AN INTERNATIONAL ORGANIZATION DEDICATED TO PROTECTING THE RIGHTS OF ALL ANIMALS

PETA

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

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September 8, 2020

Sonny Perdue
Secretary of Agriculture
U.S. Department of Agriculture
1400 Independence Ave. S.W.
Washington, DC 20250

Dear Secretary Perdue,

Thank you in advance for your time. I am writing on behalf of People for the Ethical Treatment of Animals (PETA) and our more than 6.5 million members and supporters worldwide. Based on the information presented below and enclosed, we request that the U.S. Department of Agriculture (USDA) use its regulatory authority (pursuant to the Commodity Promotion, Research, and Information Act of 1996)[1] to prohibit assessment fees—established and overseen by the agency's Agricultural Marketing Service (AMS) and paid by agricultural producers, handlers, processors, importers, and others—from going toward animal experiments funded by research and promotion (R&P) boards for the marketing of agricultural commodities.

Agricultural Commodity R&P Boards Fund Animal Tests

AMS currently oversees 21 agricultural commodity R&P boards, whose directors are appointed by the USDA.[2] Disturbingly, many of the boards use part of the assessment fees to fund cruel and deadly animal tests—including, but not limited to, ones in which animals are douched, poisoned, force-fed, starved, irradiated, bled, suffocated, beheaded, or dissected—purportedly in an attempt to establish human health claims for marketing the agricultural products and ingredients that the boards represent.

Please see Appendix A for a list of such animal experiments published between 2015 and 2019 that were funded by the various R&P boards using assessment fees, in which at least 1,554 mice, 1,030 rats, and 31 pigs were used.

Farmers Are Penalized for Failing to Pay Into the Animal Testing Fund

The R&P board assessment fees, part of which are used for animal testing activities, are overseen by AMS and levied on agricultural product stakeholders.[3] According to one researcher, "[A] commodity supplier faces stiff penalties for failing to pay an assessment or for otherwise violating the program requirements. For example, a party who fails to pay a checkoff assessment will not only be

[1]Commodity Promotion, Research, and Information Act of 1996. https://www.nodpa.com/files/checkoff_Generic_regulations_on_check-off.pdf
[2]USDA AMS. (n.d.). *Research & promotion programs*. AMS.USDA.org. https://www.ams.usda.gov/rules-regulations/research-promotion
[3]USDA. (2020). *Guidelines for AMS oversight of commodity research and promotion programs*. https://www.ams.usda.gov/sites/default/files/media/RPGUIDELINES092015.pdf

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assessed late fees and interest charges, but could also face a subsequent penalty of up to $10,000 if the party is found to have willfully violated an order of the Secretary of Agriculture."[4]

These assessment fees effectively serve as a draconian government-approved tax on America's struggling farmers. Furthermore, the animal tests funded by these assessment fees are not "vital to the welfare of persons engaged in the production, marketing, and consumption of such commodities, as well as to the general economy of the United States,"[5] which is the Congressional intent of the Commodity Promotion, Research, and Information Act of 1996, according to Sec. 512 (a)(3).

Animal Tests Fail to Advance Human Health and Are Not Required by Regulations

These animal experiments for agricultural commodities funded by the various R&P boards concern common human food ingredients (e.g., blueberries, mushrooms, and soybeans). Given that there is no toxicity concern, researchers could have safely conducted these studies on humans, which—unlike experiments on mice, rats, and other animals—would yield clinically relevant results. Also, advanced *in vitro* and computational models are widely used for researching the mechanisms and safety of the effects of food on human health. Animals are scientifically unfit "models" for human food research. Please see Appendix B for detailed critiques of the use of animal testing data for establishing human health claims.

Furthermore, there is no legal requirement to pursue animal testing, specifically, to establish human health claims for marketing agricultural products or ingredients. The U.S. Food and Drug Administration, the European Food Safety Authority, the Food Directorate of Health Canada, and others do not require studies on animals or accept them in isolation in order to make health claims. Please see Appendix C for description and analysis of relevant regulations.

R&P Boards and AMS Appear to Violate Standards for Replacing Animal Testing

The decisions by R&P boards—with oversight from AMS—to fund animal experiments are at odds with the U.S. Public Health Service's *Guide for the Care and Use of Laboratory Animals*, which includes the principle of "consideration of alternatives (in vitro systems, computer simulations, and/or mathematical models) to reduce or replace the use of animals"[6] and with the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training*, which state that "animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results."[7]

Adhering to these standards, the number of animals used in such experiments funded by R&P boards should be zero, since none of these animal tests are required by law and all can be conducted using exclusively non-animal methods. AMS is supposed to provide oversight, paid for by industry assessments, to ensure fiscal accountability and program integrity.[8] Yet animal testing wastes these funds.

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[4]Sabet, M. (2010). *Understanding the federal commodity checkoff program.* https://pennstatelaw.psu.edu/_file/aglaw/Federal_Commodity_Checkoff_Program_Michael_Sabet.pdf

[5]Commodity Promotion, Research, and Information Act of 1996.

[6]National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. (2011). *Guide for the care and use of laboratory animals.* 8th edition. https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf

[7]National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. (2011). *Guide for the care and use of laboratory animals. Appendix B: U.S. government principles for the utilization and care of vertebrate animals used in testing, research, and training.* https://www.ncbi.nlm.nih.gov/books/NBK54048/

[8]USDA AMS.

Consumers and the Food Industry Oppose Animal Testing

After discussions with PETA, dozens of major food and beverage manufacturers have established new policies against funding, conducting, and commissioning experiments on animals that are not explicitly required by law, which is the same category of tests that the R&P assessment fees are funding. These companies include Asahi Group Holdings, Ball Corporation,[9] Barilla,[10] Campbell Soup Company,[11] The Coca-Cola Company,[12] Constellation Brands, Ensuiko Sugar Refining,[13] Ezaki Glico,[14] Flowers Foods,[15] Fuji Oil Holdings, General Mills,[16] Heineken,[17] The Hershey Company,[18] House Foods Group, Ingredion, ITO EN, James White Drinks,[19] Kellogg Company,[20] Kewpie Corporation, Kikkoman,[21] Kirin Holdings, Lindt & Sprüngli,[22] Lipton,[23] McCain Foods,[24] Megmilk Snow Brand, Meiji Holdings,[25] Molson Coors Brewing Company,[26] Morinaga & Co., Nagase & Co.,[27] NH Foods, Nippon Suisan Kaisha, Nissin Foods Holdings, Ocean Spray, PepsiCo,[28] Pernod Ricard,[29] POM Wonderful LLC, Riken Vitamin, Robertet SA, Sapporo Holdings, Satake Corporation,[30] Sensient Technologies Corporation,[31] Strauss Group,[32] Suntory Holdings, Takasago International Corporation,[33] T. Hasegawa,[34] Toyo Suisan Kaisha, Welch's, Weston Foods,[35] Yakult Honsha, and others. You can see the full list on our website.[36]

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[9]Please see their policy here: https://ballcorp.gcs-web.com/static-files/b3b24f46-7ee1-4ea3-a843-98fb16928088

[10]Please see their policy here: https://www.barillagroup.com/en/groups-position/barillas-position-animal-testing-0

[11]Please see their policy here: https://www.campbellsoupcompany.com/wp-content/uploads/sites/31/2018/09/Animal-Welfare-Guidelines-Updated-092818-Clean.pdf

[12]Please see their policy here: https://www.coca-cola.ca/contact-us/faq

[13]Please see their policy here (in Japanese): https://www.ensuiko.co.jp/labo/index.html

[14]Please see their policy here: https://www.glico.com/global/rd/

[15]Please see their policy here: https://www.flowersfoods.com/company/faqs

[16]Please see their policy here: https://www.generalmills.com/en/News/Issues/animal-welfare-policy

[17]Please see their policy here: https://www.theheinekencompany.com/Contact-Us/FAQ

[18]Please see their policy here: https://www.thehersheycompany.com/content/corporate_SSF/en_us/whats-inside/transparency.html

[19]Please see their policy here: https://www.beet-it.com/beet-it-sport/science/

[20]Please see their policy here: http://crreport.kelloggcompany.com/ppm2#living

[21]Please see their policy here: https://www.kikkoman.com/en/quality/safety/productdevelopment.html

[22]Please see their policy here: https://www.lindt-spruengli.com/sustainability/ask-lindt-spruengli/

[23]Please see their policy here: https://www.unilever.com/news/news-and-features/Feature-article/2011/Unilever-commits-to-no-animal-testing-for-tea.html

[24]Please see their policy here: https://www.mccain.com/information-centre/faqs/

[25]Please see their policy here: https://www.meiji.com/global/sustainability/with-society/

[26]Please see their policy here: http://www.molsoncoors.com/en/our-story/governance-and-ethics

[27]Please see their policy here: https://www.nagase.co.jp/english/csr/compliance/other/

[28]Please see their policy here: https://www.pepsico.com/docs/album/policies-doc/pepsico-statement-on-animal-testing.pdf?sfvrsn=0

[29]Please see their policy for the company here: https://www.pernod-ricard.com/en/download/file/fid/10558/ and their policy for their suppliers here: https://www.pernod-ricard.com/en/download/file/fid/10481/

[30]Please see their policy here: https://satake-group.com/about/rd.html

[31]Please see their policy here: https://www.sensient.com/images/uploads/pdf/Animal_Testing_Policy_12_6_18.pdf

[32]Please see their policy here: https://www.strauss-group.com/wp-content/blogs.dir/3/files/sites/3/Animal-welfare-Charter-13.11.2019-3.pdf

[33]Please see their policy here: https://www.takasago.com/en/business/aromachemicals/animaltesting.html

[34]Please see their policy here: https://www.peta.org/wp-content/uploads/2018/06/T.HasegawareplytoPETAregardinganimaltesting.pdf

[35]Please see their policy here: http://www.weston.ca/en/wf/Weston-Foods_Animal_Testing_Statement_EN.pdf

[36]PETA. *Victory! Global food industry ditches deadly animal tests—see the list*. PETA.org. https://www.peta.org/features/victories-food-drink-companies-refuse-animal-tests/

The majority (52%) of U.S. adults oppose the use of animals in scientific research,[37] and 67% are concerned or very concerned about the well-being of animals in laboratories.[38] The majority (66%) of adults in European Union (EU) member states think the EU should immediately end *all* animal testing.[39] Globally, the vast majority (74%) of consumers "crave greater transparency in how companies source their products … and their stance on important issues such as animal testing."[40] In other words, animal testing does not increase the marketing and promotional appeal of agricultural commodities.

America's farmers deserve better than to be ripped off by an exorbitant assessment fee, part of which is used by R&P boards to fund crude, wasteful, and misleading experiments on animals that don't translate to useful results for humans. AMS should use its statutory authority to prohibit the use of agricultural commodity assessments to fund animal tests and instead redirect them to support more effective, ethical, and economical non-animal research that will better promote R&P boards' agricultural products.

May I please hear from you by October 6, 2020, regarding this important matter? You can contact me at FrancesC@peta.org. Thank you.

Sincerely yours,

Frances Cheng, Ph.D.
Senior Science Adviser, International Laboratory Methods
Laboratory Investigations Department

cc: Bruce Summers, Administrator, AMS (AMSAdministratorOffice@usda.gov; Bruce.Summers@ams.usda.gov)

Enclosures: Appendix A: Animal Experiments Funded by the Agricultural Marketing Service
Appendix B: Critiques of Animal Testing for Human Health Claims
Appendix C: Regulations on Human Health Claims for Foods

[37] Strauss, M. (2018, August 16). *Americans are divided over the use of animals in scientific research*. Pew Research Center. https://www.pewresearch.org/fact-tank/2018/08/16/americans-are-divided-over-the-use-of-animals-in-scientific-research/

[38] Riffkin, R. (2015, May 18). *In U.S., more say animals should have same rights as people*. Gallup. https://news.gallup.com/poll/183275/say-animals-rights-people.aspx

[39] Savanta ComRes. (2020, July 17). *Cruelty free Europe – animal testing in the EU*. https://comresglobal.com/polls/cruelty-free-europe-animal-testing-in-the-eu/

[40] Accenture. (2018, December 5). *Majority of consumers buying from companies that take a stand on issues they care about and ditching those that don't, Accenture study finds*. https://newsroom.accenture.com/news/majority-of-consumers-buying-from-companies-that-take-a-stand-on-issues-they-care-about-and-ditching-those-that-dont-accenture-study-finds.htm

Appendix A: Animal Experiments Funded by the Agricultural Marketing Service

Below is a list of descriptions of animal experiments published between 2015 and 2019 that were funded by the various research and promotion (R&P) boards overseen by the Agricultural Marketing Service (AMS) of the United States Department of Agriculture (USDA). These experiments were funded purportedly to establish human health claims for marketing the agricultural products and ingredients promoted by the R&P boards.

Hass Avocado Board

- Experimenters fed mice a high-fat diet, repeatedly force-fed them an avocado ingredient, starved them for eight hours, injected them with glucose and insulin, repeatedly bled them from their tails, killed them by suffocating them and draining their blood, and dissected them.[41]

Highbush Blueberry Council

- Experimenters repeatedly starved mice, repeatedly took their blood, repeatedly injected them with a chemical that induces menopause, douched their vaginas, fed them a high-fat diet with blueberries, injected them with insulin, and killed and dissected them.[42]
- Experimenters fed rats strawberries or blueberries; forced them to perform a series of stress-inducing psychomotor and cognitive tests, including grabbing wires while suspended, walking or balancing on accelerating rotating rods, and swimming in a maze; repeatedly injected them with a chemical; and killed and dissected them. Five rats were killed before the end of experiment owing to excessive weight loss.[43]
- Experimenters fed rats blueberries; changed their cagemates daily; repeatedly restrained them in tubes smeared with cat food with a cat in the room, inducing post-traumatic stress disorder–like symptoms in the rats; forced them to perform a stress-inducing behavioral test; and killed and dissected them.[44]
- Experimenters injected mice with cancer cells, fed them blueberries or black raspberries, and killed them.[45]
- Experimenters fed mice a high-fat diet with blueberries, took their blood, and killed and dissected them.[46]

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[41]Ahmed, N., Tcheng, M., Roma, A., Buraczynski, M., Jayanth, P., Rea, K., Akhtar, T. A., & Spagnuolo, P. A. (2019). Avocatin B protects against lipotoxicity and improves insulin sensitivity in diet-induced obesity. *Molecular Nutrition & Food Research, 63*(24), 1900688.

[42]Elks, C. M., Terrebonne, J. D., Ingram, D. K., & Stephens, J. M. (2015). Blueberries improve glucose tolerance without altering body composition in obese postmenopausal mice. *Obesity*, *23*(3), 573–580.

[43]Shukitt-Hale, B., Bielinski, D. F., Lau, F. C., Willis, L. M., Carey, A. N., & Joseph, J. A. (2015). The beneficial effects of berries on cognition, motor behaviour and neuronal function in ageing. *British Journal of Nutrition*, *114*(10), 1542–1549.

[44]Ebenezer, P. J., Wilson, C. B., Wilson, L. D., Nair, A. R., & Francis, J. (2016). The anti-inflammatory effects of blueberries in an animal model of post-traumatic stress disorder (PTSD). *PLoS One*, *11*(9), e0160923.

[45]Aqil, F., Jeyabalan, J., Kausar, H., Munagala, R., Singh, I. P., & Gupta, R. (2016). Lung cancer inhibitory activity of dietary berries and berry polyphenolics. *Journal of Berry Research, 6*(2), 105–114.

[46]Carey, A. N., Gildawie, K. R., Rovnak, A., Thangthaeng, N., Fisher, D. R., & Shukitt-Hale, B. (2019). Blueberry supplementation attenuates microglia activation and increases neuroplasticity in mice consuming a high-fat diet. *Nutritional Neuroscience, 22*(4), 253-263.

- Experimenters fed rats blueberries, restrained them in plastic tubes, rendered them cognitively impaired by irradiating them, forced them to perform confusing and stress-inducing memory tasks, killed them by cutting off their heads, and dissected them.[47]
- Experimenters fed mice a high-fat diet, cut off 70% of their stomach, starved them, injected them with glucose, took their blood, and killed and dissected them.[48]
- Experimenters fed mice a high-fat diet, repeatedly starved them, repeatedly took their blood, cut off 70% of their stomach, inserted a catheter into their arteries, and killed and dissected them.[49]
- Experimenters fed rats a high-fat diet with blueberries, repeatedly starved them, force-fed them glucose, repeatedly took their blood, and killed and dissected them.[50]
- Experimenters fed mice a high-fat diet with blueberries and killed and dissected them.[51]
- Experimenters surgically injured rats' brains, fed them blueberries, forced them to perform stress-inducing behavioral tests such as getting through mazes, and killed and dissected them.[52]
- Experimenters fed mice a high-fat diet with or without blueberries, repeatedly starved them for 16 hours, injected them with glucose and insulin, repeatedly took their blood, and killed and dissected them.[53]

Mushroom Council

- Experimenters fed rats white button mushrooms and forced them to perform several stress-inducing motor and cognitive tests, such as walking on balance beams and rotating rods and swimming through a water maze. Fourteen rats died or had to be killed early because of excessive weight loss.[54]
- Experimenters fed mice white button mushrooms, starved them for 15 hours, injected them with glucose, took their blood, and killed and dissected them.[55]

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[47]Poulose, S. M., Rabin, B. M., Bielinski, D. F., Kelly, M. E., Miller, M. G., Thanthaeng, N., & Shukitt-Hale, B. (2017). Neurochemical differences in learning and memory paradigms among rats supplemented with anthocyanin-rich blueberry diets and exposed to acute doses of ^{56}Fe particles. *Life Sciences in Space Research, 12*, 16–23.

[48]McGavigan, A. K., Garibay, D., Henseler, Z. M., Chen, J., Bettaieb, A., Haj, F. G., Ley, R. E., Chouinard, M. L., & Cummings, B. P. (2017). TGR5 contributes to glucoregulatory improvements after vertical sleeve gastrectomy in mice. *Gut, 66*(2), 226–234.

[49]McGavigan, A. K., Henseler, Z. M., Garibay, D., Butler, S. D., Jayasinghe, S., Ley, R. E., Davisson, R. L., & Cummings, B. P. (2017). Vertical sleeve gastrectomy reduces blood pressure and hypothalamic endoplasmic reticulum stress in mice. *Disease Models & Mechanisms, 10*(3), 235–243.

[50]Lee, S., Keirsey, K. I., Kirkland, R., Grunewald, Z. I., Fischer, J. G., & de La Serre, C. B. (2018). Blueberry supplementation influences the gut microbiota, inflammation, and insulin resistance in high-fat-diet-fed rats. *The Journal of Nutrition*, *148*(2), 209–219.

[51]Lewis, E. D., Ren, Z., DeFuria, J., Obin, M. S., Meydani, S. N., & Wu, D. (2018). Dietary supplementation with blueberry partially restores T-cell-mediated function in high-fat-diet-induced obese mice. *British Journal of Nutrition, 119*(12), 1393–1399.

[52]Krishna, G., Ying, Z., & Gomez-Pinilla, F. (2019). Blueberry supplementation mitigates altered brain plasticity and behavior after traumatic brain injury in rats. *Molecular Nutrition & Food Research*, *63*(15), e1801055.

[53]Liu, W., Mao, Y., Schoenborn, J., Wang, Z., Tang, G., & Tang, X. (2019). Whole blueberry protects pancreatic beta-cells in diet-induced obese mouse. *Nutrition & Metabolism*, *16*, 34.

[54]Thangthaeng, N., Miller, M. G., Gomes, S. M., & Shukitt-Hale, B. (2015). Daily supplementation with mushroom (*Agaricus bisporus*) improves balance and working memory in aged rats. *Nutrition Research, 35*(12), 1079–1084.

[55]Tian, Y., Nichols, R. G., Roy, P., Gui, W., Smith, P. B., Zhang, J., Lin, Y., Weaver, V., Cai, J., Patterson, A. D., & Cantorna, M. T. (2018). Prebiotic effects of white button mushroom (*Agaricus bisporus*) feeding on succinate and intestinal gluconeogenesis in C57BL/6 mice. *Journal of Functional Foods, 45*, 223-232.

- Experimenters fed pigs white button mushrooms, repeatedly poked their anuses, took their blood, and killed and dissected them.[56]
- Experimenters fed genetically modified mice who were prone to atherosclerosis a high-fat diet with or without shiitake or portobello mushroom, suffocated them to death and drained their blood, and dissected them.[57]

National Mango Board

- Experimenters injected mice with cancer cells, repeatedly force-fed them mango extracts, and killed and dissected them.[58]
- Experimenters fed mice a high-fat diet with mangoes, starved them, took their blood, and killed and dissected them.[59]
- Experimenters fed rats mangoes or pomegranates, fed them a chemical that induces colitis, and killed and dissected them.[60]
- Experimenters fed rats mangoes, fed them a chemical that induces colitis, and killed and dissected them.[61]
- Experimenters fed rats mango juice, repeatedly fed them a chemical that induces colitis, and killed and dissected them.[62]

National Processed Raspberry Council (Disbanded)

- Experimenters fed mice a high-fat diet with raspberries, starved them, injected them with glucose, repeatedly took their blood, and killed and dissected them.[63,64]

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[56]Solano-Aguilar, G. I., Jang, S., Lakshman, S., Gupta, R., Beshah, E., Sikaroodi, M., Vinyard, B., Molokin, A., Gillevet, P. M., & Urban, J. F. (2018). The effect of dietary mushroom *Agaricus bisporus* on intestinal microbiota composition and host immunological function. *Nutrients*, *10*(11), 1721.

[57]Kim, S. H., Thomas, M. J., Wu, D., Carman, C. V., Ordovás, J. M., & Meydani, M. (2019). Edible mushrooms reduce atherosclerosis in Ldlr−/− mice fed a high-fat diet. *The Journal of Nutrition*, *149*(8), 1377–1384.

[58]Nemec, M. J., Kim, H., Marciante, A. B., Barnes, R. C., Hendrick, E. D., Bisson, W. H., Talcott, S. T., & Mertens-Talcott, S. U. (2017). Polyphenolics from mango (*Mangifera indica* L.) suppress breast cancer ductal carcinoma in situ proliferation through activation of AMPK pathway and suppression of mTOR in athymic nude mice. *The Journal of Nutritional Biochemistry*, *41*, 12–19.

[59]Ojo, B., El-Rassi, G. D., Payton, M. E., Perkins-Veazie, P., Clarke, S., Smith, B. J., & Lucas, E. A. (2016). Mango supplementation modulates gut microbial dysbiosis and short-chain fatty acid production independent of body weight reduction in C57BL/6 mice fed a high-fat diet. *The Journal of Nutrition*, *146*(8), 1483–1491.

[60]Kim, H., Banerjee, N., Ivanov, I., Pfent, C. M., Prudhomme, K. R., Bisson, W. H., Dashwood, R. H., Talcott, S. T., & Mertens-Talcott, S. U. (2016). Comparison of anti-inflammatory mechanisms of mango (*Mangifera indica* L.) and pomegranate (*Punica granatum* L.) in a preclinical model of colitis. *Molecular Nutrition & Food Research*, *60*(9), 1912–1923.

[61]Kim, H., Banerjee, N., Barnes, R. C., Pfent, C. M., Talcott, S. T., Dashwood, R. H., & Mertens-Talcott, S. U. (2017). Mango polyphenols reduce inflammation in intestinal colitis—involvement of the miR-126/PI3K/AKT/mTOR axis in vitro and in vivo. *Molecular Carcinogenesis*, *56*(1), 197–207.

[62]Kim, H., Krenek, K. A., Fang, C., Minamoto, Y., Markel, M. E., Suchodolski, J. S., Talcott, S. T., & Mertens-Talcott, S. U. (2018). Polyphenolic derivatives from mango (*Mangifera indica* L.) modulate fecal microbiome, short-chain fatty acids production and the HDAC1/AMPK/LC3 axis in rats with DSS-induced colitis. *Journal of Functional Foods*, *48*, 243–251.

[63]Luo, T., Miranda-Garcia, O., Adamson, A., Sasaki, G., & Shay, N. F. (2016). Development of obesity is reduced in high-fat fed mice fed whole raspberries, raspberry juice concentrate, and a combination of the raspberry phytochemicals ellagic acid and raspberry ketone. *Journal of Berry Research*, *6*(2), 213–223.

[64]Luo, T., Miranda-Garcia, O., Sasaki, G., & Shay, N. F. (2017). Consumption of a single serving of red raspberries per day reduces metabolic syndrome parameters in high-fat fed mice. *Food & Function*, *8*(11), 4081–4088.

- Experimenters repeatedly starved rats, repeatedly took their blood, injected them with a chemical that induces diabetes, injected them with plant metabolites commonly found after eating raspberries, inserted a catheter into their arteries, and killed and dissected them.[65]
- Experimenters mated mice, swabbed their vaginas, fed them a high-fat diet with an ingredient common in grapes and raspberries, killed some of the babies, fed the remaining babies a high-fat diet, starved them, injected them with glucose, repeatedly took their blood, put them in a room where the temperature was 4°C for six hours, repeatedly shoved a thermometer into their rectums, killed both the mothers and babies by breaking their necks, and dissected them.[66]
- Experimenters fed mice a high-fat diet with raspberries, starved them, injected them with glucose or insulin, repeatedly took their blood, killed them by breaking their necks, and dissected them.[67]
- Experimenters forced rats to perform a series of stress-inducing psychomotor and cognitive tests, including grabbing wires while suspended, walking or balancing on accelerating rotating rods, swimming in a maze, and grabbing a metal grid while being pulled by the tail. Experimenters then took their blood, fed them raspberries, and killed and dissected them. Eighteen rats died or had to be killed early because of excessive weight loss.[68]
- Experimenters fed mice a high-fat diet with raspberries and killed and dissected them.
- Experimenters bred mice, repeatedly injected them with a chemical that induces a genetic defect, fed them a high-fat diet with raspberries, starved them, injected them with glucose, repeatedly took their blood, killed them by breaking their necks, and dissected them.[69]
- Experimenters fed mice raspberries and then a chemical that induces colitis and killed and dissected them.[70]
- Experimenters fed mice raspberries and then a chemical that induces colitis, killed them by breaking their necks, and dissected them.[71]
- Experimenters fed rats a Western diet with raspberries, repeatedly restrained them and cuffed their tails, took their blood, starved them for 18 hours, killed them by cutting off their heads, and dissected them.[72]

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[65]Savi, M., Bocchi, L., Mena, P., Dall'Asta, M., Crozier, A., Brighenti, F., Stilli, D., & Del Rio, D. (2017). In vivo administration of urolithin A and B prevents the occurrence of cardiac dysfunction in streptozotocin-induced diabetic rats. *Cardiovascular Diabetology*, *16*(1), 80.

[66]Zou, T., Chen, D., Yang, Q., Wang, B., Zhu, M. J., Nathanielsz, P. W., & Du, M. (2017). Resveratrol supplementation of high-fat diet-fed pregnant mice promotes brown and beige adipocyte development and prevents obesity in male offspring. *The Journal of Physiology*, *595*(5), 1547–1562.

[67]Zhu, M. J., Kang, Y., Xue, Y., Liang, X., González García, M. P., Rodgers, D., Kagel, D. R., & Du, M. (2018). Red raspberries suppress NLRP3 inflammasome and attenuate metabolic abnormalities in diet-induced obese mice. *The Journal of Nutritional Biochemistry*, *53*, 96–103.

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[70]Bibi, S., Du, M., & Zhu, M. J. (2018). Dietary red raspberry reduces colorectal inflammation and carcinogenic risk in mice with dextran sulfate sodium–induced colitis. *The Journal of Nutrition*, *148*(5), 667–674.

[71]Bibi, S., Kang, Y., Du, M., & Zhu, M. J. (2018). Dietary red raspberries attenuate dextran sulfate sodium-induced acute colitis. *The Journal of Nutritional Biochemistry*, *51*, 40-46.

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- Experimenters repeatedly injected genetically modified mice who were prone to diabetes with a drug that induces diabetes, fed them a high-fat diet with or without raspberries, and killed and dissected them.[73]
- Experimenters fed mice a high-fat diet with or without raspberries, forced them to perform stress-inducing behavioral tests such as going through mazes, took blood straight from their hearts, and killed and dissected them.[74]
- Experimenters fed mice a high-fat diet with or without raspberries, killed them by suffocating them and breaking their necks, and dissected them.[75]
- Experimenters fed raspberries to genetically obese rats and killed and dissected them.[76]
- Experimenters fed red raspberries to genetically obese rats, starved them overnight, killed them by suffocating them and draining their blood, and dissected them.[77]

National Watermelon Promotion Board

- Experimenters repeatedly force-fed rats watermelon or a watermelon ingredient, injected them with a carcinogen, and killed and dissected them.[78]
- Experimenters fed rats watermelon or a watermelon ingredient and took their blood.[79]
- Experimenters fed rats watermelon, took their blood, and killed and dissected them.[80]
- Experimenters fed rats an atherogenic diet with or without watermelon, suffocated them to death, took their blood, and dissected them.[81]
- Experimenters fed rats watermelon, repeatedly injected them with a carcinogen that induces colon cancer, and killed and dissected them.[82,83]

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[73]Zhao, L., Zou, T., Gomez, N. A., Wang, B., Zhu, M. J., & Du, M. (2018). Raspberry alleviates obesity-induced inflammation and insulin resistance in skeletal muscle through activation of AMP-activated protein kinase (AMPK) α1. *Nutrition & Diabetes*, *8*(1), 39.

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[79]Kalaba, M., Klarich, D. S., & Hong, M. Y. (2016). Effect of watermelon powder supplementation on colonic aberrant crypt foci formation. *FASEB Journal*, *30*(S1), lb280-lb280.

[80]Beidler, J., Hooshmand, S., Kern, M., Figueroa, A., & Hong, M. Y. (2018). Watermelon and L-arginine consumption regulate gene expression related to serum lipid profile, inflammation, and oxidative stress in rats fed an atherogenic diet. *FASEB Journal*, *31*(S1), 431–432.

[81]Hong, M. Y., Beidler, J., Hooshmand, S., Figueroa, A., & Kern, M. (2018). Watermelon and L-arginine consumption improve serum lipid profile and reduce inflammation and oxidative stress by altering gene expression in rats fed an atherogenic diet. *Nutrition Research*, *58*, 46–54.

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[83]Fesseha, M., & Hong, M. Y. (2019). Effects of watermelon consumption on cellular proliferation, and apoptosis in rat colon (P05-019-19). *Current Developments in Nutrition*, *3*(Supplement_1), nzz030.P05-019-19.

- Experimenters fed mice a high-fat diet with various parts of watermelon, starved them, injected them with glucose, repeatedly bled them from their tails, took blood straight from their hearts, killed them by breaking their necks, and dissected them.[84]
- Experimenters fed rats a high-fat diet with or without watermelon, fed them a chemical that induces colitis, starved them, suffocated them to death, and dissected them.[85]
- Experimenters fed mice a high-fat diet with various parts of watermelon and killed and dissected them.[86]

United Sorghum Checkoff Program

- Experimenters fed rats sorghum bran and a chemical that induces colitis and killed and dissected them.[87,88]

United Soybean Board

- Experimenters fed rats casein, soy protein, corn oil, soybean oil, or salmon oil and killed and dissected them.[89,90]
- Experimenters injected mice with cancer cells, repeatedly injected them with an immunosuppressive drug and other substances, repeatedly force-fed them two plant ingredients, and killed and dissected them.[91]
- Experimenters repeatedly injected a soy ingredient into mice whose ovaries had been cut out, suffocated them to death, and dissected them.[92]
- Experimenters fed or repeatedly injected a soy ingredient into genetically modified mice who were prone to cystic fibrosis, suffocated them to death, took blood straight from their hearts, and dissected them.[93]

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[84]Becraft, A. R., Sturm, M. L., Mendez, R. L., Park, S. H., Lee, S. I., & Shay, N. F. (2020). Intake of watermelon or its byproducts alters glucose metabolism, the microbiome, and hepatic proinflammatory metabolites in high-fat–fed male C57BL/6 J mice. *The Journal of Nutrition, 150*(3), 434–442.

[85]Hong, M. Y., Tseng, Y. T., Kalaba, M., & Beidler, J. (2019). Effects of watermelon powder supplementation on colitis in high-fat diet-fed and dextran sodium sulfate-treated rats. *Journal of Functional Foods, 54*, 520–528.

[86]Becraft, A., Sturm, M., Pierce, G., Mendez, R., & Shay, N. (2019). Hepatic metabolomic analysis in mice fed a high fat diet with watermelon and watermelon byproducts shows improved lipid metabolism and reduction of chronic inflammation (P06-023-19). *Current Developments in Nutrition, 3*(Suppl 1), nzz031.P06-023-19.

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[90]Maditz, K. H., Benedito, V. A., Oldaker, C., Nanda, N., Lateef, S. S., Livengood, R., & Tou, J. C. (2015). Feeding soy protein isolate and n-3 PUFA affects polycystic liver disease progression in a PCK rat model of autosomal polycystic kidney disease. *Journal of Pediatric Gastroenterology and Nutrition, 60*(4), 467–473.

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- Experimenters fed genetically obese mice a soy ingredient, suffocated them to death, and dissected them.[94,95,96]
- Experimenters fed mice a soy ingredient, suffocated them to death, and dissected them.[97]
- Experimenters fed mice soybean oil or coconut oil, starved them, took their blood, and killed and dissected them.[98]
- Experimenters injected mice with a carcinogen, fed them casein or soy protein, and killed and dissected them.[99]
- Experimenters fed genetically obese mice a soy ingredient and killed and dissected them.[100]
- Experimenters fed genetically modified mice who were prone to cystic fibrosis a soy ingredient or a laxative and killed and dissected them. Forty-nine animals died of the disease before they could be killed by the experimenters.[101]
- Experimenters repeatedly force-fed genetically modified mice who were prone to diabetes a soy ingredient, injected them with cancer cells, starved them for 15 hours, injected them with glucose and insulin, repeatedly took their blood, suffocated them to death, and dissected them.[102]

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[94]Catmull, S., Masood, F., Schacht, S., Dolan, R., Stegman, D., Leung, L., & Al-Nakkash, L. (2016). Dietary genistein rescues reduced basal chloride secretion in diabetic jejunum via sex-dependent mechanisms. *Cellular Physiology and Biochemistry*, *40*(1–2), 335–346.

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[101]Lord, R., Fairbourn, N., Mylavarapu, C., Dbeis, A., Bowman, T., Chandrashekar, A., Banayat, T., Hodges, O. A., & Al-Nakkash, L. (2018). Consuming genistein improves survival rates in the absence of laxative in ΔF508-CF female mice. *Nutrients*, *10*(10), 1418.

[102]Huang, G., Xu, J., & Guo, T. L. (2019). Isoflavone daidzein regulates immune responses in the B6C3F1 and non-obese diabetic (NOD) mice. *International Immunopharmacology*, *71*, 277–284.

Appendix B: Critiques of Animal Testing for Human Health Claims

It is widely acknowledged that animals are not suitable proxies for humans when used in biomedical research. The National Institutes of Health (NIH) strategic plan for 2016 to 2020 states, "Currently, a novel drug, device, or other medical intervention takes about 14 years and $2 billion to develop, with a failure rate exceeding 95%" (despite success during preclinical animal testing), and notes, "Petri dish and animal models often fail to provide good ways to mimic disease or predict how drugs will work in humans, resulting in much wasted time and money while patients wait for therapies."[103] Among the different categories of therapeutic targets, the average probability of success for drugs aimed at the alimentary track and metabolism specifically is estimated to be only 4.46%,[104] similar to the overall trend. Shortcomings of animal tests confound measurements and contribute to the poor translation of findings to the clinical setting—and the field of nutrition research is not immune to this issue, especially since nutrition plays an important role in many pathological conditions. If a health claim is established using animals, it has a low probability of accurate translation and reproducibility in humans, a problem recognized by several regulatory bodies. (Please see Appendix C for more details.)

Mice and rats are often the species of choice for experiments to make health claims for foods. However, rodents are scientifically unfit for human nutrition research. Some foods commonly consumed safely by humans are even toxic to them. For example, D-limonene, a terpene compound found in citrus oils (in orange and lemon peels) and mangoes, can cause renal tumors in male rats owing to the accumulation of alpha 2u-globulin, a protein synthesized exclusively by adult male rats.[105] PR toxin, a secondary metabolite from the fungus *Penicillium roqueforti* (which is used to make blue cheese), is lethal to mice and rats when ingested.[106] Persin, a fatty acid–like ingredient in avocados, can cause mastitis in lactating mice.[107]

Below are some examples of important species differences relevant to some of the most common health claim categories currently made for products on the market, such as regulating blood lipids and cholesterols, improving digestion, regulating the immune system, and producing anti-fatigue effects—which explain why using rodents to establish human health claims is ill-advised and unscientific.

Regulating Blood Lipids and Cholesterols

Bile acids play an important role in cholesterol excretion and lipid digestion and absorption. Rats lack a gallbladder and cystic duct, and the bile secreted by the liver travels to the intestine as it is made continuously and directly through the bile duct.[108] However, in humans, about half of the bile is stored in the gallbladder, where it becomes concentrated.[109] Rodents also synthesize unique bile acids called

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[103]National Institutes of Health. (2015). *NIH-wide strategic plan: Fiscal years 2016–2020.* https://www.nih.gov/sites/default/files/about-nih/strategic-plan-fy2016-2020-508.pdf

[104]Pammolli, F., Magazzini, L., & Riccaboni, M. (2011). The productivity crisis in pharmaceutical R&D. *Nature Reviews. Drug Discovery, 10*(6), 428–438.

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[106]National Center for Biotechnology Information. (2020). PubChem Compound Summary for CID 440907, PR Toxin. http://pubchem.ncbi.nlm.nih.gov/compound/440907#section=Human-Toxicity-Excerpts

[107]Oelrichs, P. B., Ng, J. C., Seawright, A. A., Ward, A., Schäffeler, L., & MacLeod, J. K. (1995). Isolation and identification of a compound from avocado (*Persea americana*) leaves which causes necrosis of the acinar epithelium of the lactating mammary gland and the myocardium. *Natural Toxins, 3*(5), 344-349.

[108]Shiojiri, N. (1997). Development and differentiation of bile ducts in the mammalian liver. *Microscopy Research and Technique, 39*(4), 328–335.

[109]Hofmann, A. F. (1999). The continuing importance of bile acids in liver and intestinal disease. *Archives of Internal Medicine, 159*(22), 2647–2658.

muricholic acids, which can have the opposite effects on farnesoid X receptor activation than human forms of bile acids do. This has major effects on cholesterol metabolism.[110]

There are also many species differences in metabolic enzymes between rodents and humans. The hepatic enzymes delta-5 and delta-6 desaturases (D5D and D6D) are important for the metabolism of fatty acids. They introduce double bonds to fatty acid chains and alter their functions. The activity of D5D is inversely related to type 2 diabetes (T2D), and the activity of D6D is directly associated with it.[111] Rats have a much higher D5D activity than humans,[112] and it is known that rodent models of T2D do not recapitulate human T2D.[113] Besides fatty acid metabolism, rodents have a unique cholesterol profile—higher high-density lipoprotein and lower low-density lipoprotein—owing to their lack of cholesteryl ester transfer proteins. This makes them resistant to diet-induced alterations in cholesterol metabolism and cholesterol-mediated pathology.[114,115] Researchers have commented that "the rat is not an appropriate human model for studies involving lipids"[116] and that "it is not possible to extrapolate directly from rat to human studies because of differences in plasma lipoprotein [cholesterol and triglycerides] metabolism between the species."[117]

Improving Digestion

Nutrients go through several stages of digestion in different organs. The gastrointestinal (GI) tracks of humans and rats differ anatomically from the mouth all the way to the large intestine.[118] In the mouth, rats lack canines and premolars. In the throat, the human pharynx connects the mouth and nasal cavity to the esophagus and larynx, whereas a rat's pharynx is divided into a respiratory region and a digestive region without an oropharynx. The stomach of a rat contains a forestomach, which is connected to the opening of the esophagus and functions to digest bacteria, and a glandular stomach, which functions more like the human stomach. There is a limiting ridge between the two stomach regions that prevents rodents from vomiting, which is a key mechanism in humans for getting rid of toxins. The large intestine of rats does not have the sigmoid designation, owing to the lack of a true pelvis, and has a relatively large cecum, which is the main site for microbial-assisted digestion (see more below). The length of other components of the GI track also differs significantly between humans and rats relative to both the length of GI subdivisions and body size, and the relative surface area of the small intestine of humans is approximately four times that of rats. These anatomic dissimilarities contribute to metabolic differences. For example, humans can absorb nutrients more efficiently than rats can because of the increased surface area of the walls within the small intestine.

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[110]Kuipers, F., Bloks, V. W., & Groen, A. K. (2014). Beyond intestinal soap—bile acids in metabolic control. *Nature Reviews. Endocrinology*, *10*(8), 488–498.

[111]Kröger, J., & Schulze, M. B. (2012). Recent insights into the relation of Δ5 desaturase and Δ6 desaturase activity to the development of type 2 diabetes. *Current Opinion in Lipidology*, *23*(1), 4–10.

[112]Stone, K. J., Willis, A. L., Hart, M., Kirtland, S. J., Kernoff, P. B. A., & McNicol, G. P. (1979). The metabolism of dihomo-γ-linolenic acid in man. *Lipids*, *14*(2), 174–180.

[113]Chandrasekera, P. C., & Pippin, J. J. (2014). Of rodents and men: Species-specific glucose regulation and type 2 diabetes research. *ALTEX*, *31*(2), 157–176.

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[115]Barter, P., & Rye, K.-A. (2011). Cholesteryl ester transfer protein inhibition to reduce cardiovascular risk: Where are we now? *Trends in Pharmacological Sciences*, *32*(12), 694–699.

[116]Siguel, E. N. (1982). Cancerostatic effect of vegetarian diets. *Nutrition and Cancer*, *4*(4), 285–291.

[117]Nishina, P. M., Schneeman, B. O., & Freedland, R. A. (1991). Effects of dietary fibers on nonfasting plasma lipoprotein and apolipoprotein levels in rats. *The Journal of Nutrition*, *121*(4), 431–437.

[118]DeSesso, J. M., & Jacobson, C. F. (2001). Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats. *Food and Chemical Toxicology*, *39*(3), 209–228.

Rats have higher needs than humans for all essential amino acids, especially those that are sulfur-containing (methionine and cysteine).[119] The digestibility of some proteins also differs between rodents and humans. For example, rapeseed protein has a digestibility of 84% to 87% in humans compared to 95% in rats, due, in part, to its resistance to human pepsin hydrolysis. Endogenous nitrogen flow in humans is 45% higher than in rats. Furthermore, the fractional protein synthesis rate is 143% per day for rats but only 22% to 50% for humans, suggesting a higher intestinal mucosa protein renewal in rats, which is evident from more efficient dietary nitrogen recycling within endogenous proteins. Studies involving protein metabolism are confounded by these differences.

The stomach pH of rodents is about 10 to 1,000 times less acidic than that of humans.[120] As a result, in rats, bacteria reside in the stomach and all throughout the GI tract, whereas in humans, bacteria are localized mainly above the stomach and below the distal ileum.[121] Bacteria metabolize nutrients and hence constantly change the composition of ingested meals, affect absorption of some nutrients, and modify the host's metabolism and immunity and many other aspects of pathophysiology.[122,123] The gut microbiota digest dietary fibers that are otherwise not digestible by humans, prevent accumulation of toxic metabolic byproducts, and facilitate fatty acid hydrolysis and uptake, to name a few functions. However, about 85% of the gut bug species in rodents are not present in humans.[124] Together with the differences in their distribution and localization, gut microbiota contribute to major species differences, especially since there are at least 10 times as many gut bacteria as human cells in the human body.[125]

Regulating the Immune System

In addition to the differences in gut microbiota mentioned above, there are many other differences between mouse and human immune systems, including the anatomy of lymphoid tissue, ratios of white blood cell types, antimicrobial peptide profiles, cytokine profiles and functions, mechanisms for crosstalk between the adaptive and innate immune systems, antibody subtypes, development and regulation of lymphocytes, and activation of clotting factors.[126] Noting differences between rodents and humans, researchers have found the following:

> The two species diverged somewhere between 65 and 75 million years ago, differ hugely in both size and lifespan, and have evolved in quite different ecological niches where widely different pathogenic challenges need to be met—after all, most of us do not live with our heads a half-inch off the ground. However, because there are so many parallels there has been a tendency to ignore differences and in many cases, perhaps,

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[119]Deglaire, A., & Moughan, P. J. (2012). Animal models for determining amino acid digestibility in humans—a review. *The British Journal of Nutrition*, *108*(S2), S273–S281.

[120]McConnell, E. L., Basit, A. W., & Murdan, S. (2008). Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. *The Journal of Pharmacy and Pharmacology*, *60*(1), 63–70.

[121]Kararli, T. T. (1995). Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharmaceutics & Drug Disposition*, *16*(5), 351–380.

[122]Janssen, A. W., & Kersten, S. (2015). The role of the gut microbiota in metabolic health. *FASEB Journal*, *29*(8), 3111–3123.

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[124]Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(31), 11070–11075.

[125]Sekirov, I., Russell, S. L., Antunes, L. C. M., & Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiological Reviews*, *90*(3), 859–904.

[126]Mestas, J., & Hughes, C. C. (2004). Of mice and not men: Differences between mouse and human immunology. *Journal of Immunology*, *172*(5), 2731–2738.

> make the assumption that what is true in mice—in vivo veritas—is necessarily true in humans. By making such assumptions we run the risk of overlooking aspects of human immunology that do not occur, or cannot be modeled, in mice.[127]

In 2013, a large and collaborative statistical analysis showed that the responses of mice following acute inflammatory stressors such as burns, trauma, endotoxin exposure, and sepsis were "close to random in matching their human counterparts" and supported the "higher priority for translational medical research to focus on the more complex human conditions rather than relying on mouse models to study human inflammatory disease."[128] A 2014 study found fundamental differences in the innate immune response between the species, stating, "While in human blood mechanisms of immune resistance are highly prevailed, tolerance mechanisms dominate for the defense against pathogenic microorganisms in mouse blood."[129]

Vitamin C is an important antioxidant and has anti-inflammatory effects as well.[130] Ascorbic acid (vitamin C for humans) is synthesized in rodents (and most other animals) in the form of L-ascorbic acid from glycogen by the enzyme L-gulonolactone oxidase. However, humans do not possess this enzyme and therefore cannot synthesize it. Instead, specific transport systems for vitamin C absorption through dietary sources have evolved for humans. Such differences between humans and rodents have led researchers to call for the abandonment of rodent use in vitamin C–related studies.[131]

Regarding influenza virus infections, "There are … a number of drawbacks of the [mouse] model that make it unsuitable for addressing certain virological questions and can render data obtained in mice difficult to translate to the human situation."[132] Viral infection is species-specific, and mice cannot naturally catch human influenza virus. Experimenters usually have to use genetically modified strains of mice who are susceptible to viral infections. The viruses used in animal experiments are often adapted through serial passage in target hosts for easy infection. The reason for this is that human influenza virus receptors (α2,6-linked sialic acids) are not abundant in the upper airways of mice, who have a different receptor (α2,3-linked sialic acids).[133] Through serial passage, the virus can adapt to the new host and become distinct from the kind that predominantly affects humans. In addition, mice do

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[127] *Ibid*.

[128] Seok, J., Warren, H. S., Cuenca, A. G., Mindrinos, M. N., Baker, H. V., Xu, W., Richards, D. R., McDonald-Smith, G. P., Gao, H., Hennessy, L., Finnerty, C. C., López, C. M., Honari, S., Moore, E. E., Minei, J. P., Cuschieri, J., Bankey, P. E., Johnson, J. L., Sperry, J. … & the Inflammation, and Host Response to Injury, Large Scale Collaborative Research Program. (2013). Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(9), 3507–3512.

[129] Zschaler, J., Schlorke, D., & Arnhold, J. (2014). Differences in innate immune response between man and mouse. *Critical Reviews in Immunology*, *34*(5), 433–454.

[130] Ellulu, M. S. (2017). Obesity, cardiovascular disease, and role of vitamin C on inflammation: A review of facts and underlying mechanisms. *Inflammopharmacology*, *25*(3), 313–328.

[131] Michels, A. J., & Frei, B. (2013). Myths, artifacts, and fatal flaws: Identifying limitations and opportunities in vitamin C research. *Nutrients*, *5*(12), 5161–5192.

[132] Bouvier, N. M., & Lowen, A. C. (2010). Animal models for influenza virus pathogenesis and transmission. *Viruses*, *2*(8), 1530–1563.

[133] Ibricevic, A., Pekosz, A., Walter, M. J., Newby, C., Battaile, J. T., Brown, E. G., Holtzman, M. J., & Brody, S. L. (2006). Influenza virus receptor specificity and cell tropism in mouse and human airway epithelial cells. *Journal of Virology*, *80*(15), 7469–7480.

not get fever—but rather hypothermia—following infection,[134] and they do not cough or sneeze, either.[135] The virus does not even transmit between mice.[136]

Producing Anti-Fatigue Effects

Mice and rats are hugely different from humans in muscle physiology and should not be used. The performance of skeletal muscles is determined largely by muscle fiber types, which are designated by myosin heavy chain (MyHC) protein isoforms expressed within. Mice and rats are the complete opposite of humans in terms of MyHC expressions.[137] Their skeletal muscle is predominantly composed of muscle fibers expressing MyHC IIb. In contrast, human skeletal muscle expresses not this protein isoform but rather MyHC I/β. (The overall MyHC isoform abundance in mice and rats is IIb > IIx > IIa > I/β, whereas in humans it is I/β > IIa > IIx.) Muscles expressing MyHC IIb tend to be larger fibers, contract faster, produce larger forces, are rich in glycolytic enzymes and tend to run on the anaerobic energy system, have low mitochondria and capillary density, and have low resistance to fatigue. Muscles expressing MyHC I/β are the complete opposite—they are smaller; contract slower; produce smaller forces; are rich in mitochondria, capillary, and oxidative capacity and hence run on the aerobic energy system; and have high resistance to fatigue.[138] (Elite runners have more/bigger muscles expressing MyHC I/β, and this can be an adaptive and acquired characteristic.)

The protein synthesis rate is also different between type II and type I muscle fibers. In response to food deprivation, there is a greater decrease in protein synthesis in type II fibers than in type I.[139] This is important because it translates to differential muscle function between mice or rats and humans under food deprivation.

Muscle glycogen, expressed relative to total body glycogen, is about 10 times lower in mice than in humans.[140] Both mice[141] and rats[142] have about five to 10 times more liver glycogen than muscle glycogen, whereas humans have three to eight times more muscle glycogen than liver glycogen.[143] Even though it is well documented that adequate muscle glycogen is important to sustain exercise in humans, accumulating evidence shows that muscle glycogen is not even necessary for mice to perform demanding muscle activities. For example, genetically modified mice completely lacking muscle

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[134]Majde, J. A., Bohnet, S. G., Ellis, G. A., Churchill, L., Leyva-Grado, V., Wu, M., Szentirmai, E., Rehman, A., & Krueger, J. M. (2007). Detection of mouse-adapted human influenza virus in the olfactory bulbs of mice within hours after intranasal infection. *Journal of Neurovirology, 13*(5), 399–409.

[135]Bouvier, N. M., & Lowen, A. C. (2010). Animal models for influenza virus pathogenesis and transmission. *Viruses, 2*(8), 1530–1563.

[136]Lowen, A. C., Mubareka, S., Tumpey, T. M., García-Sastre, A., & Palese, P. (2006). The guinea pig as a transmission model for human influenza viruses. *Proceedings of the National Academy of Sciences of the United States of America, 103*(26), 9988–9992.

[137]Haizlip, K. M., Harrison, B. C., & Leinwand, L. A. (2015). Sex-based differences in skeletal muscle kinetics and fiber-type composition. *Physiology, 30*(1), 30–39.

[138]Zierath, J. R., & Hawley, J. A. (2004). Skeletal muscle fiber type: Influence on contractile and metabolic properties. *PLoS Biology, 2*(10), e348.

[139]Goodman, C. A., Kotecki, J. A., Jacobs, B. L., & Hornberger, T. A. (2012). Muscle fiber type-dependent differences in the regulation of protein synthesis. *PloS One, 7*(5), e37890.

[140]Kasuga, M., Ogawa, W., & Ohara, T. (2003). Tissue glycogen content and glucose intolerance. *The Journal of Clinical Investigation, 111*(9), 1282–1284.

[141]Pederson, B. A., Cope, C. R., Schroeder, J. M., Smith, M. W., Irimia, J. M., Thurberg, B. L., DePaoli-Roach, A. A., & Roach, P. J. (2005). Exercise capacity of mice genetically lacking muscle glycogen synthase: In mice, muscle glycogen is not essential for exercise. *Journal of Biological Chemistry, 280*(17), 17260–17265.

[142]Baldwin, K. M., Reitman, J. S., Terjung, R. L., Winder, W. W., & Holloszy, J. O. (1973). Substrate depletion in different types of muscle and in liver during prolonged running. *American Journal of Physiology, 225*(5), 1045–1050.

[143]Ivy, J. L. (1999). Role of carbohydrate in physical activity. *Clinics in Sports Medicine, 18*(3), 469–484.

glycogen were able to run on treadmills until exhaustion, just like normal mice.[144] Genetically modified mice with over-accumulated muscle glycogen did not perform any better than normal mice did, either.[145]

In addition to glycogen, blood fatty acids and blood sugar are also important fuel sources during exercise. However, metabolism of fatty acids and glucose is significantly different in mice and rats than in humans, as explained above.

[144]Pederson, B. A., Cope, C. R., Schroeder, J. M., Smith, M. W., Irimia, J. M., Thurberg, B. L., DePaoli-Roach, A. A., & Roach, P. J. (2005). Exercise capacity of mice genetically lacking muscle glycogen synthase: In mice, muscle glycogen is not essential for exercise. *Journal of Biological Chemistry*, *280*(17), 17260–17265.

[145]Pederson, B. A., Cope, C. R., Irimia, J. M., Schroeder, J. M., Thurberg, B. L., DePaoli-Roach, A. A., & Roach, P. J. (2005). Mice with elevated muscle glycogen stores do not have improved exercise performance. *Biochemical and Biophysical Research Communications*, *331*(2), 491–496.

Appendix C: Regulations on Human Health Claims for Foods

Below are the relevant regulations regarding human health claims for foods in the European Union (EU), the United States (US), and Canada.

The EU

The European Food Safety Authority (EFSA) has several categories of health claims. General function claims "refer to the role of a nutrient or substance in growth, development and body functions; psychological and behavioural functions; slimming and weight control, satiety or reduction of available energy from the diet."[146] New function claims are "based on newly developed scientific evidence" for which "protection of proprietary data can be requested."[147] There are also claims that "refer to the reduction of disease risk or to children's development or health."[148]

For claims other than those based on the essentiality of nutrients, EFSA's requirements of scientific evidence are as follows:

> In assessing each specific food/health relationship which forms the basis of a claim, the NDA Panel [the Panel on Dietetic Products, Nutrition and Allergies] makes a scientific judgement on the extent to which a cause and effect is established between the consumption of the food/constituent and the claimed effect (i.e. *for the target group under the proposed conditions of use*) by considering the strength, consistency, specificity, dose–response, biological plausibility of the relationship and by weighing the totality of the evidence. A grade is not assigned to the evidence.
>
> *Pertinent human (intervention and observational) studies are central for health claim substantiation*. Pertinent human intervention studies are at the top of the hierarchy that informs decisions on substantiation because it is of utmost importance to show that the food/constituent can exert the claimed effect in humans and that the effect is specific for the food/constituent, an information which *can only be obtained from human intervention studies* (EFSA NDA Panel, 2011b). Human intervention (and observational) studies can also provide evidence for a dose–response relationship and for consistency of the effect (or the association) across studies. Efficacy studies in animals and non-efficacy studies in humans, animals and/or in vitro (e.g. evidence for a mechanism by which a food could exert the claimed effect) may be part of the totality of the evidence only if pertinent human studies showing an effect of the food/constituent are available [*emphasis added*].[149]

EFSA does not require animal tests or accept animal data as stand-alone evidence for establishing health claims for foods.

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[146]EFSA. (n.d.). *"General function" health claims under Article 13*. https://www.efsa.europa.eu/en/topics/topic/article13
[147]EFSA. (n.d.). *Health claims*. https://www.efsa.europa.eu/en/topics/topic/health-claims (See FAQ: What are EFSA's tasks under the Regulation?)
[148]EFSA. (n.d.). *Claims on disease risk reduction and child development or health under Article 14*. https://www.efsa.europa.eu/en/topics/topic/article14
[149]EFSA. (n.d.). *General scientific guidance for stakeholders on health claim applications*. Chapter 6.2. Claims other than those based on the essentiality of nutrients. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2016.4367

The US

The US Food and Drug Administration (FDA) defines health claims as "statements about substance/disease relationships" and defines the term "substance" as "a specific food or food component."[150] It continues, "Authorized health claims in food labeling are claims that have been reviewed by FDA and are allowed on food products or dietary supplements to show that a food or food component may reduce the risk of a disease or a health-related condition. Such claims are supported by scientific evidence and may be used on conventional foods and on dietary supplements to characterize a relationship between a substance (a specific food component or a specific food) and a disease or health-related condition (e.g., high blood pressure)."[151]

The FDA evaluates the totality of scientific evidence and would agree with the claims only having determined that the evidence is in "significant scientific agreement." The guidance document for industry[152] lists the different types of evidence in order of their strength. Human interventional studies are at the top, then observational studies, then research synthesis studies (reviews and meta-analysis), with animal and *in vitro* studies at the bottom. The guidance document clearly states, "Before the strength of the evidence for a substance/disease relationship can be assessed, FDA separates individual relevant articles on human studies from other types of data and information. FDA intends to focus its review *primarily on articles reporting human intervention and observational studies because only such studies can provide evidence from which scientific conclusions can be drawn about the substance/disease relationship in humans*" [*emphasis added*]. Furthermore, the agency states, "FDA intends to use animal and *in vitro* studies as background information regarding mechanisms that might be involved in any relationship between the substance and disease. *The physiology of animals is different than that of humans. … [T]hese studies do not provide information from which scientific conclusions can be drawn regarding a relationship between the substance and disease in humans*" [*emphasis added*]. Sections III(D) and (E) of the guidance document outline methods for evaluating and assessing the quality of studies, and only human studies are discussed. Section III(F) outlines methods for evaluating the totality of scientific evidence, and animal studies are not even mentioned.

The FDA does not require animal tests or accept animal data as stand-alone evidence for establishing health claims for foods.

Canada

The Food Directorate of Health Canada (FDHC) categorizes health claims as either disease risk reduction claims or function claims. A disease risk reduction claim is "a statement that links a food or constituent of a food to reducing the risk of developing a diet-related disease or condition" or a statement "about the treatment, or mitigation of a disease or health-related condition, or about restoring, correcting or modifying body functions." A function claim is "a statement about the specific beneficial effects that the consumption of a food or food constituent has on normal functions or biological activities of the body" or one that "describe[s] the well-established roles of energy or nutrients that are essential for the maintenance of good health or for normal growth and development."[153]

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[150]FDA. (2009, January). *Guidance for industry: Evidence-based review system for the scientific evaluation of health claims*. https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm073332.htm

[151]FDA. (2018, January 12). *Authorized health claims that meet the significant scientific agreement (SSA) standard*. https://www.fda.gov/food/food-labeling-nutrition/authorized-health-claims-meet-significant-scientific-agreement-ssa-standard

[152]FDA. (2009, January). *Guidance for industry: Evidence-based review system for the scientific evaluation of health claims*. https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm073332.htm

[153]Health Canada. (2016, May 17). *Health claims*. https://www.canada.ca/en/health-canada/services/food-nutrition/food-labelling/health-claims.html

For both types of claim, Health Canada's requirements for study designs and evidence of interest are as follows:

> **a. Human Studies**
> Health Canada's evaluation of a health claim will be based on *human studies—intervention and/or prospective observational studies*. As such, the literature search strategy should be established with a focus on retrieving human studies. *The scientific uncertainties in extrapolating non-human data to humans limit the usefulness of non-human studies, such as animal and* in vitro *studies*. A submission guided by this document should thus be based on the retrieval and evaluation of human studies. If desired, non-human studies may be used to support the discussion on biological plausibility. This is, however, optional.
>
> **b. Validity of Study Designs**
> The research design of human studies is a critical factor in interpreting the evidence for a health claim. Certain research designs can present biases that skew the interpretation of the evidence in an erroneous fashion and/or are not useful in inferring causality. Characteristics of research designs that limit the interpretation of the validity of the evidence are, for intervention studies, the absence of randomization and/or a control group. For observational studies, the use of retrospective studies (retrospective cohort, case-control), cross-sectional, and descriptive studies (ecologic, time series, demographic) does not allow determination of a causal relationship.
>
> This document provides guidance on how human studies with different research designs should be dealt with. For intervention studies, non-randomized studies may be included during literature filtering; however, their subsequent quality rating will affect their contribution to supporting consistency. For observational studies, only those with a prospective design (i.e., prospective cohort and nested case-control studies) should be included; all other observational studies should be excluded.
>
> Finally, if the subject of a health claim is a food constituent (i.e., not a food or a food category), the submission must at least include intervention studies; relevant observational studies would also be included, if available. Observational studies may be of greatest relevance for substantiation of health effects related to foods or food categories, but without intervention studies, observational studies alone generally do not allow for a causal inference to be made on the relationship between a food constituent and a health effect [*emphasis added*].[154]

FDHC does not require animal tests or accept animal data as stand-alone evidence for establishing health claims for foods.

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In summary, the EU, the US, and Canada all require human data—not animal data—to substantiate health claims for foods. Their agencies consider animal data as part of the totality of evidence but not

[154]Health Canada. (2009, March 17). *Guidance document for preparing a submission for food health claims*. Chapter 1.5: Study Designs and Evidence of Interest https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/guidance-document-preparing-submission-food-health-claims-2009-1.html#a1-5

as sufficient on its own. Some of the regulations also contain clear statements stressing the poor applicability of animal data to humans.