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EFSA Strikes Again: A Commentary on Flawed Analysis

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ABSTRACT: *In 2009, Marcia Angell, the former editor-in-chief of the New England Journal of Medicine, wrote that it was simply no longer possible to believe much of the clinical research that is published, or to rely on the judgement of trusted physicians or scientific authorities both to develop and to interpret data. We are concerned that this rather stunning indictment may increasingly describe some of the work of the regulatory agencies tasked to oversee public health, and in particular food safety. In the foregoing commentary we focus upon one recent and as yet unsettled instance involving the recommendations made by the European Food Safety Authority about food products containing Aloe vera gel and associated hydroxyanthracene (HAD) compounds. It is not our intention to suggest avenues for future research; rather, it is a call for more rigorous and precise interpretation of the best available science in support of decisions about safety. Moreover, it is our hope that the analysis of this case will illuminate the ways in which even the finest regulatory body may be prone to a significant error in a rush to judgment about novel products.*

KEYWORDS: EFSA, strikes, flawed , analysis

INTRODUCTION

The mission of the European Food Safety Authority (EFSA) is to promote **"**Safety in the food chain from farm to fork." Like the Food and Drug Administration (FDA) of the United States, EFSA's overarching goal is to serve as the responsible unit for minimizing the risk of harm in European food chains. It is declared that "We [EFSA] deliver independent and transparent scientific advice to policymakers through cooperation with our partners, and in an open dialogue with society" (https://www.efsa.europa.eu/en/about/mission-values). There is little question that EFSA is an enormously important public health institution, however, it has been subject to significant and perhaps inevitable formal criticism. Among the most scathing and well-publicized criticisms arose from Chvátalová (2019) in which the reliability of studies cited in an EFSA opinion was questioned. In addition, the body of referenced evidence EFSA drew upon was characterized as "insufficient to draw conclusions on risk." In a devastating and broad critique,

Chvátalová went on to state that "EFSA omits relevant available studies, selectively cites information, misquotes studies, fails to acknowledge uncertainties, fails to call for further research where needed, and fails to critically interpret studies and their findings."

Meyer and Hilbeck (2013) accused EFSA of double standards when it rejected a study led by Séralini, which found adverse effects in rats fed a genetically modified maize and very low levels of Roundup herbicide, while uncritically accepting at face value Monsanto's own studies on the same maize, which concluded that it was safe.

Science is imperfect and continually evolving and no scientific institution can be perfect or static either, and it is in this spirit that we add to the cry for continual self-scrutiny and improvement, especially for an entity dedicated to public safety and prosperity.

We call attention herein to significant scientific gaps in the EFSA (20 November 2018) interpretation of epidemiology putatively supporting a causal relationship between hydroxyanthracene (HAD) use and Colorectal Cancer (CRC) risk as this relates specifically to beverages containing *Aloe vera* gel…which may contain limited amounts of HADs.

Historical Context

It seems appropriate to begin with recent history. Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the evaluation of hydroxyanthracene derivatives in food. EFSA concluded that hydroxyanthracene derivatives should be regarded as genotoxic and carcinogenic unless there are specific data to the contrary, such as for Rhein, and that there is a safety concern for extracts containing any hydroxyanthracene derivatives although it was acknowledged that uncertainty persists.

The European Commission subsequently asked EFSA to assess the safety of these plant ingredients when used in foods, and provide advice on a daily *intake* not associated with adverse health effects. Based on the available data, EFSA concluded that because certain hydroxyanthracene derivatives [contained in inadequately processed *Aloe vera*] are genotoxic, it was not possible to set a safe daily intake.

These conclusions were seen by many as in line with previous assessments (shaped by the precautionary principle) on the botanical sources of these substances by other European and international bodies, including the World Health Organization, the European Medicines Agency, and, most recently, Germany's Federal Institute for Risk Assessment. The *Aloe vera* industry along with many toxicologists and public health experts have responded with a very different, evidence-based opinion contrary to that of EFSA.

EFSA's Flawed Argument

Under "Conclusion" on page 69 of the EFSA (2018) report, the agency asserted that "Epidemiological data suggest an increased risk for colorectal cancer associated with the general

use of laxatives, several of which contain hydroxyanthracene derivatives. This statement is not an accurate reflection or interpretation of the data in the studies that are cited on pages 61-63. The laxatives in the six studies reviewed were generally treated as a broad inclusive category, not broken down by chemical constituents; one study characterized laxatives as fiber or non-fiber, also without identifying chemical constituents. None of the six studies found a statistically or clinically significant association between anthranoid laxative use and colorectal cancer (CRC) risk. The studies cited fail to provide convergent validity to the toxicology research cited and discussed by EFSA.

Highlights of the six epidemiologic papers cited by EFSA; errors in interpretation by EFSA and intrinsic weaknesses of the studies

Dukas et al (2000) – looked at 17,400 US women and reported no association between any category of laxative use and CRC. The reason for laxative use and the specific type of laxative were not assessed. Subjects who used laxatives at any frequency were more likely to use aspirin, multivitamins, and hormones. There was no analysis of how these variables may have strengthened or weakened any association. It is important to note that these authors asserted that anthranoid laxatives are rarely used in the US, thus the exposure in Europe may be far greater, hence hazard and risk estimates may be significantly different between the US and Europe.

Watanabe et al (2004) – reported the results of a cohort study among middle-aged rural Japanese laxative users and non-users. A principal observation was that of increased risk for CRC in those subjects using any over-the-counter laxative use at a frequency of 2 x weekly or more. Type of laxative was not elicited [since, it is asserted, (without any cited evidence) that most laxatives are irritants that exhibit carcinogenic properties].

Kojima et al (2004) – reported a "weak nonsignificant positive association" between laxative use and the risk of colorectal cancer in Japanese men and women. Differential effects of race, ethnicity, microflora, genetics, and importantly, effects of laxative type were not investigated or discussed.

Park et al (2009) – results from a large ongoing study in the UK looked at a spectrum of antecedent and intervening variables that might modify and elucidate an association between bowel habits and colorectal cancer. During the 12-year follow-up, there was no association between laxative use and CRC. The specific laxative type was not elucidated. Zhang et al. (2013) – explored associations between bowel movement frequency, laxative use, and CRC risk from data on over 88,000 women in the Nurses' Health Study conducted in the US. There was no significant association between overall laxative use and CRC risk in the cohorts studied. The laxative type was not queried.

Citronberg et al. (2014) – looked at 10-year fiber vs non-fiber laxative use and the risk of CRC in over 75,000 subjects from the VITAL study that focused on vitamin D_3 and omega-3 fatty acids relative to cancer and cardiovascular disease. CRC risk was seen as increasing with non-fiber

laxative use and lower with fiber laxative use. Chemical constituents of the non-fiber laxatives were not reported.

Missing studies

Two key studies were missed or omitted. Nusko et al (2000) and Nascimbeni et al (2002) looked specifically at anthranoid use as a putative risk factor for colorectal neoplasia. Both prospective studies reported evidence that does not support any association between anthranoid laxative use and CRC even in the long term.

Ongoing study

An Italian group (Lombardi et al, 2020)[1] launched a systematic review and meta-analysis to critically examine more definitively and comprehensively in the style of a Cochrane review at anthraquinones (AQ) and CRC. Since there is no clear evidence of the potential association between the use of oral AQ-containing laxatives and the risk of CRC, the Italians have aimed to quantify this risk by performing the new study.

The bidirectional relationship between aloin and gut flora: The "fog of war" becomes thicker and more complex, and ought to be acknowledged

The following addresses the EFSA-neglected issue concerning the impact of the intestinal flora on the metabolism of aloins and HADs (Huang et al 2019; Sehgal et al 2013) and the modulation of toxic effects; and conversely, the impact of aloin on gut flora (Boudreau et al 2017). Boudreau et al (2017) demonstrated dose-related changes in specific families of bacteria with administration of aloin in a rodent system. They speculated that this apparent microbial dysbiosis was causally associated with the induction of colon cancer in the animals in the same fashion as what had been observed with whole leaf extracts.

A review of the human epidemiology of anthracene laxatives and possible causal linkage with CRC does not support the translation of the rat data to humans, where there is consistently little or no association with pathology. How might this be explained?Boudreau et al (2017) themselves point out that due to the β-glycosidic bond and the anthrone ring structure, as well as the hydrophilic nature of the compound, aloin is protected from acid hydrolysis in the stomach, and reaches the large intestine undigested, where bacteria metabolize aloin to yield glucose and aloe-emodin-9-anthrone which is then oxidized to aloe-emodin (with its potential mutagenic and cell-transforming properties). But we know that over time, bacterial shifts occur. In free-living animals and humans, it is tempting to speculate that future research looks at a decrease in hydrolytic activity by intestinal bacteria with the corresponding change in aloe-emodin anthrone and anthraquinone concentration and proportions…with the associated reduction in toxic impact. The post-hydrolysis absence of the sugar moiety may also decrease solubility and bioavailability with a possible reduction in toxic effects, especially systemically.

Huang et al (2019) recently elucidated possible biotransformation pathways of anthraquinones and anthrones (including aloe-emodin and emodin) by human intestinal flora *in vitro*. Ultra-performance liquid chromatography with quadrupole time-of-flight mass spectrometry was used to separate and identify 64 metabolites from nine components. Ten metabolites were identified from emodin, along with seven from aloe-emodin. Their results suggested that metabolites were generated by reduction, oxidation, hydroxylation, methylation and demethylation, hydrolysis, and acetylation. The multiplicity of metabolites produced by human gut flora lends credibility to the argument that the microbiome may serve putative detoxification functions.

Reduced systemic exposure possibly secondary to GI malabsorption

This topic was investigated by the Cosmetic Ingredient Review Board Expert Panel (2007). In a rodent model, most (75%) of consumed "anthraquinone components" from *Aloe* were recovered intact in feces while little or none was found in urine. This finding suggests that at least in some forms and combinations of *Aloe* leaf anthraquinone compounds, relatively little was absorbed. While metabolites were not assayed, the fact that much of the administered 'dose' was recovered in the feces, and not in the urine, is reassuring in terms of acceptable systemic exposure to a potentially toxic metabolite without untoward effects.

Using a human Caco-2 cell line, Liu et al (2012) investigated the poor bioavailability of emodin in humans as a result of metabolism and excretion of the emodin glucuronide and its coupling with various efflux transporters and drug metabolizing enzymes which are part of the CYP isozyme system. Interestingly, passive cell diffusion is seen as the dominant transport mechanism for emodin in the Caco-2 cell monolayers, hence the fundamental suggestion that the coupling of UDP-glucuronosyltransferases and multidrug resistance-associated protein efflux transporters causes the extensive metabolism, excretion, and low bioavailability of emodin

Omnipresence of Anthraquinones in a spectrum of common vegetable constituents in the human diet without apparent adverse consequences

The putative protective effects of the human food matrix may mitigate genotoxic risk in many cases (Mueller et al 1999). In this interesting study, cabbage, lettuce, beans, peas, and some herbs were screened for non-glycosidic anthraquinones which were present in the majority of samples at levels up to 7.6 mg/kg, i.e., 7.6 ppm. With strong epidemiologic evidence for a cancer protective effect of vegetable consumption, and taking into consideration estimated EDIs (estimated daily intake) and genotoxic data, the authors postulated that any mutagenic effects are more than compensated by protective effects so that it is possible to conclude that the studied constituents do not present a significant genotoxic risk.

The validity of 10 ppm

The National Toxicology Program and the International Aloe Science Council (IASC) have recommended that the aloin content of commercial products not exceed 10 ppm based on a four-dose animal study (Sehgal et al 2013).Since it is not at all clear what the basis for this limit of 10 ppm was, it appears that 0.5% or 5,000 ppm of whole leaf extract that resulted in no pathologic

changes was selected as the NOAEL. Aloin is about 20% of the whole leaf extract which means that 1,000 ppm of aloin is without sequelae. Applying the rule of 100 for inter- and intra-species safety means 1,000 was divided by 100, which equals 10 ppm.

Again, the principal problem with this number is the translation of animal-to-human data. Pathologic trajectories are often markedly different, especially between rodents and human subjects. Rodent models have been instrumental in garnering numerous insights into fundamental pathophysiological mechanisms that are conserved across species. Nevertheless, in key respects, rodent physiology is distinct from that of humans, and uncritical overreliance on rodent findings risks impedes the research enterprise and translational progress toward improving human health. Rodents are valuable but are often flawed representatives of humans in health and disease. Convergent validity of findings utilizing independent approaches must be appreciated before a well-tolerated and potentially valuable product is pulled from the marketplace.

Direct polysaccharide augmentation of immune/macrophage activation: Not all anthraquinones are created equal

Raw aloe gel matrix contains valuable polysaccharides, notably acemannan in addition to limited amounts of HADs. Interestingly, rhein, an anthraquinone has also been shown to activate macrophages and attenuate inflammation (Chang et al 2019). Similarly, the hydroxyanthraqionone emodin also appears to modulate macrophages and restore macrophage homeostasis (Iwanowycz 2016). Dose effects notwithstanding, conventional wisdom at this juncture is the reality of *in vitro* and *in vivo* macrophage activation by polysaccharides in diverse animal models (Zhang & Tizard 1996); Djeraba & Quere 2000) with the best explication from the literature on β-glucans. These are polysaccharides widely distributed in bacteria, algae, fungi, and plants, where they are involved in an array of biological functions. Novak & Vetvicka (2018) reviewed the evidence for various complex effects on the human body after consumption of dietary β-glucans. These included significant immunostimulation. The most studied mechanism appears to be in the augmentation of phagocytosis and proliferative activities of phagocytes–granulocytes, monocytes, macrophages, and dendritic cells. In this regard, macrophages may be considered the basic effector cells in host defense against bacteria, viruses, multicellular parasites, tumor cells, and erroneous clones of somatic cells.

It is the binding of glucan compounds to a discrete receptor that activates macrophages. The activation consists of several interconnected processes that lead to increased chemokinesis, chemotaxis, migration of macrophages to particles to be phagocytized, degranulation leading to increased expression of adhesive molecules on the macrophage surface, adhesion to the endothelium, and migration of macrophages to tissues. In addition, glucan binding also triggers intracellular processes, characterized by the respiratory burst after phagocytosis of invading cells (formation of reactive oxygen species and free radicals - hydrogen peroxide, superoxide radical, NO, HOCl [hypochlorous acid], HOI [hypoiodous acid]). This cascade leads to yet another cascade increasing the activity of hydrolytic and metabolic enzymes and signaling processes leading in turn to activation of other phagocytes and secretion of cytokines that initiate

inflammation reactions, e.g., IL-1, IL-9, TNFα (Nakashima et al 2018). Dectin-1, which is expressed on the surface of macrophages and dendritic cells, is reported to serve as the primary receptor for several glucans, which consequently have the immunostimulatory effects (Brown & Gordon 2001).

CONCLUSIONS

The challenge of regulatory oversight is to protect public health and safety while minimizing damage to innovation and growth. The inadvertent harm of the very people who are supposed to be protected, together with erosion of the public's confidence in mainstream science may well be the consequence of a rush to judgment about the potential toxicity of novel ingredients or products. The more we learn, the more we seem to be acknowledging that science is complicated and fluid, and if we are to re-capture trust, we must actively urge regulatory agencies to spend more time and funding in evaluating safety. Richard Horton (2015) went further; he has written:

…In their quest for telling a compelling story, scientists too often sculpt data to fit their preferred theory of the world…Our love of "significance" pollutes the literature with many a statistical fairy tale.

It is certainly easier at first blush to dismiss a product with complex properties as hazardous; indeed, it may be the right thing to do in some instances in which available evidence is so scant or of such poor quality that a "deep dive" is neither cost-effective nor is it likely to change a rapid assessment. However, as the *Aloe* case and others suggest, an almost reflexive decision may lead to the loss of a spectrum of products with health promoting and economic potential. Hiding behind the arguably archaic precautionary principle is simply not a defense that
is supported by clinical realities.

Conflict of Interest Declaration

The authors are paid scientific advisors to Forever Living, a producer of *Aloe*-based products, however, no funds or directives were received from the company in support of the present manuscript, and the opinions therein are those of the authors and do not necessarily reflect those of any corporate entity.

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