AN INTERNATIONAL ORGANIZATION DEDICATED TO PROTECTING THE RIGHTS OF ALL ANIMALS

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Via email: sww123@fda.gov.tw

Dear Ms. Wang,

Thank you in advance for your time. I am writing on behalf of People for the Ethical Treatment of Animals (PETA) and our 6.5 million members and supporters worldwide to provide scientific critiques regarding animal testing recommended by the Taiwan Food and Drug Administration (TFDA). **Specifically, based on the information presented below, we urge the TFDA to reevaluate its guideline on regulating blood pressure health claims for foods and remove the suggestions and acceptance of the animal test.**

Background

In 2006, the TFDA published a guideline on experiments that applicants can choose to perform to substantiate regulating blood pressure health claims for foods.[1] In this guideline, TFDA specifies that applicants should submit existing literature in support of the foods of interest and conduct the recommended human test. However, if the evidence is not substantial enough, the TFDA guideline states that applicants should conduct both the recommended human and animal tests.

The guideline recommends using genetically hypertensive rats such as the spontaneously hypertensive rats (SHR) as experimental subjects and the Wistar Kyoto rats as controls. Per the guideline, experimenters are to feed the animals the foods of interest in three dosages for at least eight weeks and measure the animals' blood pressure response using the tail-cuff method; at least four groups (one control and three experimental groups) and more than eight animals per group are required.

Below is a list of scientific limitations regarding the use of animals to substantiate human blood pressure regulation health claims.

Scientific Limitations of the Proposed Animal Test

Limitations with the SHR model

The SHR is a model of primary/essential hypertension with genetic predisposition to hypertension. Its underlying pathophysiology, however, is not similar to that in humans. This physiological difference between species is

[1] https://www.fda.gov.tw/TC/newsContent.aspx?cid=3&id=19954

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demonstrated by the variation in response to drugs for hypertension. For example, drugs that target the main system that regulates blood pressure—the renin-angiotensin system (RAS)—such as inhibitors of the angiotensin II-converting enzyme or angiotensin II receptor blockers can completely normalize the blood pressure of SHR[2] and other genetic models such as the Lyon hypertensive rats.[3] In contrast, these drugs only have limited effects on humans with primary hypertension and are not sufficient to control the condition when used alone.[4&5] This discrepancy has been explained to be due to species differences in RAS. In genetically hypertensive rats, the influence of local/tissue RAS systems is considered far more important than the circulating RAS system.[6] Therefore, any food ingredient that exerts blood pressure lowering effects through RAS is unlikely to have the same effects on rats and humans.

Another important limitation is that, genetically hypertensive rats—especially SHR—do not have adequate controls. A review of multiple studies found that the cause of phenotypic differences between SHR and their normotensive controls (most commonly, Wistar Kyoto rats) may not be the increased blood pressure or even the downstream effects of increased blood pressure, but simply be strain differences.[7] Without a proper control, one cannot carry out experiments scientifically.

Limitations with using rodents in general

Species differences in lipid metabolism

Dyslipidemia is a risk factor for hypertension, and about 60.7% - 64.3% of hypertensive individuals are found to be hypercholesterolemic.[8] Increased circulating lipid can increase the viscosity of blood and raise blood pressure, and advanced stage of dyslipidemia can result in atherosclerosis, which raises blood pressure by narrowing the blood vessels. Mice and rats however, do not usually suffer from these conditions. There are many significant species differences in lipid metabolism, which would render the use of these animals to be unscientific in dyslipidemia-related hypertension experiments. In addition, these species differences would affect any food ingredient of interest that goes through lipid metabolism.

Bile acids play an important role in cholesterol excretion, 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.[9] However, in humans, about half of the bile is stored

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[2] Baumann, M., Janssen, B. J., Hermans, J. R., Peutz-Kootstra, C., Witzke, O., Smits, J. F., & Boudier, H. A. S. (2007). Transient AT1 receptor-inhibition in prehypertensive spontaneously hypertensive rats results in maintained cardiac protection until advanced age. *Journal of hypertension*, *25*(1), 207-215.

[3] Julien, C., Bertolino, S., Medeiros, I. A., Barrès, C., & Sassard, J. (1997). Renin secretion in Lyon hypertensive rats. *Clinical and experimental hypertension*, *19*(5-6), 699-711.

[4] Heran, B. S., Wong, M. M., Heran, I. K., & Wright, J. M. (2008). Blood pressure lowering efficacy of angiotensin converting enzyme (ACE) inhibitors for primary hypertension. *The Cochrane Library*.

[5] Ruilope, L. M. (2008). Prospects for renovascular protection by more aggressive renin-angiotensin system control. *The Medscape Journal of Medicine*, *10*(Supp), S5.

[6] Paul, M., Poyan Mehr, A., & Kreutz, R. (2006). Physiology of local renin-angiotensin systems. *Physiological reviews*, *86*(3), 747-803.

[7] Nabika, T., Cui, Z., & Masuda, J. (2004). The stroke-prone spontaneously hypertensive rat: how good is it as a model for cerebrovascular diseases?. *Cellular and molecular neurobiology*, *24*(5), 639-646.

[8] Kamal, I., & Abdelkader, H. M. Dyslipidemia: the hidden sector of hypertension.

[9] Shiojiri, N. (1997). Development and differentiation of bile ducts in the mammalian liver. *Microscopy research and technique*, *39*(4), 328-335.

in the gallbladder where it becomes concentrated.[10] Rodents also synthesize unique bile acids called muricholic acids, which, for example, can have opposite effects on farnesoid X receptor activation compared to human forms of bile acids. This has major impacts on cholesterol metabolism.[11]

There are also many species differences in metabolic enzymes between rodents and humans. The hepatic enzymes delta-5 (D5D) and delta-6 desaturases (D6D) are important for metabolism of fatty acids. These enzymes 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.[12] Rats have a much higher D5D activity than humans,[13] and it is known that rodent models of T2D do not recapitulate human T2D.[14] Besides fatty acid metabolism, rodents have a unique cholesterol profile, higher high-density lipoprotein and lower low-density lipoprotein, due to their lack of cholesteryl ester transfer proteins. This makes them resistant to diet-induced alterations in cholesterol metabolism and cholesterol-mediated pathology.[15&16] Researchers have commented that "the rat is not an appropriate human model for studies involving lipids,"[17] and, "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."[18]

Species differences in nitric oxide metabolism

Nitric oxide is an important vasodilator that can be secreted by the endothelial cells in blood vessels.[19] Nitric oxide can also come from foods that are rich in nitrate (such as leafy greens and beetroots), which is converted to nitrite and further nitric oxide after ingestion.[20] In humans, about a quarter of the nitrate circulating in the bloodstream gets taken up by the salivary glands and then concentrated in saliva to about 20 times that in plasma.[21] It is then reduced to nitrite by the commensal bacteria in the oral cavity, which further increases the bioavailability of nitrite and nitric oxide. In mice and rats however, nitrate is not concentrated in saliva, and hence much higher doses of nitrate are needed to

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[10] Hofmann, A. F. (1999). The continuing importance of bile acids in liver and intestinal disease. *Archives of internal medicine*, *159*(22), 2647-2658

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

[12] 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.

[13] 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.

[14] 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.

[15] Ha, Y. C., & Barter, P. J. (1982). Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. *Comparative biochemistry and physiology. B, Comparative biochemistry*, *71*(2), 265-269.

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[18] 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.

[19] Sandoo, A., van Zanten, J. J. V., Metsios, G. S., Carroll, D., & Kitas, G. D. (2010). The endothelium and its role in regulating vascular tone. *The open cardiovascular medicine journal*, *4*, 302.

[20] Kobayashi, J., Ohtake, K., & Uchida, H. (2015). NO-rich diet for lifestyle-related diseases. *Nutrients*, *7*(6), 4911-4937.

[21] Montenegro, M. F., Sundqvist, M. L., Nihlén, C., Hezel, M., Carlström, M., Weitzberg, E., & Lundberg, J. O. (2016). Profound differences between humans and rodents in the ability to concentrate salivary nitrate: implications for translational research. *Redox biology*, *10*, 206-210.

induce similar physiological effects in mice and rats.[22] To further complicate the matter, mice and rats secrete endogenous nitrate in their upper gastrointestinal tract, but this does not happen in humans. There are also profound differences in gut microbiota that function to metabolize and affect nutrient absorption between rodents and humans. About 85% of the gut bug species in rodents are not even present in humans.[23] These differences are major confounders for any animal experiment on health foods, especially ones containing nitrate.

Species differences in Circadian rhythm

Blood pressure has a clear circadian rhythm and fluctuates mainly according to the daytime sympathetic and nighttime parasympathetic inputs in humans.[24] Mice and rats, unlike humans, are nocturnal. Their blood pressure fluctuation pattern is the opposite of humans.[25] However, one can often find rodents kept on a standard 12-hr light-dark cycle in nutritional experiments. Experimenters interact with them during the day, and interventions and measurements are carried out during the day, while the animals are supposed to be at rest. Not only does sleep disturbance increase stress,[26] measurements done in this setting cannot reflect true pathophysiology.

The circadian rhythm also affects metabolism,[27] which is important for nutritional studies. Active periods correspond to higher metabolic rate, and many metabolic substrates and hormones fluctuate in concentration and sensitivity throughout the day.

Circadian misalignment occurs in human shift workers and in mice and rats in laboratories who are forced to be experimented on during day time. Circadian misalignment increases both systolic and diastolic blood pressures and risks of hypertension and cardiovascular diseases.[28] Circadian misalignment also causes an array of metabolic disorders and increases risks of obesity, diabetes, dyslipidemia, and more.[29]

Stress

The issues with stress associated with routine laboratory procedures is not to be taken lightly because stress is a potent perturbator of blood pressure. This is of particular concern with mice and rats

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[22] Montenegro, M. F., Sundqvist, M. L., Nihlén, C., Hezel, M., Carlström, M., Weitzberg, E., & Lundberg, J. O. (2016). Profound differences between humans and rodents in the ability to concentrate salivary nitrate: implications for translational research. *Redox biology*, *10*, 206-210.

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[28] Morris, C. J., Purvis, T. E., Mistretta, J., Hu, K., & Scheer, F. A. (2017). Circadian misalignment increases C-reactive protein and blood pressure in chronic shift workers. *Journal of biological rhythms*, *32*(2), 154-164.

[29] Banks, S., Dorrian, J., Grant, C., & Coates, A. (2015). Circadian misalignment and metabolic consequences: shiftwork and altered meal times. In *Modulation of Sleep by Obesity, Diabetes, Age, and Diet* (pp. 155-164).

because they are prey animals who are easily disturbed. The day-to-day laboratory environment can increase these animals' stress and make measurements of their blood pressure inaccurate. Environmental factors, such as light,[30] noise,[31] temperature,[32] cage cleaning and transport,[33] lack of enrichment,[34] social deprivation,[35] human interaction and handling,[36] routine experimental procedures,[37] and even male experimenters,[38] all can induce stress and affect the animals' well-being and outcome of measurements.

The tail cuff method is also intrinsically inaccurate. It induces thermal stress because of thermoregulatory cutaneous blood flow in the tail and noxious stimulation from the tail compression by the occlusive cuff. It also requires restraining conscious animals in tubes, which can result in persistently elevated serum corticosterone levels.[39] Corticosterone is a stress hormone that can cause hypertension.[40]

A Note on the 3R principles

The 3R principles have a hierarchy. Replacement is the most important, followed by reduction, and last refinement. When we follow the 3R principles, we need to focus on replacement first. Considering that human tests are available and accepted by the guideline, it is clear that non-animal research methods are available and hence the replacement principle should be followed. Allowing animal tests in this case is in violation of the 3R principles and the Taiwan Animal Protection Act, which clearly states that "[o]ne shall avoid using live animals for scientific application."[41]

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[30] Azar, T. A., Sharp, J. L., & Lawson, D. M. (2008). Effect of housing rats in dim light or long nights on heart rate. *Journal of the American Association for Laboratory Animal Science*, *47*(4), 25-34.

[31] Baldwin, A. L., Schwartz, G. E., & Hopp, D. H. (2007). Are investigators aware of environmental noise in animal facilities and that this noise may affect experimental data?. *Journal of the American Association for Laboratory Animal Science*, *46*(1), 45-51.

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[41] http://law.moj.gov.tw/Eng/LawClass/LawParaDeatil.aspx?Pcode=M0060027&LCNOS=%20%2015%20%20%20&LCC=2

Conclusion

Animal tests are not justifiable for substantiating human health claims and should not be recommended or accepted by the TFDA guideline. Further, only human tests can be used to substantiate human health claims. The regulatory agencies in the United States, Canada, European Union, and others do not require or recommend animal tests to substantiate human health claims for foods.[42] Given these facts, along with the scientific limitation and cruelty associated with the animal test, we respectfully urge TFDA to remove the suggestions and acceptance of animal tests from the guideline.

You can contact me by e-mail at FrancesC@peta.org. Thank you for your consideration of our request, and I look forward to your response.

Sincerely yours,

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[42] Please see "PETA to Taiwan FDA food health claim animal testing" sent to TFDA on April 18, 2018 for details.