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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

Presented by

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Advised By

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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

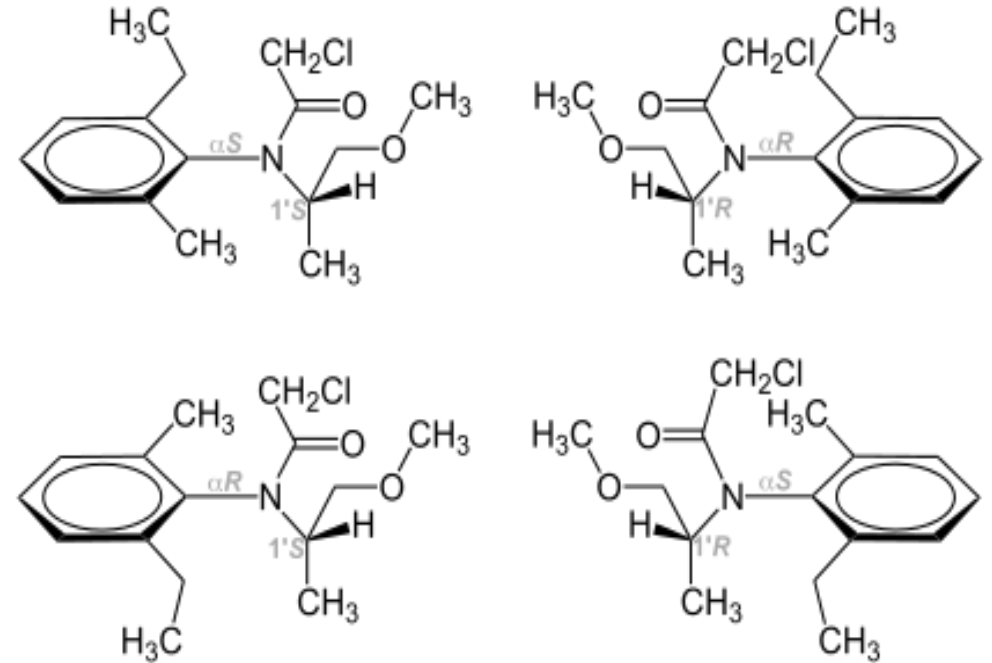
- Pre-Emergent Broad Spectrum Herbicide(1976)
 - Used to control broadleaf plants and weeds
 - Primarily agricultural or feed crops
 - Corn, Soybeans and Sorghum
 - Alternatively
 - Ornamentals, trees, shrubs, cotton, peanuts, etc.



www.epa.gov

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⁾



www.wikipedia.com

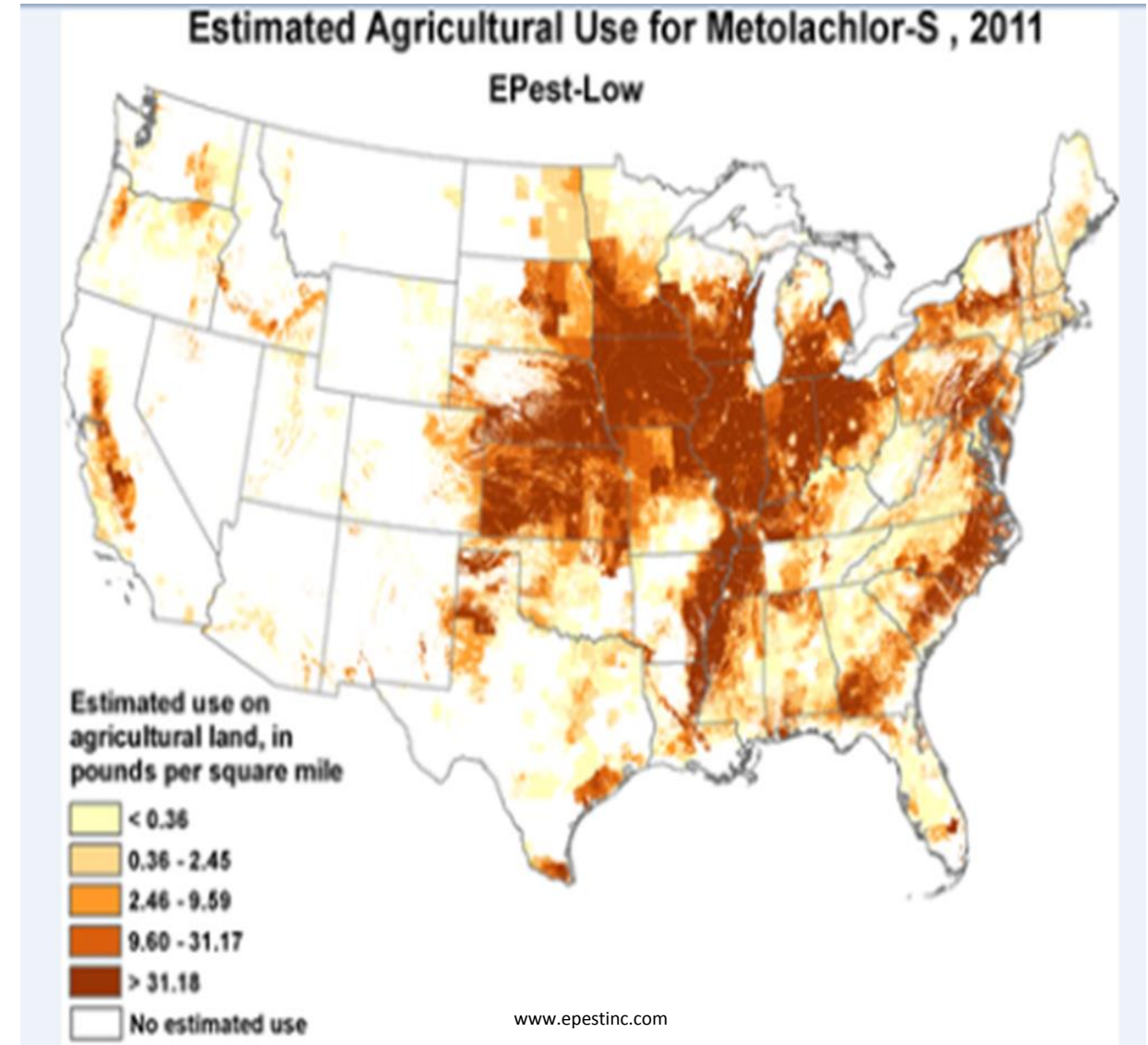
Metolachlor: Chemistry

- 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

- Toxicity (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?

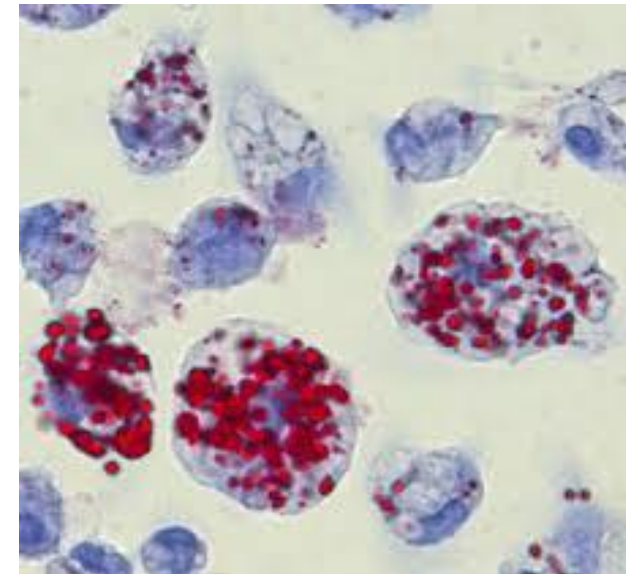


Experimental Outline

- 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)

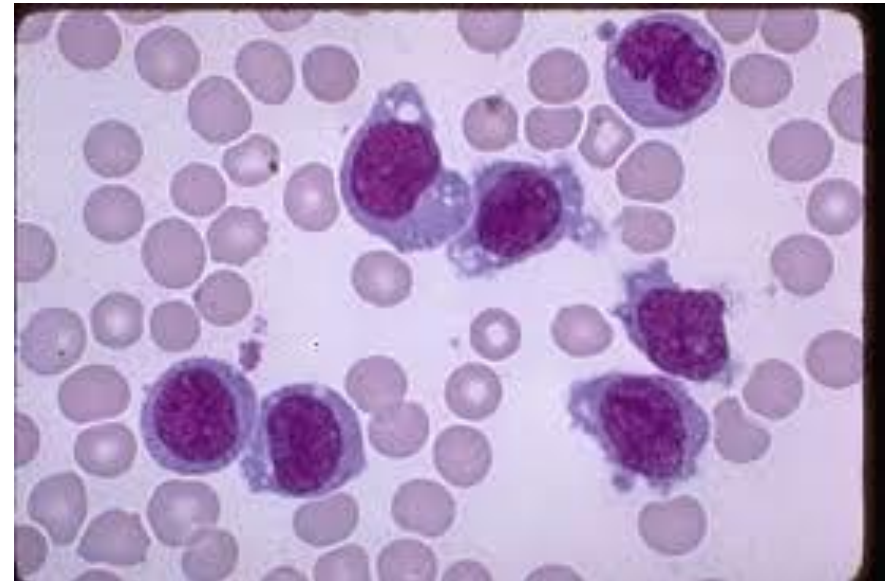
Objective I: Phagocytosis Basics

- THP-1 Cells
 - Human monocytic lineage
 - Derived from a 1 year old human male with acute leukemia patient
 - Immunohistochemistry



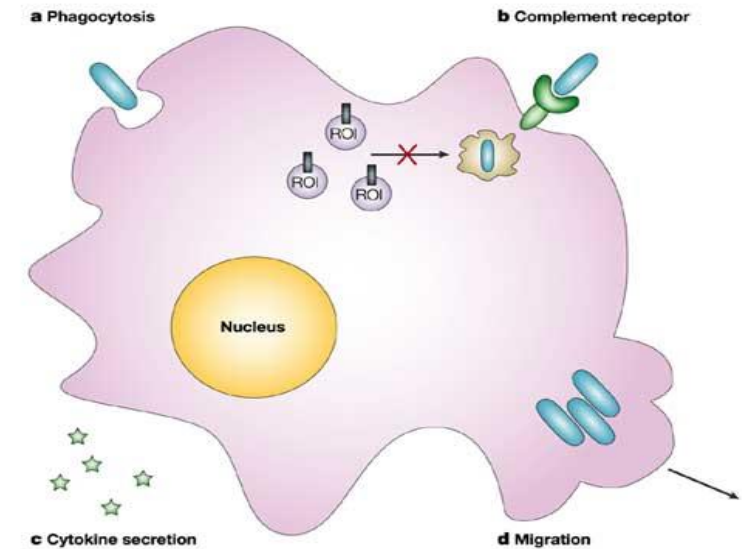
Objective I: Phagocytosis Basics

- 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
 - Phorbol Myristate Acetate
 - cellular activation (differentiation)
- FITC labeled latex beads
 - Fluorescein isothiocyanate tags and Rabbit IgG

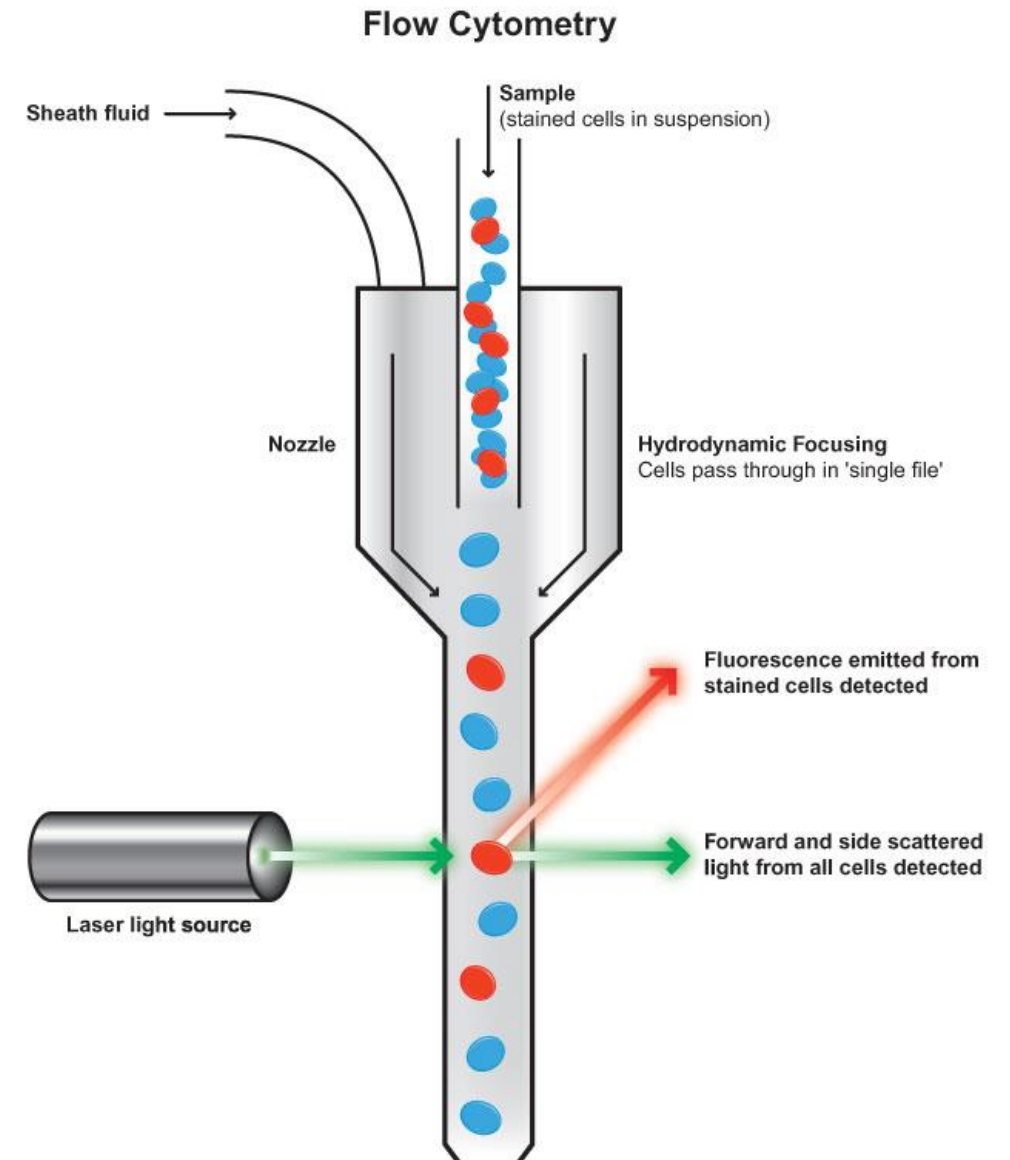
Objective I: Phagocytosis Design

- Cells maintained in media @ 37° C
- Control Group vs LPS Group ($\sim 1 \times 10^6$)
 - 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



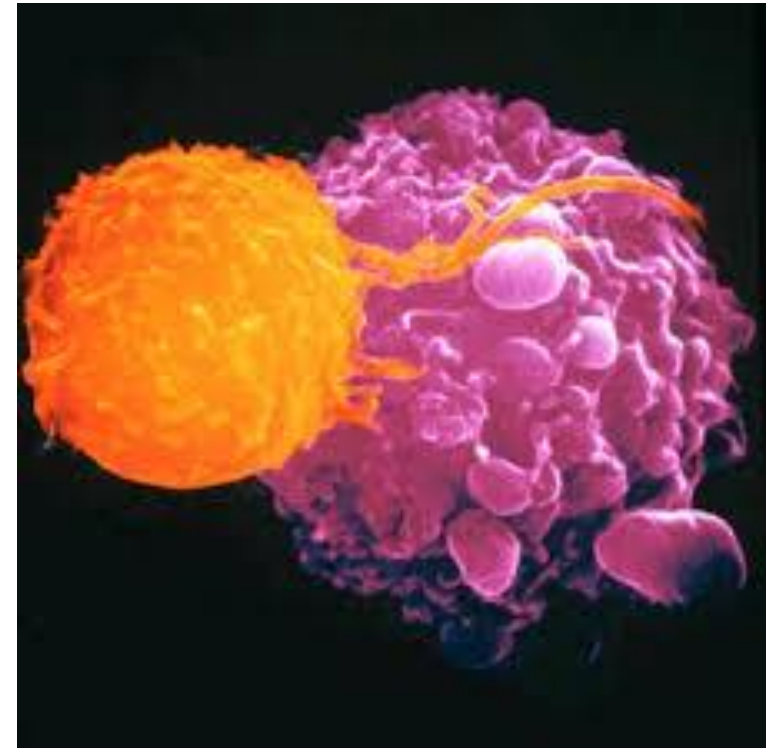
Objective I: Flow Cytometry

- 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



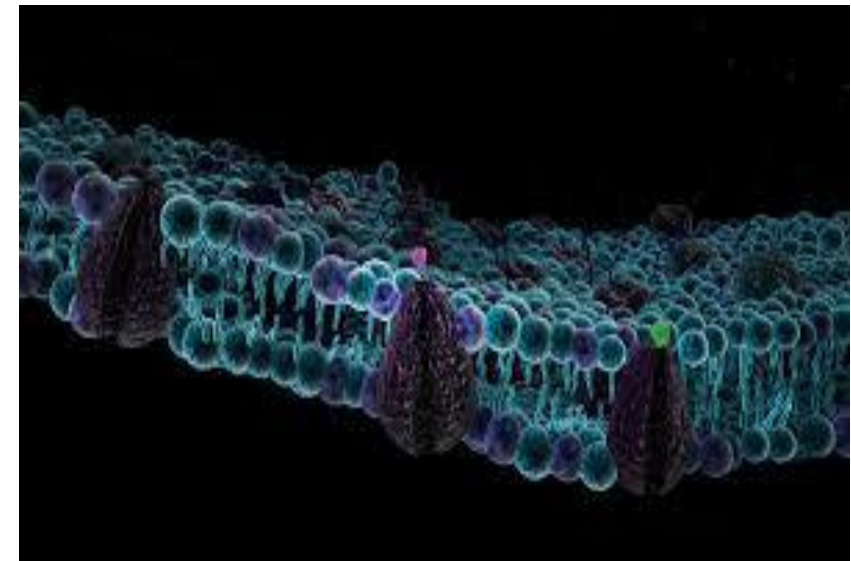
Objective II: Apoptosis Basics

- 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



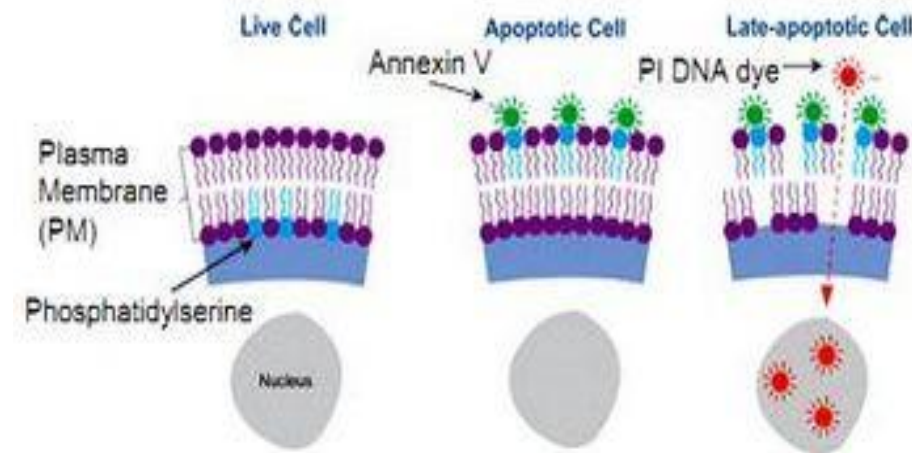
Objective II: Apoptosis Basics

- 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



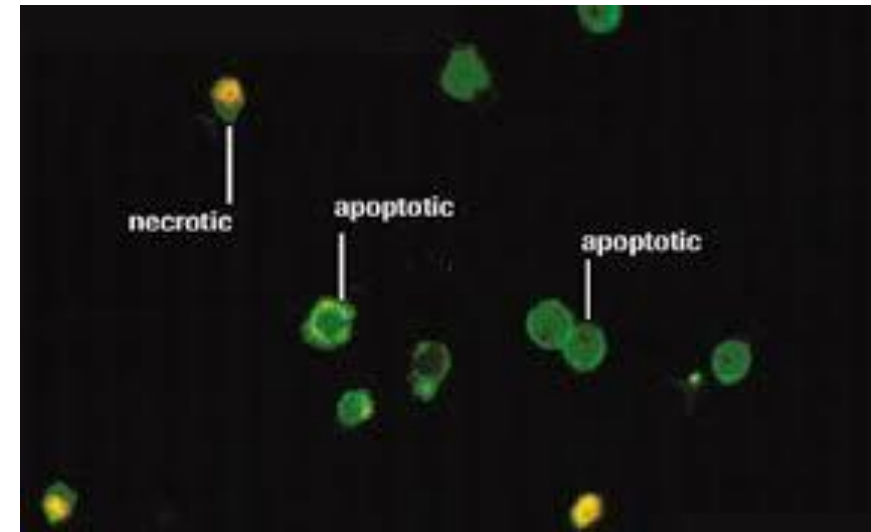
Objective II: Apoptosis Basics

- 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



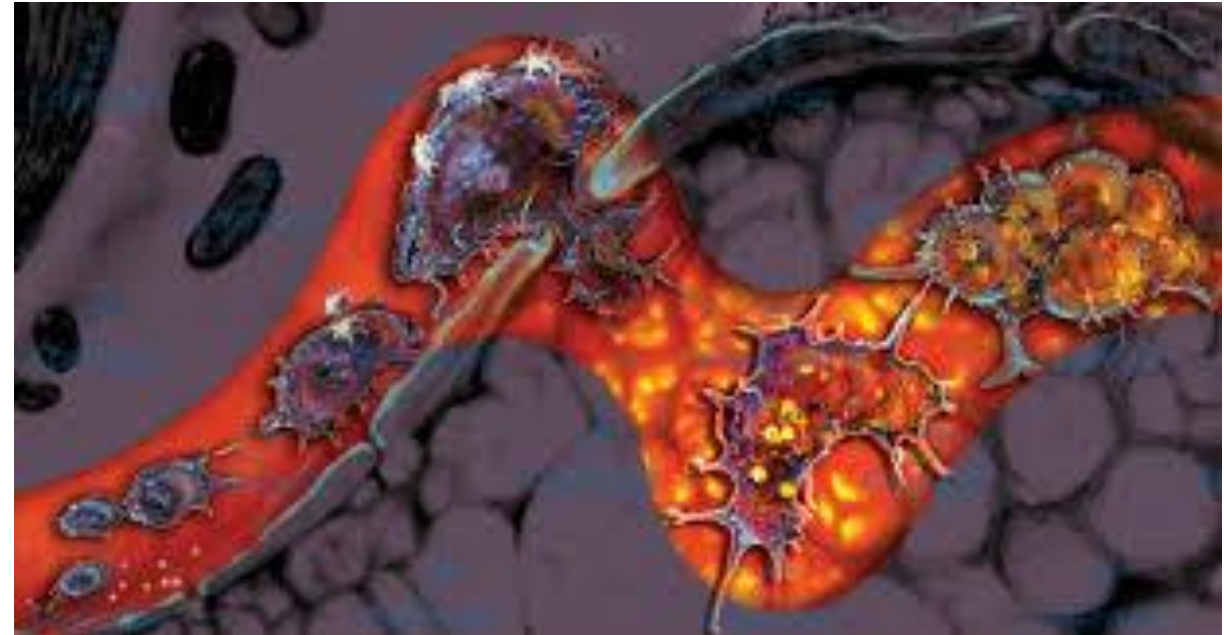
Objective II: Apoptosis Design

- Cells maintained in media in incubator 37° C
- Control Group vs LPS Group ($\sim 1 \times 10^6$)
 - 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 McClenahan
- Dr. Kavita Dhanwada
- Dr. Darrell Wiens
- Yutao (Max) Su

Citations

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