Evaluating companion dogs as biosentinels of human exposure: foods, pesticides (aka chemical mixtures)

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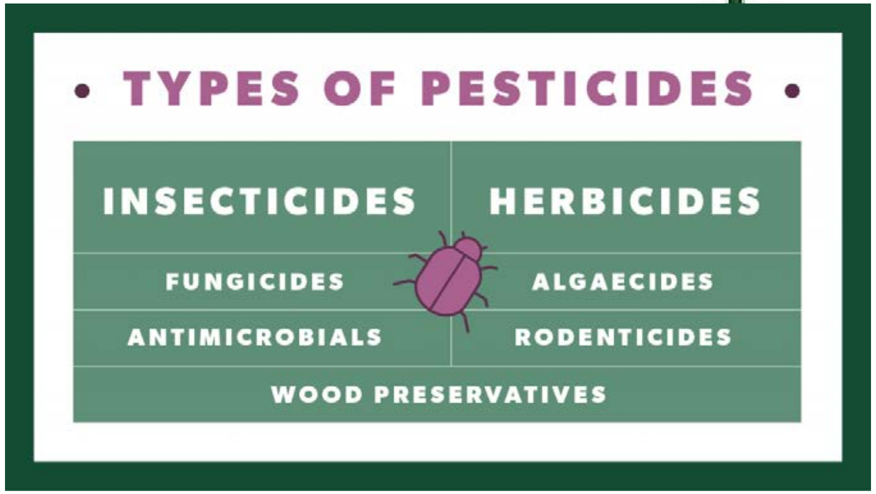
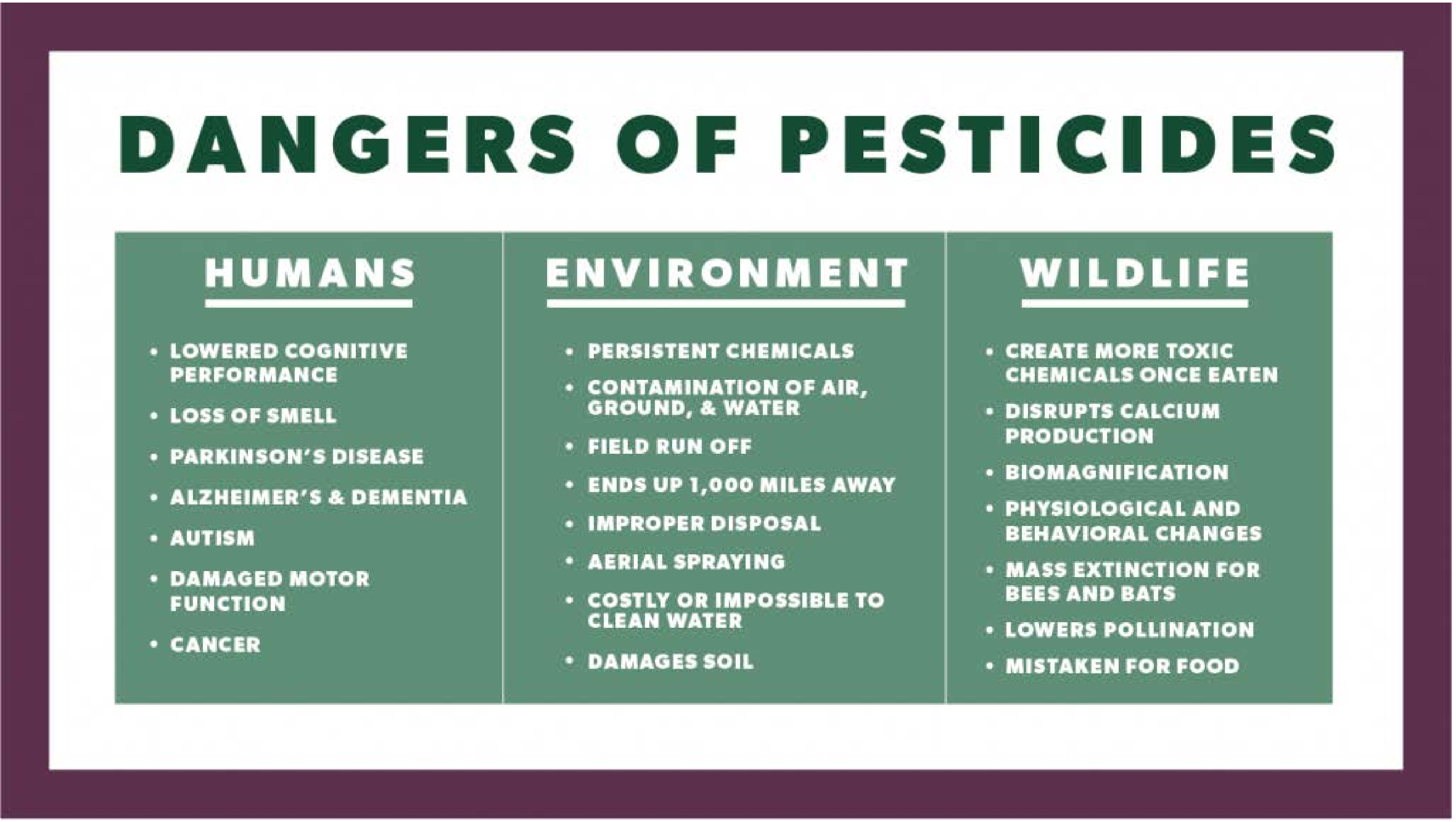
Pesticides: Indoors and Outdoors

- Drinking water contaminated by agricultural or industrial pesticide runoff
- Insecticides and rodenticides used indoors (e.g., flea sprays used in home or on pets, pesticides used in schools)
- Outdoor sprays used on lawns, gardens, playgrounds, parks, golf courses, roadsides, hydroelectric rights-of-way
- Pesticide residues on food
- Spray drift from agricultural spraying



**Glickman 1989 investigation of insecticide exposure, obesity and the risk of bladder disease in dog



Companion animals

Cancer?

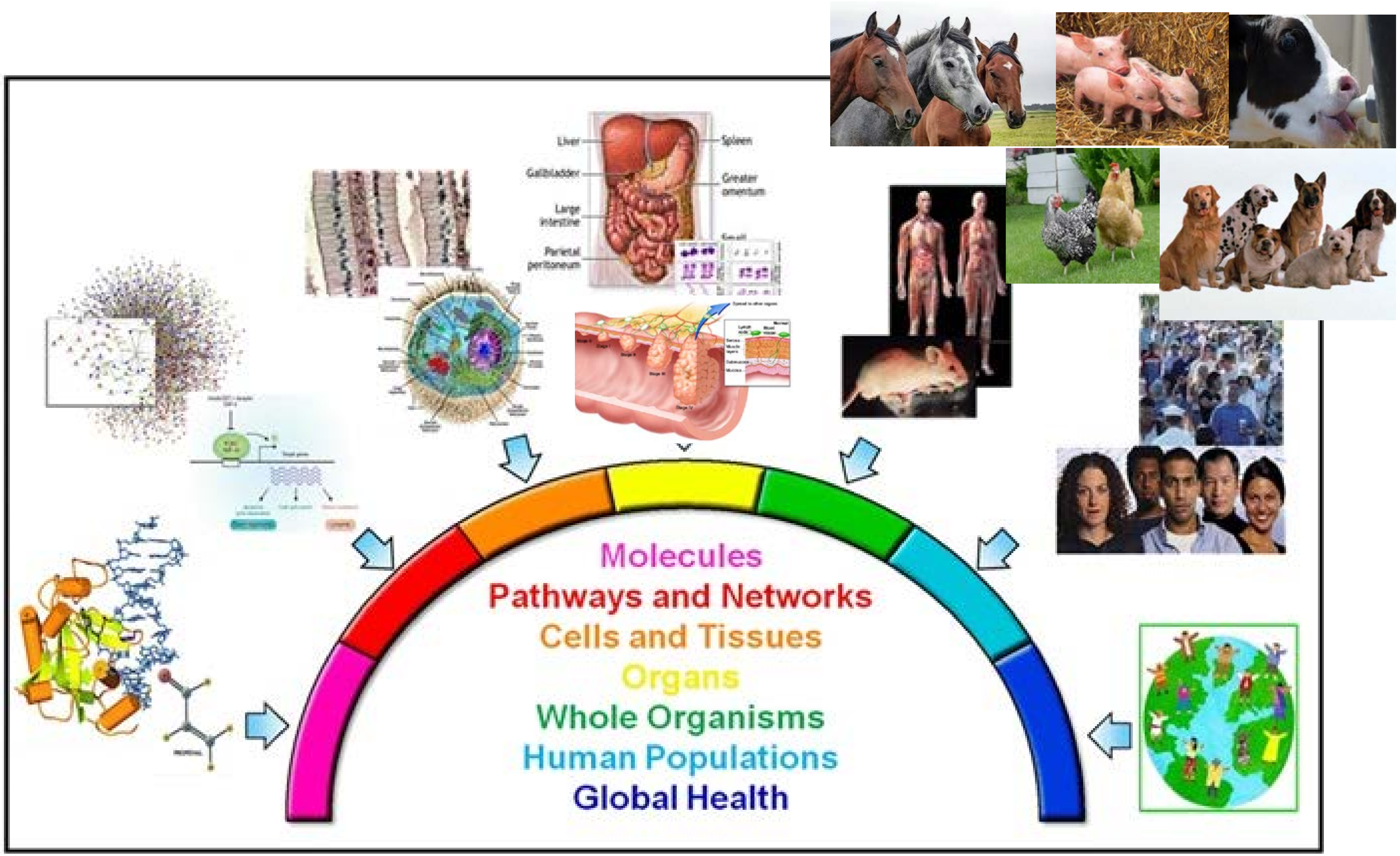
Immune dysfunction?

Endocrine Disruption?

Allergy?

Developmental or other abnormalities

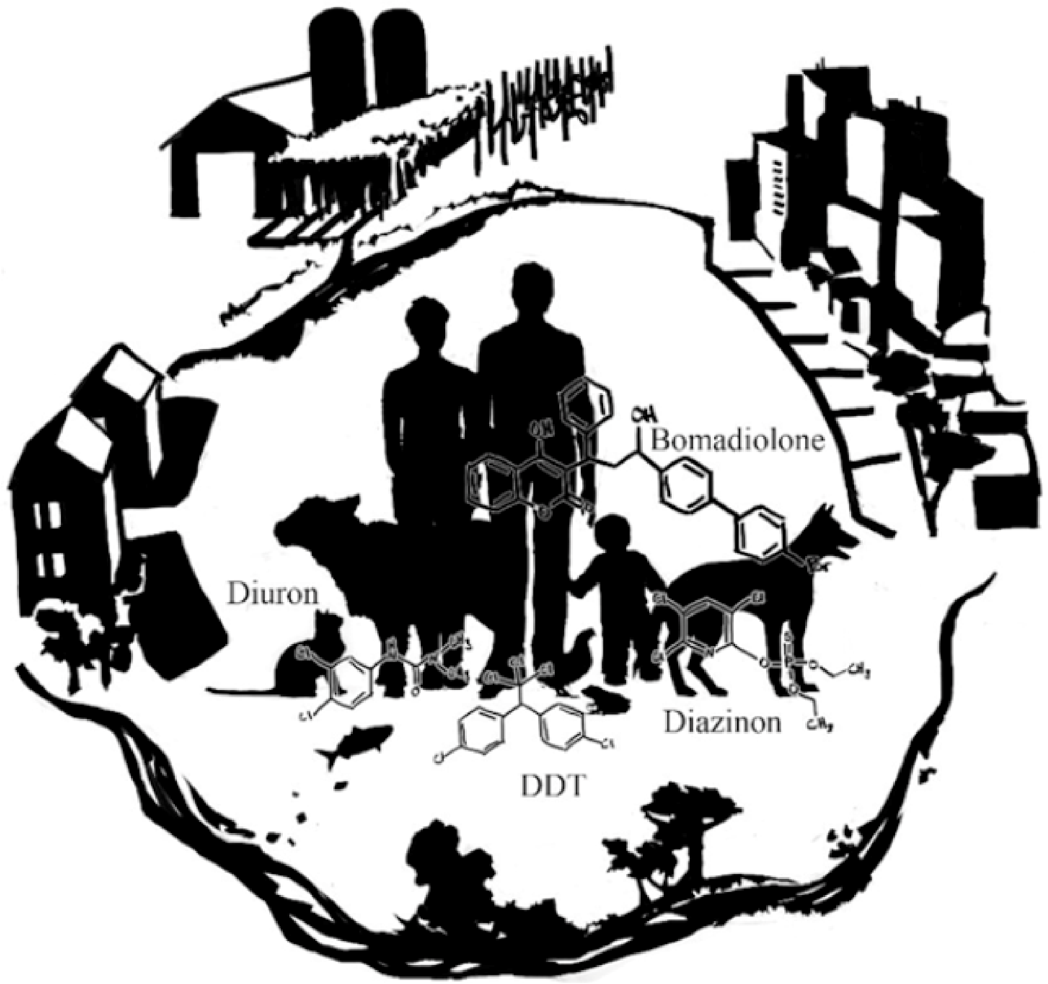

WHY THERE ARE NO PESTICIDE-SNIFFING DOGS
U.S. CUSTOMS
U.S. GRAIN
POTATOES
COTTON
IMPORT TOBACCO
JANIK



Molecules
Pathways and Networks
Cells and Tissues
Organs
Whole Organisms
Human Populations
Global Health

Distinct types and classes of pesticides that have wide-ranging sources of exposure across animals, people, and environments merit investigations with a systems approach

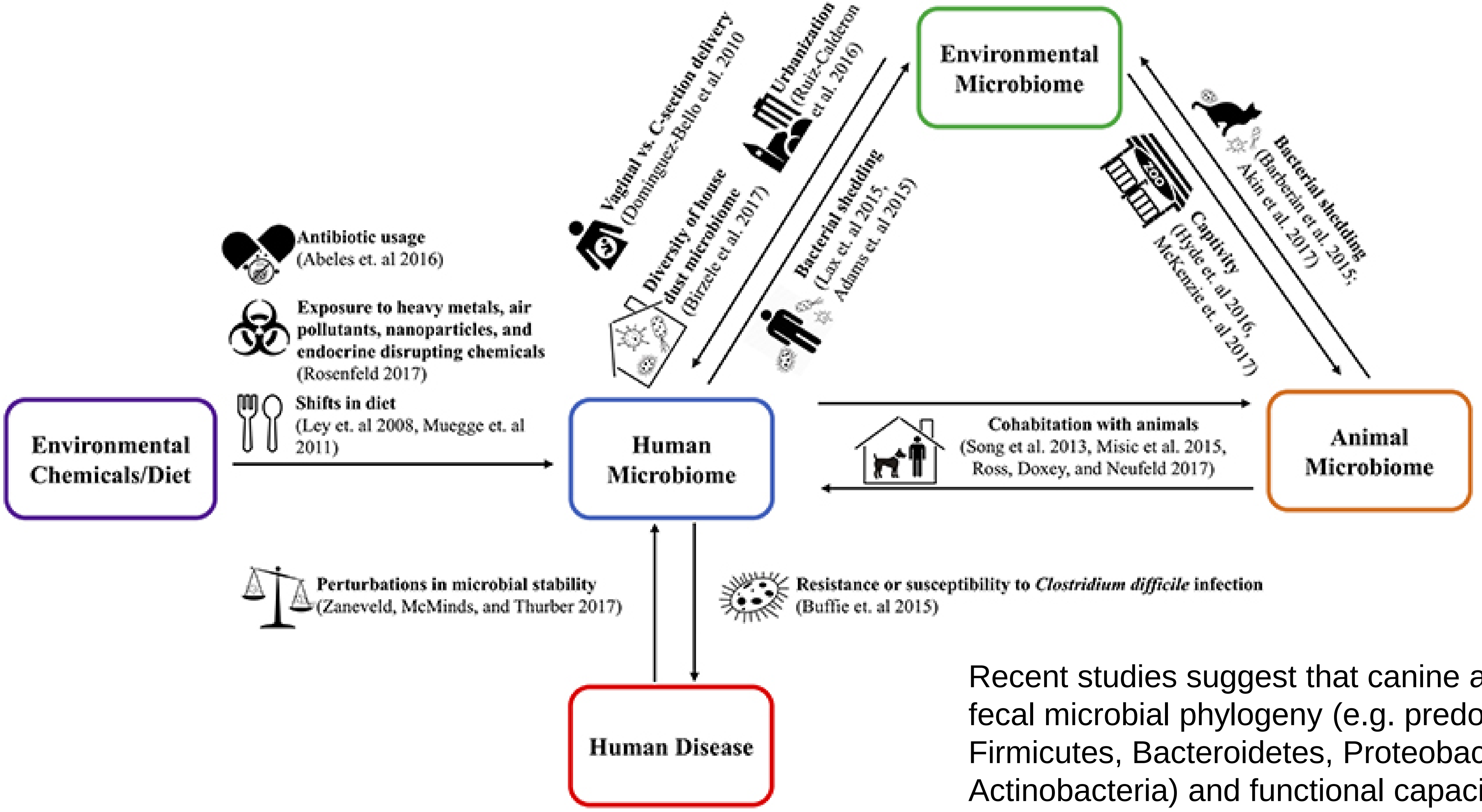
- **Testing of environments, water, and food in addition to people and pets may help identify relative levels of exposures**



Environmental and treated waters may be important sources of persistent pesticide exposures to animals and people and likely elicit different mechanisms from what is shown in controlled, single-exposure laboratory animal studies

An integrated host-microbiome response is essential to fully understand the role for bioaccumulation and/or microbial detoxification that will impact detection levels

Aslan B., Viola L., Saini S.S., Stockman J., Ryan E.P. (2020) Pets as Sentinels of Human Exposure to Pesticides and Co-exposure Concerns with Other Contaminants/Toxicants. In: Pastorinho M., Sousa A. (eds) Pets as Sentinels, Forecasters and Promoters of Human Health. Springer, Cham. https://doi.org/10.1007/978-3-030-30734-9_3



One Health Relationships Between Human, Animal, and Environmental Microbiomes: A Mini-Review
Front. Public Health, 30 August 2018
https://doi.org/10.3389/fpubh.2018.00235

Recent studies suggest that canine and feline gut fecal microbial phylogeny (e.g. predominance of Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria) and functional capacity (*major* functional groups related to carbohydrate, protein, DNA and vitamin metabolism, virulence factors and cell wall and capsule) are similar to those of the human gut!

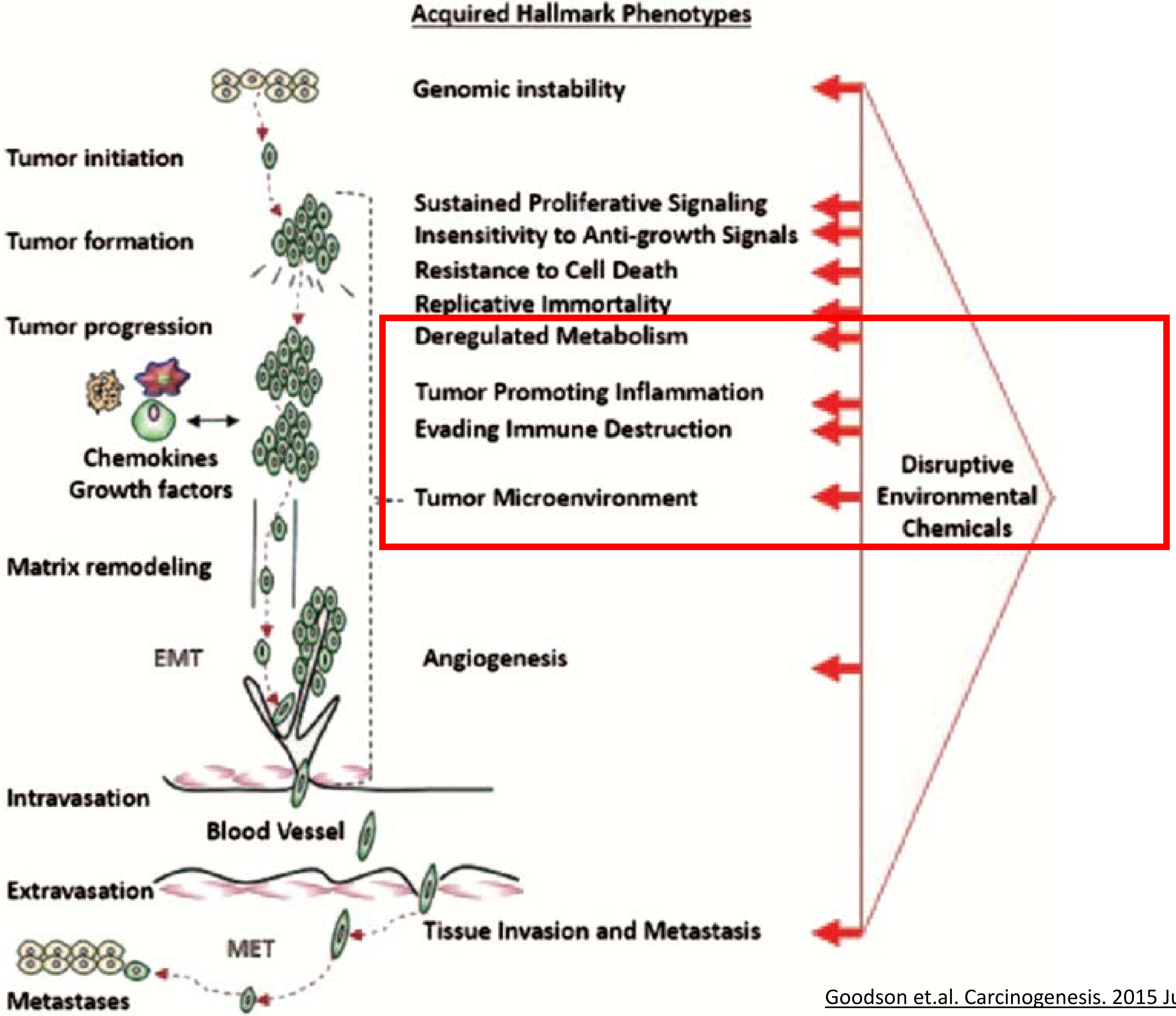
Current Recommendations for Cancer Prevention

1. **Be as lean as possible without becoming underweight.**
2. **Be physically active for at least 30 minutes every day.**
3. **Avoid sugary drinks. Limit consumption of energy-dense foods.**
4. **Eat more of a variety of vegetables, fruits, whole grains and legumes such as beans.**
5. **Limit consumption of red meats and avoid processed meats.**
6. **If consumed at all, limit alcoholic drinks to 2 for men and 1 for women per day.**
7. **Limit consumption of salty foods and foods processed with salt (sodium).**
8. **Don't use supplements to protect against cancer.**
9. ***It is best for mother breastfeed exclusively for up to 6 months and then add other liquids and foods.**
10. ***After treatment, cancer survivors should follow the recommendations for cancer prevention.**

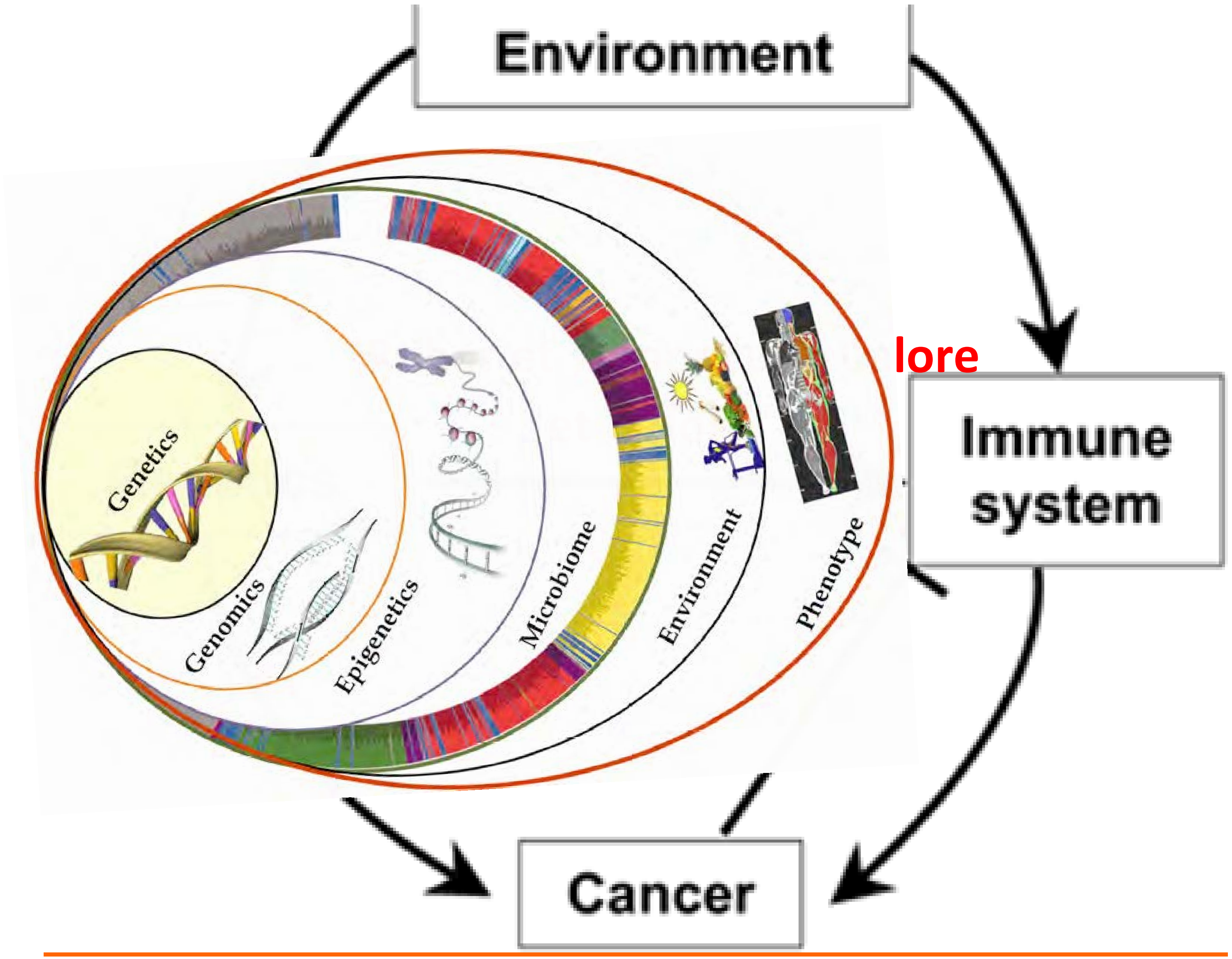
***Special population recommendations.**

11. ***do not smoke or chew tobacco.***

Following these recommendations, one third of total cancers could be prevented. Combined with eliminating tobacco use, one half of total cancers could be prevented. (AICR, 2012)

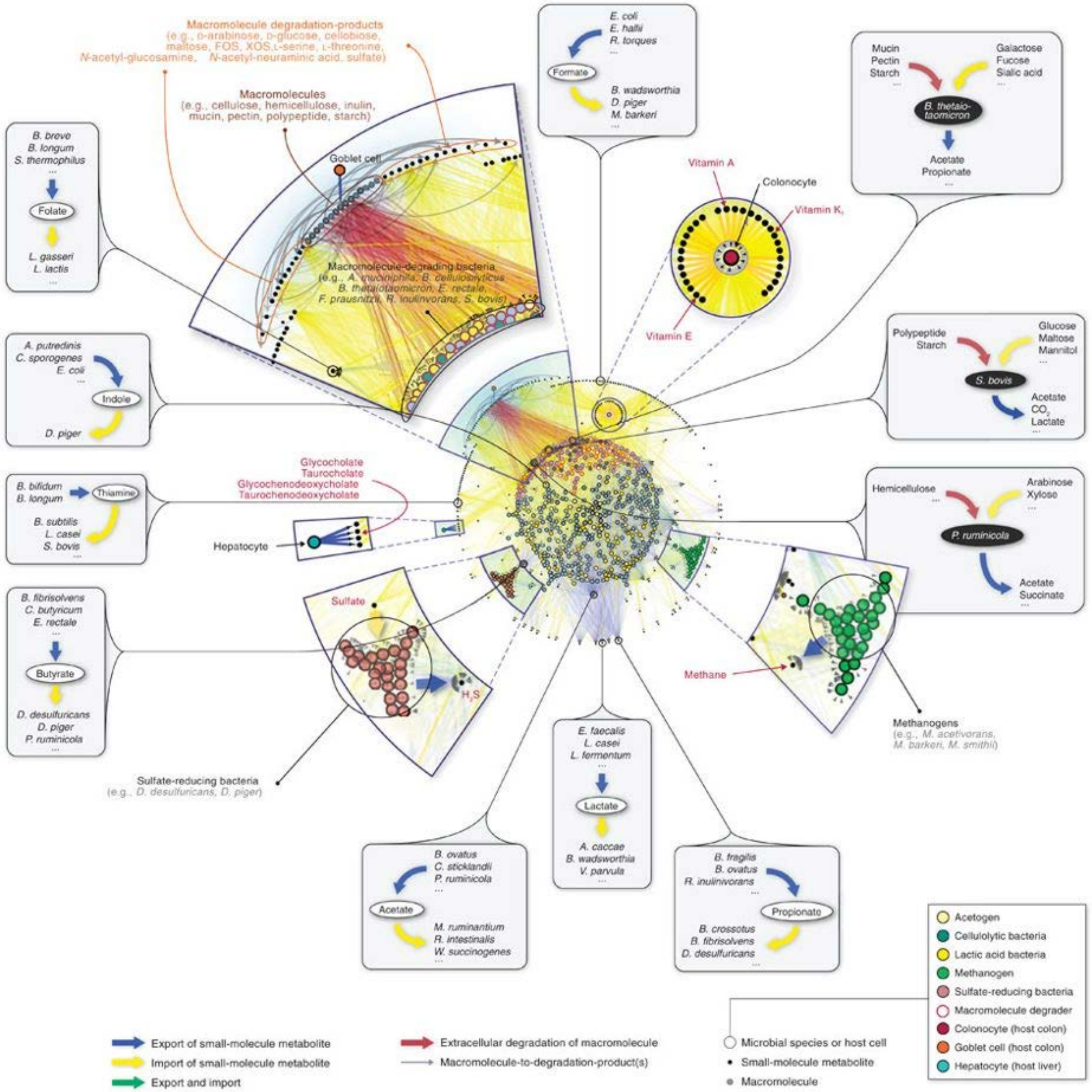


Goodson et.al. Carcinogenesis. 2015 Jun; 36(Suppl 1): S254–S296.



Environment
lore
Immune
system
Genetics
Genomics
Epigenetics
Microbiome
Environment
Phenotype
Cancer

Global landscape of the human gut microbiota organized through metabolite transport



This is also true for dogs!

Sung et. Al. Nature Communications (2017)

Review and synthesize available research in the scientific literature regarding pets and companion animal exposures to pesticides and describe instances where shared exposures may impact the health of humans.

- Oxidative stress caused by pesticides is an important mechanism through which many of the pesticides exert their harmful effects. Oxidative stress is known to cause DNA damage which in turn may cause malignancies and other disorders.

- Many pesticides have shown to modulate the gene expression at the level of non-coding RNAs, histone deacetylases, DNA methylation patterns suggesting their role in epigenetics.

Cohort Study Design.
Eighty-three (83) canines from seven different breeds were all fed the same dry extruded kibble diet for 5 weeks.

Small dogs
(34 individuals; Beagle,
Small Fox Terrier, and Miniature Schnauzer)
Mean weight of 9.3 kg (range of 6.1–15.6 kg).

Larger dogs
(49 individuals; Labrador Retriever, English Setter, Siberian Husky, and Rottweiler)
Mean weight of 31.5 kg (range of 18.4–54.4 kg).

Small dogs had a mean age of 6.8 years (range of 2.4–13.3 years) while larger dogs had a mean age of 6.2 years (range of 2.2–9.8 years).

All dogs except, Rottweilers, were housed in the same location.

Samples were handled and processed in the same manner to avoid technical variability or bias.

Background: Metabolic differences between small and large dogs

Table 2: Clinical measures with adjusted P value < 0.05 between small dogs and dogs of larger body size.

Clinical measure	Mean small	SEM small	Mean larger	SEM larger	Adj. P
DEXA tissue (gm)	9135.12	401.87	25392	569.82	$1.34E-18$
DEXA lean (gm)	6884.76	308.56	17942.89	491.91	$1.34E-18$
DEXA bone mineral content (gm)	379.58	15.99	1009.06	24.88	$1.34E-18$
Weight (gm)	9486.15	421.58	31768.72	1302.21	$2.2E-16$
DEXA fat (gm)	2250.42	145.25	7449.06	427.25	$2.91E-16$
DEXA total mass (kg)	9.51	0.42	26.40	0.59	$1.27E-11$
Bone density (gm/cm^2)	0.65	0.01	0.78	0.01	$3.07E-10$
Serum creatinine (mg/dL)	0.64	0.02	0.86	0.02	$7.80E-07$
Serum creatine kinase (IU/L)	263.88	33.51	154.04	21.37	$5.04E-05$
Serum potassium (mmol/L)	4.31	0.05	4.65	0.05	$9.33E-04$
Serum total bilirubin (mg/dL)	0.10	0.01	0.13	0.01	0.02
Serum aspartate transaminase (U/L)	35.68	1.99	27.91	1.07	0.02
Serum gamma-glutamyltransferase (g/dL)	6.93	0.43	5.94	0.36	0.02
Serum total antioxidant status (mmol/L)	1.49	0.03	1.60	0.02	0.03
Serum T3 (nmol/L)	1.06	0.04	0.94	0.03	0.04
Protein digestibility (percentage)	88.00	0.38	86.00	0.57	0.01

Mean, standard error of the mean (SEM), and adjusted P values are shown. DEXA, dual-energy X-ray absorptiometry; T3, total triiodothyronine.

Journal of Nutrition and Metabolism
Volume 2017, Article ID 4535710, https://doi.org/10.1155/2017/45

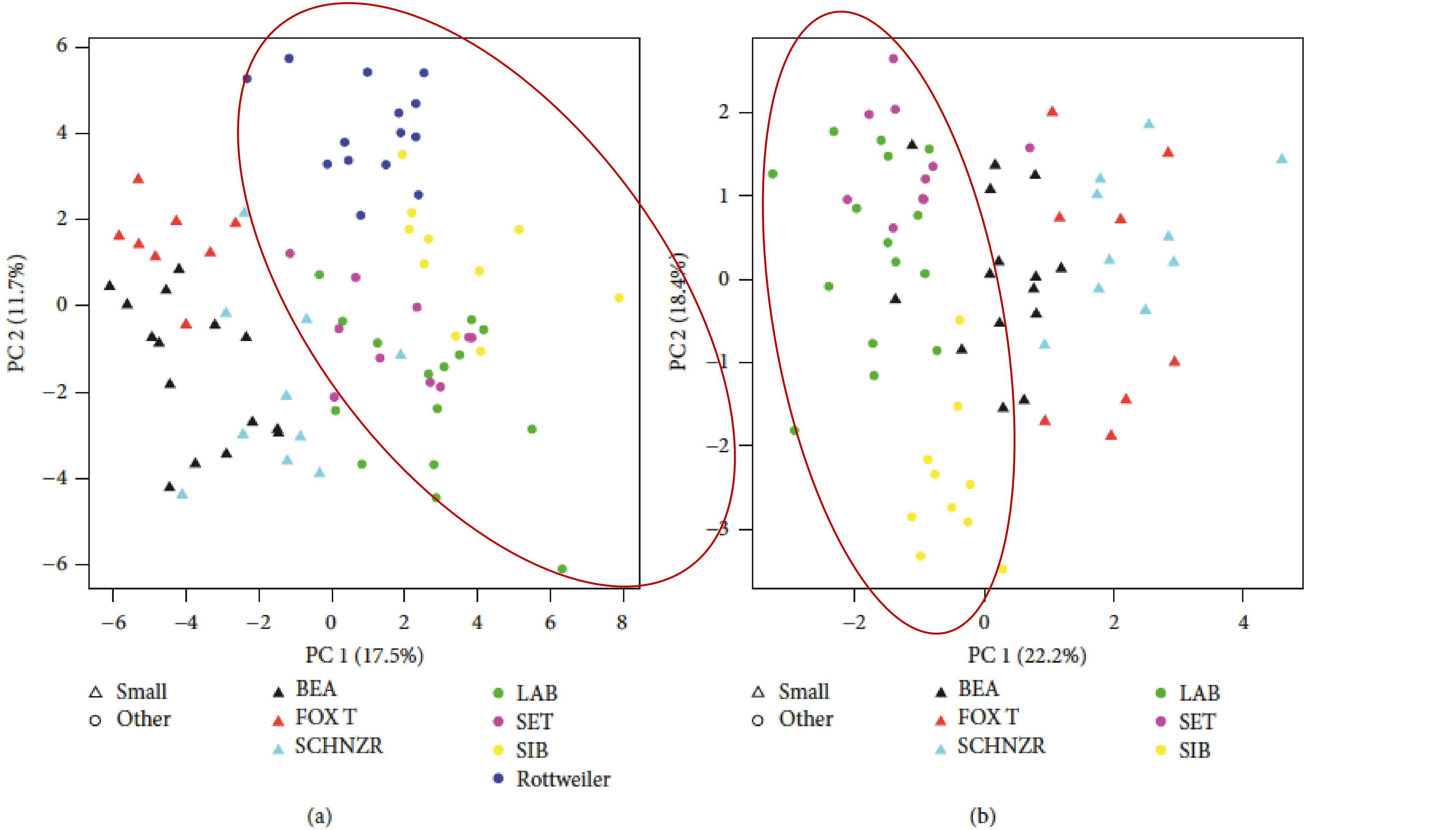


FIGURE 2: Principle component analysis using selected metabolites (a) and clinical parameters (b) between small dogs and dogs of larger (other) body size. Note that Rottweilers were not considered in the PCA generated with clinical parameters as they are missing DEXA-related parameters along with thyroid hormones T3 and T4. Body size is indicated by triangle (small) or circle (larger, other). LAB, Labrador Retriever; SET, English Setter; BEA, Beagle; FOX T, Small Fox Terrier; SIB, Siberian Husky; SCHNZR, Miniature Schnauzer.

Table 1: Continued.

Metabolite name	Ratio (small/larger)	Class	Adj. P	RI	Mass
5-Methylcytidine	1.13	Nucleotide	0.025	1388	258
N-Formylmethionine	0.89	Amino acid	0.029	1541	176.1
Bilirubin (E,E)	0.50	Cofactors and vitamins	0.031	4625	585.2
X-17299	0.83	Unnamed	0.031	1265.9	229.2
X-18156	0.79	Unnamed	0.031	1392	272.1
Palmitoyl sphingomyelin	0.84	Lipid	0.032	2524	311.3
X-16945	1.73	Unnamed	0.036	3457.9	351
Cysteine-glutathione disulfide	0.81	Amino acid	0.038	821	427.1
4-Vinylphenol sulfate *	1.29	Xenobiotics	0.040	3323	199.1
Erythritol *	0.87	Xenobiotics	0.040	1517.5	217
Dihomolinolenate (20:3n3 or 3n6)	1.19	Lipid	0.043	5600	305.4
Anthranilate	1.36	Amino acid	0.049	3213	138.1
Lysine	0.75	Amino acid	0.049	1836.7	317.2
Threitol	0.86	Carbohydrate	0.049	1513	217.1
Threonate	0.74	Cofactors and vitamins	0.049	1560.7	292.1
Glutamine	0.89	Amino acid	0.002	684	147.2
X-12010	0.72	Unnamed	0.002	1707	203.1
Glycolate (hydroxyacetate) *	0.87	Xenobiotics	0.002	1119	177
gamma-Glutamyltyrosine	0.75	Peptide	0.002	2073	311.2
X-12822	0.62	Unnamed	0.004	2786	389.1
Xylonate	0.58	Carbohydrate	0.004	1722	292
Prolylhydroxyproline	0.19	Peptide	0.005	960	229.2
Mannitol	0.33	Carbohydrate	0.005	1839	319.1
Hydroquinone sulfate *	1.57	Xenobiotics	0.005	1383	189
Ethanolamine	0.61	Lipid	0.005	1304	174.1
4-Ethylphenyl sulfate *	1.50	Xenobiotics	0.006	3570	201.1
Arabonate	0.69	Cofactors and vitamins	0.006	1736	292.1
N6-Carbamoylthreonyladenosine	0.87	Nucleotide	0.006	2656	413
Pantothenate (Vitamin B5)	1.32	Cofactors and vitamins	0.006	2218	220.1

Plasma Metabolite differences between small and large dogs:

*xenobiotics

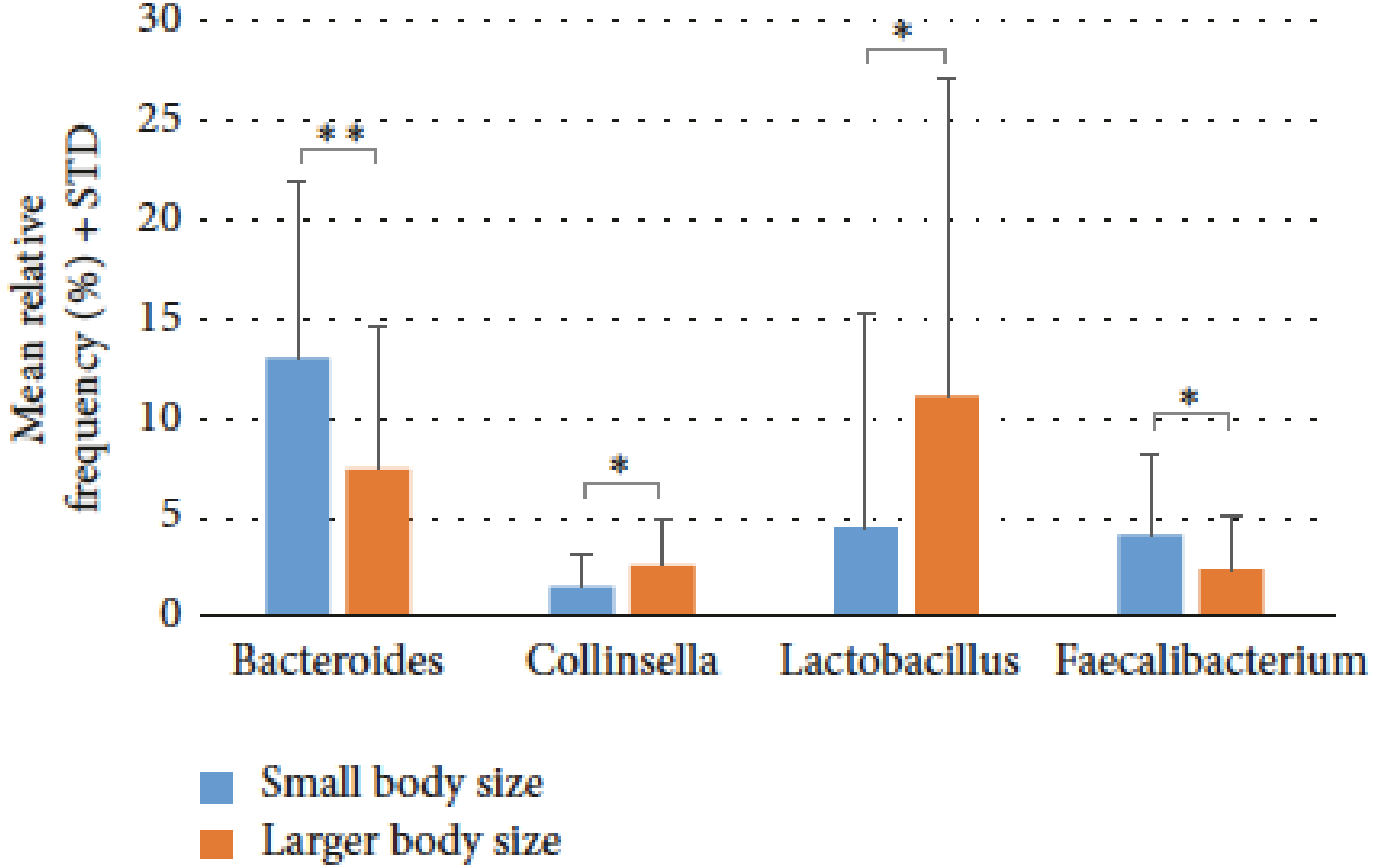


FIGURE 1: Fecal microbiota differences (genus level) between small dogs and dogs of larger body size. Microbiota differences with P value < 0.05 and relative frequencies greater than 1% are shown. $^{**}P < 0.01$; $^{*}P < 0.05$.

Journal of Nutrition and Metabolism
Volume 2017, Article ID 4535710, https://doi.org/10.1155/2017/45

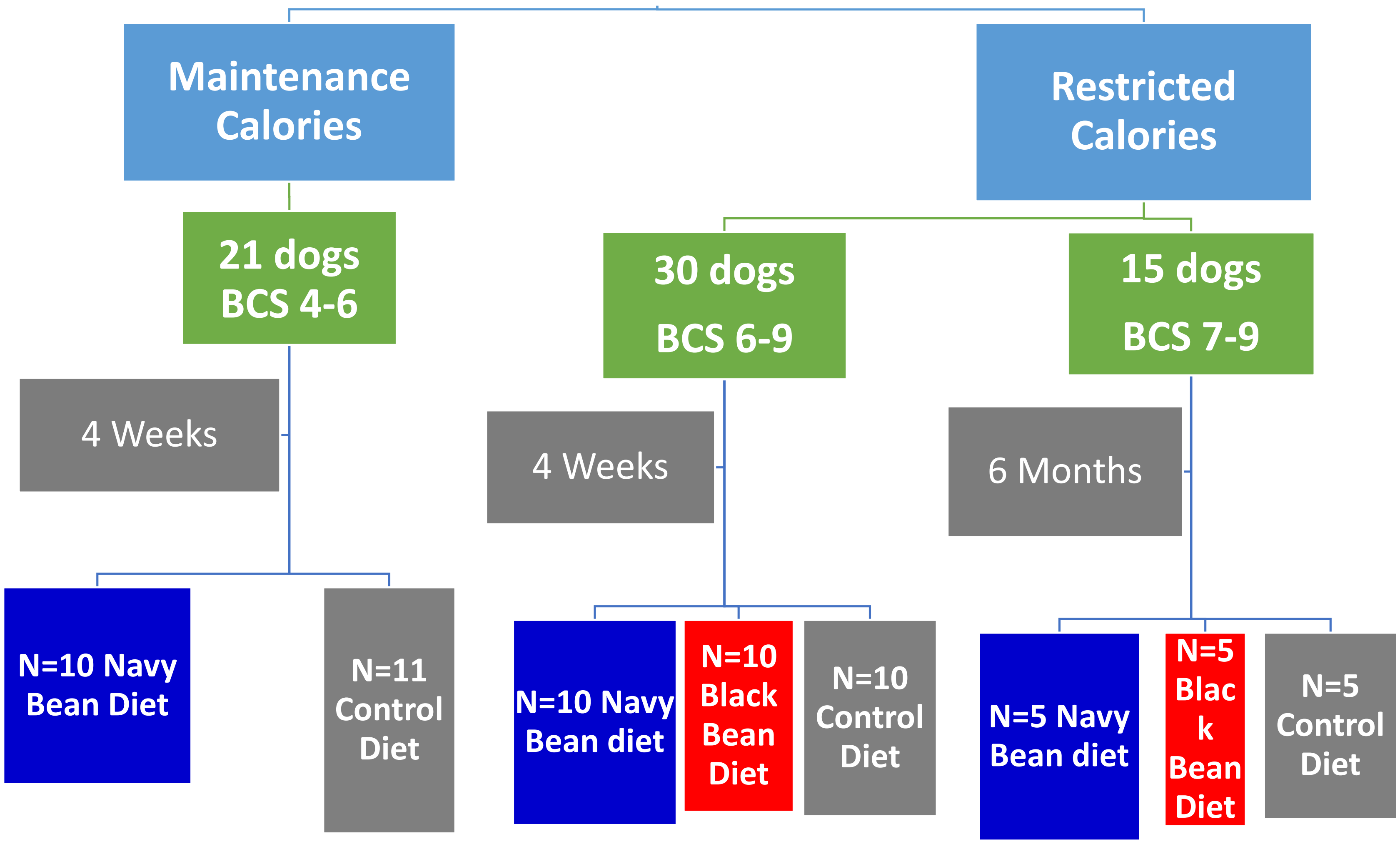
1. <u>There are multiple evidences for differences in metabolism BASED ON DOG SIZE that we must consider</u> *when evaluating the levels or impacts of pesticides or any other environmental exposures.*

Metabolic differences may influence exposure dose, immunity and risks for developing cancer in (aging) dogs and people.

2. Establishing causation, direct (and/or indirect) relationships between chemical exposures and clinical outcomes remains challenging for both humans and pets …and particularly without intentional hypothesis-driven study designs.

Is it safe for dogs to eat beans, and if so, does the consumption of beans by dogs result in metabolic shifts that may be protective of cancer?

Companion dogs (greater than 10kg) were recruited into three double-blinded, randomized, controlled dietary intervention trials evaluating the safety, digestibility, and feasibility of 25% w/w cooked bean consumption

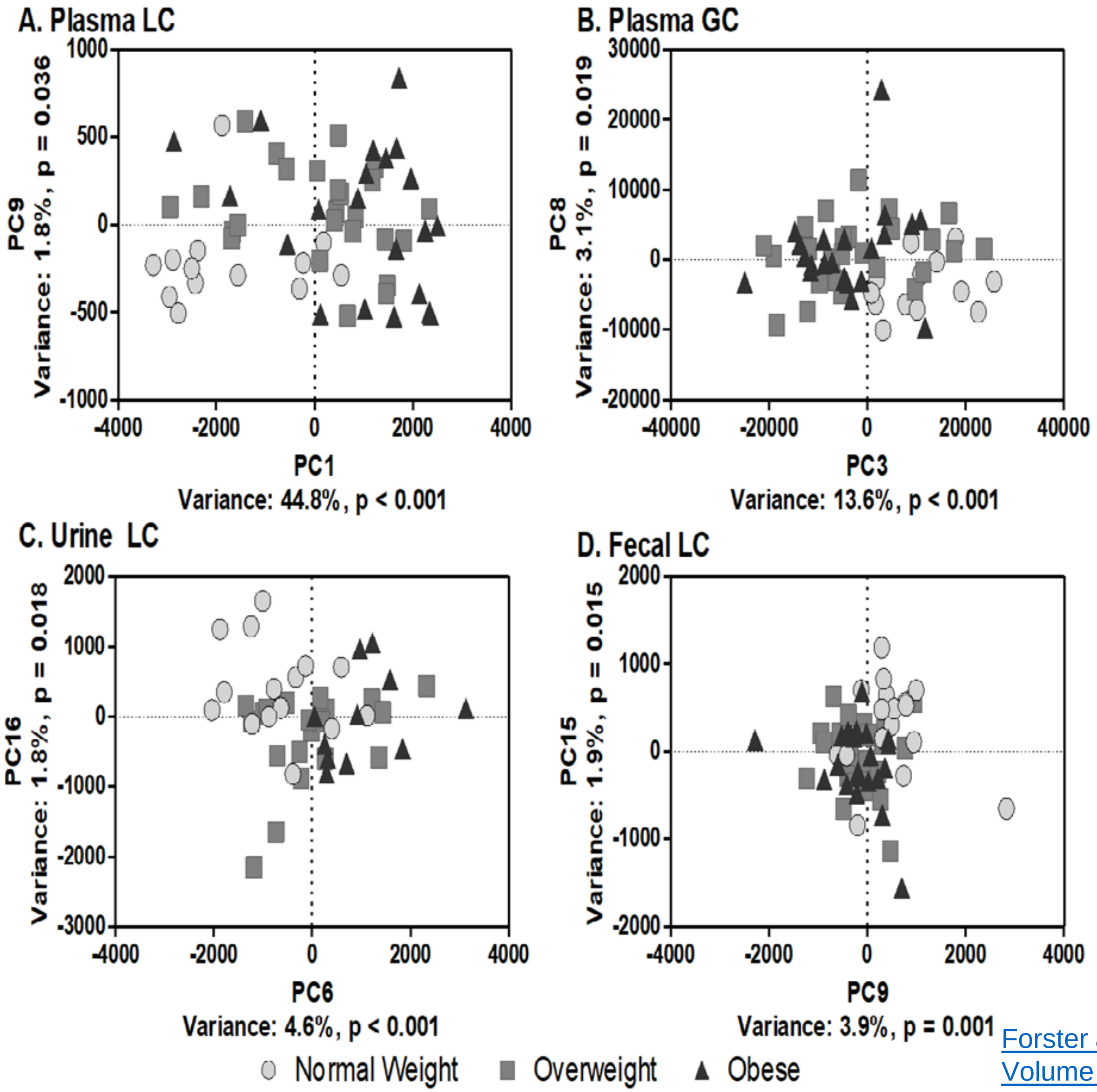


Plasma, Fecal and Urine metabolome of 66 companion dogs differ by body condition score

Annotated plasma metabolites indicated lipids responsible for separation by BCS, such as fatty acids and phospholipids.

Changes in plasma lipids in humans with increased body mass index have been previously documented and further support the hypothesis that altered energy metabolism and glucose homeostasis occur in OW and OB dogs.

*take home message: BCS may impact levels of



Forster and Stockman. Topics in Companion Animal Med Volume 33, Issue 4, December 2018, Pages 126-13

Table 2: Urine collection dates and baseline dietary intake for 21 clinically healthy dogs evaluated for pesticide exposure.

	Collection Date (2010)		Baseline Diet Major	
Dog ID	Baseline	4 Week	Ingredients	Treats and Snacks[1]
NB1	August	August	Chicken and Wheat	Biscuits, CET chews, human food
NB2	August	August	Lamb and Rice	Milk bones
NB3	August	September	Chicken and Corn	Chewie's and Purina Dog Biscuits
NB4	August	September	Meat/Bone Meal and Corn	Biscuits and table scraps
NB5	August	September	Meat/Bone Meal and Corn	Human food, bones, and rawhides
NB6	August	September	Meat/Bone Meal and Corn	Human food, bones, and rawhides
NB7	September	October	Chicken and Corn	Dog biscuits
NB8	September	October	Meat/Bone Meal and Corn	Dog biscuits
NB9	September	October	Chicken and Rice	Peanut butter
NB10	September	October	Chicken and Corn	
C1	August	August	Chicken and Corn	
C2	August	August	Chicken and Pea	Hills dental and chicken jerky treats
C3	August	September	Chicken and Rice	Rawhide chews
C4	No Sample	September	Chicken and Rice	Rawhide chews
C5	August	September	Chicken and Rice	
C6	September	October	Chicken and Corn	Milk Bones
C7	September	October	Chicken and Corn	Bread
C8	September	October	Chicken and Corn	Science diet biscuits
C9	No Sample	October	Chicken and Corn	Science diet biscuits
C10	October	November	Chicken and Rice	
C11	October	November	Chicken and Rice	

[1]As reported by owners

Ginny Forster/Dustin Brown

Multiresidue Analysis of Pesticides in Urine of Healthy Adult Companion Dogs

Genevieve M. Forster,[†,§] Dustin G. Brown,[‡,§] Gregory P. Dooley,[‡] Rodney L. Page,[†] and Elizabeth P. Ryan*[†,‡]

[†]Department of Clinical Sciences and [‡]Department of Environmental and Radiological Health Sciences, College of Veterina Medicine and Biomedical Sciences, Colorado State University, 1680 Campus Delivery, Fort Collins, Colorado 80523-1680, States

ACS Publications 14677 dx.doi.org/10.1021/es503764s | Environ. Sci. Technol. 2014, 48, 1

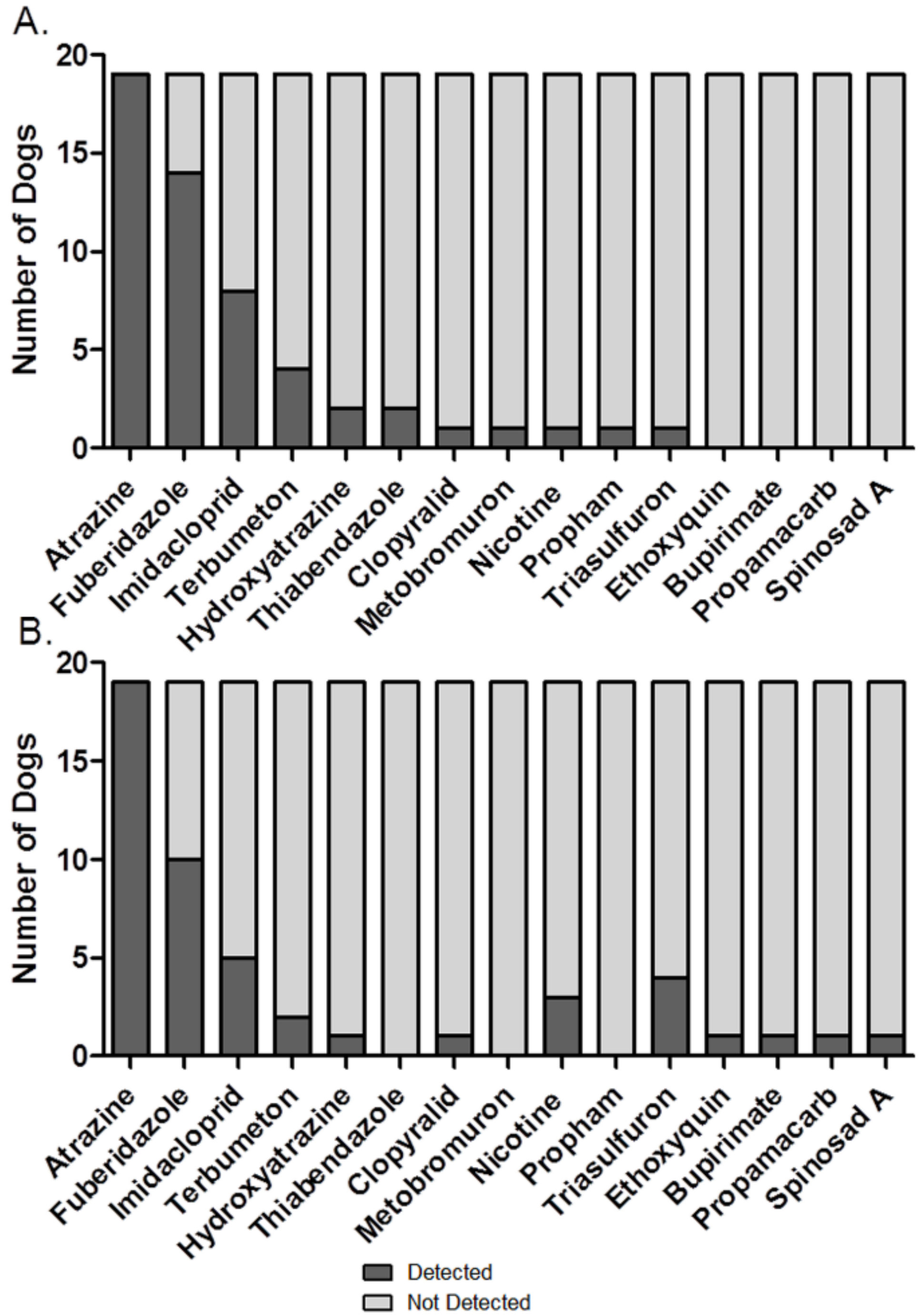
Morris Animal FOUNDATION

FLINT ANIMAL CANCER CENTER

Table 3: Identification parameters for pesticides detected in canine urine and diet samples.

Compound	Retention Time (Min)	Precursor ion (m/z)	Product ions (m/z)	Classification	EPA Registered Use
Atrazine	9.46	216.1	174.1 132.0	Herbicide	corn, sorghum, sugarcane, and residential lawns [123]
Azinphos-ethyl	11.31	346.0	137.1 97.0	Insecticide	N/A
Bromacil	8.06	261.0	205.0 187.9	Herbicide	Citrus, pineapple, and non-agricultural weed control [124]
Bupirimate	11.90	317.2	272.0 166.1	Fungicide	N/A
Clopyralid	2.00	192.0	174.0 110.0	Herbicide	Sugarbeets, mint, and wheat [125]
Ethoxyquin	11.38	218.2	174.1 148.0	Insecticide	Pears [126]
Fuberidazole	6.91	185.1	157.1 156.0	Fungicide	N/A
Hydroxyatrazine	5.61	198.0	156.1 86.0	Herbicide	N/A
Imidacloprid	4.83	256.1	209.1 175.0	Insecticide	Corn, lettuce, broccoli, apples, potatoes [127]
Metobromuron	9.15	259.0	170.0 148.1	Herbicide	N/A
Nicotine	1.13	163.1	132.1 130.0	Insecticide	Ornamental plants (poinsettias)[128]
Propamocarb	2.95	189.2	102.1 144.1	Fungicide	Ornamental, non-crop grasses, shrubs, and vines [129]
Propham	9.26	180.1	138.1 120.0	Herbicide	Legumes, flax, lettuce, safflower, lettuce, and fallow land
Propoxur	8.22	210.1	168.1 111.0	Insecticide	Indoor applications [130]
Pymetrozine	3.57	218.1	105.0 79.0	Insecticide	Fruit and vegetables [131]
Spinosad A	13.20	732.5	142.1 98.0	Insecticide	Fruit, vegetables and grains [132]
Terbumeton	10.15	226.2	170.1 114.1	Herbicide	N/A
Thiabendazole	6.57	202.0	175.0 131.1	Fungicide	Fruit, vegetables and grains [133]
Triasulfuron	8.02	402.1	167.1 141.1	Herbicide	Wheat, barley, pastures, and rangeland [134]

N/A = not currently registered for use in the U.S.

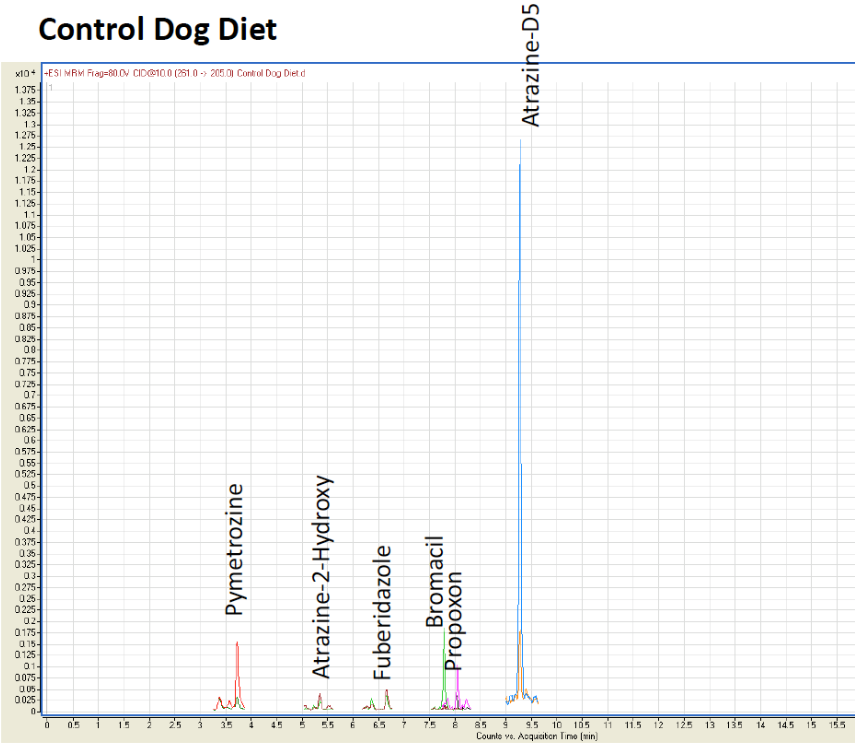


C.

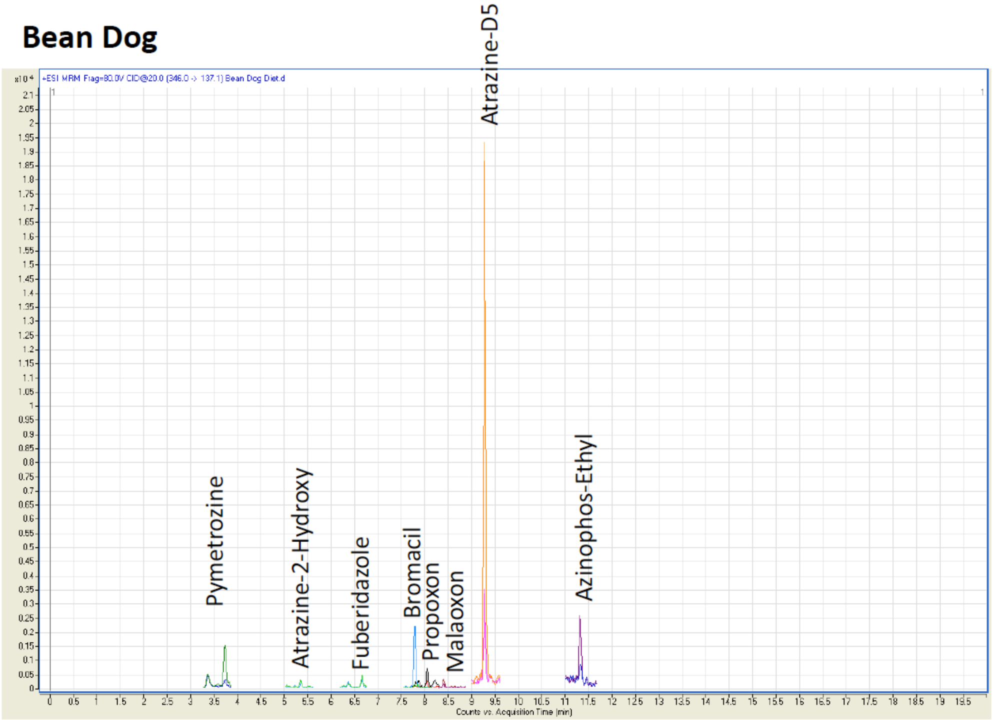
Study ID	Timepoint	Atrazine	Fuberidazole	Imidacloprid	Hydroxyatrazine	Terbumeton	Thiabendazole	Clopyralid	Metobromuron	Nicotine	Propham	Triasulfuron	Bupirimate	Ethoxyquin	Propamocarb	Spinosad A
C1	Baseline	●	●	●												
	4 Weeks	●	●	●								●				
C2	Baseline	●	●			●	●									
	4 Weeks	●	●	●												
C3	Baseline	●	●			●										
	4 Weeks	●	●										●			
C4	ND															
	4 Weeks	●	●									●				
C5	Baseline	●	●	●												
	4 Weeks	●	●	●												
C6	Baseline	●	●	●												
	4 Weeks	●														
C7	Baseline	●	●	●								●				
	4 Weeks	●		●												
C8	Baseline	●				●										
	4 Weeks	●				●										
C9	ND															
	4 Weeks	●												●		
C10	Baseline	●		●												
	4 Weeks	●	●	●								●				
C11	Baseline	●	●	●												
	4 Weeks	●	●							●						
NB1	Baseline	●	●	●	●											
	4 Weeks	●										●				
NB2	Baseline	●	●	●	●	●	●	●		●						
	4 Weeks	●	●		●			●		●						
NB3	Baseline	●	●													
	4 Weeks	●	●													
NB4	Baseline	●	●								●					
	4 Weeks	●								●						
NB5	Baseline	●	●													
	4 Weeks	●	●									●				
NB6	Baseline	●	●													
	4 Weeks	●	●													
NB7	Baseline	●														
	4 Weeks	●				●										●
NB8	Baseline	●														
	4 Weeks	●														
NB9	Baseline	●	●						●							
	4 Weeks	●														
NB10	Baseline	●														
	4 Weeks	●													●	

Some chemicals in the diet

Control Dog Diet



Bean Dog



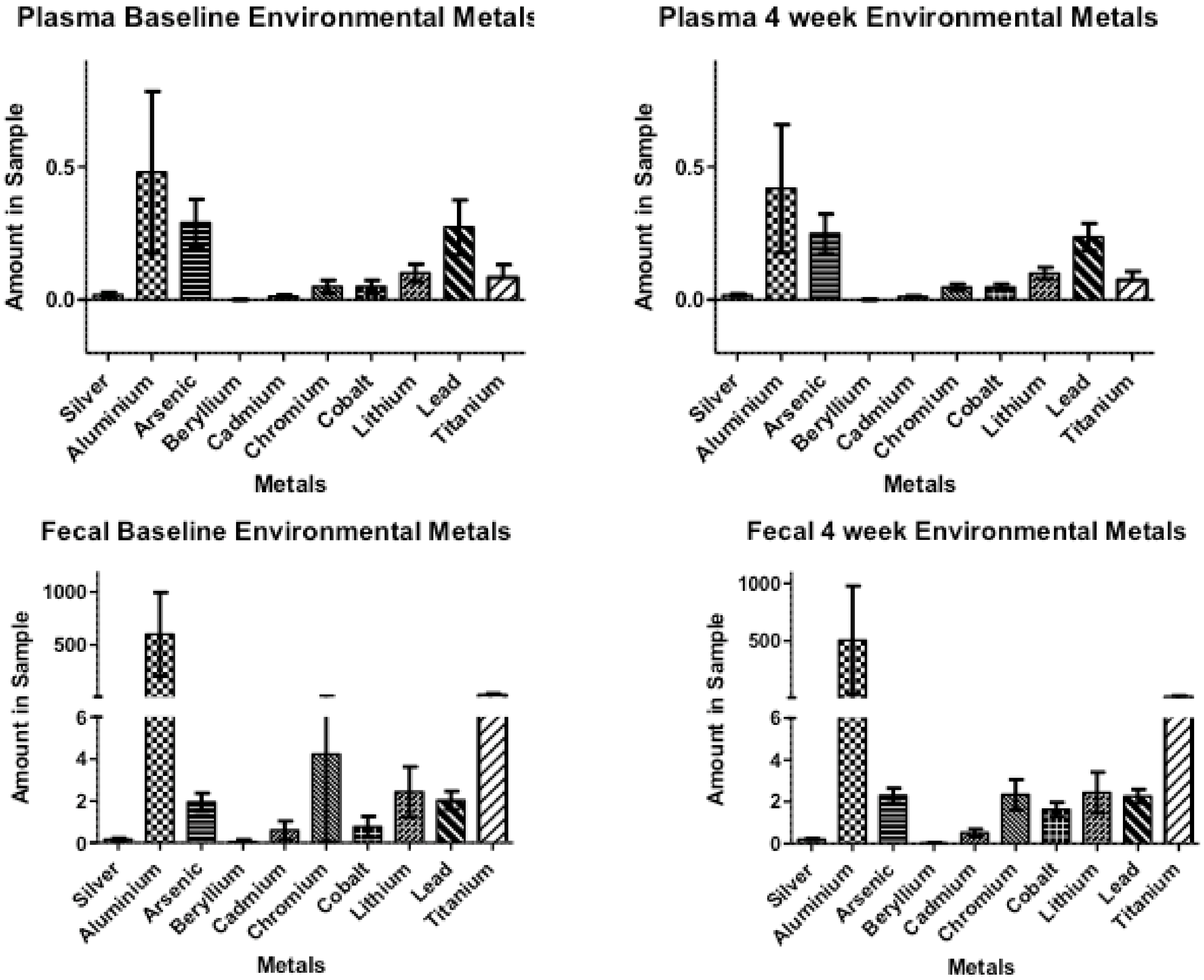
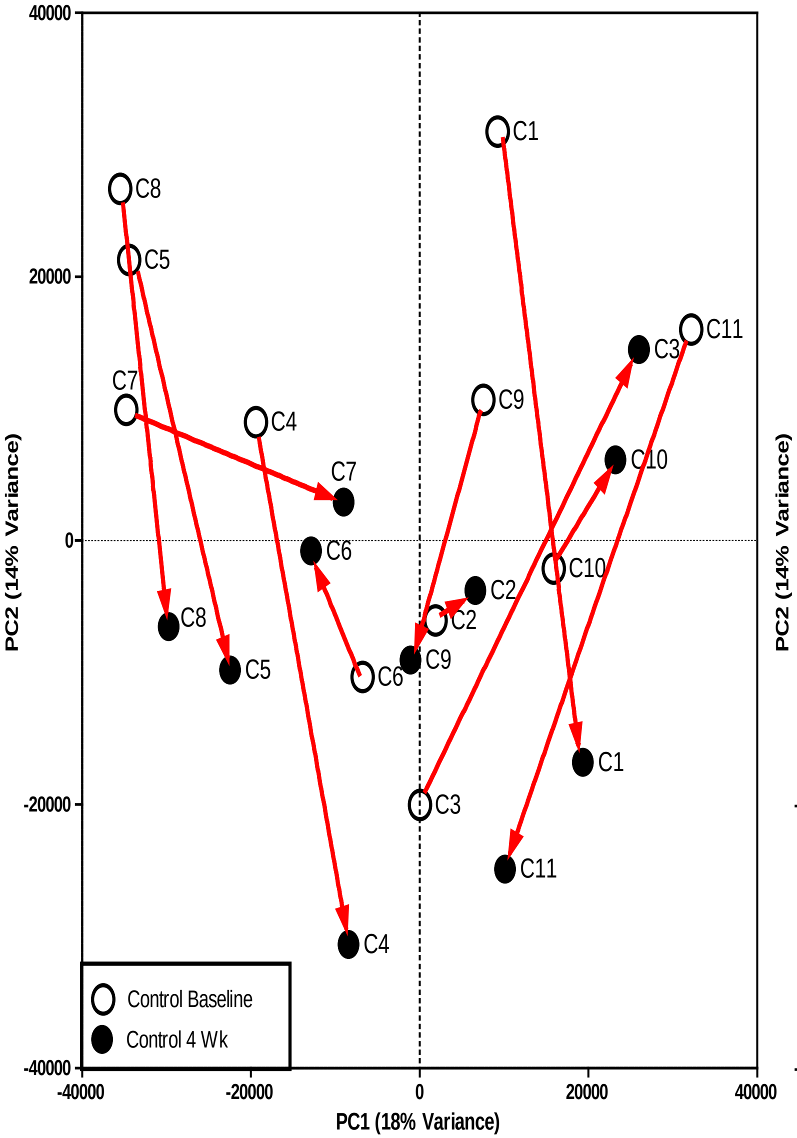

Plasma Baseline Environmental Metals
Amount in Sample
0.5
0.0
Silver
Aluminium
Arsenic
Beryllium
Cadmium
Chromium
Cobalt
Lithium
Lead
Titanium
Metals
Plasma 4 week Environmental Metals
Amount in Sample
0.5
0.0
Silver
Aluminium
Arsenic
Beryllium
Cadmium
Chromium
Cobalt
Lithium
Lead
Titanium
Metals
Fecal Baseline Environmental Metals
Amount in Sample
1000
500
6
4
2
0
Silver
Aluminium
Arsenic
Beryllium
Cadmium
Chromium
Cobalt
Lithium
Lead
Titanium
Metals
Fecal 4 week Environmental Metals
Amount in Sample
1000
500
6
4
2
0
Silver
Aluminium
Arsenic
Beryllium
Cadmium
Chromium
Cobalt
Lithium
Lead
Titanium
Metals

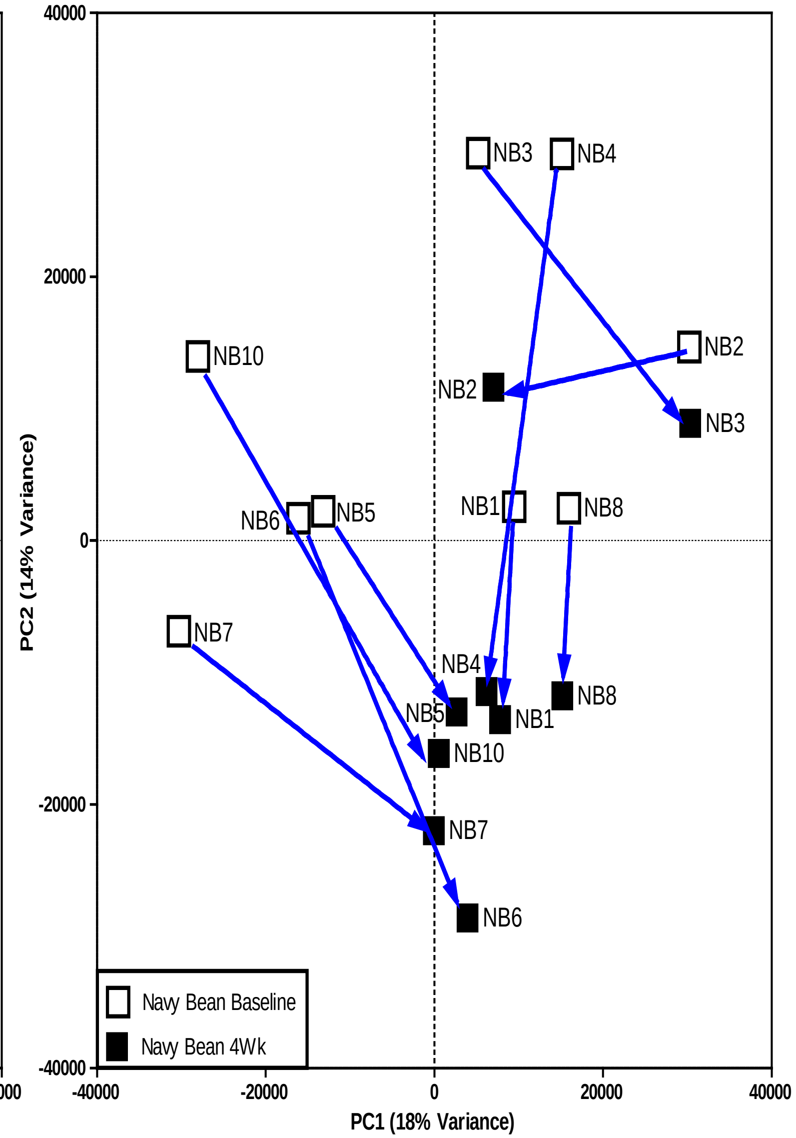
Table 4: Reported bioactivities of detected pesticides from PubChem BioAssay database.

Pesticide[1]	Bioactivity
Atrazine	Activates cAMP Responsive Element signaling (AID: 662) Activates insulin secretion (AID: 743287) Carcinogenic (AID: 1189, 1208, 1205) Inhibits the activation of platelets (AID: 1663)
Fuberidazole	Activates the AhR signaling pathway (AID:743085, 743122) Agonist of the ER-α signaling pathway (AID: 743079) Genotoxic (AID: 720516)
Imidacloprid	Inhibits cell surface uPA activity preventing formation of plasmin (AID: 540303) Inhibits tyrosyl-DNA phosphodiesterase I (AID: 686978)
Thiabendazole	Activates the AhR signaling pathway (AID: 743085) Increases SMN2 gene splice variant expression (AID: 1458) Activates 5'UTR Stem-Loop Driven Prion Protein mRNA translation (AID: 1999) Activates BRCA1 Expression (AID: 624202) Agonist of the ER-α signaling pathway (AID: 743079) Inhibits insulin promoter activity (AID: 1273) Inhibits tubulin formation (AID: 742594) Genotoxic (AID: 720516)
Clopyralid	Activates the AhR signaling pathway (AID: 743085)
Nicotine	Agonist of the nAchR (AID: 742462) Agonist of the AR signaling pathway (AID: 743036)
Bupirimate	Disruptor of mitochondrial membrane potential (AID: 720635) Antagonist of the GR signaling pathway (AID: 720725) Antagonist of the AR signaling pathway (AID: 743122, 743063, 743054) Antagonist of the PPAR-γ signaling pathway (AID: 743199) Agonist of the antioxidant response element signaling pathway (AID: 743219)
Propham	Agonist of the ER-α signaling pathway (AID: 743079)
Metobromuron	Activates the AhR signaling pathway (AID: 743122, 743085)
Ethoxyquin	Antagonist for Hypoxia Response Element Signaling Pathway (AID: 915) Inhibits Cytochrome p450 isoforms (AID: 894, 899, 883, 884, 1851) Activates Caspase-3/7 (AID: 588813) Inhibits hydroxyacyl-coenzyme-A dehydrogenase, Type II (AID: 886) Inhibits 15-human lipoxygenase 1 and 2 (AID: 881, 887) Inhibits hydroxysteroid (17-beta) dehydrogenase 4 (AID: 893) Inhibits Histone Lysine Methyltransferase G9a (AID: 504332) Inhibits vitamin D receptor (AID: 504847) Inhibits bromodomain adjacent to zinc finger domain, 2B (AID: 504333) Inhibits human tyrosyl-DNA phosphodiesterase 1 & 2 (AID: 686979, 720702) Activates heat shock response signaling pathway (AID: 743228) Agonist of the PPAR-γ signaling pathway (AID: 743094, 743140) Activates the AhR signaling pathway (AID: 743122) Antagonist of the thyroid receptor signaling pathway (AID: 743065) Agonist of the antioxidant response element signaling pathway (AID: 743202)

[1]https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi. AhR: aryl hydrocarbon receptor; AR: androgen receptor ER-α: estrogen receptor-alpha; GR: glucocorticoid receptor, nAchR: neuronal acetylcholine receptor; PPAR-δ/γ: peroxisome proliferator-activated receptor delta/gamma; uPA: urokinase plasminogen activator

Effects of Navy bean intake on the companion dog stool metabolome





Forster et. al. Current Metabolomics 2015

Effects of cooked navy bean powder on apparent total tract nutrient digestibility and safety in healthy adult dogs[1]

G. M. Forster,* D. Hill,† G. Gregory,‡ K. M. Weishaar,* S. Lana,* J. E. Bauer,§ and E. P. Ryan*[2]

*Department of Clinical Sciences, Colorado State University, Fort Collins 80523; †ADM Alliance Nutrition, Inc. Quincy, IL 62301; ‡Archer Daniels Midland Edible Bean Specialties, Decatur, IL 62525; and §Intercollegiate Faculty of Nutrition, Department of Small Animal Clinical Sciences, Texas A&M University, College Station 77843

J. Anim. Sci. 2012.90:2631–2638
doi:10.2527/jas2011-4324

Effects of Dietary Cooked Navy Bean on the Fecal Microbiome of Healthy Companion Dogs

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PLOS ONE | www.plosone.org 1 September 2013 | Volume 8 | Issue 9 | e74998

Nutritional Weight Loss Therapy with Cooked Bean Powders Regulates Serum Lipids and Biochemical Analytes in Overweight and Obese Dogs

Genevieve M. Forster[1], Cadie A. Ollila[1], Jenna H. Burton[1], Dale Hill[2], John E. Bauer[3], Ann M. Hess[4], Elizabeth P. Ryan[1]*

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J Obes Wt Loss Ther
ISSN: 2165-7904 JOWT, an open access journal
Volume 2 • Issue 8 • 1000149

Journal of Veterinary Science & Technology

Beloshapka et al., J Vet Sci Technol 2016, 7:5
DOI: 10.4172/2157-7579.1000366

Research Article Open Access

Fecal Microbial Communities of Overweight and Obese Client-Owned Dogs Fed Cooked Bean Powders as Assessed by 454-Pyrosequencing

Alison N Beloshapka[1], Genevieve M Forster[3], Hannah D Holscher[1], Kelly S Swanson[1,2]* and Elizabeth P Ryan[3,4]

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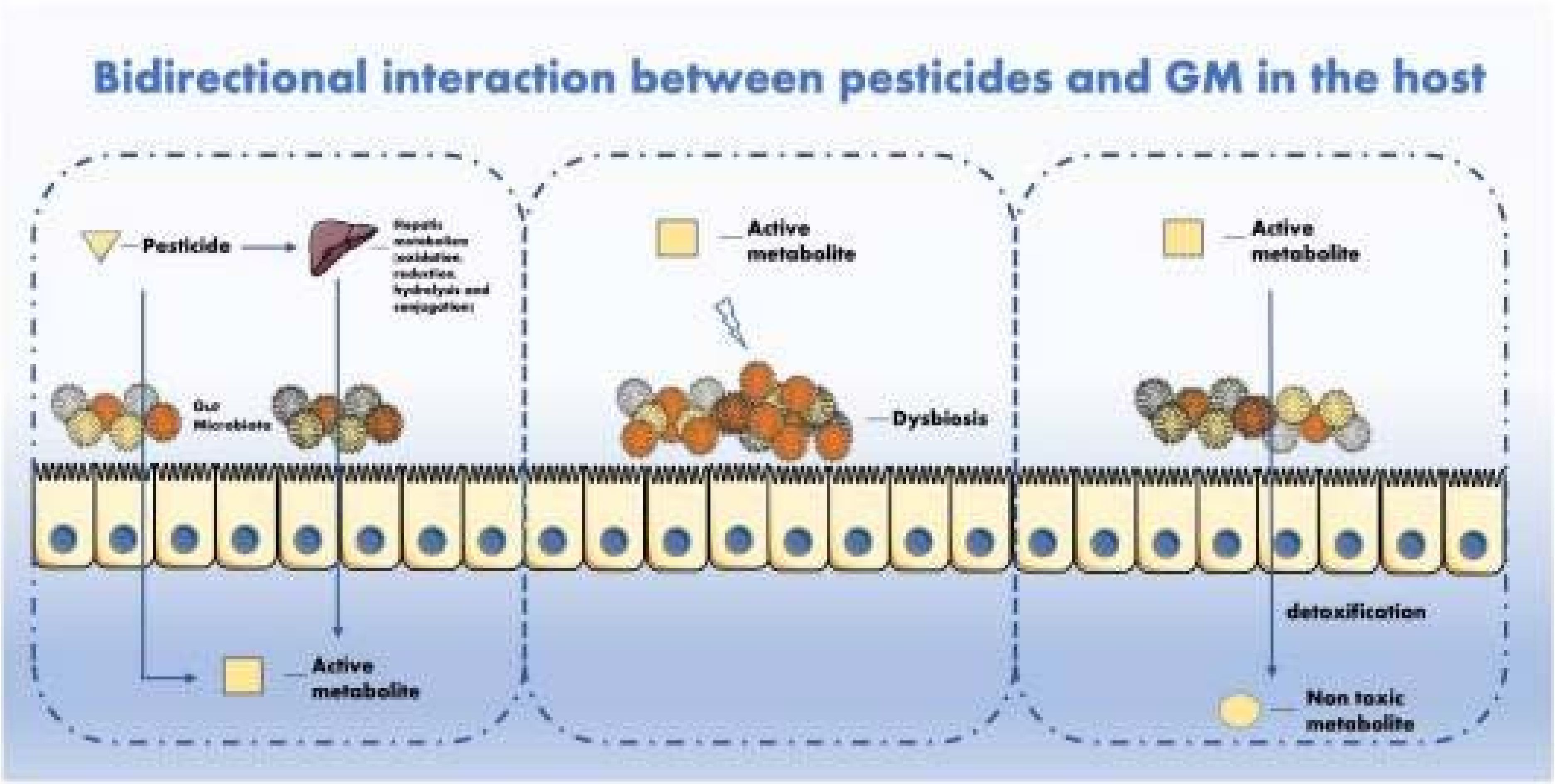
[3]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA
[4]Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, USA

Navy and black bean-based dog foods are digestible during weight loss in overweight and obese adult companion dogs

2016. Journal of Applied Animal Nutrition, Vol. 4; e4; page 1 of 11

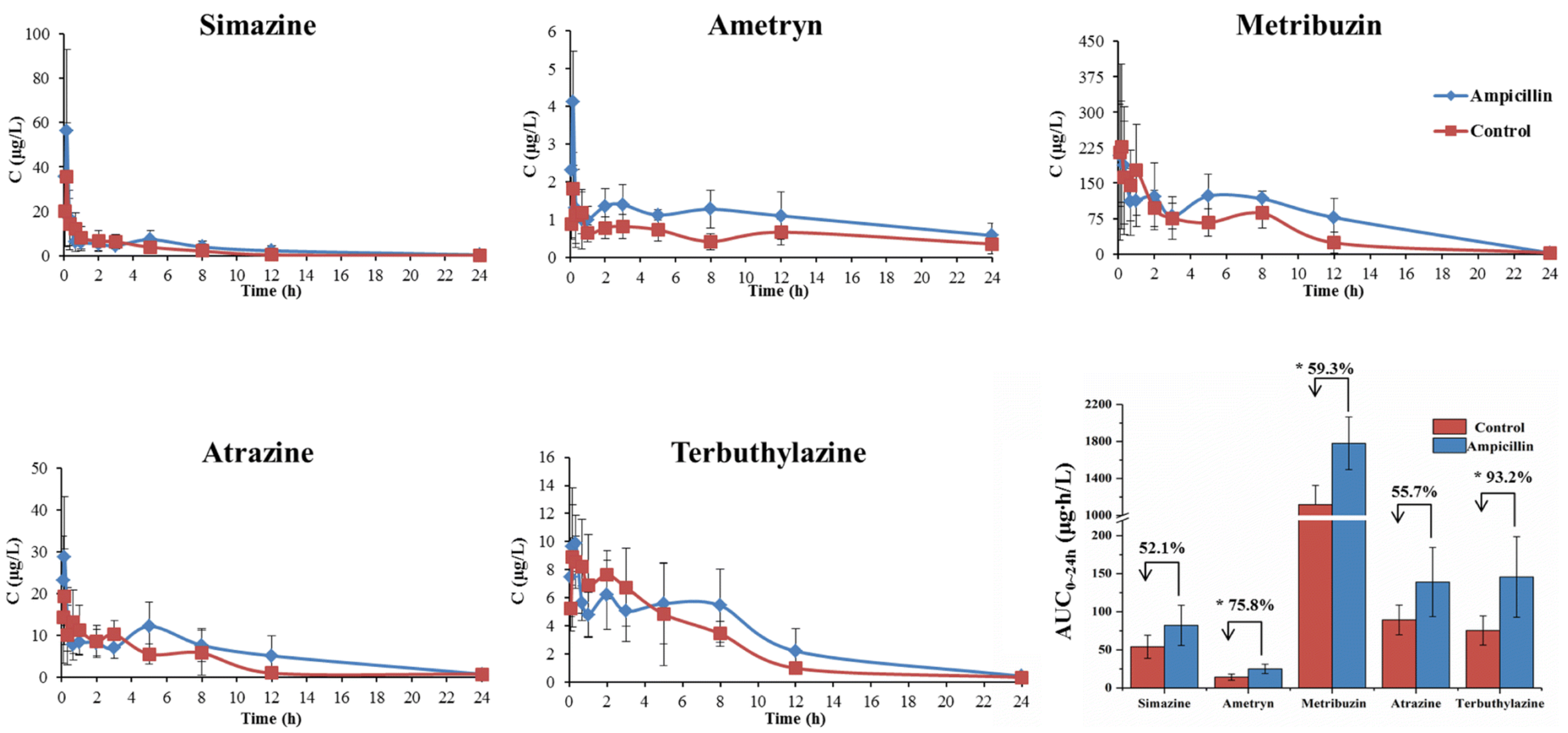
JAAN

USDA ADM



Giambò, F.; Teodoro, M.;Costa, C.; Fenga, C.
Toxicology and Microbiota: How Do Pesticides Influence Gut Microbiota? A Review.
Int. J. Environ. Res. Public Health 2021, 18, 5510.
https://doi.org/10.3390/ijerph18115510

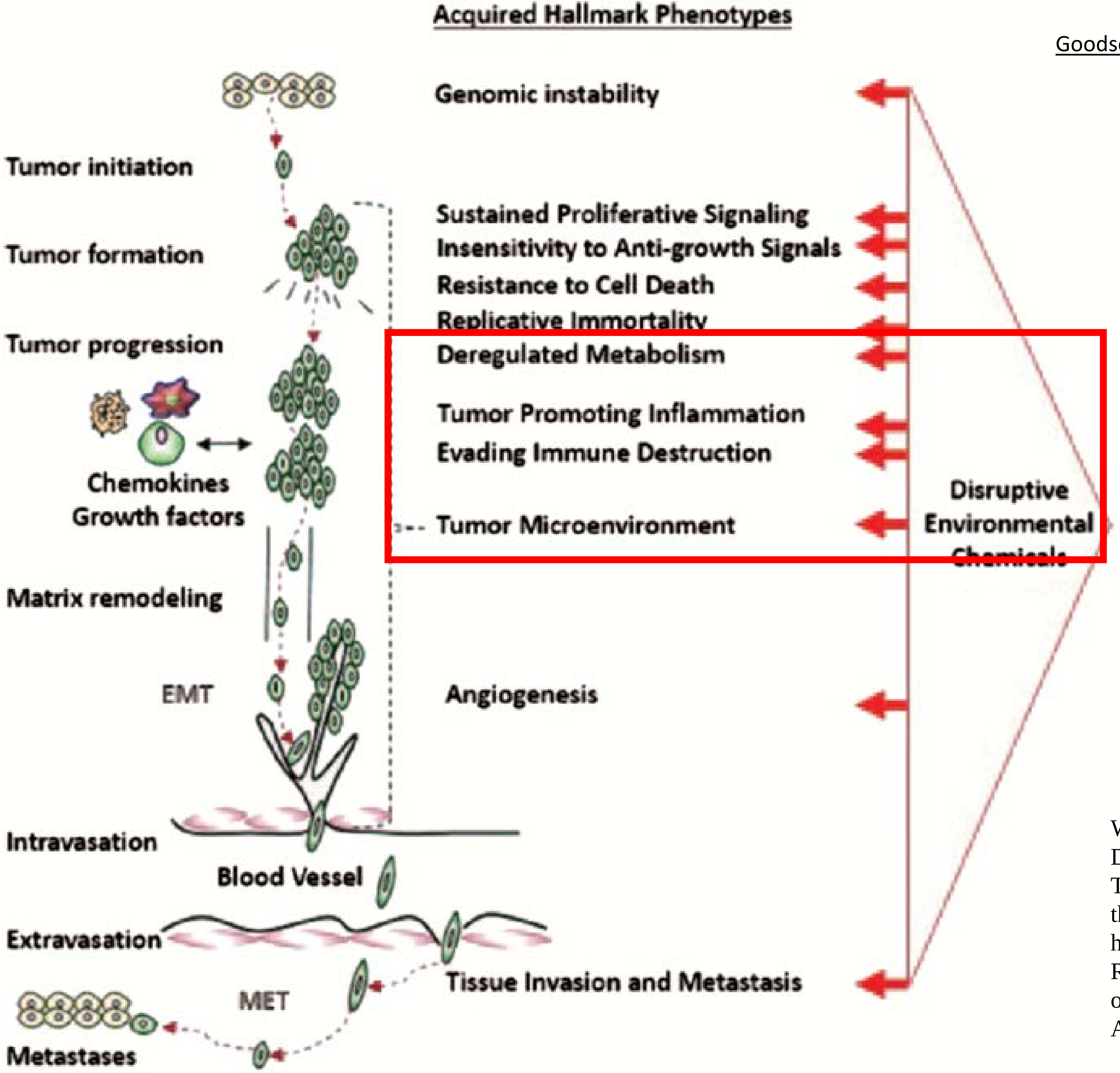
Antibiotics could increase the pesticide bioavailability and thereby may increase the pesticide exposure risk.



Effects of 7-day ampicillin treatment on the blood concentration of triazine herbicides in rats. Control rats were treated with equal volumes of water free from ampicillin. The AUC of triazine herbicides was showed in the column diagram, and the percent represents the increase rate of the AUC in the ampicillin-treated rats relative to the control rats (independent sample *t*-test, $*P < 0.05$, $n = 5$)

Zhan, J. et. al. *Microbiome* **6,** 224 (2018).

Goodson et.al. Carcinogenesis. 2015 Jun; 36(Suppl 1): S254–S296.



William H. Goodson*, Leroy Lowe, Michael Gilbertson and David O. Carpenter
Testing the low dose mixtures hypothesis from the Halifax project
https://doi.org/10.1515/reveh-2020-0033
Received March 13, 2020; accepted June 2, 2020; published online
August 24, 2020

Dysregulated metabolism	Cypermethrin	AR and ER expression, reduction of ATP and mitochondrial enzymes, mitochondrial membrane potential	LLDE (A-I) (42), NLDE (A-I) (42), NLDE (H-CL) (36,43,44)
	Acrolein	p53 activation, DNA repair inhibition, PERK phosphorylation, mitochondrial dysfunction, cell survival	LLDE (A-I, A-CL, H-PC, H-CL) (45–50), NLDE (49), threshold (46)
	Rotenone	Cell cycle, DNA damage response, proliferation, differentiation, mitochondria	LLDE (H-CL) (51–53), NLDE (H-CL) (51,53), unknown (H-CL,H-PC) (36)
	Copper	p53 activation, p21 up-regulation, cell viability	LLDE (H-CL) (54–56)
	Nickel	Neutrophil apoptosis, E-cadherin regulation, matrix metallopeptidase (MMP) production	LLDE (H-CL) (57), NLDE (H-CL) (58), Threshold (H-CL) (58)
	Cadmium	p53-dependent apoptosis, cell proliferation	LLDE (H-CL) (59), threshold (H-CL) (60)
	Diazinon	AChE activity, neuronal cytotoxicity	Unknown (A-PC) (61), LLDE (H-CL) (62), threshold (H-CL) (36)
	Iron	KRAS mutations	LLDE (A-I) (63)
	Malathion	Lymphocyte Mutations, Cytotoxicity	Unknown (H-PC, H-E) (36,64)
Tumor microenvironment	Nickel	ROS and cellular stress	NLDE (A-I) (175)
	BPA	IL-6 expression, improper DC maturation and polarization, ROS production	LLDE (A-I) (176), NLDE (A-I) (176)
	Butyltins (such as tributyltin)	NK cell inhibition	LDE (A-I) (177)
	MeHg	Chronic oxidative stress	LDE (H-PC, H-CL) (178,179)
	Paraquat	Chronic ROS production, cellular stress	Unknown (A-I) (180)
Tumor-promoting inflammation	BPA	Immune cell proliferation, proinflammatory cytokine induction	Threshold (H-PC) (36), LDE (A-I, H-CL, H-E) (122–126)
	Phthalates	Immunomodulation of macrophages, lymphocytes, eosinophils and neutrophils	Unknown (H-PC, H-CL, H-E) (36,127)
	PBDEs	Induction of pro-inflammatory cytokines (IL-6, IL8 and CRP), inhibition of anti-inflammatory cytokines (IL-10)	Threshold (H-PC, H-CL) (128–131)
	Atrazine	Immunomodulation of T cell and B cells, proinflammatory cytokines	Unknown (H-PC, A-I) (36,132,133)
	Vinclozolin	Proinflammatory cytokine induction, NF-κB activation	Unknown (H-PC, A-I) (36,134–136)
	4-NP	Proinflammatory cytokine induction, NF-κB activation, iNOS induction	Unknown (A-CL, H-CL, H-PC) (36,137,138)
Immune system evasion	Pyridaben	Chemokine signaling, TGF-β, FAK, HIF-1a, IL-1a pathways	Unknown (H-CL, H-PC, A-CL) (36,139,140), threshold (A-I) (141)
	Triclosan	Chemokine signaling, TGF-β, FAK, IL-1a pathways	Threshold (H-CL, H-PC, A-I) (36,142–144), LDE (A-I, H-CL) (145,146)
	Pyraclostrobin	Chemokine signaling, TGF-β, IL-1a pathways	Unknown (H-CL, H-PC) (36)
	Fluoxastrobin	Chemokine signaling, EGR, HIF-1a, IL-1a pathways	Unknown (H-CL, H-PC) (36)
	BPA	Chemokine signaling, TGF-β pathway	Threshold (H-PC) (36), LDE (A-I) (12), NLDE (H-CL) (147), NLDE (A-CL) (148–151), NLDE (A-I) (152–155)
	Maneb	PI3K/Akt signaling, chemokine signaling, TGF-β, FAK, IGF-1, IL-6, IL-1a pathways	Unknown (H-CL, H-PC) (36,139,156–158), LDE (A-I) (159), threshold (A-I) (139,160), threshold (A-CL, A-I) (161)

Table 3.1 Summary of pesticide sources, chemical classifications, and mechanisms of action

Pesticide name	Sources	Chemical classification	Animals exposed	Mechanism of action clinical signs	References
Brodifacoum	Ground water, biota, soil, foods	Rodenticide	Mammals	Ecchymosis, hematuria	Yu et al. (2013), Berny et al. (2010), World Health Organization (1995)
Bromadiolone	Ground water, surface water, soil, meat/food	Rodenticide	Mammals	Disruption of vitamin K pathway	Vindenes et al. (2008), Yu et al. (2013)
Chlorpyrifos	Flea control dip, ground and surface water	Organophosphate insecticide	Dogs	Musculoskeletal changes, immune system deregulation	Eskenazi et al. (1999), Rauh et al. (2006), Thrasher et al. (1993), Boone et al. (2001)
DDT	Agricultural land, sea, and river water	Organochlorine	Chickens	Seizures, tremors, low birth weight	Jayaraj et al. (2016), Arora et al. (2013), Nayak et al. (1995), Rajendran and Subramanian (1997), Pandit et al. (2002), Aulakh et al. (2006 Goel and Aggarwal (2007)
Diazinon	Home lawn, ground and surface water	Organophosphate	Dogs	Increased liver glucose release	Morgan et al. (2008), Teimouri et al. (2006), Alahyary et al. (2008), Thayer et al. (2012), Hasanuzzaman et al. (2017), Hossain et al. (2015), Bhattacharjee et al. (2012), Akan et al. (2014), Masia et al. (2015), Rahmanikhah et al. (2011)
Diuron	Agricultural runoff, ground water	Phenylurea herbicide	Fish, amphibians	Cytotoxicity, oxidative stress, enzyme induction	Moncada (2004), de Lima et al. (2011), Huovinen et al. (2015), Sunouchi et al. (2011), Rudzok et al. (2009), Marlatt and Martyniuk (2017)
Endosulfan	Dairy farms, marine/ fresh, surface, and ground water	Organochlorine	Sheep, goats	Convulsions, hypoxia	Goel and Aggarwal (2007), Soto et al. (1994), Dalvie et al. (2003), Tsiplakou et al. (2010)

M. R. Pastorinho, A. C. A. Sousa (eds.),
Pets as Sentinels, Forecasters and Promoters of Human Health,
https://doi.org/10.1007/978-3-030-30734-9_3

Pesticide name	Sources	Chemical classification	Animals exposed	Mechanism of action clinical signs	References
Fipronil	Topical flea and tick control, residential water, surface and wastewater	Phenylpyrazole	Cats, dogs	Decreased ATP production, seizures	Sengupta et al. (2014), Stone et al. (2014), Heidler and Halden (2009)
HCH	Agricultural land, river and sea water	Organochlorine	Chickens	Potential role in breast cancer	Jayaraj et al. (2016), Arora et al. (2013), Nayak et al. (1995), Rajendran and Subramanian (1997), Pandit et al. (2002), Zou and Matsumura (2003), Bronden et al. (2007), Guy et al. (2015), Aulakh et al. (2006)
Imidacloprid	Animal hairs, household use, aquatic media, i.e., ground and surface water	Neonicotinoid insecticide	Mammals, birds, fish, amphibians, reptiles	Immunosuppression, respiratory failure, prolonged sedation	Bonmatin et al. (2015), Federoff et al. (2008), Canadian Council of Ministers of the Environment (2007), Forster et al. (2014), Craig et al. (2005), Gibbons et al. (2015), Mohamed et al. (2009), Bal et al. (2012), Cardone (2015)
Linuron	Agricultural runoff, various waters	Phenylurea herbicide	Fish, amphibians	Enzyme induction	de Lima et al. (2011), Huovinen et al. (2015), Sunouchi et al. (2011)
Methaldehyde	Household	Molluscicide	Domestic animals	Ataxia, convulsions	Ruiz-Suarez et al. (2014)
Methiocarb	Household and agricultural water	Carbamate insecticide	Rabbits, rats	Musculoskeletal changes	Blazkova et al. (2009), Rodrigues et al. (2011), EPA (1994)
Paraquat	Agriculture, waters systems	Herbicide	Dogs	Lung failure	Caloni et al. (2016), Clark et al. (1966)
Permethrin	Natural waters	Pyrethroid	Cats	Loss of consciousness, metabolic acidosis	Dymond and Swift (2008), Gotoh et al. (1998)

Chemical mixtures

Challenges:

- Prioritizing mixtures
- Correct study design
- Correct statistical analysis
- Readout selection

Chemical mixtures terms

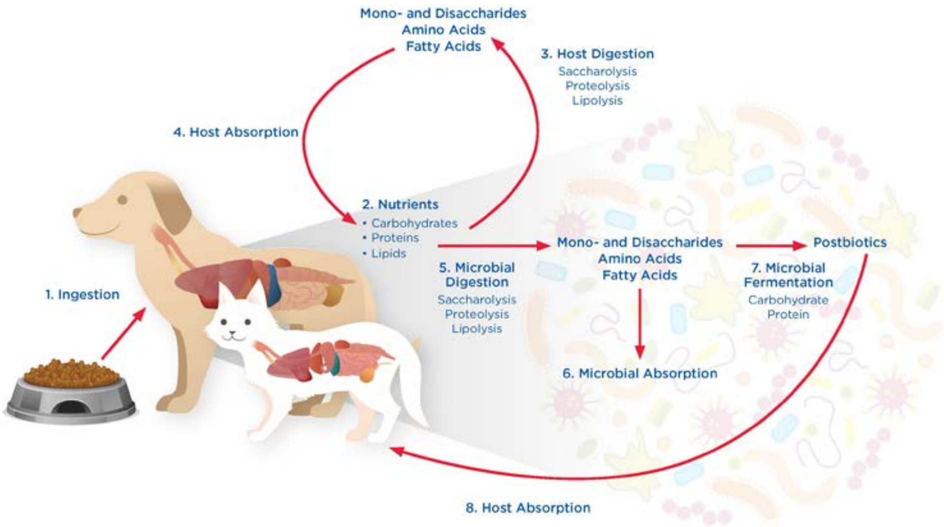
Mixture	Combo of 2 or more chemicals that may jointly contribute to effects in target population
Simple Mixture	Less than 10 chemicals
Complex Mixture	Combination of so many chemicals that composition is not fully characterized and may be variable
Similar Mixture	Same chemicals but slightly different proportions, or have most but not all chemicals in similar proportions
Components of Concern	Chemicals in mixture that likely contribute to health hazard because of concentration or joint toxicity
Indicator Chemical	Chemical selected to represent toxicity of mixture because it is characteristic, potent and has dose-response data

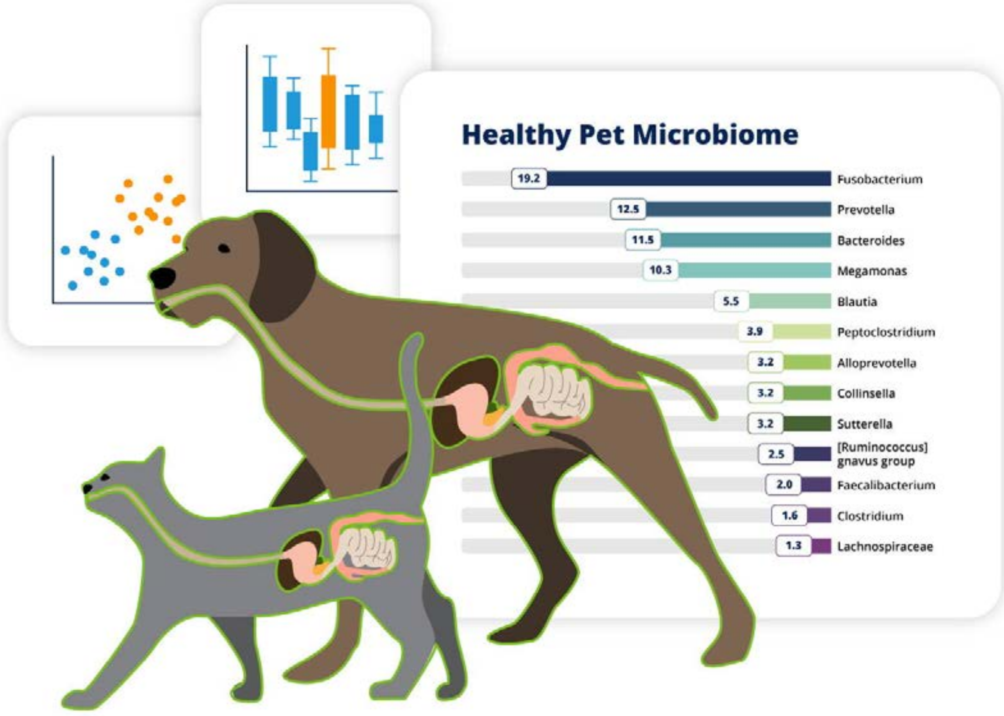
Chemical mixture interactions

Additivity	**When effect can be estimated from sum of exposure levels (weighted for potency) or effects of individual components**
Interaction	**When effect of mixture is different from additivity**
Synergism	**Effect of mixture is greater than additivity estimated based on toxicity of components**
Potentiation	**When non-toxic component increases toxicity of other chemical**
Antagonism	**When effect of mixture is less than additive**
Inhibition	**When non-toxic component decreases toxicity of other chemical**
Masking	**When components oppose or compete with, and thus counteract, each other**

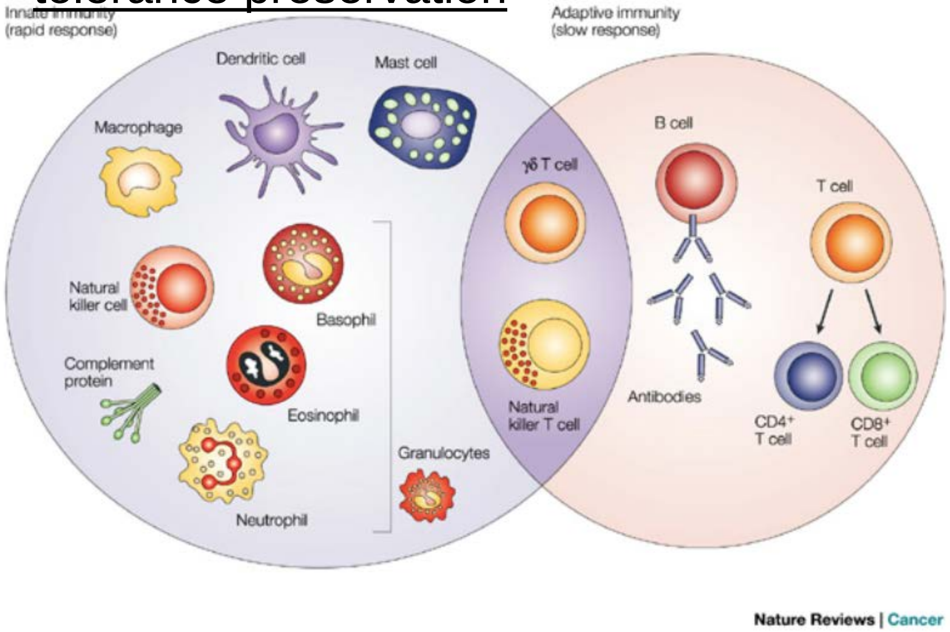
How antagonism among mixture components can complicate analysis

Basis of Interaction	Examples: Synergism of potentiation	Examples: Antagonism or inhibition
Chemical-chemical	Formation of carcinogenic nitrosamines from non-carcinogenic nitrites in stomach	Oral ammonia counteracts ingested formaldehyde
Pharmacokinetic		
Absorption		Dietary zinc inhibits lead toxicity by decreasing absorption
Distribution	Disulfiram increases lead neurotoxicity by forming complexes that enter brain	
Excretion	Probenecid decreases excretion of penicillin, increasing effect	
Metabolism		Selenium shifts metabolism to detoxification and decreases toxicity of 2-acetylaminofluorene in liver





The host microbiota and the immune system must communicate to maintain a balance between the inflammatory response activation and the <u>immune tolerance preservation</u>



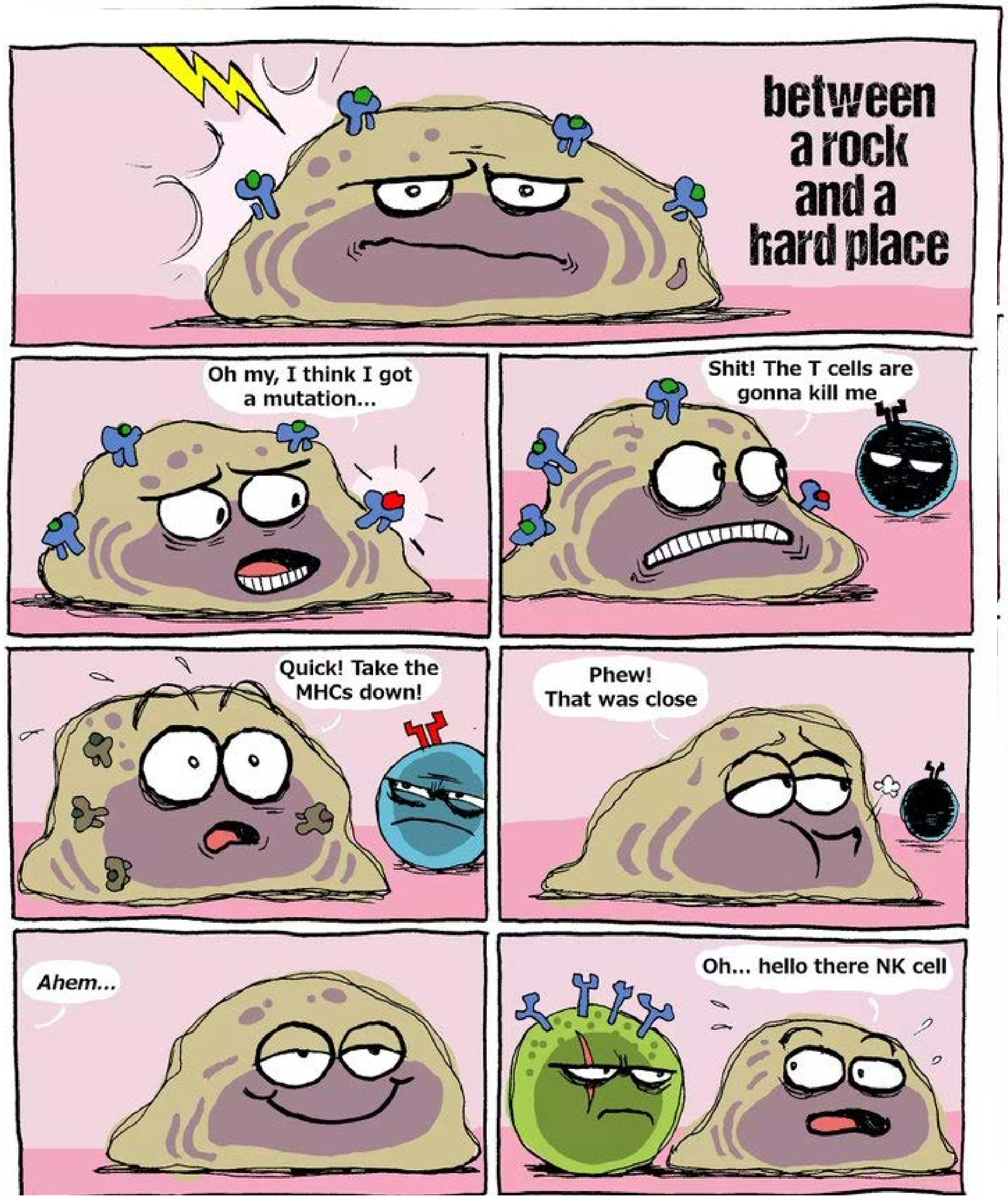
A lack of control in pro-inflammatory and anti-inflammatory bacteria (causing an imbalance between Th17 and T-regulatory cells) establishes a dysbiotic state.

Immunological intolerance results in a loss of homeostasis that can promote a pro-neoplastic inflammatory environment through chronic inflammation, immune evasion and immune suppression

Permethrin- induced changes in the expression of genes (in fish) associated with the immune response

Testable Hypothesis in dogs:

Does exposure to pesticide- permethrin impact tumor immune evasion strategies such as downregulation of MHC-1



The transcriptome-wide effects of exposure to a pyrethroid pesticide on the Critically Endangered delta smelt Hypomesus transpacificus. **Vol. 28: 43–60, 2015**
doi: 10.3354/esr00679

a-BHC
a-Chlordane
Alachlor
Aldrin
Atrazine
b-BHC
Chlorobenzilate
Chloroneb
Chlorothalonil
cis-Permethrin
d-BHC
Dacthal
Dieldrin
Endosulfan I
Endosulfan II
Endosulfan sulfate
Endrin
Endrin aldehyde
Etridiazole
g-BHC
g-Chlordane
Heptachlor
Heptachlor epoxide (Isomer B)
Methoxychlor
p,p'-DDD
p,p'-DDE
p,p'-DDT
Simazine
trans-Nonachlor
trans-Permethrin

TABLE 1 Signalment of the enrolled dogs

	Breed	Sex	Age (years)	Body weight (kg)	Clinical/clinico pathological signs
Lymphoma dogs					
1	Jack Russell Terrier	m	7	10	Splenomegaly;
					Increased CRP, LDH
2	Labrador Retriever	m	9	43	Splenomegaly; hepatomegaly;
					Increased LDH
3	Dachshund	m	10	8	Increased LDH
4	Jack Russell Terrier	m	6	8	Mild thrombocytopenia;
					Spleno-hepatomegaly
					Increased ALP, GGT, ALT, AST, LDH
5	Beagle	f	8	16	Increased LDH
6	Labrador Retriever	f	9	36	Increased ALP
7	Belgian Shepherd	m	7	34	–
8	Hound	f	6	23	Splenomegaly
9	Beagle	m	9	17	–
10	Mixed	f	9	20	Increased ALP, ALT, AST, LDH
11	Doberman	f	10	34	Increased CRP, LDH
12	Mixed	m	8	45	Mild anaemia;
					Increased ALP, CRP
Healthy dogs					
13	Boxer	m	5	31	
22	Pit Bull Terrier	mc	4	30	
23	Boston Terrier	fs	3	12	
24	Labrador Retriever	mc	2	36	
14	Boxer	f	7	24	
32	Mixed	fs	11	40	
25	Dachshund	mc	6	5	
15	Boxer	f	4	22	
16	Boxer	f	9	31	
28	Mixed	mc	7	26	
29	Labrador Retriever	mc	10	29	
30	Beagle	mc	5	9	
17	Boxer	f	3	24	
20	Mixed	f	4	26	
21	Mixed	f	4	22	
18	Boxer	f	4	25	
19	Boxer	f	8	29	
26	Brittany Spaniel	mc	11	21	
27	Jack Russel terrier	mc	9	10	
31	Weimaraner	mc	11	27	
33	Mixed	fs	10	30	

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPR: C-reactive protein; f, female; fs, spayed female; LDH, lactate dehydrogenase; m, male; mc, castrated male.

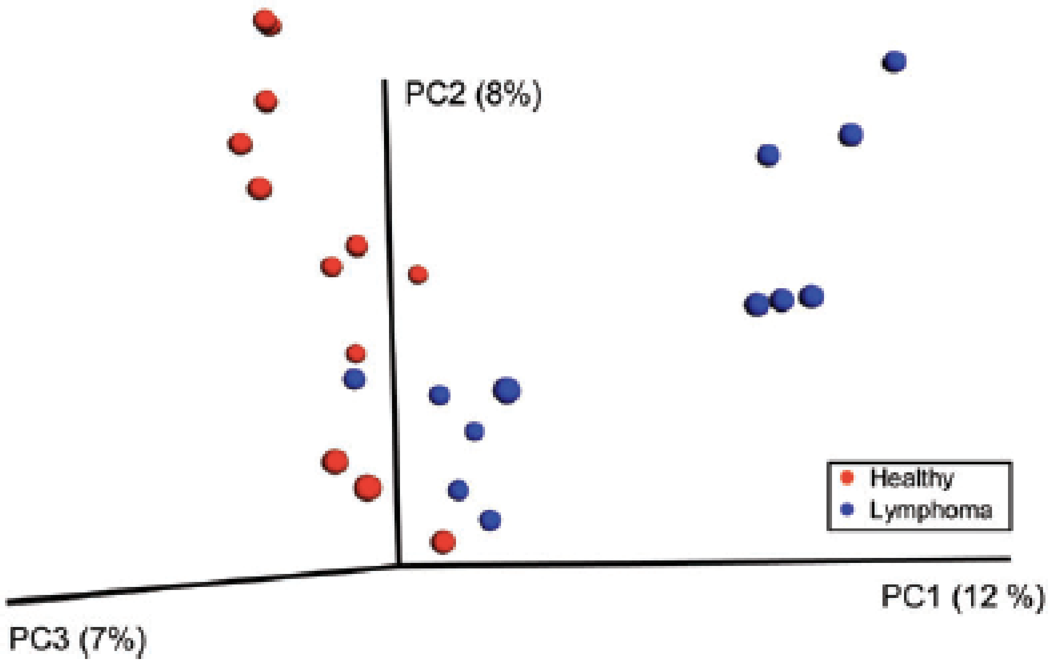


FIGURE 2 Principal coordinates analysis of unweighted UniFrac distances of 16S rRNA gene. Analysis of similarity revealed clustering between dogs with lymphoma and healthy control dogs (R = .33, P = .001)

Vet Comp Oncol. 2017;1–7.

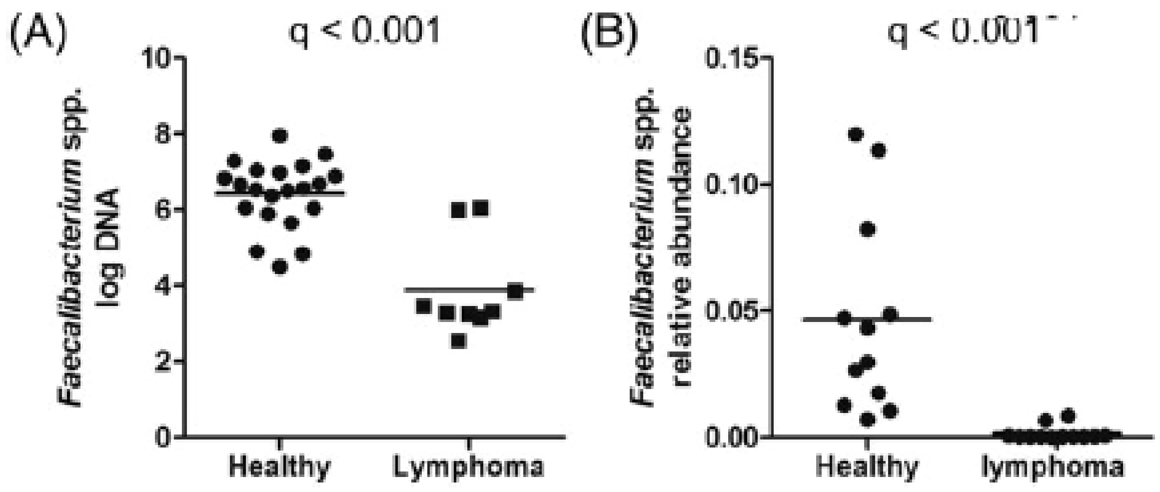
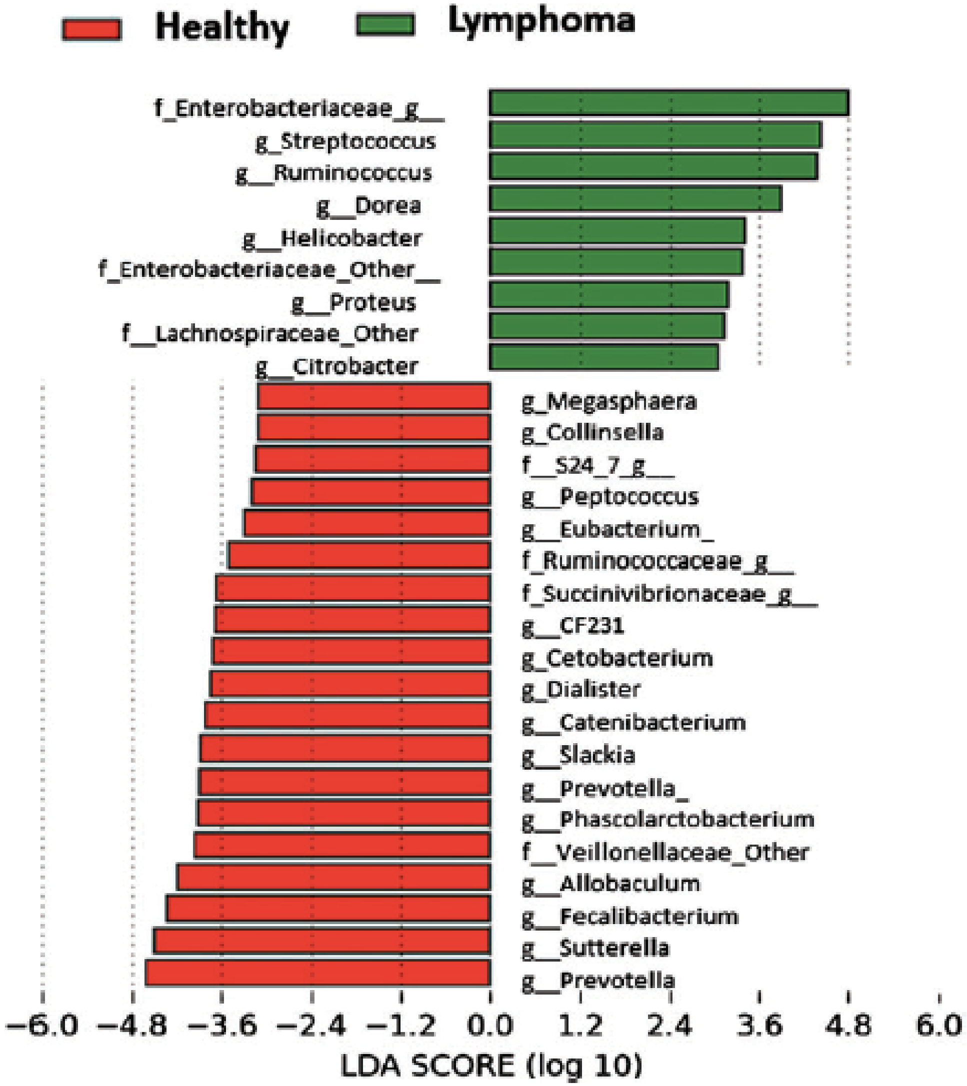
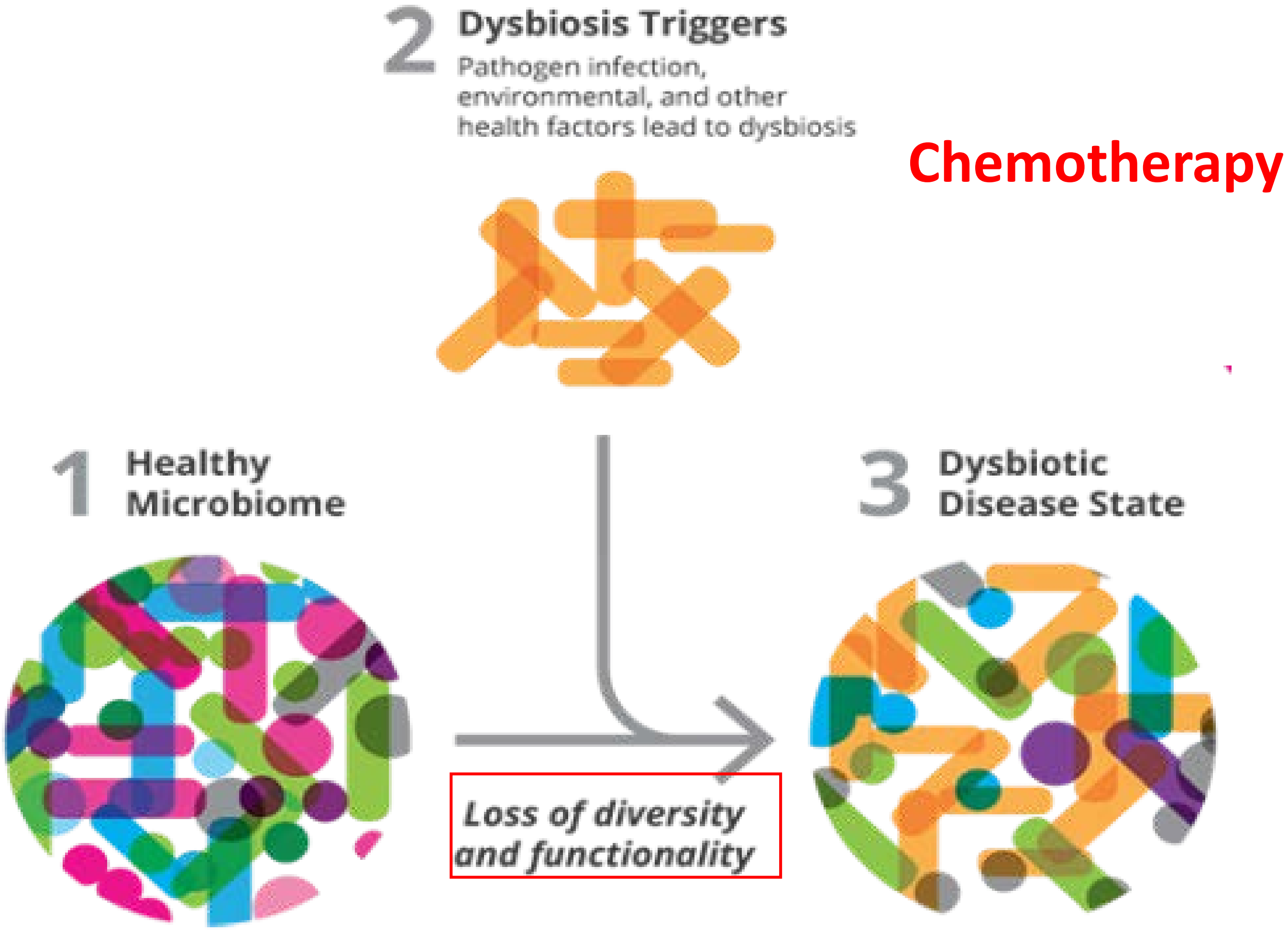


FIGURE 4 Comparison of the abundance of *Faecalibacterium* spp. based on qPCR analysis and Illumina sequencing analysis. Bars represent the mean log DNA (qPCR data) or mean relative abundance (sequencing data)



Does Chemotherapy negatively impacts the gut microbiome in dogs?

If yes, can diet help recover this dysbiosis?
Or Are there chemical exposures that are responsible for this dysbiosis?



ACC clinical trials core
Jonathon Stockman, DVM
Hend Ibrahim, MD, PhD
Nora Jean Nealon, PhD, DVM

Image adapted from Seres Therapeutics, 2017

Rice bran and navy beans have prebiotic functions and support microbiota metabolism for cancer control and prevention in people

Brown Rice

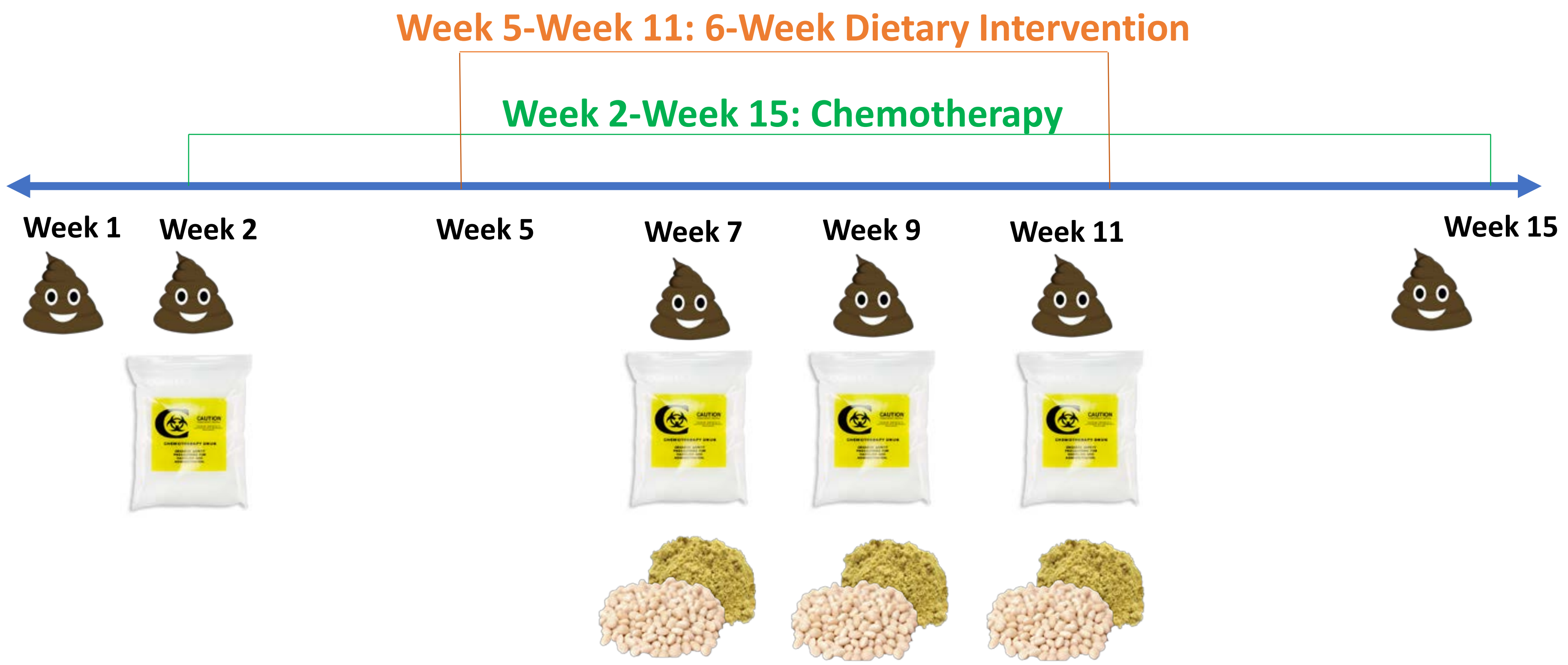
+

Rice Bran

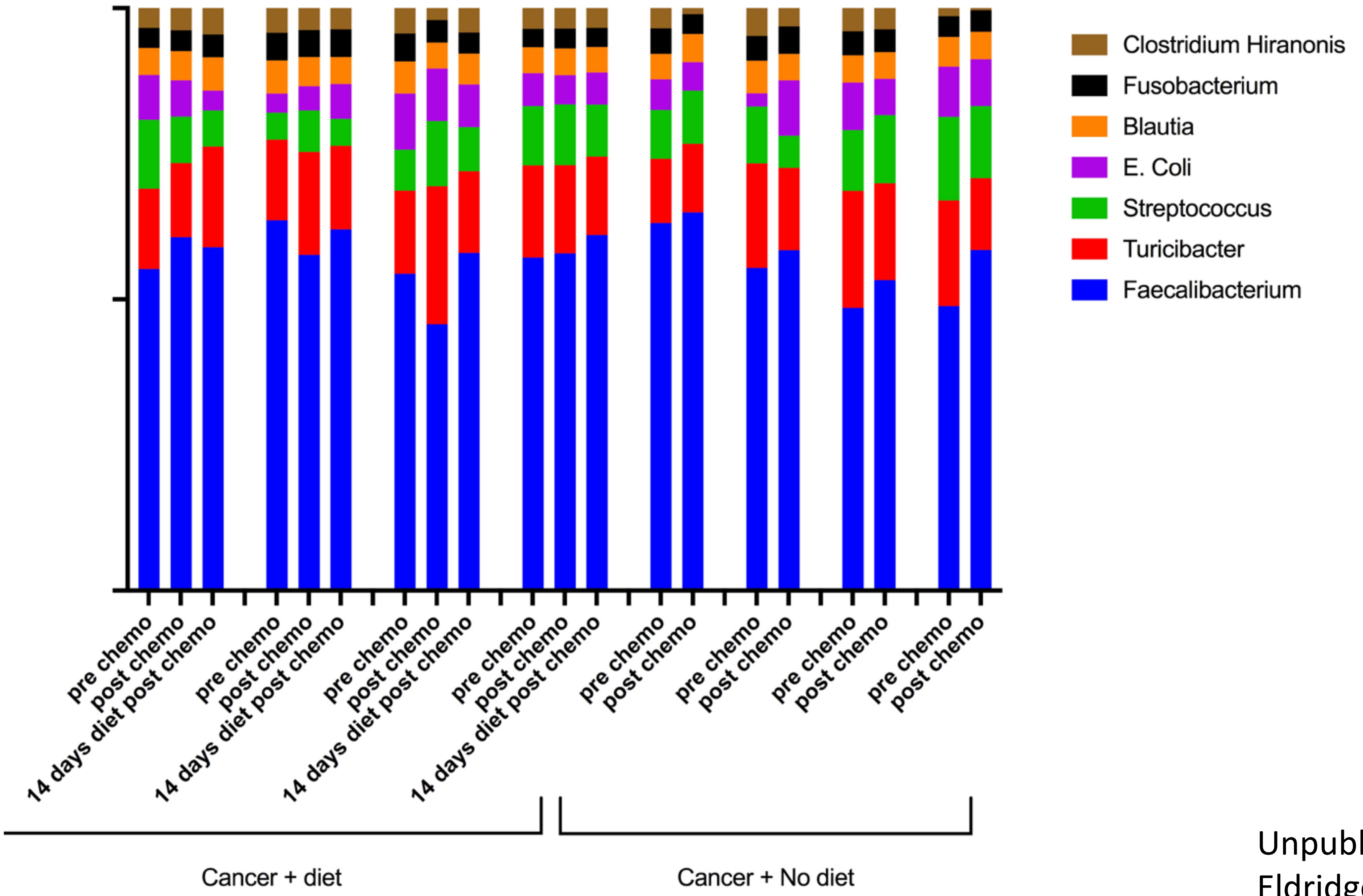
Navy beans

These ingredients are safe for use in dogs!

Study Timeline

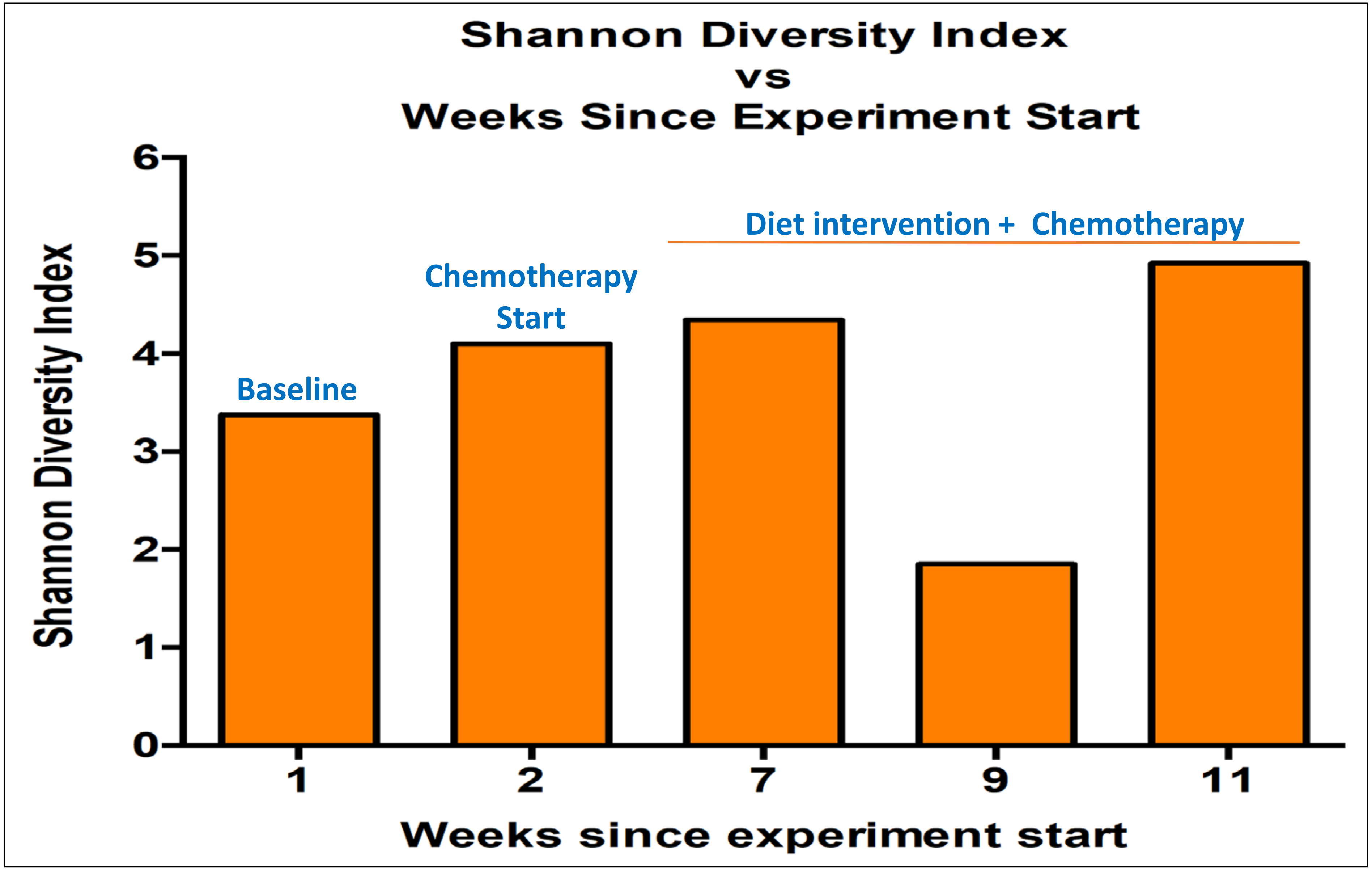


STUDY DIET:
20% w/v cooked beans, 5% heat-stabilized rice bran

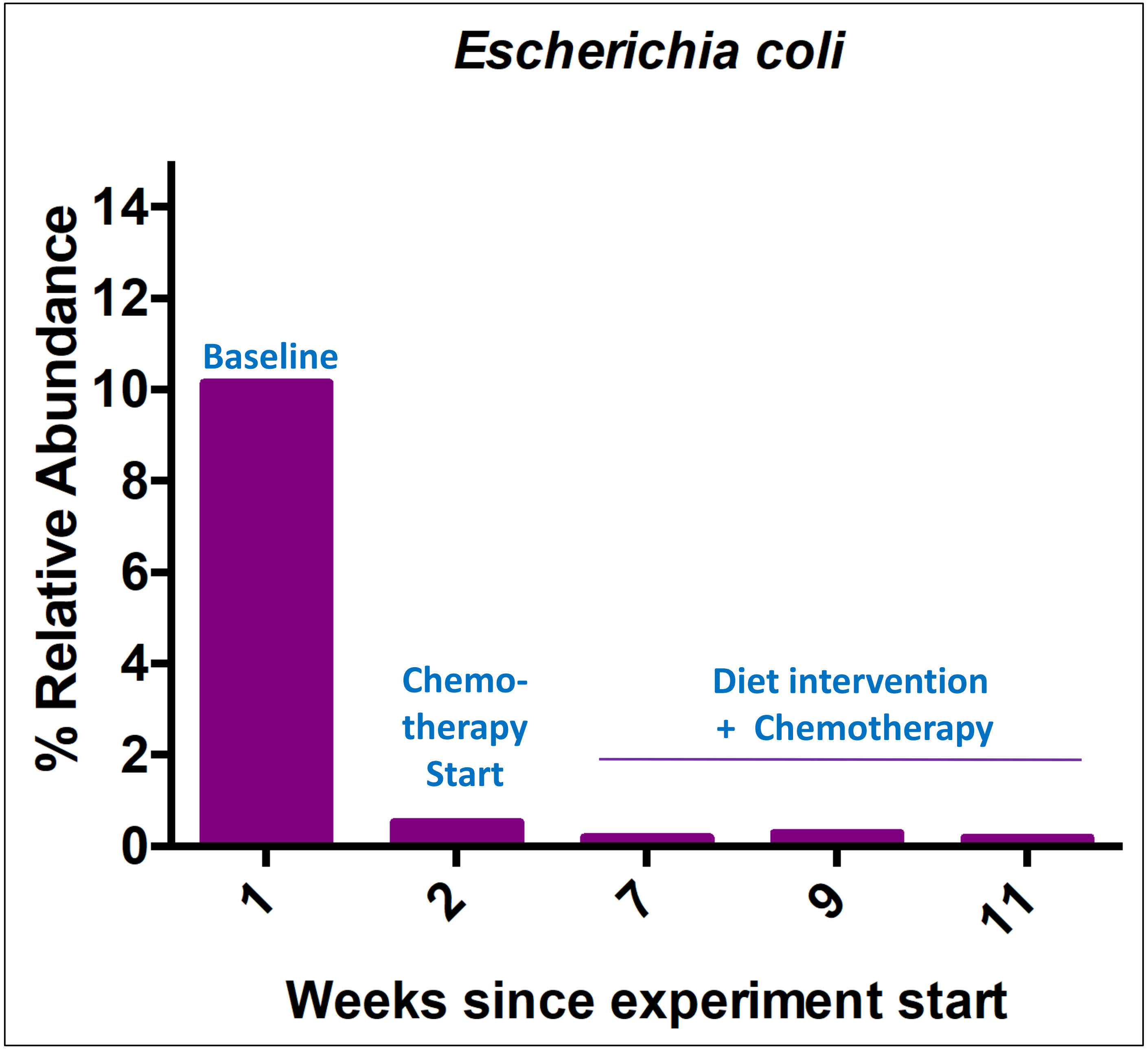


Unpublished data (Ryan lab)
Eldridge Foundaition support

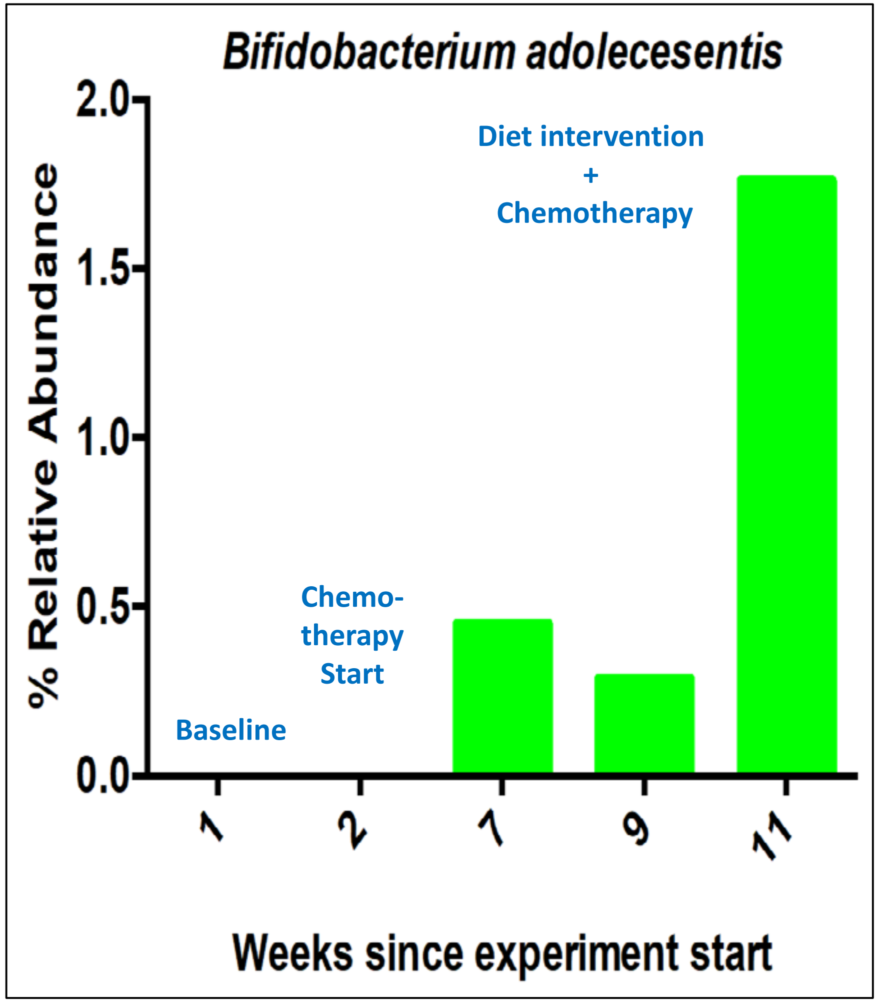
Gut microbial diversity increased over time

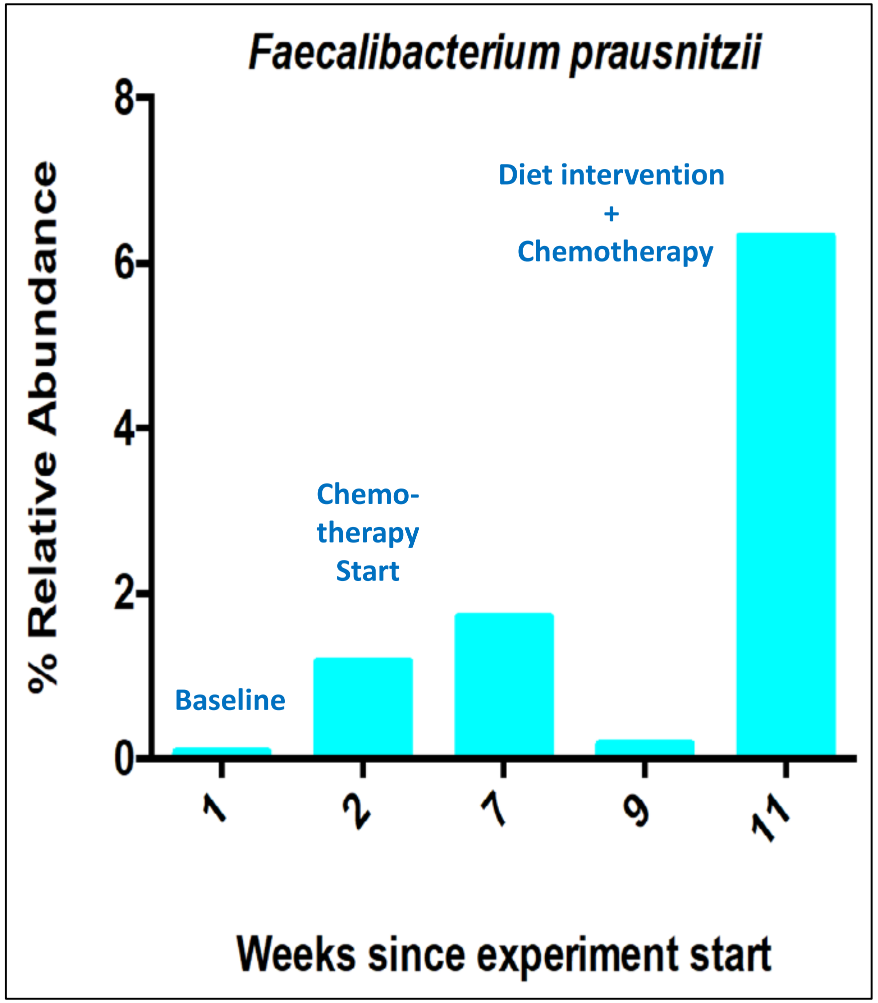


E. Coli did not increase during dietary intervention



Probiotic commensals increased during dietary intervention





To what extent, microbiota mediate the disease-causing effects of the environmental chemicals?

Are the effects of the environmental chemicals on gut microbiota highly depend on sex and age

Exposure to environmental chemicals during various stages of life causes alterations in the gut microbiome that may be associated with changes in health, including immune dysfunction and cancer.

The altered carbohydrate and lipid metabolism are promising intervention targets

The field of gut microbiome toxicology is still relatively new, which is why many studies have only reported changes in the gut microbiota composition and not on the mechanisms by which chemicals interfere with gut microbiota.

Toxicology and Nutrition Lab at CSU

Extramural Funding/Research Support Acknowledgements

- National Institutes of Health, NCI R01
- USDA-NIFA: “Dietary Exposure Biomarkers of Rice Bran and Bean intake across the lifespan”
- Norharvest Dry Bean Health Research
- Industry partnerships: ADM Alliance Nutrition, Edible Bean Specialties, Bush Brothers, Rice Bran Technologies
- USAID Legume Innovation Lab, subaward
  - (Dietary Exposure Biomarker s of Cowpea intake in children and women)