Advancing the ball: Using guinea pigs to study perfluorinated alkyl substances (PFAS)

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Hundreds of studies, and dozens of agencies, have attempted to estimate risks to human health posed by perfluorinated alkyl substances (PFAS). Essentially none of these studies, guidelines, or regulations has been based on evidence of health effects in humans exposed to PFAS (as all of us are, to greater or lesser extents).

At the same time, guidelines for allowable levels of PFAS in drinking water and other media are exceptionally stringent — making perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), for example, appear to be much riskier to human health than arsenic, mercury, benzene, and countless other, better characterized chemicals.

As a scientific matter, such stringency seems unjustified. Part of the problem is that, to date, PFAS have been studied almost exclusively in laboratory rats and mice; and these rodent-species, unfortunately, are quite poor models for humans — at least when it comes to this class of compounds.

To rectify this situation, we propose a simple solution: Bring back the guinea pig as the laboratory model of choice.

Our reasoning is as follows.

PFAS are "peroxisome proliferators", working through peroxisome proliferator-activated receptors (PPARs) to cause biological effects that may vary according to animal-species. As noted by Bell and colleagues (1998), "The guinea pig models the human response to peroxisome proliferators, where other rodents differ fundamentally in their regulation of hepatic lipid metabolism." Because of these differences, guinea pigs are employed preferentially in research focused on developing drugs, such as fibrates, used to treat people who have abnormally high levels of lipids in their blood (that is, hyperlipidemia) in order to prevent atherosclerosis and other diseases (Vázquez et al., 1995; West and Fernandez, 2004; Fernandez and Volek, 2006).

With regard to the potentially toxic effects of fibrates, PFAS, and other chemicals that activate PPAR, Dr. Chris Corton and his colleagues (2018) also note that "guinea pigs and non-human primates are better human surrogates than mice and rats because of differences in PPAR expression and activity." More generally, Corton et al. (2018) analyze the "striking differences in species responses" with regard to how different animal-species react to the clinical and toxic effects of various activators of PPAR-alpha

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activators.

As to the liver tumors and other adverse hepatic effects caused by peroxisome proliferators, Corton and co-investigators (2000, 2014 & 2018) argue convincingly that both qualitative and quantitative risk assessment should be based not on results from studies in rats and mice, but instead on results from studies in animal-species such as the guinea pig (and, when available, primates, including of course humans).

In many other, potentially relevant respects, guinea pigs are known to be better biological models for humans than are other rodents, such as rats and mice.

For example, as noted by Burns (1957), "Man, other primates and guinea pig are the only mammals that are known to be unable to synthesize L-ascorbic acid ; thus they require vitamin C in their diet to prevent scurvy."[1] Humans, monkeys, and guinea pigs are therefore susceptible to adverse health-conditions caused or exacerbated by deficiencies of vitamin C, while rats and mice are not.

Vitamin C is an antioxidant, acting *in vivo* to counter the potentially toxic effects of oxidation by hydroxyl and peroxyl radicals formed from the metabolism of dietary fats and other chemicals (Padayatty et al., 2003). Humans and guinea pigs with low (but still "adequate") levels of vitamin C are thus susceptible to the adverse effects of "oxidative stress," believed to be a major risk factor for the development of cardiovascular disease, cancer, immune system dysfunction, and other diseases linked to chronic inflammation (Ross, 1993; Santilli et al., 2015; Siti et al., 2015; Carr & Maggini, 2017; Shenoy et al., 2018; Ang et al., 2018).

In rats, mice, and most other mammals (but, again, not in humans nor in guinea pigs), vitamin C is biosynthesized (from glucose) in animals' livers: the enzyme required for the last synthetic step is missing from the liver of humans and of guinea pigs (Loewus et al., 1960; Nishikimi et al. 1992, 1994).

The livers of guinea pigs and human livers are alike in still other respects. In particular, the human metabolism of lipids, cholesterol, and many other important molecules are well modeled by guinea pigs, and poorly modeled by rats and mice.

More generally, as summarized by Podell et al. (2017) in their paper describing a guinea pig model of human type 2 diabetes that more closely mimics various aspects of the human syndrome than available rat and mouse models:

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[1] That was the state of knowledge as of 1957. More recently, a few other mammalian species, including the capybara and various species of bats, have also been found to be unable to biosynthesize Vitamin C (Birney et al., 1976; Cui et al., 2011; Padayatty & Levine, 2016).

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"In addition to inflammatory changes induced by high-fat and high-sugar diets (Fernandez and Volek, 2006; Ye et al., 2013), the guinea pig is widely regarded for research in specific diseases, including cardiovascular disease, atherosclerosis and arthritis, as well as a number of infectious diseases that have been linked as comorbidities with diabetes (West and Fernandez, 2004; Madsen et al., 2008; Padilla-Carlin et al., 2008). The guinea pig is crucial for development of new vaccines, particularly because of its immunological and pathological similarities in response to a number of infectious diseases of humans (Hickey, 2011). Additionally, the guinea pig, more so than any other rodent, shares commonalities with human lipid metabolism, including cholesterol metabolism and transport, with a greater proportion of cholesterol carried in association with low-density lipoproteins (Ensign et al., 2002; Fernandez et al., 1999; Ye et al., 2013)."

And an earlier review (West and Fernandez, 2004) noted:

". . . cholesterol and lipoprotein metabolism in guinea pigs has remarkable similarities to that of human metabolism (28). These analogies include: 1) high LDL-to-HDL ratios (25); 2) higher concentrations of free compared to esterified cholesterol in the liver (2); 3) similar intravascular processing of plasma lipoproteins (20,30,62); 4) comparable rates of hepatic cholesterol synthesis (66), esterification (26) and catabolism (67); 5) higher HDL concentrations in females compared to males (69); 6) similar plasma lipid profiles in ovariectomized guinea pigs compared to postmenopausal women (69); and 7) decreases in triacylglycerol (TG) concentrations and increases in plasma HDL cholesterol (HDL-C) with prolonged exercise (22). Due to these similarities and others, it is easy to understand why guinea pig responses to drug treatment have been shown to mimic human alterations in cholesterol and lipoprotein metabolism."

Guinea pigs are also used by researchers studying the causes of, and treatments for, diseases such as non-alcoholic fatty liver disease (NAFLD — a major cause of liver disease worldwide; Younossi et al., 2016; Perumpail et al., 2017; Ipsen et al., 2018). "Unlike mice and rats," note Ipsen and colleagues (2018), "guinea pigs naturally resemble the human lipoprotein profile and develop human-like NASH [non-alcoholic steatohepatitis] histopathology, dyslipidemia, and hepatic oxidative stress when fed a Western diet . . . ."

As our last example, in a study comparing the toxicity and the efficacy of a drug used for metal chelation (1,2-diethyl-3-hydroxypyridin-4-one; "CP94") in rats and in guinea pigs, Porter and colleagues (1993) reported that "CP94 was highly effective at mobilizing liver iron in rats but showed toxicity at higher doses, whereas in the guinea-pig the compound lacked toxicity but was ineffective at mobilizing liver iron." They added "[t]he lack of both efficacy and toxicity in the guinea-pig may therefore be explained by the rapid inactivation of CP94 by glucuronidation. This metabolism of CP94 in the guinea-pig is closer to humans than the rat, suggesting that both the efficacy and

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toxicity of this compound in humans may also be limited by glucuronidation."

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Overall, then, we would urge the health risk-assessment community to generate and/or rely upon the best toxicity data it can, employing test-species thought to be most like humans in relevant biological respects.

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