or both, were studied in rats and rabbits. In rats, crambene at 1 mmol/kg caused severe pancreatic apoptosis without clinical toxicity, while higher doses were hepatotoxic and more lethal. Doses of the epithionitrile were nephrotoxic and hepatotoxic and higher doses were lethal too. In rabbits, the two compounds were more toxic than in rats, and the effect of crambene on the pancreas was minimal. For rats the subtoxic dose of the two compounds was estimated to be 1 mmol/kg while that for rabbits was lower.

Quantitation of phalaris alkaloids throughout a growing season in Victoria, Australia

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Phalaris aquatica is a summer dormant pasture grass that has significant importance in livestock pasture systems. During growth seasons it provides high quality high biomass forage for livestock that is tolerant to water logging and heavy grazing. During dormancy (summer) it can persist throughout drought due to its deep root system and dormant bulbs. However, phalaris has been implicated in a poisoning of sheep and cattle in Australia. The phalaris toxicities commonly observed are: sudden death neurological (SDN) and staggers. Fatally toxic levels of ammonia have been reported in cases of SDN but the toxin that causes hyperammonia has not been identified. Similarly, the staggers type syndrome is presumed to be caused by several different alkaloids. However, the abundance of these compounds in pasture has not been thoroughly investigated.

This project investigated the changes in abundance of five potentially toxic alkaloids in leaves and stems of phalaris grass over a growing season in Victoria, Australia. The concentration of toxins was then compared to the concentration detected in a few samples collected during outbreaks of toxicity in sheep in the same region. Pasture samples were collected every two weeks from February to June in 2016. The quantities of norharmane, hordenine, gramine, DMT, and 5-MeO DMT were investigated in each sample using HPLC-MS. Only minimal amounts of norharmane (<0.3 ug/g) was found, while hordenine and gramine were more abundant, and DMT and 5-MeO DMT were the most abundant. Details of alkaloid variation and the implications of these finding for graziers will be discussed.

Is tremetone really the toxin in white snakeroot and rayless goldenrod that is responsible for causing milk sickness?

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White snakeroot (*Ageratina altissima*) and *Isocoma* spp. contains the putative toxin tremetone and produces a disease called "trembles" or "milk sickness". However the toxicity of tremetone has not been demonstrated *in vivo*. Data from several studies have failed to confirm that tremetone is the singular toxin in white snakeroot and *Isocoma* spp. and have raised the possibility that there are potentially other toxin(s) that either are directly responsible or act synergistically with tremetone to cause trembles and milk sickness. Evidence for this hypothesis was obtained through several studies of dosing many different collections of dried, ground plant material by oral gavage to Spanish goats. These studies included; 1) *Isocoma tenuisecta* that contained 4 to 20 times more tremetone than other toxic plant populations yet, did not produce disease, 2) when different plant populations were dosed there was no correlation between tremetone concentrations and toxicity of plant, 3) white snakeroot that had been dried and stored for five years remained as toxic as fresh plant from the same collection even though over 80% of the tremetone had been lost from the plant during storage, 4) extracts from white snakeroot containing molar equivalents of tremetone, as fresh ground plant, did not produce disease, and 5) nursing neonates became poisoned even though tremetone was not detected in the milk. These studies demonstrate the need to definitively identify the toxin in white snakeroot and *Isocoma* spp. so that risk assessments of rangelands and pastures where these plants grow can be determined.

Asphodel exposure of sheep in Algeria

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Asphodel or Common onion weed (*Asphodelus tenuifolius* Cav., family Asphodelaceae) is native to southern Europe, northern Africa and western Asia. It has been naturalised in Australia, New Zealand, tropical Asia, south western United States of America, southern parts of south America and South Africa. In the northern part of the algerian Sahara desert near Laghouat a flock of 600 mixed ages sheep was grazing partially cleared bushes. 50 animals were observed with nervous symptoms of staggering , incoordination, ataxia, paralysis and falling down when stimulated to move. They remained weak and recumbent in the sitting dog position though they were responsive and lively. All affected animals died within three months. The abundant presence of Common onion weed in the area and the clinical similarity with the *Trachyandra divaricata* (Jacq.)Kunth (Branched onion weed, Asphodelaceae) intoxication led us suspect a toxic encephalopathy due to this plant. Necropsy and brain histology were performed. Severe lung lesions were suspected to be due to repeated false route. Brain histology revealed lipofuscine inclusions in the neurons : golden brown bodies with haemalun-eosin staining, positive Periodic Acid Schiff staining, negative Ziehl staining and a self fluorescent dye. As lipofuscin accumulation is the consequence of oxydizing processes an antioxidant treatment (vitamins E and C, Selenium) could be tried but was not set up for economic reasons. Ataxia in sheep is well known by desert nomad shepherds who observe sporadic cases during dry winters. 4000 sheep out of 20 flocks were exposed in 2017. More than 1000 sheep were euthanized. *Asphodelus tenuifolius* Cav. poisoning looks amazingly like *Trachyandra divaricata* (Jacq.)Kunth poisoning.

Metabolic profiling of cytotoxic steroidal saponins in five Australian *Panicum* species

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Up to 500 species of *Panicum* (panic grasses) are recognised worldwide, of which 24 species are indigenous to Australia and nine introduced. Recently, some of the introduced summer-growing annual *Panicum* spp. have been identified with high prevalence across southern Australia. Certain *Panicum* species have also been implicated in severe outbreaks of hepatogenous photosensitization in livestock, characterized by crystal-associated cholangiohepatopathy. The causal compound(s) responsible for liver dysfunction in livestock ingesting *Panicum* spp. have been previously identified as steroidal saponins. Although metabolic profiling of *Panicum* spp. has been undertaken in the USA and NZ, the saponins implicated in toxic outbreaks in Australia have not yet been identified. In this study, fresh shoot tissue from three introduced (*P. capillare*, *P. hillmanii* and *P. gilvum*) and two native (*P. decompositum* and *P. effusum*) species were extracted in methanol and analysed by UPLC/QTOF mass spectrometry. The relative abundance of key steroidal saponins was determined based on specific molecular features including retention time, mass spectra and fragmentation pattern when compared to saponin analytical standards. Distinct qualitative and quantitative differences were observed in the saponin profiles of the *Panicum* species evaluated, including in the type, number and abundance of the saponins detected, such as protodioscin and pseudoprotodioscin. *In vitro* cytotoxicity assays of shoot extracts collected from *Panicum*-related photosensitization outbreaks will be performed to further identify key steroidal saponins and saponin profiles associated with hepatogenous photosensitization in grazing livestock.

Investigating photosensitisation in livestock resulting from ingestion of the pasture legume *Biserrula pelecinus L.*

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Biserrula pelecinus L. is an annual legume native to the southern Mediterranean. It was first introduced to Australia in 1991 as a potentially valuable rotational pasture species for livestock production. It produces large quantities of biomass, exhibits drought tolerance and is effective for weed suppression in pasture rotations. However, despite proving to be a valuable addition to the pasture toolbox, producers in NSW and WA have reported a limiting factor to uptake: incidence of severe photosensitization when grazing sheep on biserrula pastures. Biserrula photosensitivity, anecdotally, appears to be associated with non-senescent foliage and shows an increased severity of clinical signs in young animals grazing green tissues; however, the pathogenesis of this photosensitization and the metabolite(s) responsible are, as yet, unknown. Studies reported in this project have identified that both commercially available cultivars of biserrula, 'Casbah' and 'Mauro' can cause outbreaks of primary photosensitization. This work identified that fresh foliar extracts were photosensitizing, and that this activity diminished greatly with drying. Both cultivars were found to be equally bioactive, and photocytotoxic activity was associated with extracts from field-grown biserrula at all stages of plant growth until senescence. Biochemical analysis using fractionated extracts, bioactivity-guided metabolic profiling is described as an experimental approach.

The effect of drying temperature on the tremorgenicity of *Ipomoea asarifolia* in mice

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Several *Ipomoea* species, including *Ipomoea asarifolia,* have been shown to cause a tremorgenic syndrome in cattle, goats, and sheep. The prominent clinical signs observed in these animals included muscle tremors. Recent work suggests that the tremorgenic compounds in *Ipomoea* species are indole diterpenes. In order to further characterize and identify the individual tremorgenic compounds in the various *Ipomoea* species, the development of a rodent model is needed. Initial studies in our laboratory were conducted using plant material that was dried at 40^{o}C and dosed orally to mice via their chow. Adult mice dosed with plant processed in this manner showed no tremors, even when the plant constituted 40% of their diet. Subsequent chemical analyses of the plant material discovered that drying at 40^{o}C resulted in a significant decrease in the amount of indole diterpenes compared to plant material that was freeze dried. Consequently studies were performed again using plant material that was freeze dried. Using plant material processed in this manner, mice demonstrated noticeable tremors, even with doses as low as 10% of the diet. This work demonstrated the heat labile nature of the indole diterpenes and the importance of properly processing plant material for toxicology studies.