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The Effects of Metolachlor Exposure in THP-1 Alveolar and Monocyte and Macrophage Cellular Functions

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Effects of Metolachlor Exposure on THP-1 Alveolar Monocyte and Macrophage Cellular Functions

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Introduction

- Analysis of Metolachlor
  - History
  - Chemistry
  - Application
  - Environmental Fates and Concerns
- Experimental Design (Objective I, II & III)
  - Procedure
  - Expectations
- Acknowledgments
Metolachlor: History

• Pre-Emergent Broad Spectrum Herbicide (1976)
  ▫ Used to control broadleaf plants and weeds
  ▫ Primarily agricultural or feed crops
    • Corn, Soybeans and Sorghum
  ▫ Alternatively
    • Ornaments, trees, shrubs, cotton, peanuts, etc.
Metolachlor: Chemistry

- **Chemical Activity**
  - Inhibits long-chain fatty acid synthesis
  - Unintended consequences uncertain

- **Character**
  - Primarily an odorless, clear to amber colored liquid
  - Can be found in granular forms
  - 21 known degradates (3)
Metolachlor: Chemistry

• Aliases
  ▫ Trade Names
    • Bicep®, CGA-24705®, Dual®, Pennant® and Pimagram®
  ▫ Alone or Herbicidal Cocktail (3)
    • Combined with atrazine, cyanazine and fluometuron
Metolachlor: Application

• Historically
  ▫ 60 million lbs. in U.S. annually \(^{(2)}\)

• Recommended Application
  ▫ Ground application
  ▫ Aerial, irrigation and chemigation

• Prohibited Applications
  ▫ Greenhouses and enclosed areas
  ▫ Peaty, sandy or loamy soils
  ▫ Fruit bearing trees or vines
  ▫ Grazing areas
Metolachlor: Environmental Fates

- **EPA Classifications**
  - **Soil**
    - Persistent to Moderately Persistent
    - Mobile to Highly Mobile
  - **Ground Water**
    - Primary source of exposure
    - Considerable contamination to ground water – found in over 20 states
      - .08 – 850 ppb found in various water sources (.078-849.03 μg/L)
  - **Air Contamination**
    - Volatilized – Ontario watershed findings ~5ng/L
    - Dust Contamination - ~50% of 39 homes in Iowa study had measurable levels
      - Improper PPE pre and post application
Metolachlor: Health Concerns

• Toxicty (EPA)
  ▫ Generally low level of toxicity in acute tests
  ▫ Toxicity Category III (oral and inhalation routes)
  ▫ Toxicity Category IV (eyes or skin)
• Animal testing - High levels of exposure
  ▫ Dogs - Low birth and body weight
  ▫ Rabbits- Increased liver and kidney size
  ▫ Rats - Carcinogenic- liver nodules and carcinomas in females
• Humans (New Jersey) - Correlation
  ▪ Low birth weights (2010)
Metolachlor: Health Concerns

• Symptoms of Metolachlor Poisoning
  ▫ High levels of exposure
    • Eye or skin irritation, cramps, shortness of breath, weakness, sweating and diarrhea
  ▫ Prolonged exposure
    • Anemia, hypoxemia, convulsions and jaundice
Experimental Questions

• Effects on human alveolar leukocytes
  - Inhibition of normal cellular function
  - Provocation of an erratic function

• Specific Function
  - How will metolachlor effect cells?
    • Phagocytosis?
    • Apoptosis?
    • Necrosis?
Experimental Outline

- **Objective I: Monocyte/Macrophage Phagocytosis Assay**
  - Measuring the effects of Metolachlor on human alveolar monocytes and macrophages via flow cytometry
- **Objective II: Apoptosis Assay**
  - Measuring possible effects of Metolachlor on the apoptotic pathway of monocytes and macrophages
- **Objective III: Reactive Oxygen Species (ROS) Assay**
- (Will not be discussed)
Objective I: Phagocytosis Basics

• THP-1 Cells
  ▫ Human monocytic lineage
  ▫ Derived from a 1 year old human male with acute leukemia patient
  ▫ Immunohistochemistry
Objective I: Phagocytosis Basics

- Monocytes
  - Develop in bone marrow and migrate to various body tissues
  - Alveolar Monocyte
    - Immune Defense
      - 1st line of contact (sentinel cells)
    - Phagocytosis
    - Inflammation
Objective I: Phagocytosis Basics

- Macrophages
  - Mature monocytes
  - Phagocytosis
  - Cytokine secretion
  - Migration – lymph nodes (acquired immunity)
Objective I: Phagocytosis Tools

- LPS
  - Lipopolysaccharide
    - Activates cellular function
- PMA
  - Phorbal Myristate Acetate
    - cellular activation (differentiation)
- FITC labeled latex beads
  - Fluorescein isothiocyanate tags and Rabbit IgG
Objective I: Phagocytosis Design

- Cells maintained in media @ 37° C
- Control Group vs LPS Group (~1 x 10^6)
  - Metolachlor exposures at 50ppb, 100ppb, 500ppb and 1,000ppb along with a positive and negative control
  - Three time trials
    - 24, 48 and 72 hours
- Differentiated trials
  - FITC labeled beads
    - Added 24 hours from completion of trial
    - Cells subjected to assay treatment
    - Cells washed and suspended in assay buffer
Objective I: Flow Cytometry

- Cells subjected to flow cytometer
  - Cells are funneled through one at a time
  - Laser passed through cell
    - Fluorescent tag
    - Forward and side scattered light
    - Recorded data
  - Allows the determination of FITC presence
    - Presence indicates the cells with normal fxn
    - Standard level comparison
Objective II: Apoptosis Basics

- Apoptosis is the process of highly regulated cellular death
  - ~ 50-70 billion cells die everyday in an adult human
  - (of ~ 37.2 trillion)
  - Apoptosis promotes normal development
    - Homeostasis
    - Counterpoint to cell proliferation
    - Can remove any unwanted or damaged cells

- Necrosis is the process of premature cell death
  - Caused by outside factors
    - Severe damage, toxins, infections
    - Inflammatory response that can block phagocytic fxn
    - Can damage surrounding tissues
Objective II: Apoptosis Basics

- Phosphatidylserine
  - An important phospholipid found in cells
  - Oriented towards the cytosolic side of cellular membrane

- Initiation of apoptosis
  - PS is acted upon by flippase
  - Reverse orientation and signal macrophages
Objective II: Apoptosis Basics

- Apoptosis assay
  - Exploitation of the presence phosphatidylserine
  - PS binds to Annexin-V stain
    - Annexin-V is conjugated with a fluorochrome
  - Propidium Iodide
    - Reacts with nucleic acid
    - Leaky cell membranes
      - Necrosis or late apoptosis
- Florescence is detectable via flow cytometry
Objective II: Apoptosis Design

- Cells maintained in media in incubator 37°C
- Control Group vs LPS Group (~1 x 10^6)
  - Metolachlor exposures at 50ppb, 100ppb, 500ppb and 1,000ppb along with a positive and negative control
  - Three time trials
    - 24, 48 and 72 hours
  - Annexin-V exposure (PI)
    - Cells incubated for 15 minutes in darkness, 37°C
    - Cells washed, centrifuged and suspended
    - Subjected to flow cytometer for quantitation
Measures of Significance

- Data Sets
  - 6 experiments per trial
    - 3 replicates
  - Average Experimental Mean
    - Normalized due to cell/cell variation
    - Comparison using ANOVA
    - $\alpha=.05$
    - P-value and significance
    - Post-hoc analysis (Tukey)
Expected Results

- It is likely that Metolachlor will affect function in these cells
  - Specifically, I think that it will impair normal function
  - Higher levels of exposure
    - Phagocytosis
    - Apoptosis - uncertain

- Previous studies, readings and initial experimental results
Accolades Thus Far

- Dr. David McClanahan
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- Dr. Darrell Wiens
- Yutao (Max) Su
Citations

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