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Exploring Pesticide Effects on Hematopoiesis and the Thymus

Jada Mari C. Young and Jennifer O. Manilay, PhD

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Exploring Pesticide Effects On Hematopoiesis and the Thymus

Jada Mari C. Young and Jennifer O. Manilay, PhD
School of Natural Sciences, University of California, Merced

Abstract

INTRODUCTION: Hematopoiesis in the bone marrow (BM) produces red blood cells, platelets, or various white blood cells. Common lymphocyte progenitors in the BM can migrate to the thymus to form T lymphocytes, a type of immune cell. In some cases, bone marrow failure (BMF) arises from impairments in hematopoiesis and results in the inability to produce necessary blood cells. California's Central Valley has a high exposure to pesticides due to agriculture. Past research shows correlations between leukemia and high pesticide exposure, but surprisingly, there has been little published research regarding the direct effects of pesticides on BMF. This study aims to use mouse models to aid our understanding of the molecular effects of two pesticides, abamectin and pyraclostrobin, on hematopoiesis. In previous studies, abamectin led to weight loss while pyraclostrobin led to weight gain.

HYPOTHESIS: We hypothesize that changes in the BM due to pesticide exposure may result in lower numbers of T lymphocytes.

METHODS: We exposed 8-week-old C57BL/6 mice to pyraclostrobin or abamectin for 14 days via intraperitoneal injections and monitored their health with routine weighing and complete blood cell analysis using a Hemavet cell counter. After 14 days, we collected BM and spleen cells for flow cytometric analysis on a ZE5 Cell Analyzer and the thymus for histology.

RESULTS: We expect to see a decrease in T lymphocytes in the periphery and impairments in the thymus structure.

CONCLUSION: For future work, this study hopes to uncover underlying mechanisms of BMF, possible disease mitigation strategies, and encourage safer policies for pesticide use.

Research Question

1. How do pesticides affect hematopoiesis and T lymphocytes in the bone marrow?
2. What changes will occur on the thymus when exposed to pesticide?

Background

Hematopoiesis and T-Cell Maturation

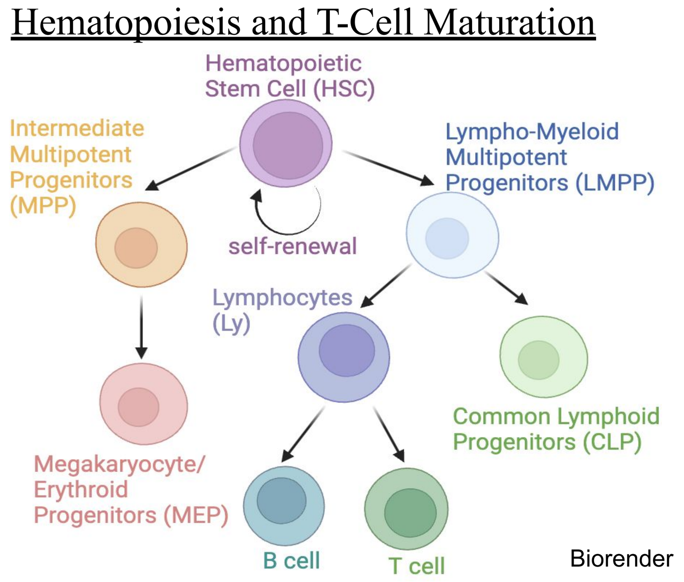

Figure 1. *Hematopoiesis Pathway*[2]

- HSCs can either self-renew or follow the MPP pathway or LMPP pathway.
- T cells are derived along the LMPP pathway, specifically through the CLPs.
- T cell precursors (CLP) develop in the BM or fetal liver, which migrate to the thymus for maturation (CD4 helper or CD8 cytotoxic).
- CD8 T cells secrete cytokines and kill pathogens. If a T cell expresses high self-reactivity, it undergoes apoptosis or become Tregs[1]

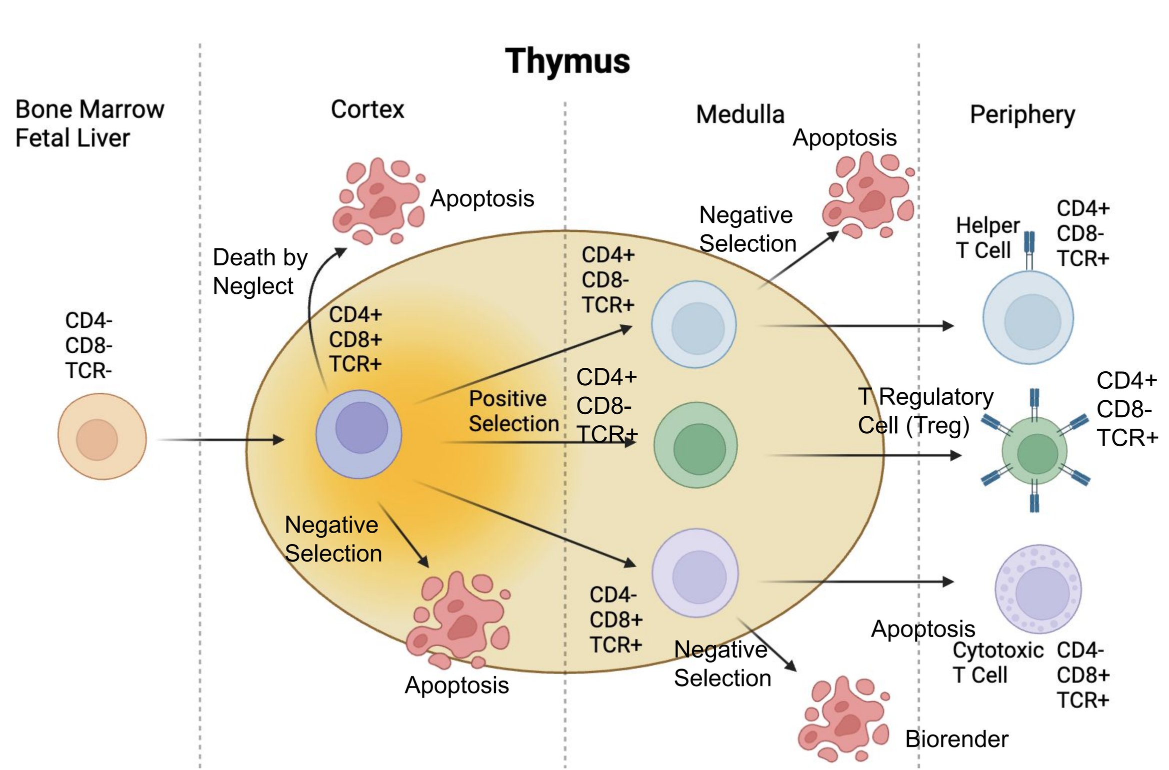

Figure 2. *T-Cell Maturation Pathway*

Methods

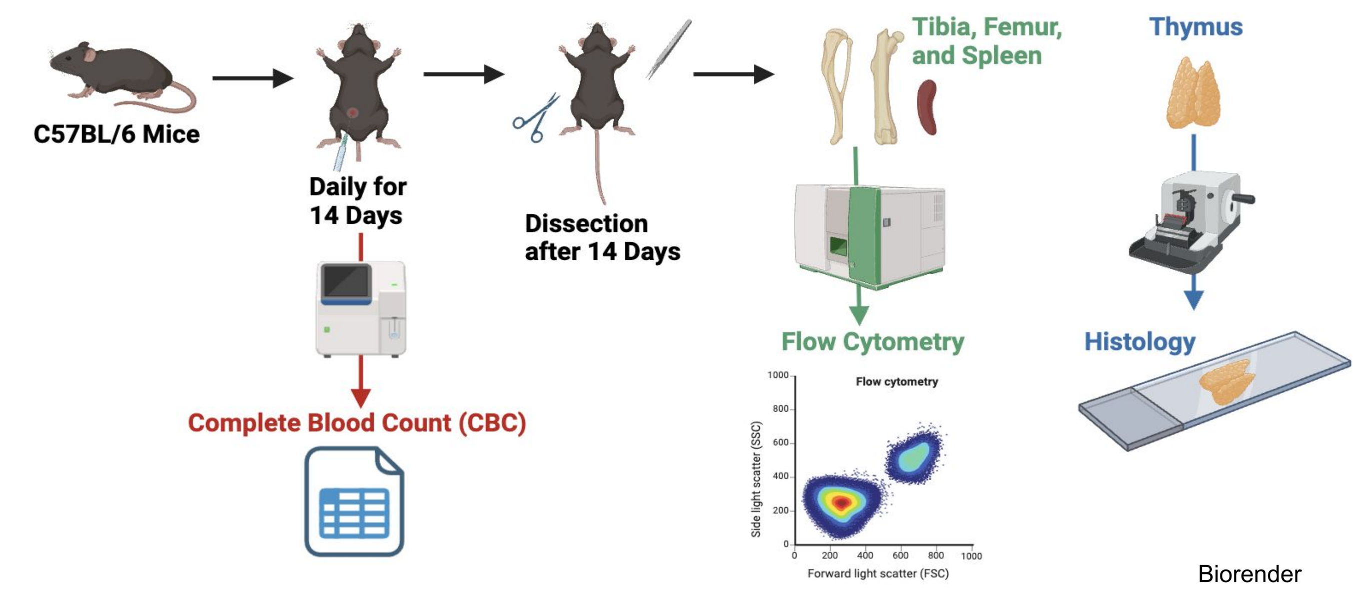

Figure 3. *Overview of Methods*

Pesticide Effect on Bone Marrow, Weight, and the Thymus

- High pesticide usage leads to public health concerns due to pesticides' effects; BM is sensitive to pesticide effects and can lead to hypoplastic/aplastic marrow failure[3]
- **Abamectin** (insecticide) leads to weight loss[5] with unknown effects on the thymus
- **Pyraclostrobin** (fungicide) leads to weight gain due to triglyceride accumulation and blocking of Electron Transport Chain (ETC) III[6]
  - Arises from mitochondrial dysfunction, leading to metabolic syndrome
  - Leads to atrophy in the thymus, specifically a decrease in size[4]

Overview of Complete Blood Count (CBC), Flow Cytometry, and Histology

- **CBC** to analyze changes in blood cell parameters, such as white blood cells
- **Flow cytometry** to analyze cell populations in the bone marrow
- **Histology** to analyze anatomical changes in the thymus, a primary lymphoid organ consisting of two lobes.

Results

- Independent-samples T-test was used to examine Lymphocyte Percentage differences between mice in control groups, and mice in pyraclostrobin or mice in abamectin groups
- Since this is a pilot experiment and doesn't have sufficient repeat experiments, it was decided that p values less than 0.1 but approaching 0.05 are statistically significant

Pyraclostrobin

- There were significant differences in the lymphocyte % between mice in the control group and the pyraclostrobin group at Day 14, p = 0.047
- Day 0 has been disregarded since no significant changes should occur on the first day of treatment

Table 1. *CBC Data for Pyraclostrobin BM, Lymphocyte Percentage*

		n	Mean	p
Day 0	Control	4	51.87	0.077
	Pyraclostrobin	5	58.11	
Day 14	Control	4	66.32	0.047**
	Pyraclostrobin	5	61.36	

Note. ** p < 0.05

- There were significant differences between CD4+ CD8- control and pyraclostrobin treated groups, p = 0.026
- There were significant differences between CD4- CD8- control and pyraclostrobin treated groups, p = 0.086

Table 2. *FACS Data for Pyraclostrobin BM*

	Control		Pyraclostrobin		
	Mean	SD	Mean	SD	p
T-Cells	6.99E+05	8.78E+04	5.21E+05	1.18E+05	0.173
CD4+ CD8-	1.52E+05	5.56E+03	1.01E+05	1.60E+05	0.026**
CD4- CD8-	1.36E+05	1.71E+04	1.12E+05	4.27E+05	0.086**
CD4- CD8+	3.08E+05	9.34E+03	2.07E+05	1.23E+05	0.352

Note. ** p < 0.1

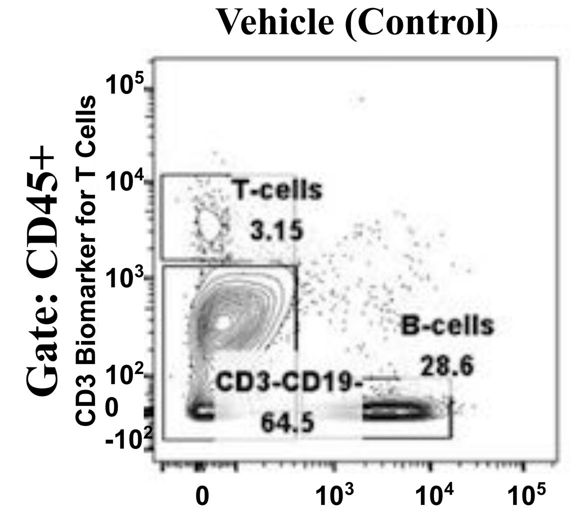

Figure 4. *Flow Plot of CD45+ in the Vehicle Group*

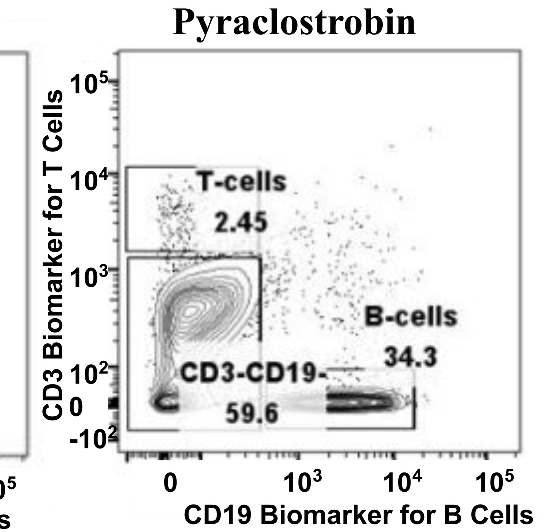

Figure 5. *Flow Plot of CD45+ in the Pyraclostrobin Group*

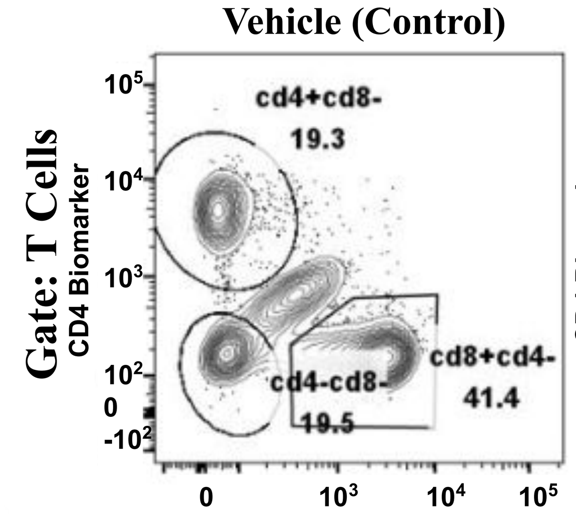

Figure 6. *Flow Plot of T Cells in the Vehicle Group*

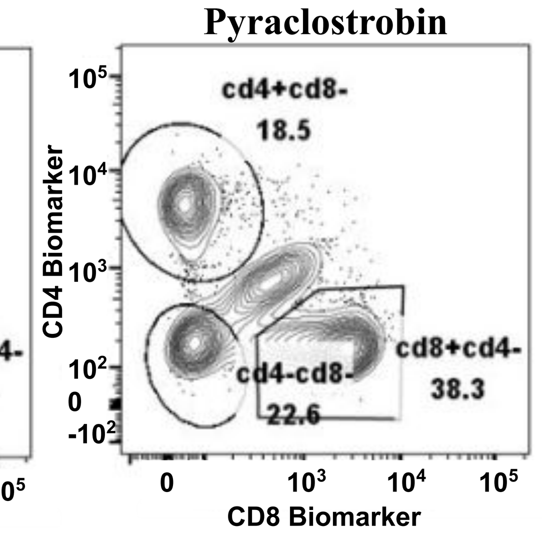

Figure 7. *Flow Plot of T Cells in the Pyraclostrobin Group*

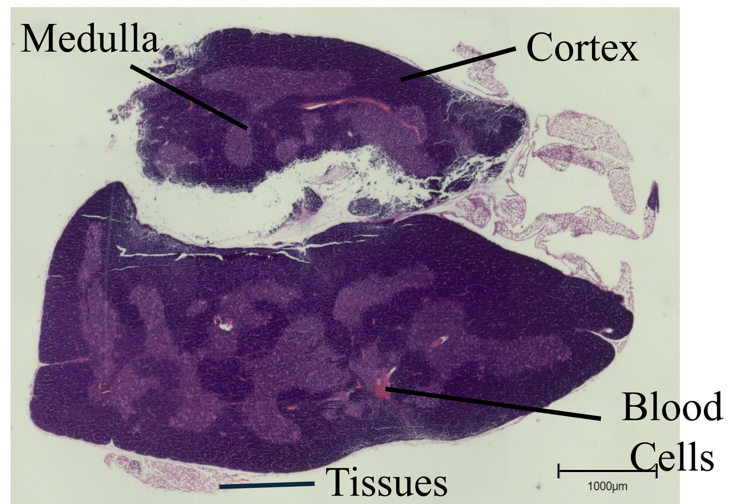

Figure 8. *Thymus 10, Control Group, 4X magnification*

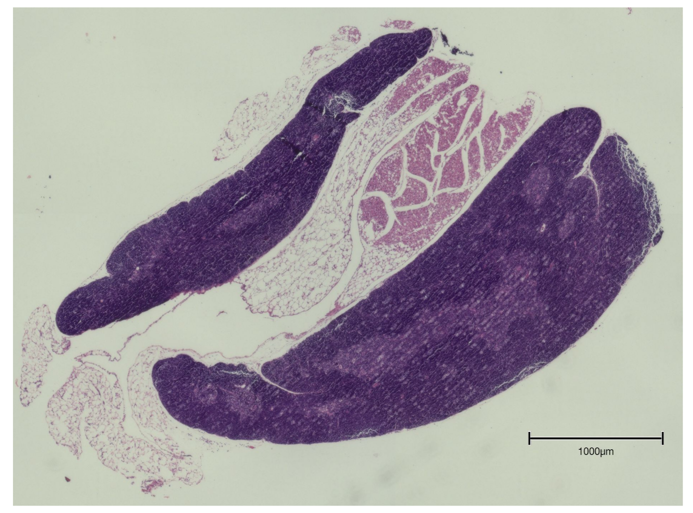

Figure 9. *Thymus 30, Pyraclostrobin Group, 4X Magnification*

- Pyraclostrobin-treated thymus is smaller

Abamectin

- There were no significant differences between the control group and the abamectin treated groups
- Abamectin-treated thymus were difficult to cut
  - Unsure about specific mechanisms leading to difficulty in cutting

Table 3. *CBC Data for Abamectin BM, Lymphocyte Percentage*

		n	Mean	p
Day 0	Control	4	56.08	0.358
	Abamectin	5	58.57	
Day 14	Control	4	48.77	0.134
	Abamectin	5	55.18	

Note. ** p < 0.05

Table 4. *FACS Data for Abamectin BM*

	Control		Abamectin		
	Mean	SD	Mean	SD	p
T-Cells	7.31E+05	2.93E+05	1.69E+07	2.77E+07	0.493
CD4+ CD8-	1.82E+05	6.94E+04	2.51E+06	4.05E+06	0.497
CD4- CD8-	1.94E+05	4.57E+04	6.57E+06	1.09E+07	0.492
CD4- CD8+	2.72E+05	2.02E+05	5.61E+06	9.20E+06	0.493

Note. ** p < 0.1

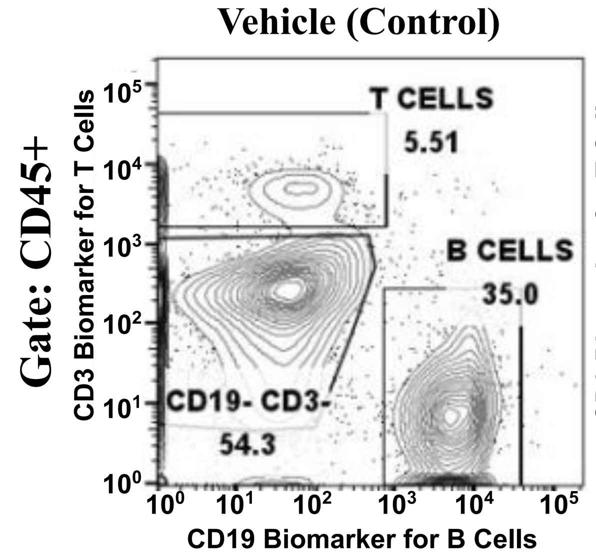

Figure 10. *Flow Plot of CD45+ in the Vehicle Group*

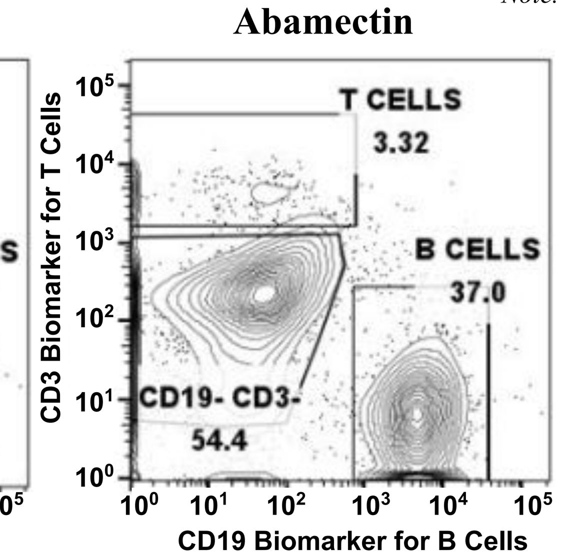

Figure 11. *Flow Plot of CD45+ in the Abamectin Group*

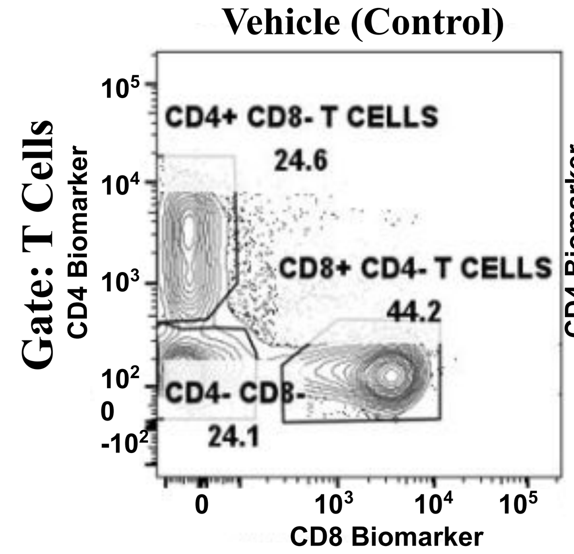

Figure 12. *Flow Plot of T Cells in the Vehicle Group*

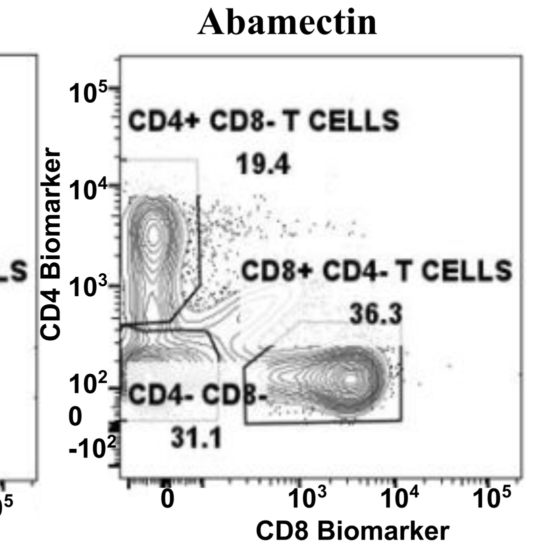

Figure 13. *Flow Plot of T Cell in the Abamectin Group*

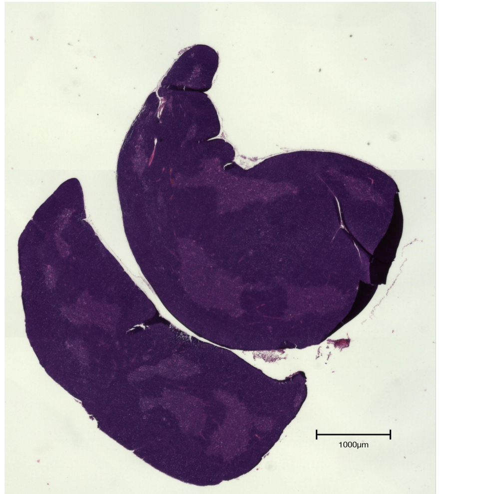

Figure 14. *Thymus 1, Control Group, 4X magnification*

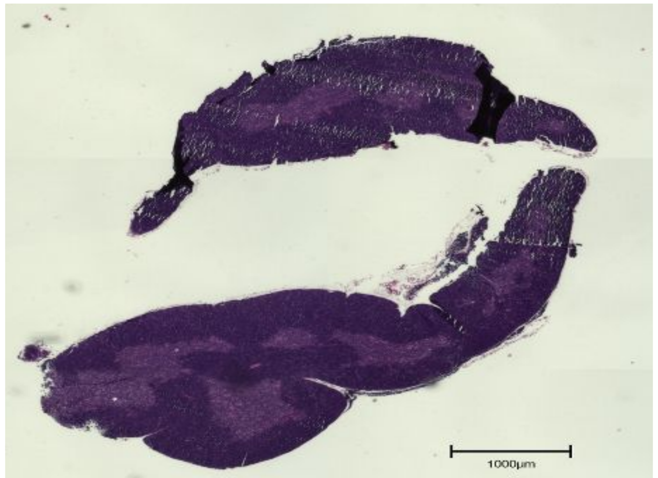

Figure 15. *Thymus 30, Abamectin Group, 4X Magnification*

Conclusion

- Pyraclostrobin CBC data shows a decrease in lymphocytes at day 14 and statistically significant (p < 0.05)
- Abamectin CBC data at day 14 shows no lymphocyte changes
- By using flow cytometry, specific T cell populations are determined
  - CD4+ CD8- and CD4- CD8- T cell populations in BM were decreased in pyraclostrobin-treated mice
- Longer exposure may be required to observe changes in other parameters/cell populations

Future Directions

- Use same experimental setup with different pesticides and/or different aged mice
- Use different exposure method instead of injections (i.e., dermal)
- Perform a more in-depth experiment on the effects of abamectin or pyraclostrobin on mice, such as longer exposure
- Quantify the thymus subsets by FAC
- Enumerate and test function of Th1, Th2, Th17 and Tregs in the peripheral immune organs

References

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