CITY OF BOULDER
CITY COUNCIL AGENDA ITEM

MEETING DATE: January 15, 2019

AGENDA TITLE
Consideration of a motion to accept the December 11, 2018 Study Session Summary for the Discussion on Prairie Dog Working Group Phase 2 Recommendations

PRESENTERS
Jane S. Brautigam, City Manager
Yvette Bowden, Director of Parks and Recreation (PR)
Dan Burke, Interim Director of Open Space and Mountain Parks (OSMP)
Chris Meschuk, Interim Director of Planning,
Steve Armstead, Interim Deputy Director, OSMP
Keri Konold, Community Relations Officer, OSMP
John Potter, Resource and Stewardship Manager, OSMP
Joy Master, Natural Lands Program Coordinator, PR
Val Matheson, Senior Urban Wildlife Coordinator, Planning
Andy Pelster, Agricultural Stewardship Supervisor, OSMP
Heather Swanson, Senior Wildlife Ecologist, OSMP
Pat Comer, Prairie Dog Working Group Member
Dan Brandemuehl, Prairie Dog Working Group Member
Carse Pustmueller, Prairie Dog Working Group Member

EXECUTIVE SUMMARY

This agenda item provides a summary of the December 11, 2018 City Council Study Session on the work of the Prairie Dog Working Group. This summary marks the end of the Prairie Dog Working Group efforts and staff will move forward will further analysis of the Working Group's Phase 2 recommendations, including analysis of funding and staffing requirements; plan and policy change process needs; and trade-offs with other city priorities such as:

- Ecological (other species and communities)
- Sustainable Agriculture
- Parks Development.

STAFF RECOMMENDATION

Suggested Motion Language:

Staff requests council consideration of this matter and action in the form of the following motion:

Motion to accept the December 11, 2018 Study Session Summary for the Discussion on Prairie Dog Working Group Phase 2 Recommendations

BACKGROUND

Background information can be found in the December 11, 2018 Study Session Memorandum and materials found here:

https://bouldercolorado.gov/city-council/council-documents

ADDITIONAL INFORMATION

Though the PDWG did discuss but did not recommend the use of Delta Dust on prairie dog relocation receiving sites, some council members suggested it's use. Attachment B provides additional information on Delta Dust that was provided by the city's IPM Coordinator.

NEXT STEPS

Staff is scheduled to return to council on May 7, 2019 with additional information on how to operationalize Prairie Dog Working Group Phase 2 recommendation.

ATTACHMENTS

- Attachment A: Summary of the December 11, 2018 Prairie Dog Working Group study session
- Attachment B: Considerations for the use of Deltamethrin (Delta Dust) for plague management in prairie dog colonies and preliminary literature review for assessment of Deltamethrine.

Dec.11, 2018 Study Session Summary
Prairie Dog Working Group (PDWG) Recommendations

PRESENT:
City Council: Mayor Suzanne Jones, Council Members Bob Yates, Cindy Carlisle, Jill Adler Grano, Lisa Morzel, Mirabai Kuk Nagle, and Sam Weaver.

Staff Presenters: Val Matheson, Urban Wildlife Conservation Coordinator (P); Heather Swanson, Senior Wildlife Ecologist (OSMP); Andy Pelster, Agricultural Stewardship Supervisor (OSMP).

Working Group Member Presenters: Pat Comer, Dan Brandemuehl, Carse Pustmueller

OVERVIEW:
The study session provided the opportunity for staff and PDWG community members to present the final prairie dog management recommendations, initial implementation and analysis, and proposed future analysis to council. The study session was facilitated by Heather Bergman of Peak Facilitation and provided council members the opportunity provide feedback on shaping future analysis.

SUMMARY OF PRESENTATION:
Staff and community working group members presented the working group final products and distinguished between which management recommendations could be implemented now or in the near term, and which recommendations would require more analysis on tradeoffs, and staffing and funding impacts prior to operationalizing the recommendations. The presentation included:

- PDWG background and selection process
- Phase 1 Recommendations and implementation update
- Phase 2 Recommendations
    - Initial Staff Analysis
    - Board Feedback
    - Initial Implementation
    - Future Analysis/Next Steps

DISCUSSION SUMMARY
<u>PDWG Phase 1 prairie dog management recommendations: clarifying questions</u>

- **Why is there a backlog of prairie dogs needing to be relocated?**
  The city (OSMP, PW, PR) has approximately 900 acres designated for removal due to many years without conducting relocations, a lack of receiving site availability, feasibility of what can get done in a year and very high populations currently.
- **What is the survival rate of prairie dogs being relocated to the Southern Grasslands?**
  Damyonavich has had high survival, Waneka did not with most of the relocated colony dying about a year after relocation to suspected plague (not confirmed). Delta Dust is applied on the sending site and each prairie dog is sprayed with insecticide prior to

release to prevent flea transmission to the new site, but receiving sites are not treated with Delta Dust (but did receive plague vaccine in 2018).

- **What is the prairie dog carrying capacity of the Southern Grasslands?**
  Hard to determine carrying capacity but it is likely prairie dogs have historically already chosen best spots for them. The map in the council packet shows the olive color where prairie dogs have been in the past and it may be good receiving sites, but the vegetation needs to be analyzed on an annual basis. Grassland Plan includes target occupation of 10-26% of Grassland Preserves.
- **Is it now possible to relocate into areas that haven't had prairie dogs before?**
  The Southern Grasslands has had more than 2% occupation in the past so there is already capacity there to relocate into areas that have had prairie dogs before. It is not possible under the Grassland Plan to relocate to areas in the Southern Grasslands that have not had prairie dogs before.

<u>PDWG Phase 2 prairie dog management recommendations</u>

Irrigated Agriculture

Several council members had questions about irrigated agriculture and conflicts with prairie dogs. The method of key-lining was explained as one of the techniques to restore degraded agriculture fields. Staff described a property where the process of key lining, and reseeding with compost was effective in reclaiming and revegetating an area that had become degraded due to a variety of factors including overgrazing by cattle and prairie dogs. There is approximately 7,000 acres of irrigated agricultural lands owned by the Open Space and Mountain Parks department of which about 15% is impacted by prairie dogs.

Grassland Conservation Fund

Clarity around the intent of the grassland conservation dedicated fund was discussed and described as a recommendation to bring in resources from multiple sources though not defined as part of the PDWG process.

Future Analysis

Some council members requested expedited analysis of developing a Conservation Fund, and a Plague Management Plan. In addition, identifying timeline and plan for addressing the 950 acres of irrigated agriculture that is inhabited by prairie dogs; timeline and tradeoffs of barrier installation to mitigate conflicts: how that would be phased; an analysis of approaches to creation of the grassland conservation fund; ensure our IMP specialists are involved in the analysis of the use of Delta Dust and it's impact on other species.

<u>Additional Comments</u>

Some council members made prairie dog management suggestions that were outside of the scope of the PDWG recommendations. They included:

- an accelerated strategic recovery plan for the 960 acres of degraded agricultural fields;
- the use of Delta Dust on Southern Grasslands, or in conjunction with relocations on receiving site;

- the use of key-lining as a technique for managing prairie dog colonies; and
- leverage the expertise of willing community members to continue the work involved with analyzing and implementing the PDWG Phase 2 prairie dog management recommendations.

City of Boulder Integrated Pest Management

Considerations for the Use of Deltamethrin (Delta Dust) for Plague Management in Prairie Dogs

What is it? Deltamethrin belongs to the pyrethroid family of insecticides. It can enter the body through ingestion, absorption through the skin or mucous membranes of sensory organs and via inhalation. Deltamethrin acts as a neurotoxin and affects the central nervous system through sodium channel disruption of the nerve cells. It has a rapid knockdown effect in insects and death is thought to be due to irreversible damage to the nervous system (Rehman et al., 2014).

How is it used? Delta Dust, a formulation of deltamethrin, is currently used by the city prior to prairie dog relocations at sending sites to kill fleas in burrows that could potentially carry sylvatic plague (*Yersinia pestis*) to new receiving sites. This treatment is required by Colorado Parks and Wildlife permitting for prairie dog relocation. Approximately four to five grams is applied to each prairie dog burrow to kill fleas.

Potential issues: Deltamethrin is a broad-spectrum neurotoxin that can harm non-target species. Widespread use has also resulted in resistance in multiple insect species, including fleas. A recent study showed that resistance can develop within five to six years of annual treatments. In addition, treatment with deltamethrin does not guarantee that plague will not kill the majority of a colony. In a new study, 95% of a prairie dog colony died after deltamethrin was applied for three years.

Background:

Successful integrated pest management relies on as much knowledge as possible about the pest, the ecosystem and the interactions of different organisms within it. Therefore, it's important to gain as thorough of an understanding as possible of the complexity of the plague cycle and how different plague management tools could impact the overall ecosystem as well as plague dynamics.

Deltamethrin is applied to reduce transmission of the bacterium, *Yersinia pestis* or sylvatic plague, by controlling fleas, which can transmit the pathogen. Not all fleas are infected with plague and other transmission pathways exist that do not involve flea transmission (Richgels et al, 2016). However, higher flea populations in prairie dogs are associated with plague epizootics and application of deltamethrin has been shown to improve prairie dog survival (Biggins et al., 2010). Although infected fleas are an important factor for plague transmission, a model predicted that flea transmission is not the driver for epizootics and that a short-term reservoir, including other mammals, infected prairie dog carcasses, fleas or a combination are all possible reservoirs for epizootics (Webb et al., 2006). A later study/model found that higher northern grasshopper mouse populations harboring prairie dog fleas are associated with prairie dog plague die-offs the following year (Salkeld et al, 2010), underscoring the complexity of the transmission cycle.

It is assumed that sylvatic plague is endemic/enzootic (continuously present) within Boulder's grasslands system. Animals that experience enzootic plague exposure have been shown to develop natural immunity, although this

varies depending on the species, the genetics of the individual animal, climate and other factors (Gage and Kosoy, 2005; Ben Ari et al, 2011). Studies indicate that natural immunity appears to be developing in prairie dogs in areas of enzootic plague exposure. Black-tailed prairie dogs from Colorado, Texas and South Dakota were challenged by injection with *Y. pestis*—at the time of the study, South Dakota was plague-free. Nearly all of the prairie dogs from South Dakota died from the plague injection, but overall survival rates of prairie dogs from Colorado and Texas, where plague is endemic, were 50-60% (Rocke et al., 2011). A study of Gunnison's prairie dogs in Arizona compared a colony that had survived plague epizootics to a neighboring colony that had been severely affected by plague. When injected with lethal doses of *Y. pestis*, animals from both colonies survived—70% from the colony that had done better under epizootics and 60% from the colony that had been more affected (Busch et al., 2013). The animals in both of these studies had a single injection, whereas animals in the wild may have multiple exposures to *Y. pestis*. However, these studies do suggest that prairie dogs with enzootic plague are developing some level of natural immunity.

Integrated Pest Management (IPM) Objective:

The city's IPM policy "uses a 'whole systems approach,' looking at the target species as it relates to the entire ecosystem." Management options or a combination of options are assessed for the best management of the pest, while decreasing potential impacts to the environment, non-target species and overall ecosystem function, following the IPM process that requires the least impactful options are first considered with pesticide use as the last resort. When a pesticide is considered, the least toxic pesticide is applied in a manner that targets the pest most effectively, while limiting exposure to other species and the environment.

Deltamethrin Risk Assessment:

A preliminary risk assessment of deltamethrin, including abstracts of scientific publications can be found here. This document provides information about the potential environmental impacts of Delta Dust. Insects are in precipitous decline around the world, placing entire ecosystems at risk. Beneficial insects are potentially impacted by the use of deltamethrin on Boulder's open space. Boulder County is home to over 550 species of bees alone, of which 70% are solitary bees—the majority of which are ground-nesting. Many of these species nest in the ground around prairie dog colonies (personal communication, Virginia Scott, University of Colorado, Boulder). Many beetles and other invertebrates inhabit the areas in and around prairie dog burrows. Several of these species have disappeared or at risk. Therefore, any use of a broad-spectrum insecticide in natural areas must be carefully considered and weighed against other options.

Preliminary Literature Review for Assessment of Deltamethrin

Background

Deltamethin belongs to the pyrethroid family (type II) of insecticides. It can enter the body through ingestion, absorption through the skin or mucous membranes of sensory organs or via inhalation. Deltamethrin acts as a neurotoxin and affects the central nervous system through sodium channel disruption of the nerve cells. It has a rapid knockdown effect in insects. Due to its widespread use, resistance has already been seen in multiple insect species, including fleas.

The European Union's format for regulatory information is well-organized and includes interpretation of deltamethrin's relative toxicity and environmental fate and is provided for reference.

The Literature

In most pesticide assessments, including agency/regulatory assessments, representative organisms are used in a range of well-defined tests that are most often conducted by the pesticide registration applicant. The data are used to extrapolate the potential effects for other species in a variety of exposure scenarios. Since other species may or may not respond differently than the test subject organisms, assessments are limited by the available data and by the testing protocol. Most of these studies are focused on acute toxicity, where test subjects are exposed to the pesticide active ingredient through oral, dermal or inhalation exposures. Some tests may look at chronic exposures of individuals or multi-generational studies. Independent studies may show similar results in different species and reach similar conclusions using different study protocols or they may arrive at different conclusions than the regulatory studies.

A gap in the regulatory testing that independent literature may address is sublethal exposures that can affect a wide range of behavioral, physiological and learning performance measures. These impacts can affect the individual, population or ecosystem and therefore, consideration of sublethal effects is important for understanding how a pesticide could potentially affect an ecosystem. Some studies measure indirect impacts of pesticides, where a predator may be affected by the effect the pesticide has on its prey. When a thorough assessment is conducted, all of this information must be considered as a whole to attempt to understand how the use of a pesticide may affect human and environmental health. For prairie dog plague management, most studies with deltamethrin are aimed at efficacy for flea reduction and prairie dog survival. There are a few studies in prairie dog colonies that measure non-target impacts.

The majority of the literature that was found in this preliminary search examines sublethal effects. A brief overview is provided under each heading with a corresponding appendix, which contains abstracts of the referenced studies (please disregard codes, which are for staff use to locate copies of the original studies). Each publication is also linked to a source on the internet. The full text of many of these papers is copyright protected. The city has copies of each publication that is referenced. When the text is freely available, the full studies can be obtained through the links. When the text is not available online, paper copies of the publications will be provided upon request.

This review is a work in progress and is incomplete. If new uses of deltamethrin are proposed for city properties, this information will be used, along with an analysis of regulatory data, to prepare an ecological risk assessment.

Direct Effects - Vertebrates

I. Human Studies

Non-human animals are generally used to estimate human health effects (see studies for non-human vertebrate studies below), but when pesticides are in wide use, researchers can correlate health issues to either known exposure or with body load – often measured as urinary or blood metabolites. Pyrethroids are frequently found in human urine, though deltamethrin is not as commonly found as other pyrethroid insecticides. Children tend to have higher amounts of urinary metabolites than adults, which have been correlated with memory and learning issues. Studies in Chinese men showed a correlation with pyrethroid metabolites in urine and alteration in reproductive hormones, semen quality and sperm DNA.
(See Appendix A for study abstracts and links to original papers).

II. Non-human studies- Physiological Effects

A. Effect on Nervous System

Studies show a range of effects from behavioral changes to neurotransmitter alteration in the brain and nerve cell degeneration at low doses that can be persistent and impact generations. One study measured dermal exposure to mimic occupational exposure in humans. Results showed brain injury and changes to sensitivity of dopamine transport in the brain. Another study showed that prenatal exposure in rats impacted the brains and livers of offspring. Low exposure to zebrafish embryos also affected dopamine function and affected locomotor skills.
(See Appendix B for study abstracts and links to original papers).

B. Effect on Immune System

The first paper (full text available) in this section reviews the various pathways affected by deltamethrin that result in immunosuppression. Another study showed that deltamethrin-treated mice had increased infection with yeast. Deltamethrin also alters antioxidant enzymes in mice, which can lead to oxidative stress – one way that that immune system can be compromised.
(See Appendix C for study abstracts and links to original papers).

C. Effect on Reproduction, Development and Endocrine Disruption

Deltamethrin exposure reduces the weight of liver, kidney and testes in rats. As seen in human studies, it also alters reproductive hormones in male rats. It decreases sperm count and motility. A review paper shows that pyrethroids bioaccumlate in fish and act as endocrine disruptors in fish and mammals. Pyrethroid metabolites can have more endocrine disruption activity than the parent compounds. They also interfere with immune response. Deltamethrin may also increase thyroid hormones. Prenatal exposure to deltamethrin in rats produced multiple birth defects in offspring, as well as necrosis of the brain, liver and intestine. Chicken eggs exposed to deltamethrin had higher mortality, significant decrease in body weight and skeletal malformations.
(See Appendix D for study abstracts and links to original papers).

D. Effects on DNA and Carcinogenicity

Low doses of deltamethrin caused DNA damage in a particular test – the “commet assay.” Tests in root cells showed that deltamethrin binds with DNA and causes chromosome damage. Long-term dermal exposure of deltamethrin in mice causes benign skin tumors. It did not show other tumor-promoting activity.
(See Appendix E for study abstracts and links to original papers).

E. Toxicity to Non-Targets

Pyrethroids are considered to be low toxicity to birds. However, registration testing is conducted on two representative species – bobwhite quail and mallard duck. A study with the pyrethroid beta-cyfluthrin is

included, because it tests acute toxicity in three bird species and shows high toxicity in one, indicating that some species of bird may be significantly more sensitive to pyrethroids than registration testing may indicate. (See Appendix F for study abstracts and links to original papers).

Direct Effects – Invertebrates

Insecticides are developed to control insects, so non-target invertebrate species are particularly vulnerable. Although invertebrates may recolonize an area after initial mortality from acute toxicity to a pesticide, the results of sublethal effects to behavior and physiology can be more difficult to assess, but can be important for ecosystem function.

Deltamethrin affects the ability of spiders to escape from predators by reducing walking speed making them more likely to be eaten by carabid beetles. The density of three species of biological control insects (important predators) was reduced by 100% in deltamethrin treatments and did not begin to increase until 21 days after spraying. Deltamethrin reduced egg production in honeybees, lengthened the time spent as larvae and decreased the rate they developed to adults. In a species of beneficial wasp (lays wasp eggs in pest insect eggs), sublethal doses of deltamethrin altered sex pheromone communication and altered the ability of females to choose hosts and lay eggs. In lab studies, earthworm survival was affected with increasing dose of deltamethrin and showed more toxicity in soil conditions, indicating that deltamethrin metabolites may be more toxic to earthworms than the parent compound.
(See Appendix G for study abstracts and links to original papers).

Indirect Effects

Mountain plovers rely on prairie dog colonies for nesting sites. Links to some background papers are included in the appendix. A long-term study (7 years) compared mountain plover nest survival in prairie dog colonies treated with deltamethrin compared to colonies with no history of deltamethrin and found a strong negative correlation for nest survival in deltamethrin-treated colonies. The researcher concludes that lower nest survival was due to food availability from fewer insects in treated colonies. A master's thesis looked at deltamethrin effects on arthropods in prairie dog burrows in a 2 year study using pitfall traps and examining feces of deer mice that feed on arthropods. A decline was seen in deer mice during the second year, though effects seemed minimal. Beetles had reduced numbers before the second year of treatment, but returned to a similar number after treatment. A study where deltamethrin was sprayed to control grasshoppers in grasslands measured diet and reproductive success of chestnut-collared longspurs – grasshoppers make up approximately 85% biomass of nestling diet. Spraying reduced grasshoppers by 93%, but parent birds switched to other food sources. Clutch size and nestling survival were similar, but egg success was lower in sprayed plots.
(See Appendix H for study abstracts and links to original papers).

Appendix A – Deltamethrin and Pyrethroid Human Studies

(H2) Effects of non-occupational environmental exposure to pyrethroids on semen quality and sperm DNA integrity in Chinese men

Abstract: Observations in several western and Asiatic countries point toward a decline in semen quality which may be associated with environmental exposures. To investigate the effect of environmental exposure to pyrethroids on sperm DNA integrity and semen quality, 240 men were recruited from an infertility clinic through the clinic following strict eligibility screening. Urinary 3-phenoxybenzoic acid (3-PBA) concentration, semen quality, and sperm DNA integrity were evaluated. After adjustment for potential confounders, a significant inverse correlation was observed between the urinary 3-PBA level and the sperm concentration (β = −0.27, 95%CI: −0.41 to −0.12, P < 0.001). Moreover, we also found a significant positive correlation between urinary 3-PBA level and sperm DNA fragmentation (β = 0.27, 95%CI: 0.15–0.39, P < 0.001). Our results suggest that non-occupational environmental pyrethroids exposure may have a negative impact on sperm DNA integrity and semen quality in Chinese males.

(H5) The relationship of 3-PBA pyrethroids metabolite and male reproductive hormones among non-occupational exposure males

Abstract: Many pesticides possess hormonal activities and have been classified as endocrine disrupting chemicals. Synthetic pyrethroids are one kind of the most common pesticides used in the world. In the present study, we explored the association between serum reproductive hormone levels and urinary creatine (CR) adjusted concentration of 3-phenoxybenzoic acid (3-PBA), a general metabolite of pyrethroids, in Chinese adult men. The study subjects (n = 212) were from the affiliated hospitals of Nanjing Medical University. By using GC–MS, urinary 3-PBA level of each subject was measured and adjusted by urinary CR. Blood samples were collected for measuring the serum levels of reproductive hormones, including follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), testosterone (T) and prolactin (PRL). All the subjects had detectable levels of 3-PBA in their urine samples. The median concentration of 3-PBA was 0.815 μg $g^{-1}$of CR. The results showed that there was positive associations between the levels of serum LH and 3-PBA (p = 0.013) but negative associations between E2 and 3-PBA level (p = 0.022), and the adjusting p-value was 0.044 for LH and E2, which suggested that pyrethroids are capable of disrupting the male endocrine function. In adult men, urinary 3-PBA levels were associated with increased LH and reduced E2 levels. On a population level, these reductions show potential public health importance because of widespread exposure to these pesticides.

(H7) Urinary Concentrations of Metabolites of Pyrethroid Insecticides in the General U.S. Population: National Health and Nutrition Examination Survey 1999–2002 (full text)

Abstract: Background: Pyrethroid insecticides are the most commonly used residential insecticides in the United States. Objectives: Our objective was to assess human exposure via biomonitoring to pyrethroid insecticides in a representative sample of the general U.S. population ≥ 6 years of age. Methods: By using isotope-dilution high-performance liquid chromatography/electrospray chemical ionization/tandem mass spectrometry, we measured five urinary metabolites of pyrethroid insecticides in 5,046 samples collected as a part of the 1999–2002 National Health and Nutrition Examination Survey (NHANES). Univariate, multivariate, and Pearson correlation analyses were performed using SUDAAN and SAS software, incorporating the appropriate sample weights into the analyses. Multivariate analyses included age, sex, race/ethnicity, creatinine, fasting status, and urine collection time as covariates. Results: We detected 3-phenoxybenzoic acid (3PBA), a metabolite common to many pyrethroid insecticides, in more than 70% of the samples. The least-squares geometric mean (LSGM) concentration (corrected for covariates) of 3PBA and the frequency of detection increased from 1999–2000 (0.292 ng/mL) to 2001–2002 (0.318 ng/mL) but not significantly. Non-Hispanic blacks had significantly higher LSGM 3PBA concentrations than did non-Hispanic whites and Mexican Americans in the 2001–2002 survey period and in the combined 4-year survey periods but not in the 1999–2000 survey period. Children had significantly higher LSGM concentrations of 3PBA than did adolescents in both NHANES periods and than adults in NHANES 1999–2000. Cis- and

trans-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid were highly correlated with each other and with 3PBA, suggesting that urinary 3PBA was derived primarily from exposure to permethrin, cypermethrin, or their degradates. Conclusions: Pyrethroid insecticide exposure in the U.S. population is widespread, and the presence of its metabolites in the urine of U.S. residents indicates that children may have higher exposures than adolescents and adults.

(H4) Pyrethroids: Exposure and health effects – An update

Abstract: Synthetic pyrethroids are present in numerous commercial insecticide formulations and have extensive indoor and outdoor applications worldwide, including agricultural, public, residential, and veterinary usages for pest control. Pyrethroid use has increased continuously in recent years. The aim of this review is to provide updated and comprehensive information on human exposure and potential hazards associated with this class of pesticides. An initial keyword search in the PubMed database was conducted to identify relevant articles. Were taken into considerations only the studies published in the last decade that have assessed exposure and health effects of pyrethroids in human populations. Literature review shows that exposure evaluations increasingly focus on biomonitoring and that a large number of recent epidemiological studies pertain to the effects of pyrethroids on male fertility and prenatal development.

The main metabolites of pyrethroids have frequently been detected in urine samples from the general population, confirming widespread exposure of children and adults to one or more pyrethroids. Non-occupational exposure to pyrethroids mainly occurs through ingestion of residues in food, or ingestion of or dermal contact with contaminated house dust or surface-adhering particles, following domestic use. Although clinical features resulting from acute accidental exposure to pyrethroids are well described (e.g., paraesthesiae, and respiratory, eye and skin irritation), information regarding their chronic effects at low concentrations is both limited and controversial. Several recent epidemiological studies have raised concerns about potentially adverse effects on sperm quality and sperm DNA, reproductive hormones, and pregnancy outcomes. Early neurobehavioural development after in utero exposure is discussed. Further research is needed to clarify the possible risks associated with long-term environmental exposure to pyrethroids.

(H8) Urinary Metabolites of Organophosphate and Pyrethroid Pesticides and Neurobehavioral Effects in Chinese Children

Abstract: Organophosphate (OP) and pyrethroid (PYR) pesticides are widely used in China. However, few studies have investigated the neurobehavioral outcomes of Chinese children exposed to low levels of OP and PYR. We investigated urinary metabolite levels and their association with exposure characteristics and the neurobehavior of children. For all children, biomarker measurements were made in the same interval relative to neurobehavioral testing. We analyzed the morning urine samples of 406 children aged 3–6 years from Nanjing, China. The Kruskal–Wallis and Wilcoxon rank sum tests were used to identify the associations between urinary metabolite levels and exposure characteristics. Multiple linear regression models were used to test the associations between urinary metabolite levels and neurobehavioral test scores after adjusting for covariates (e.g., sex, age, and education expense). The detection of 3,5,6-trichloropyridinol (TCP) and 3-phenoxybenzoic acid (3-PBA) in the urine was positively associated with living areas adjacent to agricultural fields and using indoor mosquito repellent incense. These two metabolites were negatively associated with the soaking time of fruits and vegetables. When treated as dichotomous variables, TCP was significantly associated with arithmetic test scores in adjusted models, and 3-PBA was significantly associated with the scores on the Chinese Binet and arithmetic tests. When treated as a continuous variable, higher urinary 3-PBA levels were significantly associated with lower cancellation test scores. Our findings suggest that exposure to organophosphate and pyrethroid pesticides may have a significant impact on children's working memory and verbal comprehension.

Appendix B – Deltamethrin Effects on Nervous System

(N27) **Neurobehavioral toxicology of pyrethroid insecticides in adult animals: A critical review** (full text)
Abstract: Pyrethroids are pesticides with high selectivity for insects. In order to identify strengths and gaps in the database for pyrethroid neurobehavioral toxicology, we have critically analyzed the data from peer-reviewed literature. This review includes dose–response data that have been recently generated demonstrating consistent findings for low-dose, acute, oral exposure to pyrethroids in small rodents. All pyrethroids tested (i.e., about twenty compounds), regardless of structure, produce a decrease in motor activity in a variety of test protocols. The range of relative potencies varies more than two orders of magnitude, and thresholds for motor activity were found well below doses that produce overt signs of poisoning. Six compounds (allethrin, permethrin, *cis*-permethrin, deltamethrin, cypermethrin, and fenvalerate) impair schedule-controlled operant responding, seven compounds (pyrethrum, bifenthrin, *S*-bioallethrin, permethrin, β-cyfluthrin, cypermethrin, and deltamethrin) decrease grip strength, and two compounds (deltamethrin and α-cypermethrin) produce incoordination using the rotarod. In addition, while compounds lacking an α-cyano group (e.g., cismethrin, permethrin, bifenthrin) induce an increase in acoustic-evoked startle response amplitude, cyano compounds (e.g., deltamethrin, cypermethrin, cyfluthrin) produce the opposite outcome. Other endpoints (e.g., tremor intensity, sensory response) have been only occasionally explored. A synthesis of the neurobehavioral evidence relating to the action of pyrethroids indicates that some differences in the experimental findings across compounds are also present in the low-effective dose range. For risk assessment purposes, a strategy that takes into account data from an array of neurobehavioral endpoints is needed to capture the heterogeneity of pyrethroid-induced adverse effects and accurately inform policy decisions.

(R5) **Influence of dermal exposure to the pyrethroid insecticide deltamethrin on rat brain microanatomy and cholinergic and dopaminergic neurochemistry**
Deltamethrin is a pesticide largely used. Acute toxicity of this compound was extensively investigated, whereas less information is available on the effects of subchronic and/or chronic exposure to deltamethrin or on the effects of its dermal absorption. Sparse data are also available on deltamethrin neurotoxicity. This study has assessed in the rat the effects of dermal application of deltamethrin (30 mg/kg/day in cyclohexane for 4 weeks to the skin of the back of the neck) on microanatomy of cerebrocortical areas (frontal cortex and hippocampus) and on cholinergic and dopaminergic neurotransmission markers. Treatment with deltamethrin caused nerve cell loss and the appearance of signs of neuronal sufferance primarily in layer III of frontal cortex as well as in the dentate gyrus and to a lesser extent in the CA1 and CA3 subfields of hippocampus. Deltamethrin induced also astrogliosis. Cholinergic neurotransmission markers investigated in frontal cortex, hippocampus and striatum were acetylcholine (ACh), the synthesizing and catabolic enzymes choline acetyltransferase and acetylcholinesterase and the high affinity ACh uptake system labeled with [^{3}H]hemicholinium-3. These markers were unaffected by deltamethrin administration. Dopamine and the dopamine plasma membrane transporter labeled with [^{3}H]GBR 12935 were unaffected by treatment with deltamethrin in frontal cortex and decreased significantly in hippocampus and striatum. These findings indicate that dermal exposure to the pyrethroid insecticide deltamethrin using an administration module mimicking a possible long-lasting occupational skin contact is accompanied by cerebrocortical injury and loss of hippocampal and striatal dopamine and dopamine transporter. The sensitivity of dopaminergic system in our experimental model suggests that dermal exposure to deltamethrin could represent a risk factor for Parkinson's disease.

(R7) **Long lasting effects of prenatal exposure to deltamethrin on cerebral and hepatic cytochrome P450s and behavioral activity in rat offspring**
Prenatal exposure to different doses (0.25, or 0.5 or 1.0 mg/kg corresponding to 1/320th or 1/160th or 1/80th of LD_{50}) of deltamethrin to the pregnant Wistar rats from gestation day 5 to 21 were found to produce a dose dependent increase

in the activity of cytochrome P450 (CYP) dependent 7-ethoxyresorufin-*O*-deethylase (EROD), 7-pentoxyresorufin-*O*-dealkylase (PROD) and *N*-nitrosodimethylamine demethylase (NDMA-*d*) in brain and liver of offspring postnatally at 3 weeks. The increase in the activity of cytochrome P450 monooxygenases was found to be associated with the increase in the mRNA and protein expression of xenobiotic metabolizing CYP1A, 2B and 2E1 isoenzymes in the brain and liver of offspring. Dose-dependent alterations in the parameters of spontaneous locomotor activity in the offspring postnatally at 3 weeks have indicated that increase in cytochrome P450 activity may lead to the accumulation of deltamethrin and its metabolites to the levels that may be sufficient to alter the behavioral activity of the offspring. Interestingly, the inductive effect on cerebral and hepatic cytochrome P450s was found to persist postnatally up to 6 weeks in the offspring at the relatively higher doses (0.5 and 1.0 mg/kg) of deltamethrin and up to 9 weeks at the highest dose (1.0 mg/kg), though the magnitude of induction was less than that observed at 3 weeks. Alterations in the parameters of spontaneous locomotor activity in the offspring postnatally at 6 and 9 weeks, though significant only in the offspring at 3 and 6 weeks of age, have further indicated that due to the reduced activity of the cytochrome P450s during the ontogeny, the pyrethroid or its metabolites accumulating in the brain may not be cleared from the brain, thereby leading to the persistence in the increase in the expression of cerebral and hepatic cytochrome P450s in the offspring postnatally up to 9 weeks. The data suggests that low dose prenatal exposure to pyrethroids has the potential to produce long lasting effects on the expression of xenobiotic metabolizing cytochrome P450s in brain and liver of the offspring.

(N6) **Developmental Deltamethrin Exposure Causes Persistent Changes in Dopaminergic Gene Expression, Neurochemistry, and Locomotor Activity in Zebrafish** (full text)

Abstract: Pyrethroids are commonly used insecticides that are considered to pose little risk to human health. However, there is an increasing concern that children are more susceptible to the adverse effects of pesticides. We used the zebrafish model to test the hypothesis that developmental exposure to low doses of the pyrethroid deltamethrin results in persistent alterations in dopaminergic gene expression, neurochemistry, and locomotor activity. Zebrafish embryos were treated with deltamethrin (0.25–0.50 µg/l), at concentrations below the LOAEL, during the embryonic period [3–72 h postfertilization (hpf)], after which transferred to fresh water until the larval stage (2-weeks postfertilization). Deltamethrin exposure resulted in decreased transcript levels of the D1 dopamine (DA) receptor (*drd1*) and increased levels of tyrosine hydroxylase at 72 hpf. The reduction in *drd1* transcripts persisted to the larval stage and was associated with decreased D2 dopamine receptor transcripts. Larval fish, exposed developmentally to deltamethrin, had increased levels of homovanillic acid, a DA metabolite. Since the DA system is involved in locomotor activity, we measured the swim activity of larval fish following a transition to darkness. Developmental exposure to deltamethrin significantly increased larval swim activity which was attenuated by concomitant knockdown of the DA transporter. Acute exposure to methylphenidate, a DA transporter inhibitor, increased swim activity in control larva, while reducing swim activity in larva developmentally exposed to deltamethrin. Developmental exposure to deltamethrin causes locomotor deficits in larval zebrafish, which is likely mediated by dopaminergic dysfunction. This highlights the need to understand the persistent effects of low-dose neurotoxicant exposure during development.

(N8) **Effects of sub-chronic exposure to deltamethrin on shuttle-box avoidance and contents of amino acid neurotransmitters in hippocampus of mice** (full text)

The purpose of this study was to evaluate the effects of sub-chronic exposure to deltamethrin in lower doses on the acquisition of a two-way avoidance task and the levels of amino acid neurotransmitters in hippocampus of mice measured using shuttle-box and LC-MS/MS system. Deltamethrin was given to mice respectively at doses of 0.46, 0.92, 1.80 mg/kg BW daily for 60 days by gavage. Deltamethrin was found to decrease the number of avoidance responses, increase response latency, and increase glutamate levels in the 0.92 and 1.80 mg/kg BW-dose group. As revealed by electron microscopy, in 0.92 and 1.8 mg/kg-dose group mice, morphology of cells were changed and degeneration and necrosis morphological characteristics obviously were appeared. Collectively, results from this study suggest that

deltamethrin may have cumulative effects in mice following repeated dosing of deltamethrin using moderately effective doses, beside Na^+ and Ca^{2+} channels as well as Na^+ and Ca^{2+}-dependent glutamate release, may be involved with neurotoxic action of deltamethrin.

Appendix C – Deltamethrin Impact on Immune System

(N30) **Mechanism of Deltamethrin induced Immunotoxicity: Current and Future Perspectives** (full text)
The immune system is the most vulnerable system regarding toxicity of pesticides. Any alteration in the immune functions makes an individual immunocompromised and more susceptible to cancer, infections, autoimmunity and allergies. Deltamethrin is the most popular type 2 pyrethroid insecticide which is widely use in agriculture and home due to restriction on the organophosphate insecticides. Due to their extensive use, it becomes an increasingly serious source of chemical pollution. We all are exposed to deltamethrin through inhalation, ingestion and dermal contact. It has been demonstrated that deltamethrin alters the immune response signalling pathways, but its mechanism of immunotoxicity is still an open question for researchers to be explored. Thus, herein we tried to understand the mechanism of deltamethrin induced immunotoxicity. Possibilities of deltamethrin induced other immunotoxic signalling pathways have also been discussed and should be considered in future studies. Further, current challenges and future perspectives have been also discussed.

(R1) **Deltamethrin Increases Candida albicans Infection Susceptibility in Mice**
Abstract: Deltamethrin, an alpha-cyano type II synthetic pyrethroid insecticide, is used to control a wide range of insects on a variety of crops and vectors of diseases. Deltamethrin has been previously reported for its immunotoxic effects and therefore its exposure may affect the host resistance to infection and tumour challenge. Effect of exposure of deltamethrin on host resistance to *Candida albicans* infection was examined in Swiss albino mice. The objective of this study was to investigate the modulatory action of deltamethrin in *C. albicans* infected mice. The dose of deltamethrin was initially tested and selected from our previous study (18 mg⁄ kg). Percentage of infection in deltamethrin treated animals increased faster when compared to that of the controls. Deltamethrin exposure along with *C. albicans* infection caused alteration of humoral immune response. The number of colony forming unit in liver and spleen were also found to be significantly increased in the treated group. The results from our present study suggest that deltamethrin exhibits an immunosuppressive effect and has a negative impact on host resistance to *C. albicans* infection.

(R2) **Deltamethrin-Induced Oxidative Stress and Mitochondrial Caspase-Dependent Signaling Pathways in Murine Splenocytes**
Abstract: Deltamethrin (DLM) is a well-known pyrethroid insecticide used extensively in pest control. Exposure to DLM has been demonstrated to cause apoptosis in various cells. However, the immunotoxic effects of DLM on mammalian system and its mechanism is still an open question to be explored. To explore these effects, this study has been designed to first observe the interactions of DLM to immune cell receptors and its effects on the immune system. The docking score revealed that DLM has strong binding affinity toward the CD45 and CD28 receptors. In vitro study revealed that DLM induces apoptosis in murine splenocytes in a concentration-dependent manner. The earliest markers of apoptosis such as enhanced reactive oxygen species and caspase 3 activation are evident as early as 1 h by 25 and 50 mM DLM. Western blot analysis demonstrated that p38 MAP kinase and Bax expression is increased in a concentration-dependent manner, whereas Bcl 2 expression is significantly reduced after 3 h of DLM treatment. Glutathione depletion has been also observed at 3 and 6 h by 25 and 50 mM concentration of DLM. Flow cytometry results imply that the fraction of hypodiploid cells has gradually increased with all the concentrations of DLM at 18 h. N-acetyl cysteine effectively reduces the percentage of apoptotic cells, which is increased by DLM. In contrast, buthionine sulfoxamine causes an elevation in the percentage of apoptotic cells. Phenotyping data imply the effect of DLM toxicity in murine splenocytes. In brief, the study demonstrates that DLM causes apoptosis through its interaction with CD45 and CD28 receptors, leading to oxidative stress and activation of the mitochondrial caspase-dependent pathways which ultimately affects the immune functions. This study provides mechanistic information by which DLM

causes toxicity in murine splenocytes. VC 2014 Wiley Periodicals, Inc. Environ Toxicol 31: 808–819, 2016.
Keywords: deltamethrin; oxidative stress; Bcl-2; Bax; caspase-3; apoptosis

(R8) **The modulatory effect of deltamethrin on antioxidants in mice**
Background - Deltamethrin is a α-cyano pyrethroid insecticide used extensively in pest control. Although initially thought to be least toxic, a number of recent reports showed its toxicity in mammalian and non-mammalian laboratory and wildlife animal species. In fish, it is a potent oxidative stress-inducing agent. We studied the oxidative stress-inducing effect of deltamethrin in mice. Methods - Male Swiss albino mice were orally administered 2 doses of deltamethrin viz., 5.6 and 18 mg/kg body weight (bw), for 15 days. Results - Both the doses of deltamethrin significantly induced lipid peroxidation (LPO) in liver and kidney. Along with the induction of LPO, activities of vital antioxidant enzymes such as glutathione peroxidase (GPx), glutathione *S*-transferase (GST) and catalase (CAT) were also suppressed in both the tissues. Glutathione (GSH) level was also decreased. GSH decrease was more pronounced in kidney than the liver. Conclusion - Toxicity of many chlorinated and organophosphate insecticides is mediated by the reactive oxygen species (ROS). Findings of the present investigation also suggest a role for ROS in deltamethrin toxicity. An increased LPO indicates that these ROS might have caused degradation of biomembrane in deltamethrin-exposed animals.

(N11) **How Deltamethrin Produces Oxidative Stress in Liver and Kidney** (full text)
Abstract: Deltamethrin (DEL) is a synthetic pyrethroid widely used as an insecticide. The aim of our study was to determine the effect of a single exposure of female albino Swiss mice to DEL (at doses of 8.3 mg/kg, 20.75 mg/kg, or 41.5 mg/kg) on parameters of liver and kidney function and activities of antioxidant enzymes in these organs. The activity of alanine transaminase (ALT) in the blood sera of the experimental animals was not significantly elevated after exposure to DEL. Asparagine transaminase (AST) activity was significantly higher in the groups exposed to the moderate and the highest dose of DEL. The levels of creatinine in the blood sera of the experimental animals did not significantly differ among the groups. The activities of superoxide dismuthase (SOD) and glutathione peroxidase (GPx) were significantly reduced in the livers of mice exposed to the highest dose of DEL in comparison with controls. In the kidneys, however, the SOD and GPx activities were significantly elevated after exposure to the highest dose of DEL. In conclusion, DEL produces oxidative stress in the livers and, to a lesser degree, the kidneys of exposed animals

Appendix D – Deltamethrin reproductive, developmental and endocrine disruption effects

See the Human Health section for endocrine/reproductive studies in humans.

(R3) **Dose Dependent Effect of Deltamethrin in Testis, Liver, and Kidney of Wistar Rats** (full text)

Abstract: **Objectives:** Deltamethrin is a synthetic pyrethroid insecticide used worldwide in agriculture, household pest control, protection of foodstuff, and disease vector control. Although initially thought to be least toxic, a number of recent reports showed its toxic effects in mammalian and non-mammalian animal species. The current study was performed to assess the dose-dependent deltamethrin toxicity on testes, liver, and kidney of male Wistar rats. **Materials and Methods:** Twenty-four rats were divided in four groups of 6 each. Group A served as normal control. Group B, C, and D were administered with different doses (2 or 3 or 6 mg/kg corresponding to 1/30 th or 1/20 th or 1/10 th of LD 50, respectively) of deltamethrin for 28 days. **Results:** Deltamethrin exposure caused a significant reduction in weight of reproductive organs, decrease in sperm count, sperm motility, serum testosterone (T), follicle stimulating hormones (FSH), and luteinizing hormones (LH) in testis. Glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione S transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx) were decreased in testis, liver and kidney of exposed rats. Deltamethrin exposure significantly increased sperm abnormalities in testis. Significant increase in lipid peroxidation (LPO) level was observed in testis, liver and kidney. Deltamethrin also caused histological alterations in testes, liver, and kidney. **Conclusions:** The results indicated that deltamethrin at a dose of 6 mg/kg exerts significant harmful effects on testes, liver and kidney as compare to 2 mg and 3 mg/kg. The study concluded that the system toxicity induced by deltamethrin was dose dependent.

(N19) **Pyrethroid Pesticides as Endocrine Disruptors: Molecular Mechanisms in Vertebrates with a Focus on Fishes**

Abstract: Pyrethroids are now the fourth most used group of insecticides worldwide. Employed in agriculture and in urban areas, they are detected in waterways at concentrations that are lethally and sublethally toxic to aquatic organisms. Highly lipophilic, pyrethroids accumulate in sediments and bioaccumulate in fishes. Additionally, these compounds are demonstrated to act as endocrine disrupting compounds (or EDCs) in mammals and fishes, and therefore interfere with endocrine signaling by blocking, mimicking, or synergizing endogenous hormones through direct receptor interactions, and indirectly via upstream signaling pathways. Pyrethroid metabolites have greater endocrine activity than their parent structures, and this activity is dependent on the enantiomer present, as some pyrethroids are chiral. Many EDCs studied thus far in fish have known estrogenic or antiestrogenic effects, and as such cause the inappropriate or altered expression of genes or proteins (i.e., Vtg–vitellogenin, Chg–choriogenin), often leading to physiological or reproductive effects. Additionally, these compounds can also interfere with other endocrine pathways and immune response. This review highlights studies that focus on the mechanisms of pyrethroid biotransformation and endocrine toxicity to fishes across a broad range of different pyrethroid types, and integrates literature on the *in vitro* and mammalian responses that inform these mechanisms.

(R4) **Effects of commercial formulations of deltamethrin and or thiacloprid on thyroid levels in rats**

Deltamethrin (DEL) and thiacloprid (THIA) are the two commonly used synthetic insecticides applied either separately or as a mixture. The aim of this study was to assess thyroid stimulating hormone (TSH) and the serum levels of thyroid hormones exposure to these compounds in rats. The animals were orally gavaged with a single dose of DEL (15 mg/kg), THIA (112.5 mg/kg) or DEL + THIA (15 + 112.5 mg/kg) for 24 h (acute treatments) or DEL (3 mg/kg per day), THIA (22.5 mg/kg per day) or DEL + THIA (3 + 22.5 mg/kg per day) for 30 days (subacute treatments). Although all independent and combined treatments with DEL and THIA changed the levels of TSH, these alterations were not significant. Statistically significant increases in free triiodothyronine (FT3) and free thyroxine (FT4) serum hormone levels were observed in the independent treatment with THIA and the combined treatment with DEL and THIA for 30 days. The results of this study suggest that *in vivo* exposure to subacute treatments of commercial formulations of THI and mixture of DEL + THIA increased serum FT3 and FT4 levels in rats. Further studies are required to determine the effects of

endocrine disruptors and potential health risks of these insecticides in human, especially in children because of the importance of these hormones during growth and development.

(R10) **Teratogenic Effect of Sublethal Doses of Deltamethrin in Mice** (full text)

Abstract: Present study was conducted to evaluate the teratogenic effects of sublethal concentrations *viz.,* 19.36, 9.7, and 4.8µg/g BW of deltamethrin, which was administered orally to the pregnant mice on day 6 of gestation. The fetuses were recovered on day 18 of gestation. The morphological studies revealed abnormalities including sacral hygromae, microcephally, micromelia, open eyelids, microphthalmia, exophthalmia, cryptophthalmia, anophthalmia, droopingwrist, kyphosis, and short tail. Fetal resorptions increased with the increasing dose. Morphometric analysis showed an overall significant ($P<0.001$) reduction in bodyweight, crown rump length, brain and eyes circumferences, lengths of hind and forelimbs and tail size. The histological studies showed malformations including defective nasal pouch, nasal septum with atrophied inferior cochlea, missing of eye ball, pericranial hydrocephaly, cleft palate, degeneration of jaw muscles. Tissue necrosis of brain, liver and intestine were also observed as compared to vehicle control.

(R7) **Effect of prenatal exposure of deltamethrin on the ontogeny of xenobiotic Cytochrome P450s**

Abstract: Prenatal exposure to different doses (0.25, or 0.5 or 1.0 mg/kg corresponding to 1/320th or 1/160th or 1/80th of LD_{50}) of deltamethrin to the pregnant Wistar rats from gestation day 5 to 21 were found to produce a dose dependent increase in the activity of cytochrome P450 (CYP) dependent 7-ethoxyresorufin-*O*-deethylase (EROD), 7-pentoxyresorufin-*O*-dealkylase (PROD) and *N*-nitrosodimethylamine demethylase (NDMA-*d*) in brain and liver of offspring postnatally at 3 weeks. The increase in the activity of cytochrome P450 monooxygenases was found to be associated with the increase in the mRNA and protein expression of xenobiotic metabolizing CYP1A, 2B and 2E1 isoenzymes in the brain and liver of offspring. Dose-dependent alterations in the parameters of spontaneous locomotor activity in the offspring postnatally at 3 weeks have indicated that increase in cytochrome P450 activity may lead to the accumulation of deltamethrin and its metabolites to the levels that may be sufficient to alter the behavioral activity of the offspring. Interestingly, the inductive effect on cerebral and hepatic cytochrome P450s was found to persist postnatally up to 6 weeks in the offspring at the relatively higher doses (0.5 and 1.0 mg/kg) of deltamethrin and up to 9 weeks at the highest dose (1.0 mg/kg), though the magnitude of induction was less than that observed at 3 weeks. Alterations in the parameters of spontaneous locomotor activity in the offspring postnatally at 6 and 9 weeks, though significant only in the offspring at 3 and 6 weeks of age, have further indicated that due to the reduced activity of the cytochrome P450s during the ontogeny, the pyrethroid or its metabolites accumulating in the brain may not be cleared from the brain, thereby leading to the persistence in the increase in the expression of cerebral and hepatic cytochrome P450s in the offspring postnatally up to 9 weeks. The data suggests that low dose prenatal exposure to pyrethroids has the potential to produce long lasting effects on the expression of xenobiotic metabolizing cytochrome P450s in brain and liver of the offspring.

(N7) **Effect of deltamethrin containing formulation on developing chick embryo: morphological and skeletal changes**

Abstract: The teratogenicity of a commercial formulation of the insecticide deltamethrin (Decis®) in chick embryos was evaluated. Fertilized eggs of *Gallus domesticus* were immersed in aqueous emulsions of deltamethrin at concentrations of 12.5 mg L-1, 25 mg L-1 and 50 mg L-1 for 60 min at 37◦C on 4th day of incubation. Two control groups of eggs were used: One group was immersed in distilled water (vehicle) and the second group was kept as untreated to study background toxicity. On embryonic day 16; recovered embryos were evaluated for mortality rate, wet body weight, gross morphological and skeletal malformations. The result revealed that embryonic mortality markedly increased after administration of deltamethrin. The significant decrease ($p\leq0.05$) in wet body weight and significant increase ($p\leq0.05$) in percentage of abnormal survivors was observed in dose dependent manner. A spectrum of external and skeletal

malformations was exhibited by deltamethrin treated embryos. These finding suggests that deltamethrin exhibits embryotoxic and teratogenic effects in the developing chick embryos.

Appendix E – Deltamethrin effect on DNA and carcinogenicity

(H3) **In vitro genotoxic effects of the insecticide deltamethrin in human peripheral blood leukocytes: DNA damage ('comet' assay) in relation to the induction of sister-chromatid exchanges and micronuclei**

Abstract: Deltamethrin, a synthetic dibromo-pyrethroid insecticide, is extensively used in agriculture, forestry and in household products because of its high activity against a broad spectrum of insect pests (both adults and larvae), its low animal toxicity and its lack of persistence in the environment. Data on the genotoxicity and carcinogenicity of deltamethrin are rather controversial, depending on the genetic system or the assay used. The aim of this study was to further evaluate the potential genotoxic activity of deltamethrin. The in vitro genotoxicity of deltamethrin has been evaluated by assessing the ability of the insecticide to damage DNA (as evaluated using the single-cell microgel-electrophoresis or 'comet' assay) or induce sister-chromatid exchanges (SCE) and micronuclei (MN) in human peripheral blood leukocytes. All treatments were conducted with and without the presence of an external bioactivation source (±S9mix). The results indicate that deltamethrin, in the presence of metabolic activation (+S9mix), is able to induce DNA damage (double- and single-strand breaks, alkali-labile sites and open excision repair sites) as revealed by the increasing tail moment values observed with increasing doses. The frequency of SCE and MN were not statistically increased in deltamethrin-treated cells as compared to controls, both with and without S9mix. However, lower deltamethrin doses were tested, as compared to 'comet' assay, because of cytotoxicity.

(P3) **Evaluation of cytogenetic effects of deltamethrin in root meristem cells of *Allium sativum* and *Allium cepa*: A possible mechanism of chromosome damage**

To establish the use of *Allium sativum* as a sensitive test model for genotoxicity, cytogenetic effects of commercially formulated deltamethrin were examined through chromosomal and mitotic aberrations in the root meristem cells of *A. sativum* and *Allium cepa*. Ultraviolet (UV) and Fourier transform infrared (FTIR) spectral measurements were also carried out to understand the interaction of deltamethrin with DNA. Test concentrations of deltamethrin 0.06, 0.12, 0.25, 0.5, 1, or 2mg kg_1 were mixed in soil and the garlic cloves/onion bulbs were placed over deltamethrin contaminated soil. Some roots were sampled at 48 h exposure and other intact roots were washed thoroughly with distilled water and left for 48 h recovery in normal soil. Cells analyzed immediately after the exposure showed a significant, concentration-dependent inhibition of mitotic index (MI) and induction of mitotic and chromosomal aberrations (MA and CA) in both the test systems. The observed CA and MA were relatively higher in *A. sativum* system compared to A. cepa. The 48 h recovery period reduced the effect of the test compound on % aberrations; however, cells exposed to 1 and 2 ppm showed a significant frequency of aberrations despite the recovery period. Data indicate that higher concentrations of deltamethrin induce CA and MA in *A. sativum* and *A. cepa*. The present study demonstrates greater sensitivity of *A. sativum* versus *A. cepa* and may be used as a sensitive and reliable test system for environmental monitoring. A bathochromic shift observed in UV absorption spectra reveals that deltamethrin binds with DNA. Role of vibrational modes of the active site in the recognition (via polarization) and reaction of deltamethrin with DNA was described. Based on data of valence charge distributions on the atoms of deltamethrin, spectroscopic studies and structural properties, a possible mechanism was proposed for the interaction of deltamethrin with DNA resulting in chromosomal damage.

(R9) **Tumourigenic studies on deltamethrin in Swiss albino mice**

Abstract: Deltamethrin, an α-cyano type II synthetic pyrethroid insecticide is used to control a wide range of insects on a variety of crops. Deltamethrin is reported to cause many adverse effects on non-target species. Deltamethrin is reported to cause DNA damage and micronuclei induction in human lymphocytes. It is highly toxic for other organisms such as aquatic invertebrates, fish and Daphnia. About the tumorigenic risk (both tumour initiating and promoting) associated with deltamethrin exposure, very few reports are available in literature. In the present set of investigations,

deltamethrin has been evaluated for its tumorigenic and co-carcinogenic (tumour initiating and tumour promoting) potential following long term dermal exposure in Swiss albino mice. The results revealed that deltamethrin has only tumour initiating potential in both the sexes of Swiss albino mice, initiated with deltamethrin and promoted by standard tumour promoter, 12-*O*-tetra decanoyl phorbol-13-acetate (TPA). In the single dose initiated mice (deltamethrin 4 mg/kg body weight, once only), 44% males and 43% females developed benign skin tumours. A much higher incidence of tumorigenesis was recorded in multiple dose initiated animals (deltamethrin 4 mg/kg body weight, three times per week for 3 weeks), where 71% male and 75% female mice developed tumours at the site of application of deltamethrin. Deltamethrin exposure failed to show any tumour promoting and complete tumorigenic potential at all the three tested dose levels.

Appendix F – Deltamethrin non-target toxicity

(N10) **Formulated Beta-Cyfluthrin Shows Wide Divergence in Toxicity among Bird Species** (full text)

Abstract: It is generally assumed that the toxicity of pyrethroid insecticides to birds is negligible, though few species have been tested. The oral acute toxicity of formulated beta-cyfluthrin was determined for canaries (*Serinus* sp.), shiny cowbirds (*Molothrus bonariensis*), and eared doves (*Zenaida auriculata*). Single doses were administered to adults by gavage. Approximate lethal doses 50 (LD_{50}) and their confidence intervals were determined by approximate D-optimal design. Canaries were found to be substantially more sensitive to formulated beta-cyfluthrin (LD_{50}= (170 $\pm$ 41) mg/kg) than the other two species tested (LD_{50} = (2234 $\pm$ 554) mg/kg and LD_{50} = (2271 ± 433) mg/kg, resp.). The LD_{50} obtained for canaries was also considerably lower than typical toxicity values available in the literature for pyrethroids. This study emphasizes the need for testing a broader range of species with potentially toxic insecticides, using modern up and down test designs with minimal numbers of birds.

[Staff note: Pyrethroids are generally thought to be non-toxic to birds and there are few studies outside of the regulatory required studies. Although this paper is about a different pyrethroid than deltamethrin, it could represent similar gaps.]

(N22) **Toxicity of Pesticide Deltamethrin to Fish** (full text)

Deltamethrin is widely used and most effective pesticide based on pyrethroid preparations. It is extensively used in agriculture, for controlling pests, insects and vectors of endemic diseases, protecting seeds during storage and fighting household insects because of their low environmental persistence. However, deltamethrin is found to be highly toxic to various non-targeted aquatic organisms including fish. Contributing factor to the sensitivity of fish to deltamethrin exposure seems to be its high rate of gill absorption due to the lipophilicity. The main mode of its action is neurotoxicity, and its capacity to induce oxidative stress or alteration of antioxidant system and lipid peroxidation. Thus, the main aim of this study is to review the toxic effect of synthetic pyrethroid, deltametrin in fish.

Appendix G – Deltamethrin direct effects to invertebrates

(N2) **The toxic effect of deltamethrin on linyphiid and erigonid spiders in connection with ambient temperature, humidity, and predation**

Abstract: The first part of this study concerns the effect of temperature and air humidity on the toxicity of deltamethrin to the erigonid Oedothorax apicatus. The second part concerns the effect of deltamethrin on behavior of linyphiids and erigonids with respect to their ability to escape from predators and to select between unfavorable (dry) and favorable (moist) habitat conditions. The toxic effect of deltamethrin was highest at the combination of high temperature and low air humidity. It was concluded that the spider's sensitivity to drought is increased by this pyrethroid. Affected spiders, however, are less able to select moist habitat conditions than unaffected ones. Walking speed of spiders was decreased by exposure to deltamethrin and their predation by carabid beetles was increased. It was concluded that the effect observed under field conditions is the result of a combination of neurological, physiological, and behavioral disturbance.

(N13) **Lethal and sublethal effects of seven insecticides on three beneficial insects in laboratory assays and field trials**

Abstract: Lethal and sublethal effects of insecticides on target and non-target arthropods are a concern of pest management programs. *Cycloneda sanguinea*, *Orius insidiosus* and *Chauliognathus flavipes* are important biological control agents for aphids, whitefly, lepidopterus eggs, thrips and mites. All three test species were subjected to a toxicity study using the insecticides acephate, bifenthrin, chlorantraniliprole, chlorpyrifos, deltamethrin, imidacloprid, and thiamethoxam. Experiments were done in the lab and field. In the laboratory we evaluated the mortality and sublethal effects of the concentration that killed 20% of the population (LC_{20}) on feeding, repellence and reproduction of the species tested. The lethal effects of these insecticides at the recommended doses was evaluated in the field. Concentration-response bioassays indicated chlorantraniliprole had the lowest toxicity, while chlorpyrifos and acephate were the most toxic. Test species exposed to filter paper surfaces treated with pyrethroids, neonicotinoids and organophosphates were repelled. On the other hand, test species were not repelled from surfaces treated with chlorantraniliprole. Chlorantraniliprole therefore seemed to be the least dangerous insecticide for these three beneficial arthropod test species.

(staff notes: quotes from paper: “The insecticides from the organophosphate group (acephate and chlorpirifos) and the pyrethroids (bifenthrin and deltamethrin) were the insecticides that most reduced test species densities. In general the test species were reduced in the days after spraying, with populations of O. insidiosus and C. flavipes reduced by 100% (Fig. 3 B,C). The populations did not increase again until 21 d after spraying (Fig. 3).”

(N9) **Effects of sublethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development of the honeybee Apis mellifera ligustica**

Abstract: Bifenthrin and deltamethrin have been widely used as pesticides in agriculture and forestry and are becoming an increasing risk to honeybees. The honeybee, Apis mellifera ligustica, is widely recognized as a beneficial insect of agronomic, ecological, and scientific importance. It is important to understand what effects these chemicals have on bees. Effects of two pesticides at sublethal concentrations on fecundity, growth, and development of honeybees were examined with the feeding method for a three-year period (2006-2008). It was shown that both bifenthrin and deltamethrin significantly reduced bee fecundity, decreased the rate at which bees develop to adulthood, and increased their immature periods. The toxicity of bifenthrin and deltamethrin on workers of Apis mellifera ligustica was also assessed, and the results from the present study showed that the median lethal effects of bifenthrin and deltamethrin were 16.7 and 62.8 mg/L, respectively.

(N18) **Partial compensation of the sublethal effect of deltamethrin on the sex pheromonal communication of Trichogramma brassicae**

Abstract: Pyrethroid insecticides are widely used and lead to a sizable environmental pollution that could interfere with the population biology of insects. *Trichogramma* is a beneficial insect used in biological control and which natural populations contribute to the control of Lepidopterus pests. In this work, we determined the effect of a sublethal dose of deltamethrin on the sex pheromonal communication of *Trichogramma*. The dose used (LD 0.1) induces no detectable

mortality (the theoretical mortality is only one insect over 1000) and can be a good representation of contamination by this insecticide from environmental pollution. The insecticide was shown to have opposite effects on the sex pheromonal communication of *Trichogramma*, depending on which sex was exposed (Delpuech, J.M., Legallet, B., Terrier, O., Fouillet, P., 1999. Chemosphere 38, 729–739). We show that, when both sexes are simultaneously exposed to the insecticide, this effect is only partially neutralized. The mean response of treated males responding to the sex pheromone from treated females is not significantly different from that of controls, but the kinetics of their response is not the same. When both sexes are treated, the response of males to the sex pheromone is lower at the beginning but their response does not decrease during time contrary to controls and becomes finally higher than that of controls. Therefore, the sublethal effect of deltamethrin in the field can be either advantageous or disadvantageous depending on the difficulty in finding females and their scarcity.

(N20) **The sublethal effects of deltamethrin on Trichogramma behaviors during the exploitation of host patches**
Abstract: *Trichogramma* and parasitoids as a whole are key species because they regulate natural populations of other insects. As any non-target species, this parasitoid can be exposed to insecticides by environmental pollution. This study identified the effects of an LD 20 of deltamethrin (a pyrethroid) on the behavior of *Trichogramma brassicae* females infesting a patch of host eggs. The study found that females that survived exposure to the insecticide infested fewer host eggs; spent more time on unsuitable, previously infested host eggs; and infested more previously infested host eggs than controls. The insecticide also induced an increase in antennal and ovipositor rejection of previously infested host eggs. These results are discussed in the light of the mode of action of pyrethroid insecticides. The findings of the study highlight sublethal effects that reduce the fitness of parasitoids and that could consequently modify the equilibrium of natural ecosystems.

(N14) **Long-term toxic effects of deltamethrin and fenvalerante in soil**
Abstract: In this study, the long-term toxic effects of pyrethroids on the earthworm *Eisenia fetida* were evaluated. Earthworms were exposed to moist filter paper and soil for 14 days to evaluate the survival, exposed to soil for 56 days to assess the reproductive success and for 28 days to identify the cytotoxicity. Results showed that the earthworm survival rate decreased with increasing the concentration of either deltamethrin or fenvalerate in both filter paper test and soil test. No worms survived at 602.15 μg cm^{-2} of deltamethrin and 0.86 μg cm^{-2} of fenvalerate in the filter paper test, however 100–125 mg kg^{-1} of both chemicals resulted in the maximum mortality of 90% in the soil test. The CYP3A4 enzyme activity responded significantly to deltamethrin and fenvalerante in soil at low concentration levels, however, the toxicity response of worms under the long-term exposure conflicted with the degradation of deltamethrin and fenvalerate in soil, indicating the possible formation of more toxic pyrethroid metabolites. This study gave an insight into the toxicological effects profile of pyrethroids for a better risk assessment of pyrethroids deltamethrin and fenvalerante in soil.

(N3) **Assessment of Potential Sublethal Effects of Various Insecticides on Key Biological Traits of The Tobacco Whitefly, *Bemisia tabaci*** (full text)
Abstract: The tobacco whitefly Bemisia tabaci is one of the most devastating pests worldwide. Current management of B. tabaci relies upon the frequent applications of insecticides. In addition to direct mortality by typical acute toxicity (lethal effect), insecticides may also impair various key biological traits of the exposed insects through physiological and behavioral sublethal effects. Identifying and characterizing such effects could be crucial for understanding the global effects of insecticides on the pest and therefore for optimizing its management in the crops. We assessed the effects of sublethal and low-lethal concentrations of four widely used insecticides on the fecundity, honeydew excretion and feeding behavior of B. tabaci adults. The probing activity of the whiteflies feeding on treated cotton seedlings was recorded by an Electrical Penetration Graph (EPG). The results showed that imidacloprid and bifenthrin caused a reduction in phloem feeding even at sublethal concentrations. In addition, the honeydew excretions and fecundity levels of adults feeding on leaf discs treated with these concentrations were significantly lower than the untreated ones. While, sublethal concentrations of chlorpyrifos and carbosulfan did not affect feeding behavior, honeydew excretion and fecundity of the whitefly. We demonstrated an antifeedant effect of the imidacloprid and bifenthrin on B. tabaci,

whereas behavioral changes in adults feeding on leaves treated with chlorpyrifos and carbosulfan were more likely caused by the direct effects of the insecticides on the insects' nervous system itself. Our results show that aside from the lethal effect, the sublethal concentration of imidacloprid and bifenthrin impairs the phloem feeding, i.e. the most important feeding trait in a plant protection perspective. Indeed, this antifeedant property would give these insecticides potential to control insect pests indirectly. Therefore, the behavioral effects of sublethal concentrations of imidacloprid and bifenthrin may play an important role in the control of whitefly pests by increasing the toxicity persistence in treated crops.

(Staff note: although this study examines the effect bifenthrin, another pyrethroid, it examines sublethal impacts that should be considered for non-target insects in prairie dog colonies. It also shows that sublethal physiological effects occur in both different trophic levels of insects.)

Appendix H – Deltamethrin indirect effects to non-target species

(N16) **Mountain Plover responses to deltamethrin treatments on prairie dog colonies in Montana**

Abstract: Pyrethroid insecticides containing deltamethrin provide broad spectrum insect control that can adversely affect food supplies of insectivorous birds. I hypothesized that this could result in lowered nest survival for a ground-nesting insectivorous bird, the Mountain Plover (Charadrius montanus), which preferentially nests on prairie dog colonies. I studied Mountain Plover nest survival in 2003–2010 at a small cluster of black-tailed prairie dog (Cynomys ludovicianus) colonies in north-central Montana. Three colonies were treated with deltamethrin to control fleas and limit the spread of plague; four untreated colonies served as controls. I monitored 412 plover nests during the 8 year study (264 on treatment colonies and 148 on control colonies) and found a strong negative effect of deltamethrin treatments on nest survival (bDust = -1.24, 95 % CI was -2.00 to -0.48) in the years following the actual treatment (2004–2006). I conclude that the observed treatment effect most likely occurred because of changes in insect (food) availability for the plover, and this in turn lowered nest survival because adults spent more time off nests or switched to less desirable insect prey. These results lend support to the need to consider the indirect effects of insecticide treatments on non-target species and suggest a potential conflict in current plague management strategies for prairie dogs.

**Other studies (not pesticide-related) showing importance of prairie dog colonies to mountain plover health:
(N5) Density and abundance of mountain plovers in Northeastern Montana
(N17) Mountain plover responses to plague in Montana

(N1) **Ecological Effects of Deltamethrin Insecticide in Prairie Dog Colonies** (M.S. Thesis – full text)
In North America, plague is a relatively novel disease, causing many species to be highly to moderately susceptible to infection. The plague bacterium, Yersinia pestis, is spread between mammals by fleas (Siphonaptera), and, during an outbreak, causes entire prairie dog (Cynomys spp.) colonies to die off. These outbreaks are of particular conservation concern as they have slowed the recovery of the endangered black-footed ferret (Mustela nigripes) which feeds almost entirely on prairie dogs. In many areas where black-footed ferrets have been reintroduced, prairie dog colonies are treated with deltamethrin, a pytheroid insecticide that reduces flea populations and therefore reduces the spread of plague. Although this treatment has been successful at increasing survival of ferrets and prairie dogs while preventing outbreaks of the disease, little is known about the secondary effects of this treatment. I aimed to determine the effects of deltamethrin of non-target flea and arthropod populations to assess impacts to small mammals present on prairie dog colonies. Fleas, insects, and small mammals were assessed on prairie dog colonies at Wind Cave National Park, SD and Custer State Park, SD across six pairs of treated and untreated grids. In both 2013 and 2014, four small mammal trapping sessions occurred resulting in the capture of 146 and 280 deer mice (Peromuscus maniculatus) and the collection of 167 and 67 fleas respectively. Mouse fecal samples were collected from captured mice to analyze the consumption of arthropods and pit fall traps were placed throughout grids and opened for 3 sessions annually to assess the abundance of arthropods on the grids. Deltamethrin was shown to reduce the likelihood of flea infestation in mice on grids treated with deltamethrin in 2013 but not 2014 when overall infestation was low. Due to the large amounts of insects collected, specimens are still being identified to family. Three major beetle families, Scarabaeidae, Tenebrionidae and Carabidae, were pulled from 2014 samples and tallied. Scarabaeidae beetles were found in reduced numbers on treated grids, before the second annual treatment but returned to similar number after treatment. iii Fecal sample analysis revealed that deer mice on treated and untreated grids did not vary in their consumption of arthropods in either year. Analysis of mouse survival and population in frequentist and Bayesian frameworks revealed minimal to no change in mouse populations between treated and untreated areas as a small decline was noted in one session from one analysis. Overall, deltamethrin has a positive impact by reducing the fleas present on small mammals with minimal and non-lasting negative effects on arthropod populations. These effects did not impact mouse populations present on treated areas, supporting the use of deltamethrin. However, deltamethrin should be used with caution as minor declines observed in arthropod and mouse populations could be magnified with repeated uses. To maximize the benefits deltamethrin should be applied in years of high flea populations while forgoing treatment in low population years to reduce any negative impacts.

(N12) **Indirect effects of the pyrethroid insecticide deltamethrin on reproductive success of chestnut-collared longspurs**
An experiment was conducted to determine whether spraying with a broad-spectrum pyrethroid insecticide in grassland habitat for the control of grasshoppers could affect nesting songbirds through the removel of insect food resources. Three 81 ha plots were sprayed at the recommended rate of Decis 5F (6.25 g deltamethrin ha−1). Paired control plots remained unsprayed. The density of (Acrididae) grasshoppers was monitored throughout the spring and summer. The nests of chestnut-collared longspurs (Calcarius ornatus) were monitored to determine the nest and nestling survival rates, size at fledging and food habits. Attributes of parental foraging were quantified. Food selection by parents and consumption by nestlings were measured using oesophageal ligatures. Grasshoppers accounted for >85% of the biomass of the nestling diet to spraying and this proportion increased throughout the season in unsprayed plots. Applications of Decis 5F initially reduced the grasshopper density by 93%. After spraying, parent birds

switched to other arthropod taxa less affected by insecticide application; the overall biomass fed to nestlings was not significantly reduced although the acridid proportion declined to <30%. The weight and skeletal size of the nestlings at fledging was unaffected. Parent birds in sprayed plots flew no further to feed their nestlings at a similar rate to that of birds in the control plots. The clutch size and nestling survival were similar between the sprayed and unsprayed plots after Decis 5F application, but egg success was lower in the sprayed plots compared to the control plots (67 versus 87%, $p < 0.05$)