PQRI Workshop:
TiO2 Use in Pharmaceuticals
Global Regulatory and Technical Challenges
June 13-14, 2023

Institute for Integrative Toxicology
MICHIGAN STATE UNIVERSITY

Evaluation of the Immunologic and Intestinal Effects of Dietary E 171 (Food Grade Titanium Dioxide) Consumption

Lance K. Blevins, Ph.D.

Institute for Integrative Toxicology

Michigan State University
East Lansing, MI, USA

blevin24@msu.edu

Disclosures

Research Support

Michigan State University
(Center for Research on Ingredient Safety)

Titanium Dioxide Manufacturer's Associate

Grocery Manufacturer's Association

International Color Manufacturer's Association

Presentation Outline

- Brief background on TiO2
- Briefly comment on recent policy and the science behind it
- Presentation of our findings
- Provide some final thoughts

Toxicology of TiO2

Toxicity is highly dependent on route of exposure

- Respiratory – possible human carcinogen (IARC Group 2B)
- Dermal - No known toxicity
- Oral – Controversial?

  National Toxicology Program – 2 yr dietary doses as high as 50,000 ppm (5% of diet) no preneoplastic or neoplastic lesions (1979).

  European Food Safety Authority bans the use of E 171 in food in 2021

In the US, the **dietary** intake of **TiO2** is **estimated** to be 1-2 mg/kg body weight per day for children and 0.2-0.7 mg/kg body weight per day for other age groups

Summary of EFSA Scientific Opinion (March 25, 2021)

- E 171 <50% of constituent particles <100 nm.
- Particles <30 nm amount to less than 1% of particles by number in E 171.

Therefore, studies with particles <30 nm were considered of limited relevance.

- GI absorption of E171 particles is **low** but may accumulate in the body.
- Studies on general organ toxicity did not indicate adverse effects with either E 171 up to a dose of 1000 mg/kg bw/day or TiO2 NP (30 nm) up to the highest dose of 100 mg/kg/day.
- Observation of **potential** immunotoxicity and inflammation with E 171 and **potential** neurotoxicity with TiO2 NP together with the **potential** of aberrant crypt foci with E 171, may indicate adverse effects.

Summary of EFSA Scientific Opinion (March 25, 2021)

- Panel concluded that TiO2 particles have **potential** to induce DNA strand breaks and chromosomal damage but not gene mutations.
- "**No** clear correlation was observed between the physico-chemical properties of TiO2 particles and the outcome of either in *in vitro* or *in vivo* genotoxicity assays. A concern of genotoxicity of TiO2 particles that may be present in E 171 could therefore **not** be ruled out."
- "**No** appropriately designed study was available to investigate the potential carcinogenic effects."

Conclusion

"Based on all the evidence available, a concern for genotoxicity could not be ruled out and given the many uncertainties, the Panel concluded that E 171 can no longer be considered as safe when used as a food additive."

LA Times + Follow

California bill targeting 'toxic' chemicals in Skittles, other snacks passes first hurdle

Story by Vanessa Arredondo • Yesterday 7:01 PM

ABC 7 Los Angeles + Follow

CA Assembly approves bill to ban key ingredients in Skittles, other foods and candy

Story by ABC7.com staff • Tuesday

DailyMail.com

First-of-its-kind junk food ban moves one step closer in California: State assembly passes bill to axe cancer-linked chemicals found in Skittles and Sour Patch Kids

- If signed by Gov Newsom, the additive ban would be the first of its kind in the US
- Food manufacturers would have until January 2025 to make ingredient changes
- READ MORE: Risky food additives deemed too risky in the EU but not in the US

What is the Science that is Driving Health Concerns of TiO2 (E 171)

Urrutia-Ortega et al. 2016	Increased tumors by E 171 in mice in colitis cancer model (azoxymethane + DSS), decreased colonic goblet cells; decreased colonic IL-2, **TNFa**, IFNg, **IL-10**, GM-CSF	Administered in **water**; single dose (5 mg/kg/day 5 d/wk gavage)
Bettini et al. 2017	Immunologic alterations and increased aberrant crypt foci (ACF) by E 171 following treatment of rats with DNA reactive intestinal carcinogen dimethylhydrazine (DMH).	Administered in **water**; two doses 200 µg/kg, 10 mg/kg; 7 or 100 d)
Blevins et al. 2019	No immune alterations and no ACF or tumors due to E 171 after treatment of rats with or without DMH	Administered in **diet**; three doses (40,400, 5000 ppm; 7 or 100 d)
Talamini et al. 2019	Increase in inflammatory markers by E 171 in IL-1b in stomach and intestine; increase in circulating IL-6 (mice)	Administered in **water**; single dose (5 mg/kg/day 3 d/wk oral)
Pinget et al. 2019	Reduced colonic mucin 2 gene, increase CD8 T cells and macrophages, IFNg and IL-17 mRNA by E 171 in mice	Admin. in **water**; three dose (2, 10, 50 mg/kg/day; drinking water)
Han et al. 2020	Trend toward decreased circulating GM-CSF and IgM by E 171 in mice	Administered in **water**; three dose (10, 100, 1000 mg/kg; 90 d gavage)

Method of Sonication can Significantly Affect Dispersion and Size Distribution of TiO2 Nanoparticles

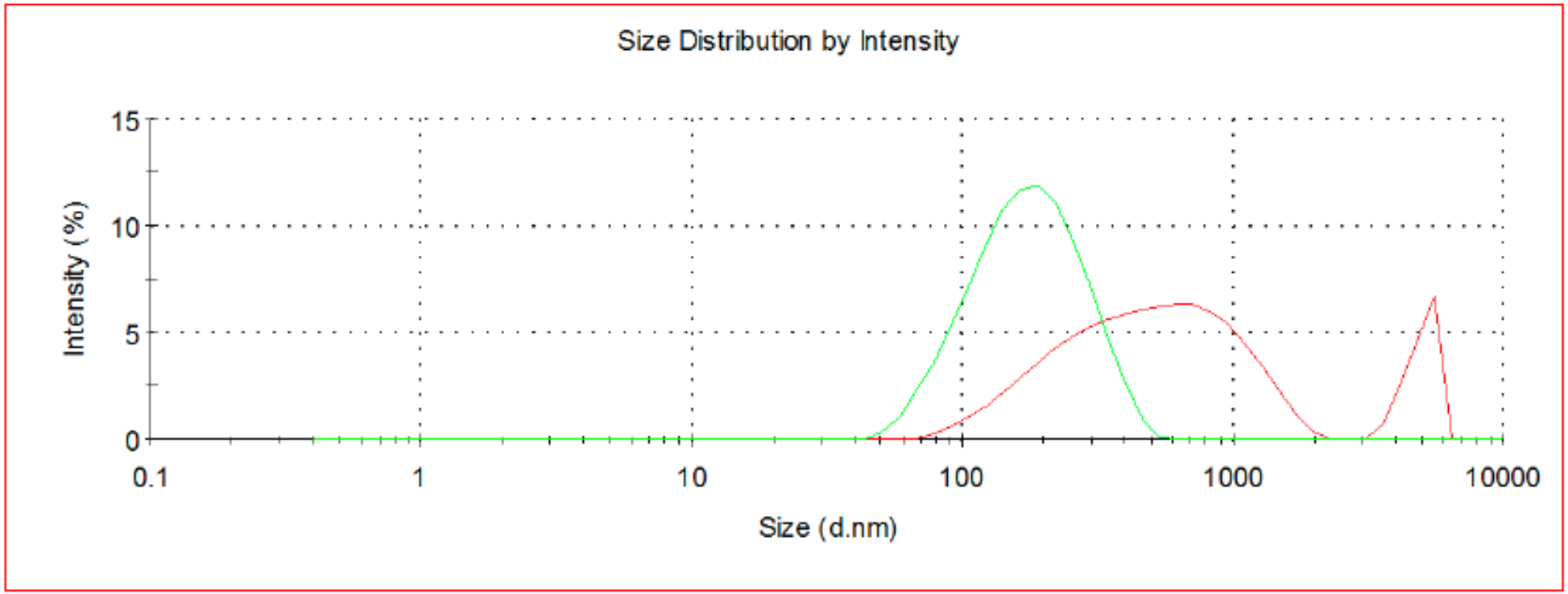


Figure 30. Comparison of DLS size distribution by intensity for NM-105 dispersed in MilliQ-water by using ultrasonic bath (red) and ultrasonic tweeter (green).

JRC Science and Policy Report: Titanium Dioxide, NM-100, NM-101, NM-102, NM-103, NM-104, NM-105,: Characterisation and Physico-Chemical Properties (European Commission 2014)

What is the Science that is Driving Health Concerns of TiO2 (E 171)

Urrutia-Ortega et al. 2016	Increased tumors by E 171 in mice in colitis cancer model (azoxymethane + DSS), decreased colonic goblet cells; decreased colonic IL-2, **TNFa**, IFNg, **IL-10**, GM-CSF	Administered in **water**; single dose (5 mg/kg/day 5 d/wk gavage)
Bettini et al. 2017	Immunologic alterations and increased aberrant crypt foci (ACF) by E 171 following treatment of rats with DNA reactive intestinal carcinogen dimethylhydrazine (DMH).	Administered in **water**; two doses 200 µg/kg, 10 mg/kg; 7 or 100 d)
Blevins et al. 2019	No immune alterations and no ACF or tumors due to E 171 after treatment of rats with or without DMH	Administered in **diet**; three doses (40,400, 5000 ppm; 7 or 100 d)
Talamini et al. 2019	Increase in inflammatory markers by E 171 in IL-1b in stomach and intestine; increase in circulating IL-6 (mice)	Administered in **water**; single dose (5 mg/kg/day 3 d/wk oral)
Pinget et al. 2019	Reduced colonic mucin 2 gene, increase CD8 T cells and macrophages, IFNg and IL-17 mRNA by E 171 in mice	Admin. in **water**; three dose (2, 10, 50 mg/kg/day; drinking water)
Han et al. 2020	Trend toward decreased circulating GM-CSF and IgM by E 171 in mice	Administered in **water**; three dose (10, 100, 1000 mg/kg; 90 d gavage)

What is the Science that is Driving Health Concerns of TiO2 (E 171)

Urrutia-Ortega et al. 2016	Increased tumors by E 171 in mice in colitis cancer model (azoxymethane + DSS), decreased colonic goblet cells; decreased colonic IL-2, **TNFa**, IFNg, **IL-10**, GM-CSF	Administered in **water**; single dose (5 mg/kg/day 5 d/wk gavage)
Bettini et al. 2017	Immunologic alterations and increased aberrant crypt foci (ACF) by E 171 following treatment of rats with DNA reactive intestinal carcinogen dimethylhydrazine (DMH).	Administered in **water**; two doses 200 µg/kg, 10 mg/kg; 7 or 100 d)
Blevins et al. 2019	No immune alterations and no ACF or tumors due to E 171 after treatment of rats with or without DMH	Administered in **diet**; three doses (40,400, 5000 ppm; 7 or 100 d)
Talamini et al. 2019	Increase in inflammatory markers by E 171 in IL-1b in stomach and intestine; increase in circulating IL-6 (mice)	Administered in **water**; single dose (5 mg/kg/day 3 d/wk oral)
Pinget et al. 2019	Reduced colonic mucin 2 gene, increase CD8 T cells and macrophages, IFNg and IL-17 mRNA by E 171 in mice	Admin. in **water**; three dose (2, 10, 50 mg/kg/day; drinking water)
Han et al. 2020	Trend toward decreased circulating GM-CSF and IgM by E 171 in mice	Administered in **water**; three dose (10, 100, 1000 mg/kg; 90 d gavage)

SCIENTIFIC REPORTS

OPEN

Received: 13 June 2016
Accepted: 06 December 2016
Published: 20 January 2017

Food-grade TiO_2 impairs intestinal and systemic immune homeostasis, initiates preneoplastic lesions and promotes aberrant crypt development in the rat colon

Sarah Bettini[1], Elisa Boutet-Robinet[1], Christel Cartier[1], Christine Coméra[1], Eric Gaultier[1], Jacques Dupuy[1], Nathalie Naud[1], Sylviane Taché[1], Patrick Grysan[2], Solenn Reguer[3], Nathalie Thieriet[4], Matthieu Réfrégiers[3], Dominique Thiaudière[3], Jean-Pierre Cravedi[1], Marie Carrière[5,6], Jean-Nicolas Audinot[2], Fabrice H. Pierre[1], Laurence Guzylack-Piriou[1] & Eric Houdeau[1]

Background on Bettini et al. 2016 (Experimental Design)

Model: Adult male Wistar rats

Exposure

- 7 days oral gavage NM-105 or E 171 (10 mg/kg) **in water** (10 rats/group)

- Pretreated with 1,2 dimethylhydrazine. E171 (200 µg/kg or 10 mg/kg) in **drinking** water for 100 days. Used for flow cytometry and cytokine assay for gut inflammation and ACF assessments.
(11- 12 rats/group)

- Ex vivo T activation using isolated cells from spleen and Peyer's Patches

Background on Bettini et al. 2016

Changes in immune parameters after E 171 (titanium dioxide) administration

Peyer's Patches

- Increase in **dendritic cells** after 7-day treatment
- Decrease in **T regulatory cells** after 7-day and 100-day treatment
- Decrease in activated **T helper cells** after 7-day and 100-day treatment

Colonic Mucosa

- Increase in pro-inflammatory **TNFa** after 100-day treatment.
- Increase in anti-inflammatory **IL-10** after 100-day treatment.
- Increase in the chemokine **IL-8** after 100-day treatment.

Ex vivo stimulation of T cells after 7-day administration

- Peyer's Patch: Decreased **IFNg**
- Spleen: Increase **IFNg** and **IL-17**

Food and Chemical Toxicology 133 (2019) 110793

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Evaluation of immunologic and intestinal effects in rats administered an E 171-containing diet, a food grade titanium dioxide (TiO_2)

Lance K. Blevins[a], Robert B. Crawford[a], Anthony Bach[a,b], Michael D. Rizzo[a,c], Jiajun Zhou[a,d], Joseph E. Henriquez[a,e], D. M. Isha Olive Khan[a,e], Sera Sermet[a], Lora L. Arnold[f], Karen L. Pennington[f], Nathalia P. Souza[f], Samuel M. Cohen[f,g], Norbert E. Kaminski[a,b,e,*]

[a] *Institute for Integrative Toxicology, Michigan State University, East Lansing, MI, USA*
[b] *Center for Research on Ingredient Safety, Michigan State University, East Lansing, MI, USA*
[c] *Cell and Molecular Biology Program, Michigan State University, East Lansing, MI, USA*
[d] *Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI, USA*
[e] *Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI, USA*
[f] *University of Nebraska Medical Center, Omaha, NE, USA*
[g] *Havlik-Wall Professor of Oncology, USA*

Background on Blevins et al. 2019 (Experimental Design)

Model: Adult male Wistar rats (15 rats/group)

Exposure

- 7 day **dietary** E171 (0, 40, 400 and 5,000 ppm). Used for flow cytometry and cytokine assays
- 100 day **dietary** E171 (0, 40, 400 and 5,000 ppm) with and without 1,2 dimethylhydrazine. Used for flow cytometry and cytokine assays for gut inflammation and ACF assessments.
- All determination performed in a blinded manner!

E 171 Analysis (Blevins et al. 2019)

Performed by 2 independent laboratories using 2 different methods

Scanning electron microcopy

particles <36% of TiO_2 <100 nm in diameter;
average diameter: 100-115nm

Volume/mass based approach:

particles 1-2% of TiO_2 <100nm in diameter
average diameter: 150 nm

Good concordance between two labs using the two methodologies

E 171 Diet used in Blevins et al. 2019

- All diets containing E 171 prepared by Dyets (Bethlehem, PA).
- E 171 administered in irradiated Certified Purina 5002R33 at a concentration of 40, 400 and 5000 ppm.
- Homogeneity and concentration of E 171 in the diet were analyzed by Eurofins Food Chemistry Testing US.

0 ppm dose (22.3 ± 1.2; 23.7 ± 1.8 ppm)

40 ppm dose (59.6 ± 1.1; 61.0 ± 2.6 ppm)

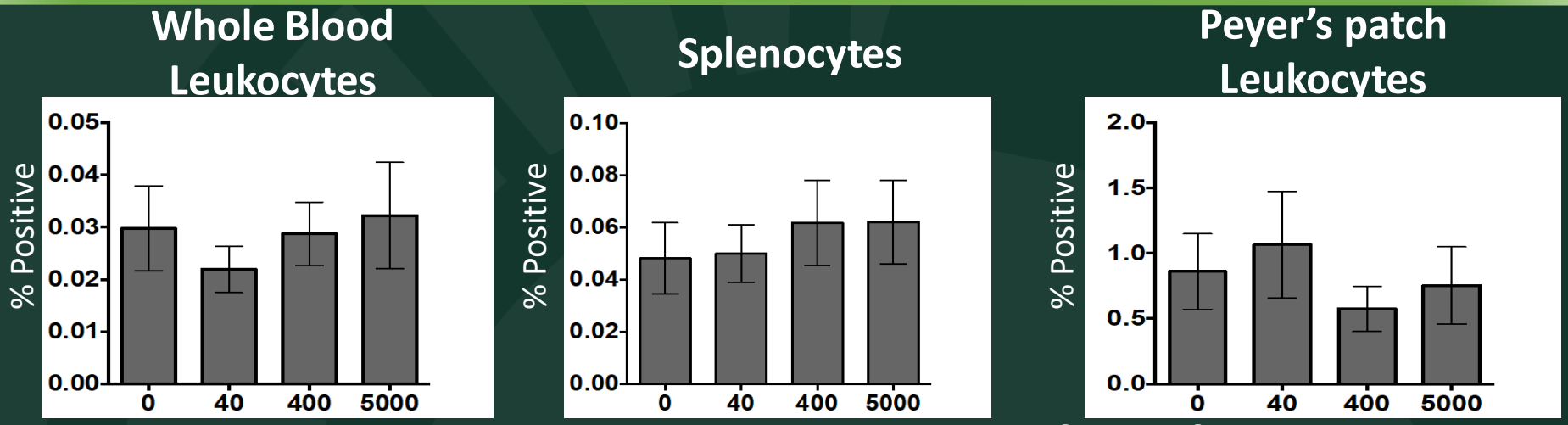
400 ppm dose (384 ± 8; 387 ± 13 ppm)

5,000 ppm dose (4310 ± 132; 4610 ± 160 ppm)

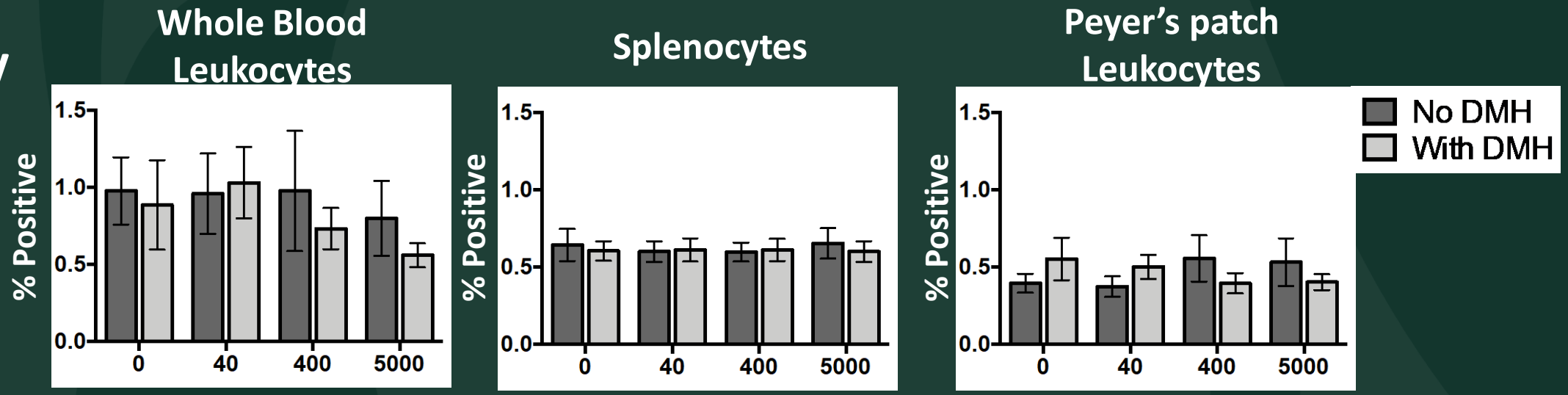
Dendritic Cells

(CD11b/c$^+$, CD103$^+$, MHCII$^+$)

7-Day

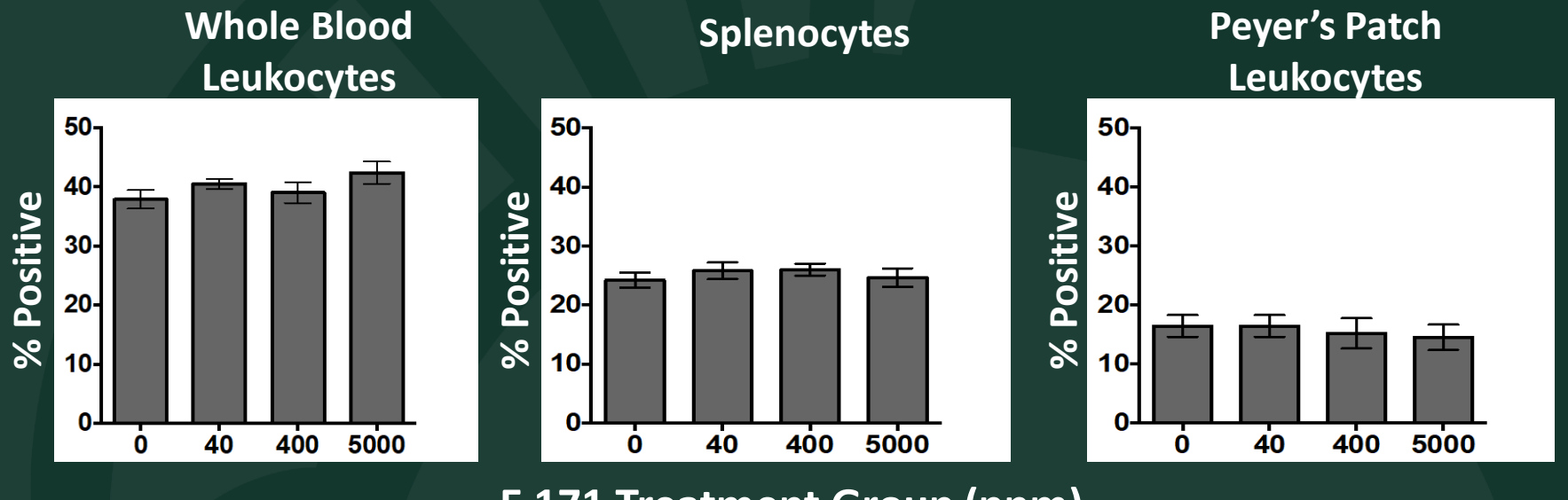


100-Day

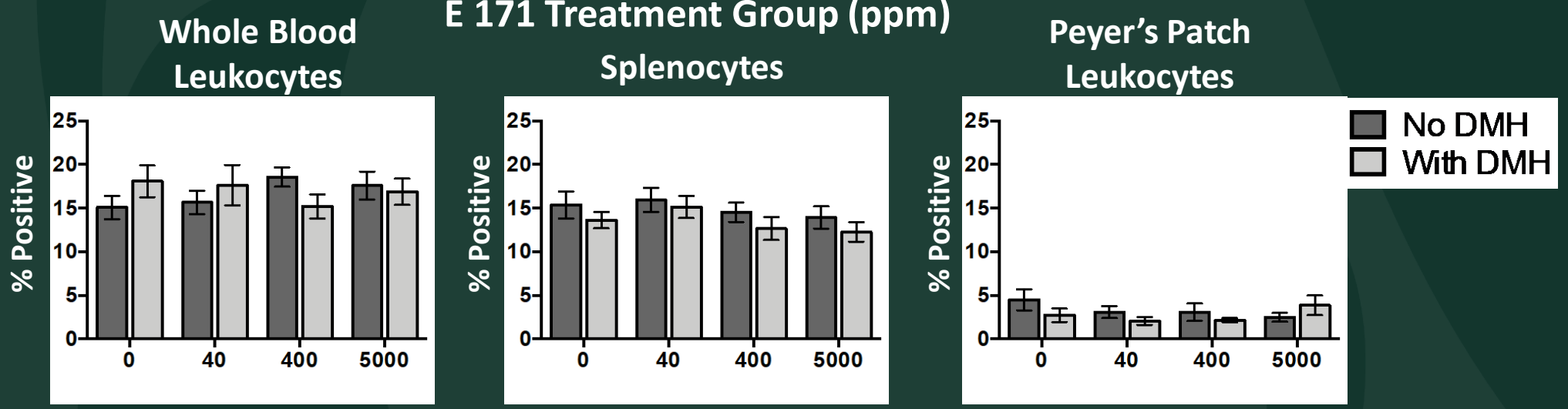


Total T_{helper} ($CD4^+$)

7-Day



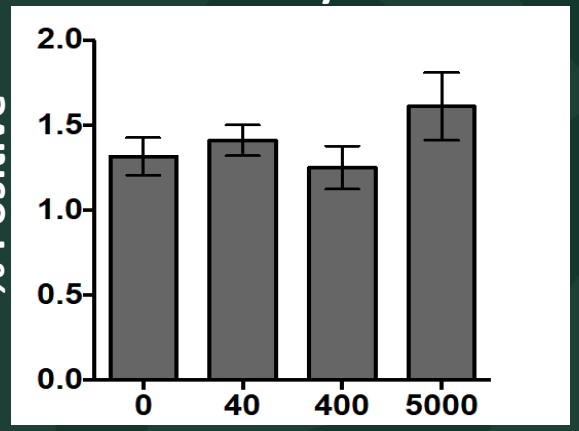
100-Day



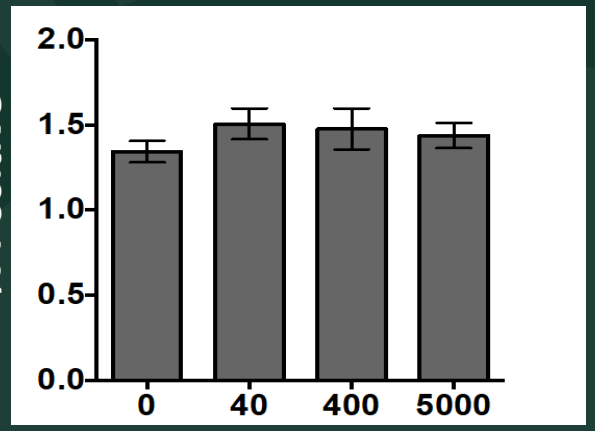
Activated T_{helper} ($CD4^+$, $CD25^+$)

7-Day

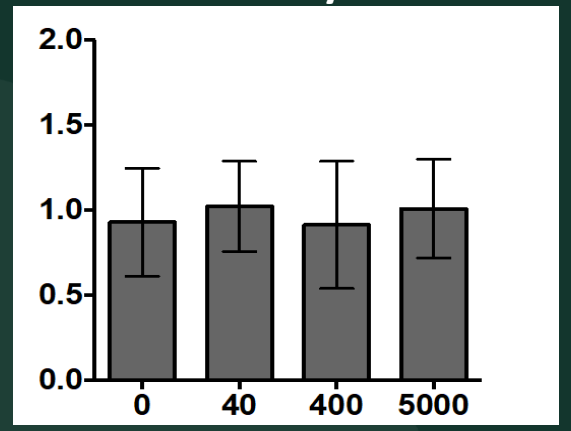
Whole Blood Leukocytes



Splenocytes



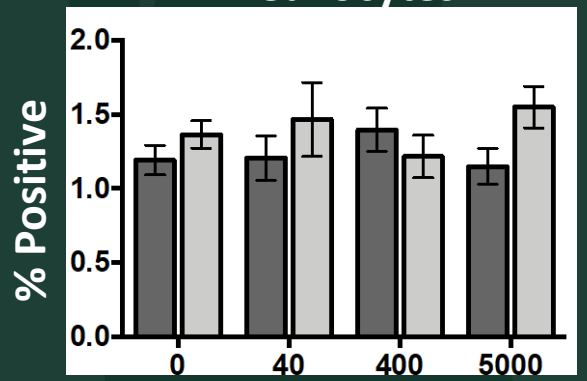
Peyer's Patch Leukocytes



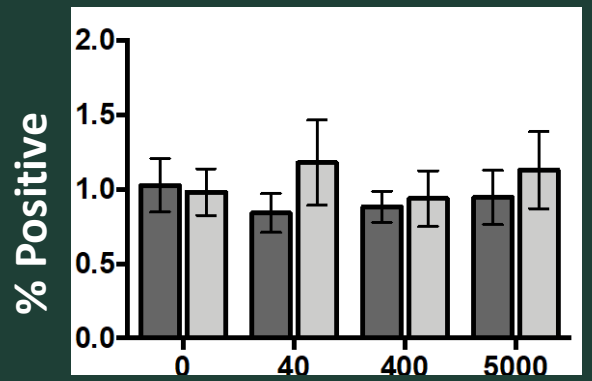
E 171 Treatment Group (ppm)

100-Day

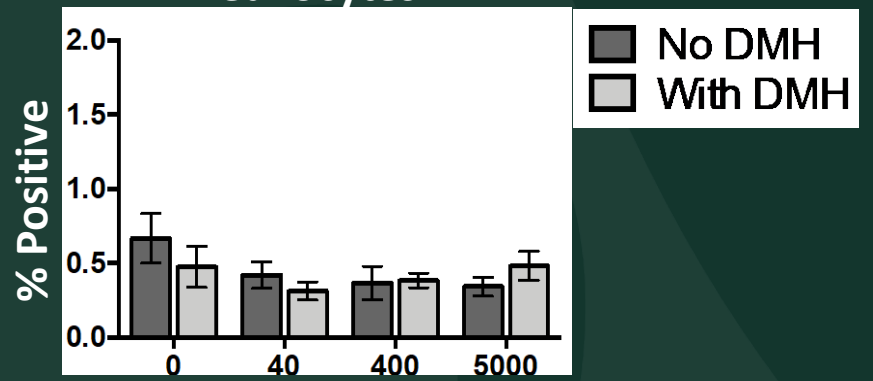
Whole Blood Leukocytes



Splenocyte



Peyer's Patch Leukocytes

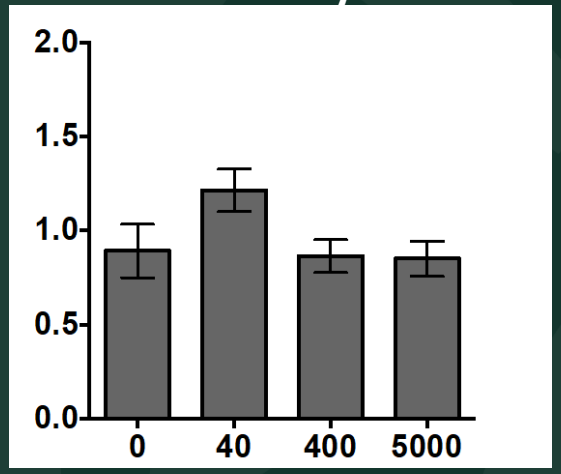


E 171 Treatment Group (ppm)

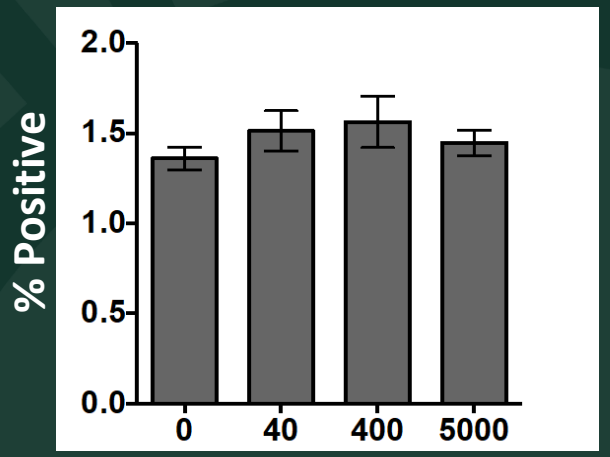
Total T_{reg} ($CD4^+$, $FoxP3^+$)

7-Day

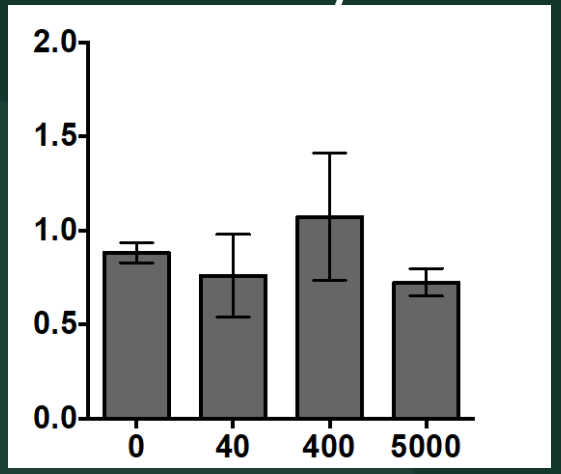
Whole Blood Leukocytes



Splenocytes



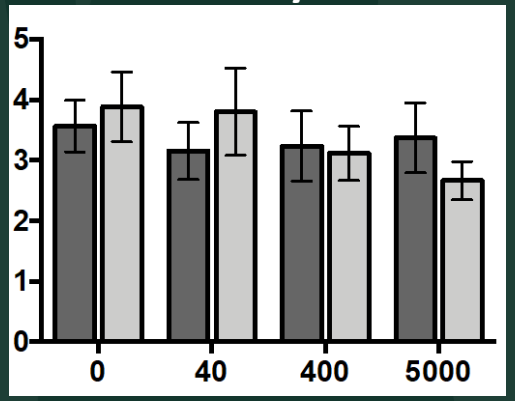
Peyer's Patch Leukocytes



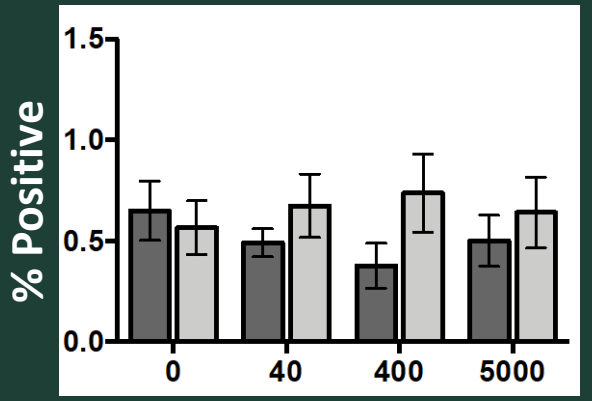
E 171 Treatment Group (ppm)

100-Day

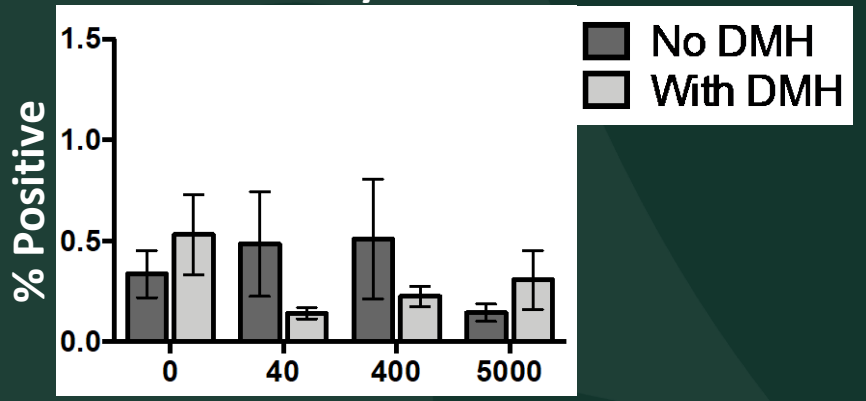
Whole Blood Leukocytes



Splenocyte



Peyer's Patch Leukocytes

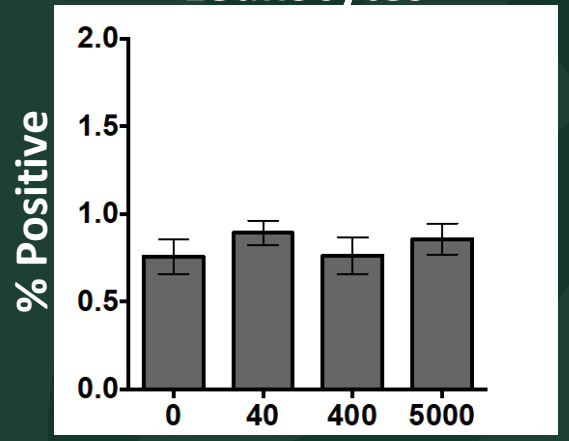


E 171 Treatment Group (ppm)

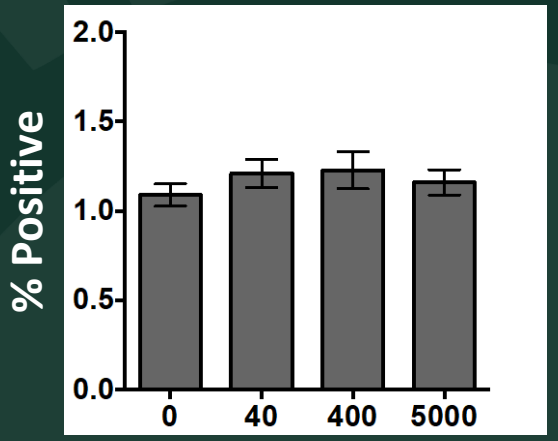
Activated T_{reg}
($CD4^+$, $CD25^+$, $FoxP3^+$)

7-Day

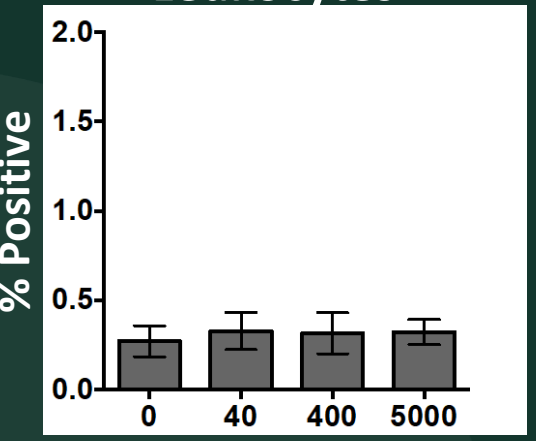
Whole Blood Leukocytes



Splenocytes



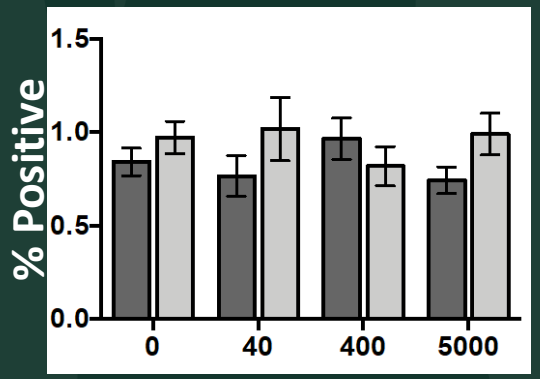
Peyer's Patch Leukocytes



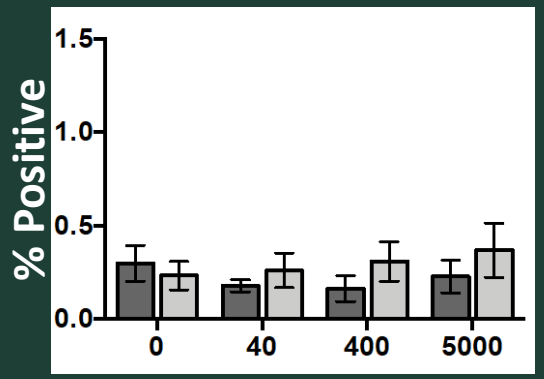
E 171 Treatment Group (ppm)

100-Day

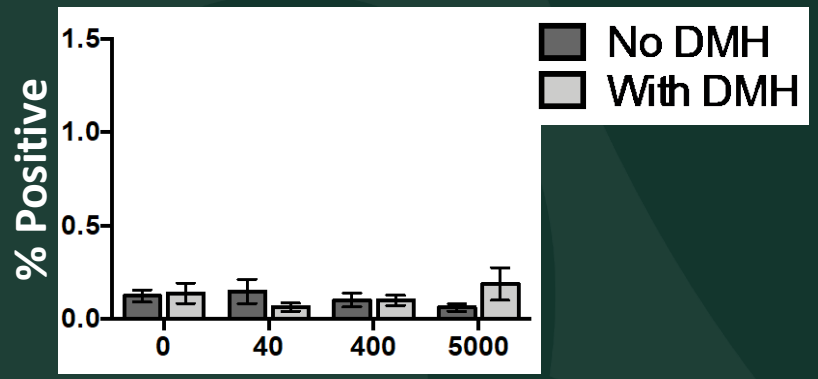
Whole Blood Leukocytes



Splenocytes

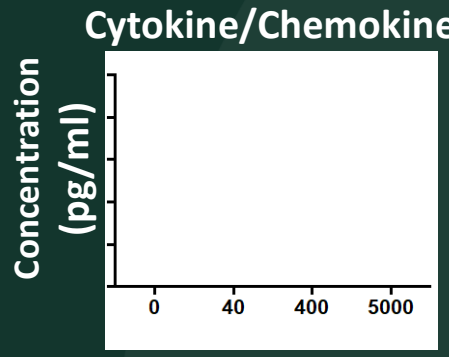


Peyer's Patch Leukocytes



E 171 Treatment Group (ppm)

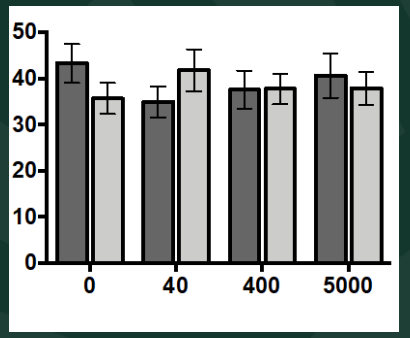
Plasma Cytokine/Chemokine Levels (100-Day)



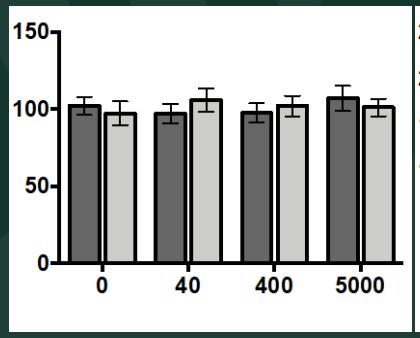
Not Detected

IL-10, IL-18, IL-33, GM-CSF

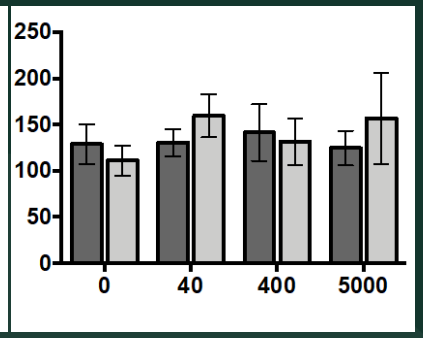
IL-1a



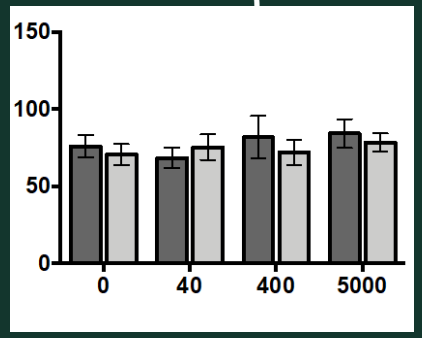
IL-1b



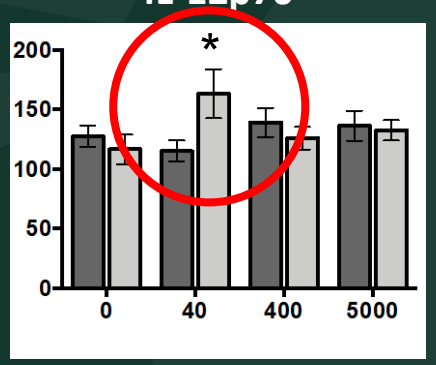
IL-6



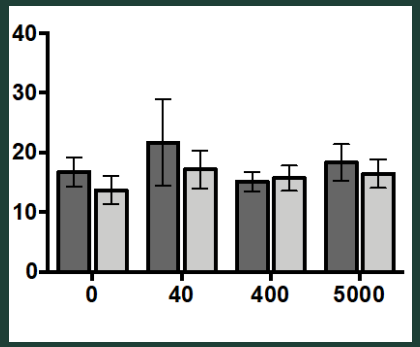
IFN-γ



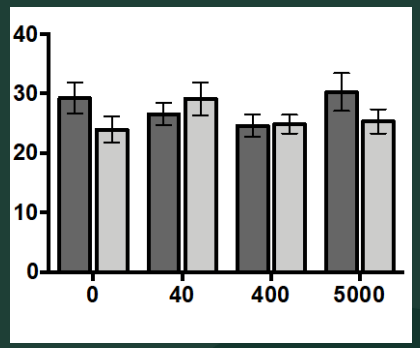
IL-12p70



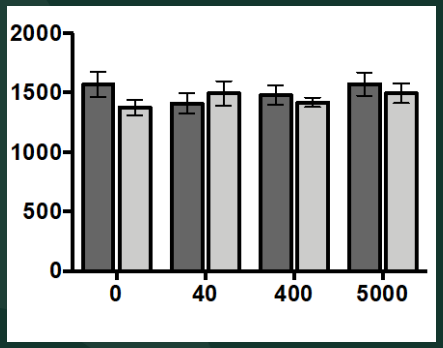
IL-17A



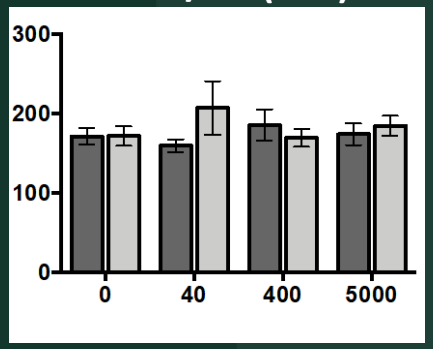
TNF-α



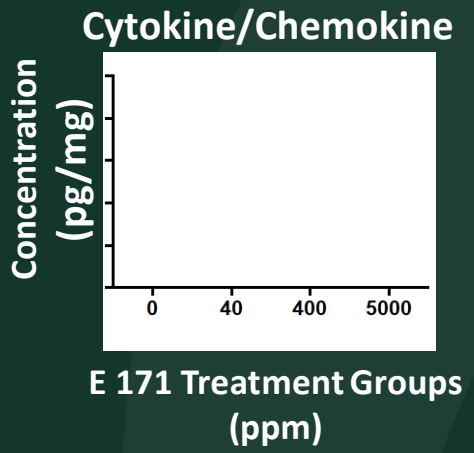
CCL2/MCP-1



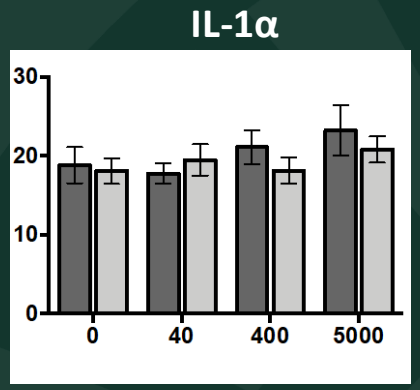
CXCL1/KC (IL-8)

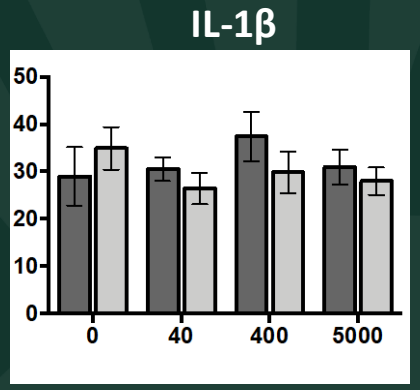


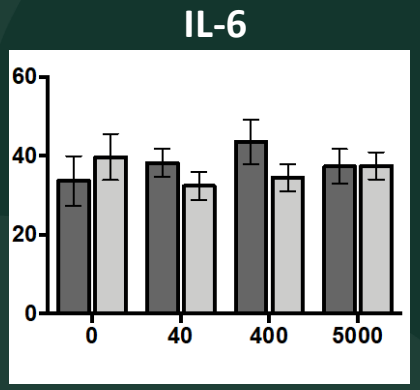
Small Intestine (Jejunum) Cytokine/Chemokine Levels (100-Day)

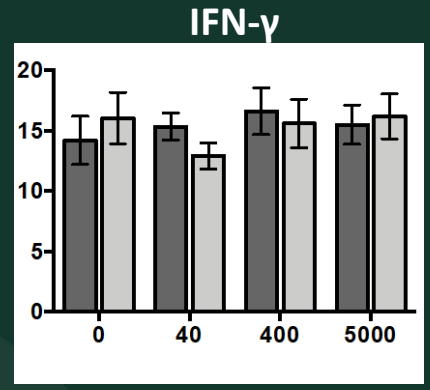


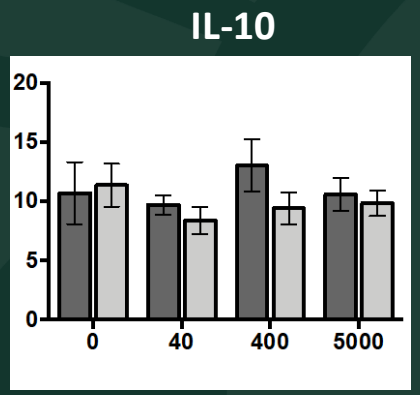
Not Detected
GM-CSF

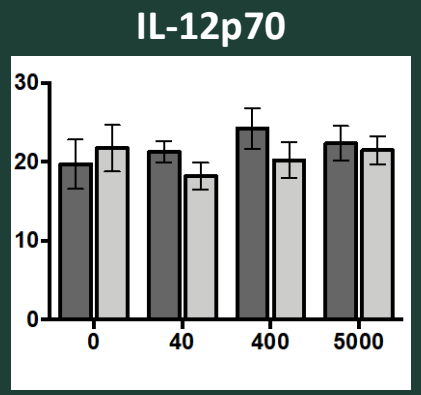


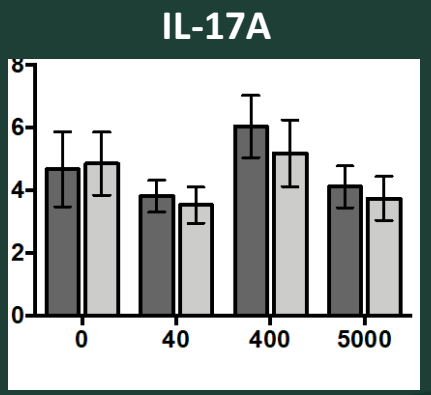


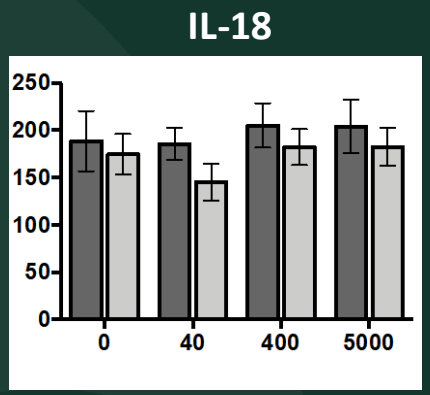


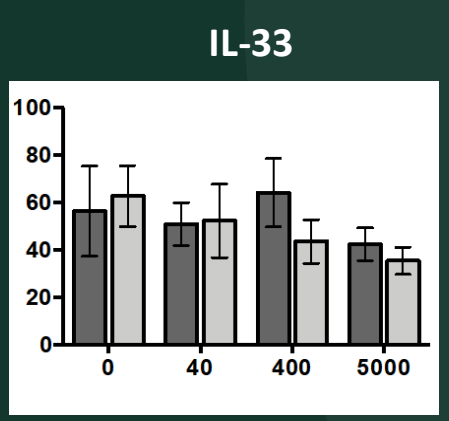


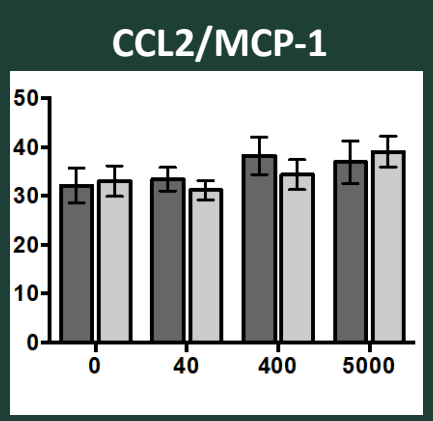


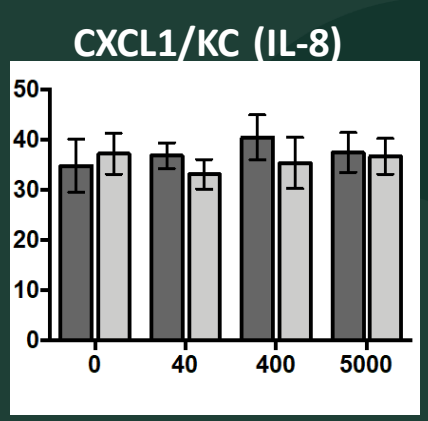


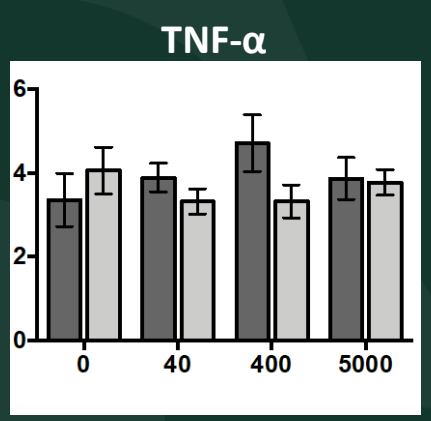




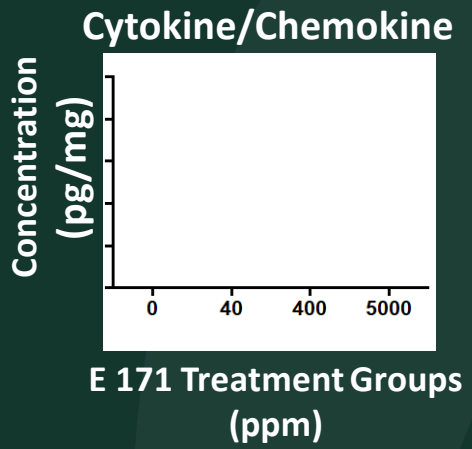








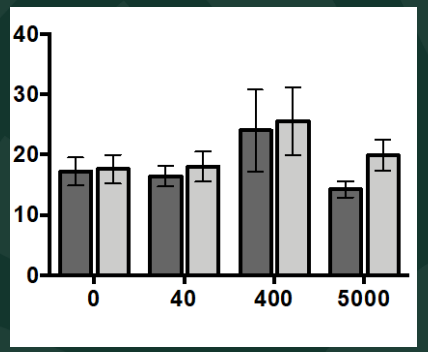
Colon Cytokine/Chemokine Levels (100-Day)



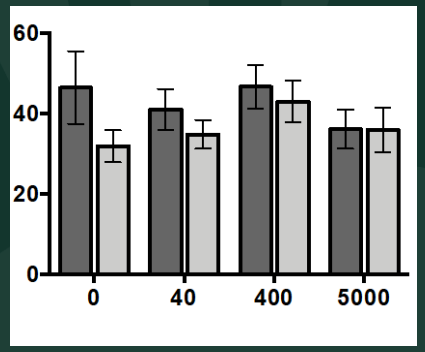
Not Detected
TNF-α, GM-CSF

No DMH
With DMH

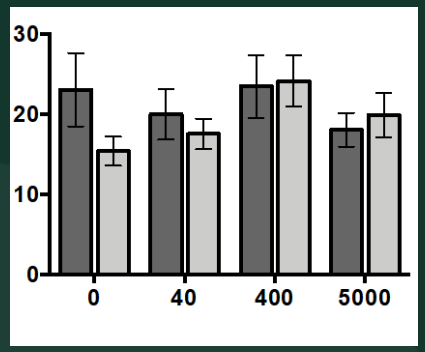
IL-1α



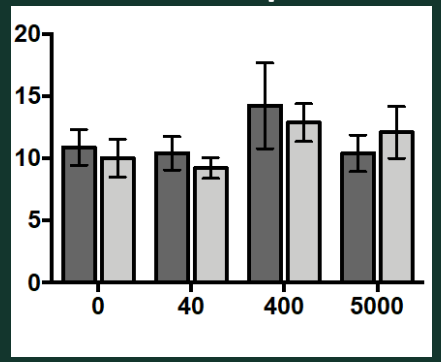
IL-1β



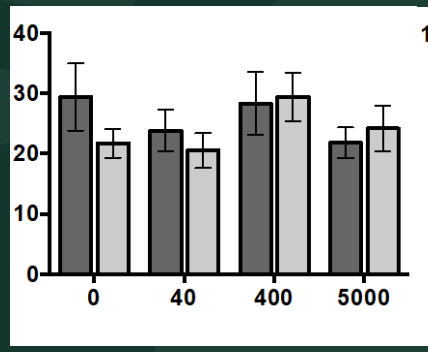
IL-6



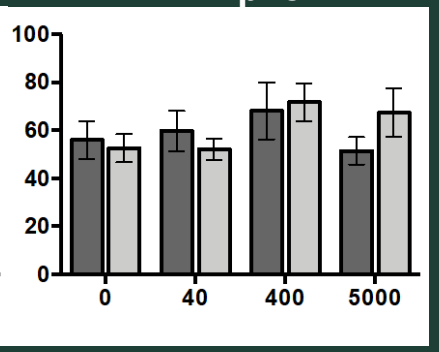
IFN-γ



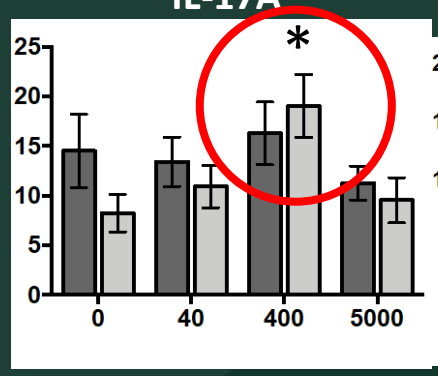
IL-10



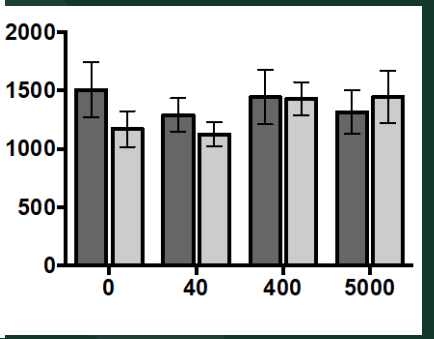
IL-12p70



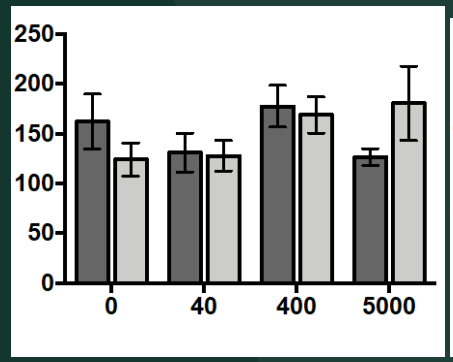
IL-17A



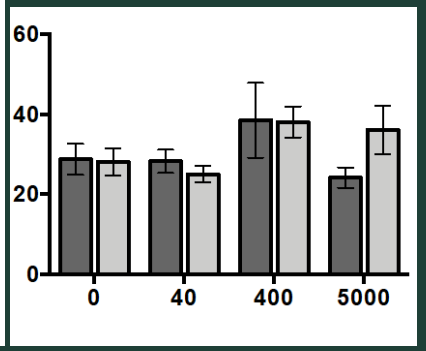
IL-18



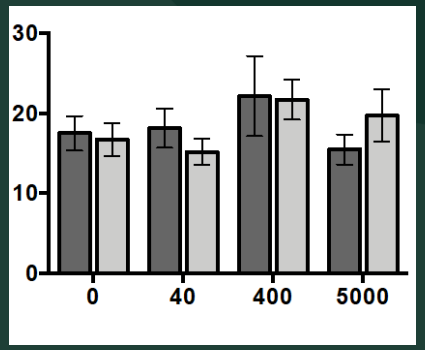
IL-33



CCL2/MCP-1

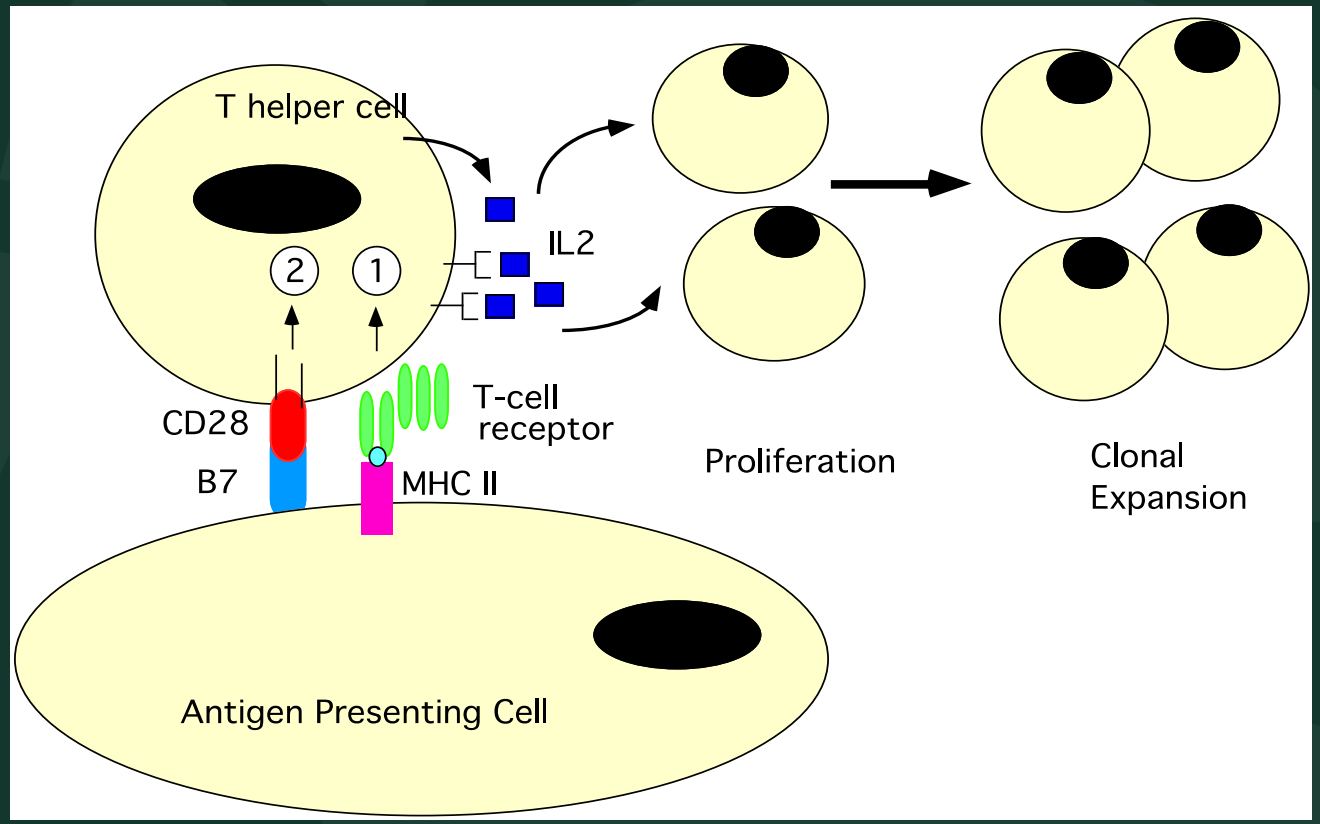


CXCL1/KC (IL-8)

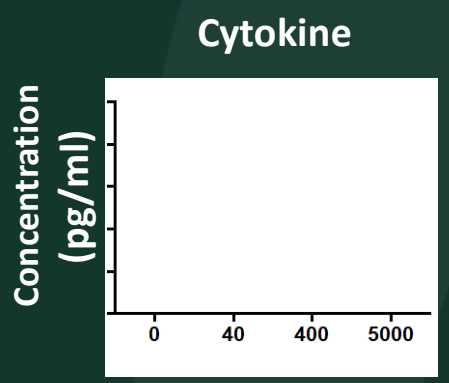


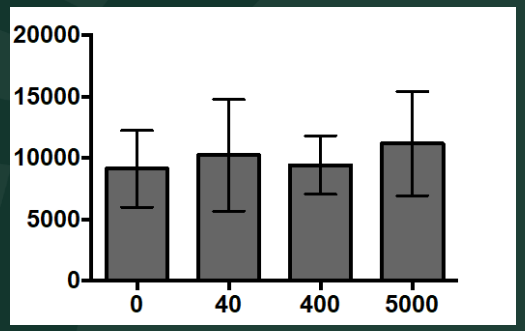
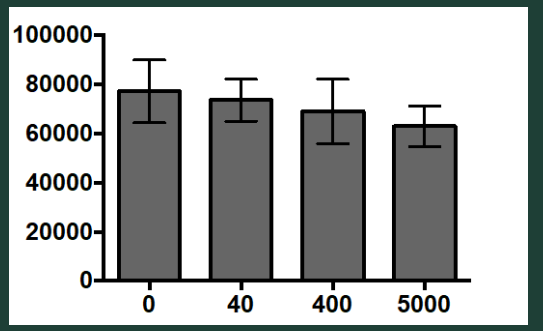
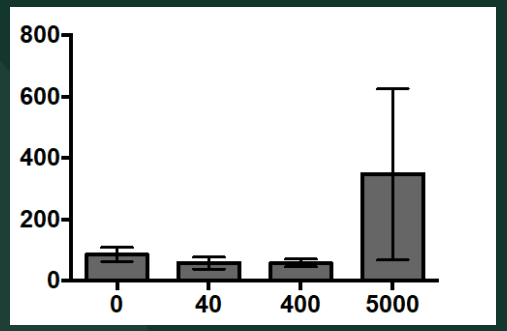
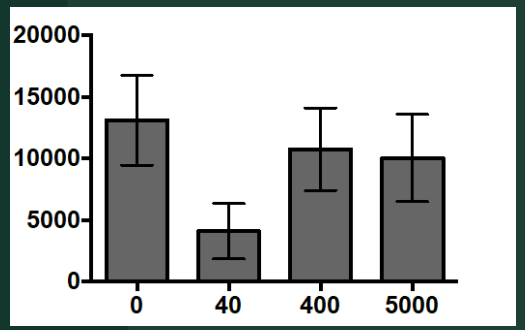
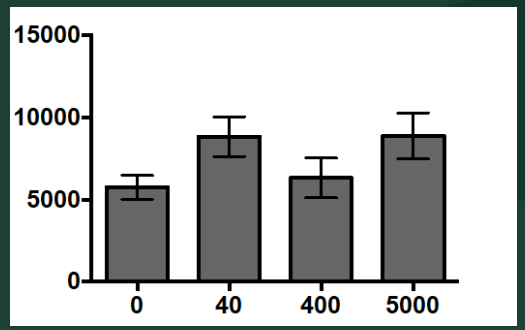
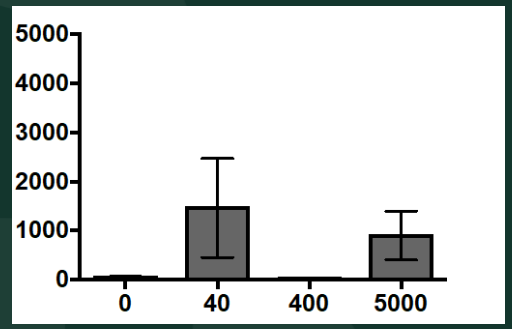
Ex Vivo Stimulation of T cells

Activation via TCR and CD28



Ex Vivo Stimulation of T cells



	Whole Blood Leukocytes	Splenocytes	Peyer's Patch Leukocytes
IFN-γ			
IL-17A			

Conclusions (Blevins et al 2019)

Dietary E 171 for 7 or 100-days caused **no** changes in the percentages of dendritic cells, T helper cells (resting or activated) or T regulatory cells (resting or activated) in whole blood, spleen or Peyer's patches.

Dietary E 171 for 7 or 100-days caused **no** significant changes in inflammatory cytokines/chemokines in whole blood, jejunum and colon after 7 and 100-days of E 171 administration with the exception at 100-days in:

- IL-17A in colon (400 ppm E 171+DMH)
- IL-12p70 in plasma (40 ppm E 171+DMH)

There was **no** E 171 treatment-related changes in T cell-derived IFNg or IL-17 after ex vivo stimulation of T cells from either Peyer's patches or spleen.

There were **no** increases in ACF due to E171 treatment at 100 days of exposure.

Final Thoughts

- Should regulatory decisions be made using studies with a questionable experimental design?
- If an agent produces a statistically significant change in a biological endpoint, should the change immediately be interpreted as an “adverse effect”?
- How should animal models of disease be used in assessing toxicity and in making regulatory decisions?
- Is a harmonized framework for assessing the toxicity of nanomaterials needed?

Acknowledgement

Michigan State University

(Center for Research on Ingredient Safety)

Lance Blevins

Robert Crawford

Anthony Bach

Michael Rizzo

Jiajun Zhou

Joseph Henriquez

Isha Khan

Sera Sermet

University of Nebraska Medical Center

Samuel Cohen

Lora Arnold

Karen Pennington

Nathalia Souza