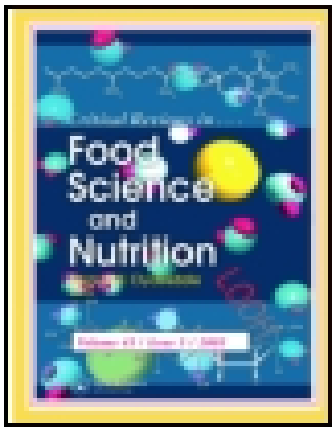

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Nutrition and health—transforming research traditions

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Nutrition and health – transforming research traditions

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Abstract

In this contribution we show that current scientific methodologies used in nutrition science and by regulatory agencies, such as the randomised control trial, limit our understanding of nutrition and health as they are to crude to capture the subtle pleiotropic nature of most nutrients. Thereby, regulatory agencies such as the European Food Safety Authority curb the development of scientific knowledge and industrial innovations within the nutritional field. In order to develop insights into the health impact of certain food and food-components, we need to realise that health is adaptation set within a homeostatic range. Increased performance of health, i.e. the maximum stimulation of health, typically seems 30-60% greater than the control group, with a width of no more than about a factor of ten, clarifying the difficulty of documenting responses of food-endogenous components within the homeostatic range of healthy people. A strategy to record subtle responses of food components is the summation of procentual effects of relevant health outcomes. We illustrate this approach with the action of flavanols on vascular health, specifically endothelial function.

Introduction

Health claims related to food products and the scientific elucidation process thereof will be the focus of our contribution to the discussion concerning appropriate methods of researching the relationship between food and human health and the scientific evaluation and grading of outcomes of primary research. European Regulation 1924/2006EC on nutrition and health claims made in commercial communications regarding foods and foodstuffs provides the regulatory setting (Regulation 1924/2006). We will, however, not just reflect on this regulation and its implementation, e.g. in terms of scientific assessments, as such. More importantly, we will provide a context for the assessment of food components that goes beyond the current methods that are deemed compulsory to protect the European consumer (e.g. against misleading information) and we will attempt to clarify the role of nutrition in human health.

In Regulation 1924/2006EC, roughly two types of health claims are defined: claims related to *reduction of disease risk and children's development and health* (article 14) and *other claims* (article 13) concerning the (physiological) role of a nutrient or other substance in growth, development and the functions of the body (13.1a); psychological and behavioural functions (13.1b) and any additions of claims to the list referred to in paragraph 3 based on newly developed scientific data (13.5). Assessing these article 13 and 14 claims is a matter of primary scientific inquiry and subsequent assessment of available scientific evidence.

In this contribution, we will to some extent elucidate the connection between human health and nutrition, with specific reference to polyphenols, by taking a methodological look at the ways in

which current scientific practices in the nutritional sciences operate. Further, we offer a perspective on nutrition and human health that features well-known pharmacological and toxicological concepts. Moreover, methodological concepts in science that seems superiorly equipped to reveal the connection between nutrition and health are discussed. Finally, we demonstrate that the EFSA and the EC have created a scientific and regulatory cul-de-sac with respect to research into nutrition and health by favouring certain methodologies. Limiting scientific methodology *a priori* impedes scientific and industrial innovation, might well hinder human health, and carries a contradiction that makes all regulatory efforts and advice related to food and health immaterial.

Benefit-Risk assessment - European Food Safety Authority approach

In order to delineate the regulatory contours of scientific investigation into nutritional benefits, we first present European Food Safety Authority's take on the assessment of benefits and risks of nutrition. As the gatekeeper of the European food market's safety, EFSA's assessment methodology effectively underlies all assessments done so far (Scientific Opinion EFSA, 2010). A key element in its approach is the mirroring of the conventional *risk* assessment paradigm when assessing the *benefits* of food. Therefore, 'food-safety' *here* is not so much a procedure of elucidating risks posed to human health but is focussed on assessment of the ostensible benefits of certain foods and food components. These benefits are twofold. They might result from the *stimulation* of physiological processes such as growth, development and bodily functions *and* the *reduction* of disease risks. Figure 1 provides a schematic representation of EFSA's views.

Figure 1

The EFSA indicates some ostensible defects in this emulation. It is stated that, in general, a *benefit* imparts an advantage to something or someone. As a result, adverse and positive health effects

of the consumption of some food product or component are opposite terms. However, it is remarked that there is no *term* for the *inherent* potential of an agent (nutrition in this case) to cause beneficial effects on health that would correspond to the term hazard as applied in risk assessment. Also, the term benefit is not regarded as to correspond to its ostensible opposite risk, which describes the *probability* of an adverse effect. Nevertheless, in line with the definition of risk, a benefit is considered by the EFSA to consist of the *probability* of a positive effect on health (Scientific Opinion EFSA, 2010). The reduction of a risk will also be considered as a benefit.

Overall, the following definition of benefit is proposed by the EFSA: 'the probability of a positive health effect and/or the probability of a reduction of an adverse health effect in an organism, system (future generations), or (sub)population, in reaction to exposure to an agent.' In the context of Regulation 1924/2006EC's article 14, risk would indicate 'disease risk'. Figure 1 above gives an impression of EFSA's 'mirror image' risk-benefit comparison process (Scientific Opinion EFSA, 2010).

To connect dietary patterns with human health and thereby assess benefits and risks, methods such as observational epidemiologic studies, intervention trials (Randomised Controlled Trials – RCTs), models and simulations, *in* and *ex vivo* animal and human studies, *in vitro* research, and the like, have been devised. Specific endpoints might comprise of the number of healthy life years and life expectancy, motor-, cognitive-, neurologic- and metabolic function, wellbeing, satiety and hunger, and the like (Asp *et al.*, 2003). From a political, regulatory and mainstream scientific point of view, the RCT is generally regarded as the 'gold standard' for connecting food and health (Cummings *et al.*, 1985).

A clinical RCT roughly comprises of a group of volunteers receiving medical treatment parallel to a control group not receiving the same treatment, but a placebo. Comparing both groups, in a properly designed and executed trial (Schulz *et al.*, 2010), gives insight in the efficacy –can the intervention work?- or effectiveness –does the intervention work when used in normal practice?- of the treatment under scrutiny (Zwarenstein *et al.*, 2008).

Before we deliberate on the methods that are denoted as the primary scientific means to elucidate risks and benefits, we first will delve into the food-endogenous polyphenols, illustrative of the intricate relation between nutrition and human health.

Polyphenols: flavanoids

Flavonoids are a class of polyphenols that are abundantly present in our daily diet, and are widely recognized to beneficially affect human cardiovascular health (Hooper *et al.*, 2008; Boots *et al.*, 2008). The basic structure of the flavone backbone is given in figure 2 below.

Figure 2

Unsurprisingly, the *explanations* for the functional effects of flavonoids on the vascular endothelium change over time. As more knowledge on vascular health comes to the fore, the intrinsic health-beneficial character of flavonoids and sub-classes of flavonoids such as flavanols, becomes more evident and health markers can be more clearly and specifically defined.

The first notion that was reported on was that free radicals are damaging to the vascular tissue and that flavonoids could act as scavengers (Van Acker *et al.*, 1996). This scavenging quality accordingly might prevent capillary fragility, among others. Subsequently, flavonoids were recognized as inhibitors of the formation of oxygen radicals (reactive oxygen species, ROS) by inhibiting the enzymes NADPH oxidase and xanthine oxidase (Weseler and Bast, 2010). ROS af-

fect intracellular redox signalling, which provokes the activation of transcription factors and subsequently leads to inflammation. For the inflammatory response to be active in the vascular tissue, adhesion of inflammatory cells should occur. Flavonoids are known to prevent this adhesion (Abou El Hassan *et al.*, 2003). The inflammasome is thus inhibited by flavonoids in various ways.

It is further increasingly recognized that phase II metabolites, i.e. glucuronides and methylated metabolites, are also biologically active (Steffen *et al.*, 2008). Most recently, epigenetic modulation by dietary flavonoids, e.g. by inhibition of DNA methyltransferases, has been shown to fine-tune the genomic influence on physiology (Link, *et al.*, 2010). Alkylating and epigenetic effects induced by polyphenols might easily be misconstrued as no more than toxic actions against which we should guard ourselves one way or the other. This 'toxicity', however, is considered to form the basis for the multitude of beneficial physiological effects observed for these compounds (Boots et al, 2007).

These numerous, seemingly insignificant, yet potentially beneficial, changes that occur upon consumption of various groups of polyphenols, either through food or food-supplements, do not promptly lead to strong physiological outcomes as e.g. an immediate hypotensive effect, an augmented memory, direct endurance improvement, and the like. That of course is fortunate; food can be consumed without the common pharmacological restraints. It is not without reason that through the ages, food suitable for human consumption has been selected for –apart from taste, smell, nutritious value and digestive qualities- the absence of strong physiological impacts after consumption, ethanol probably being one of the few exceptions.

Indeed, the past hundred years or so have shown an increased health status of large populations around the world, partly because of an improved diet (e.g. Heaney, 2008). The question then is how we can link the '*ensemble*' and interplay of micro-effects (elucidated through molecular mechanistic studies) with the macroscopic health effects (epidemiology). Toxicology, aiming at elucidating the causes of adverse effects, struggled with this problem already quite some time and offers insights and limitations that might be applied to the analysis of beneficial health capacities of food-endogenous chemical compounds. Below, we will illustrate this by focussing on stochastically acting genotoxic carcinogens and combinatory toxicology.

Of combinatory-toxicology and the one-hit model

Stochastically acting genotoxic carcinogens interact with DNA. Every singly chemical interaction is called 'a hit'. The assumption that a linear relation exists between exposure (i.e. dose) and DNA-toxicity is based on the notion that the chance of an effective hit, i.e. a hit eventually leading to cancer, is linearly related to the dose.

To be able to linearly extrapolate high-dose effects to low levels of exposure presumes that DNA-damage is in fact a stochastic process in which there is either DNA damage or not. In other words, probability, rather than the degree of DNA-damage itself in terms of measurable results, determines carcinogenicity. A second presumption in this model is that no threshold exists below which a compound is not active. In other words, the one-hit model implicates that every exposure other than zero (even as low as one molecule or ionising photon) always creates a certain risk to the cell's health and thereby implicitly to the whole organism. A standard probability of failure of the cell's ability to repair the damage to its DNA within a certain range of damaging events –the homeostatic capacity of organisms- seems to be reckoned with in the non-threshold

model. Whether this standard probability of failure of the cell's repair mechanisms by definition results in non-threshold linearity remains unclear.

Non-stochastically acting genotoxic carcinogens, e.g. compounds that act on DNA-repair or DNA-replication are deemed to show a threshold value below which no damage is done, or rather observed. Also the non-genotoxic carcinogens that may enhance the cancer-forming process without direct or indirect damage to the DNA will have a threshold-value below which no effect is deemed to arise (Dutch Health Council report A10/07, 2010).

In the practice of *combining* the effects of potential toxic compounds, typically, the effects are added. In governance and risk management, subsequently, precaution typically prevails. Hence, both for compounds that show a threshold response and for compounds for which a linear no-threshold dependency on dose is assumed, adding the separate effects is usually applied. This approach could well be mirrored in the benefit-assessment procedure, as we have depicted in figure 1.

Indeed, for compounds, like dietary components with multiple effects, adding these separate effects could correspondingly be employed to define a 'beneficial capacity'. Typically, this is not done, however. Studies defining health aspects of foodstuffs tend to focus on separate, isolated compounds and isolated 'cause-effect'-related identifiable phenomena.

To be sure, adjoining these detached events to explain a biological effect on a molecular basis obviously is quite tricky. Conversely, in toxicology, stochastically acting genotoxic carcinogens, as portrayed in the one-hit model, are curbed by DNA repair systems, detoxifying biotransformation pathways and compounds that activate those kind of biological structures.

Regardless, combi-tox methodology, clearly as an unsatisfactory and incomplete rule, assembles one-hit toxic responses to attain a toxic risk evaluation. Similarly, for compounds that possess a 'beneficial capacity', a linear addition most likely will not fully suffice. Feedback and adaptation processes will hamper simple linear extrapolation of the effects that occur within a homeostatic range. As a matter of fact, this has already been recognized in the 1970s.

From linearity to biphasic dose-responses – biological performance and plasticity

Indeed, the US FDA acknowledged the need to *validate* linearity at low dose predictions for carcinogens using 2-AAF (2-acetylaminofluorene) as a model carcinogen. However, this effort revealed that the analysis of risks lower than only one individual in one hundred was not practically achievable for carcinogens within chronic animal bioassays. Thus, they referred to this study, performed with 24,000 mice, as the Effective Dose (ED01) study, also known as the mega-mouse study (Bruce *et al.*, 1981).

The failure of this study to adequately test linearity at low-dose modelling, despite the use of unmatched resources, lead to a continued reliance on non-validated hypothetical models for risk assessment of chemical carcinogens. An important irony that ties in with our observations concerning the biological limitations of adding either risks or benefits in a merely linear fashion, is that a detailed re-analysis of the US FDA/2-AAF study by an expert panel of the US Society of Toxicology revealed an unequivocal non-linear dose-response for bladder cancer with risks decreasing below the non-exposed control group at low exposure doses. The authors remark that if 'we insist that it is possible to construct low-dose extrapolations for carcinogens in terms of increases in overall probability of tumor, then we are forced to use additive models. In such a case,

the ED01 study provides more than evidence of a "threshold". It provides statistically significant evidence that low doses of a carcinogen are beneficial.' (Bruce *et al.*, 1981)

Obviously, this is an unsettling conclusion that so far has been undervalued or even ignored. Only in the late 1990s, unbiased evaluative criteria were defined to assess the existence of *biphasic* dose-responses, such as tentatively found in the mega-mouse study. These lead to the development of a large database that would aid in the empirical assessment of the *biphasic* dose-response model. By and large, it seems that biphasic dose-responses are a reproducible phenomenon that is general, being independent of biological model, endpoint measured and chemical class/physical stressor agent (Calabrese and Baldwin, 1997; Calabrese and Blain, 2005, Calabrese *et al.*, 2006). These biphasic dose-responses are presented as a low-dose stimulation and a high-dose inhibition, and they display consistent quantitative features including the magnitude of the stimulation, the width of the stimulation and their relationship to the zero equivalent point (i.e., threshold).

Biphasic responses may be seen as adaptive reactions, mediating cellular stress involved in a plethora of preventive, reparative and signalling activities. From the fundamental problem-orientated scientific perspective, the biphasic dose-response curve seems useful in elucidating the relation between food and health, although we haven't touched on the homeostatic range, i.e. the *biological plasticity* thereof. By and large, the maximum stimulation of studied biological systems, the *performance* of the homeostatic range, typically seems 30-60% greater than the control group, with a width of no more than about a factor of ten (Calabrese and Baldwin, 1998; Calabrese *et al.*, 2007). This observation is critical since it clarifies why responses within the homeostatic range of healthy people are not easy to document with respect to food-endogenous

components such as flavonoids. That is, since the maximum stimulation is modest, it requires the use of rigorous study designs along with adequate statistical power in order to observe the relatively small performance signals.

Dose-responses *within* the homeostatic range may occur via a direct stimulation or via a compensatory response. This may be detected by the inclusion of a time component in the assessment of the dose-response. Many published studies emphasize either the use of multiple doses or numerous time points for repeat measures, usually not both. In the case of a compensatory response, the chemical agent initially causes a disruption in homeostasis or a minor –subclinical-level of toxicity. This effect typically induces a compensatory response to repair the biological damage. In response to such a disruption in homeostasis, biological systems typically have been observed to overcompensate in their reparative responses, overshooting the measured control group's value (Calabrese and Baldwin, 2001).

In the case of direct stimulation, the agent stimulates the biological response *without* the initial decline in endpoint response. This is commonly seen in various receptor-based pharmacological systems. Of particular importance are the observations that the quantitative features of the dose-response within the homeostatic range are similar whether they originate from either a direct stimulation or an over-compensatory stimulation. This suggests that the dose-response maxima of either type of induced stimulation are constrained by limits of biological (phenotypic) plasticity (Izem and Kingsolver, 2005; Pigliucci at al., 2006; Calabrese and Mattson, 2011).

These findings have important implications for the food industry, as it may be possible to achieve a maximum stimulatory homeostatic response with the use of certain quantities of bioactive food-ingredients. Food as a means to enhance homeostatic performance, within the confines

of biological plasticity, is now within range of scientific investigation. A recent example of human performance enhancement was documented with sodium nitrate supplementation (Bailey *et al.*, 2009; see also Lundberg *et al.*, 2011). During exercise at a fixed moderate work rate, increased NO_3^- intake resulted in improvements in muscle oxygenation and a significant reduction in pulmonary O_2 uptake. Bailey *et al.* state that reduction in the O_2 cost by some 19% of submaximal cycle exercise following dietary supplementation with inorganic nitrate (in the form of a natural food product) cannot be achieved by any other known means, including long-term endurance exercise training.

Polyphenols II – en route to quantifying performance

Application of the performance concept to our example of certain polyphenols, illustrates the potential thereof. The effect of polyphenols in cardiovascular health might be followed with risk factors.

Table 1.

Biomarkers with a high signal to noise ratio might be selected. Interestingly, generally accepted biomarkers function in situations where there is an apparent aberrant pathogenic state, which needs to be fixed such as plasma cholesterol levels (Griffiths *et al.*, 2002). Therefore, there is a clear need for more subtle biomarkers that are operative in the homeostatic range of *healthy* individuals. Quantification and defined consequences of epigenetic alterations induced by polyphenols might be of great value in this respect (Link *et al.*, 2010). Increased flow mediated dilatation (FMD) is recognized as a biomarker for enhanced endothelial function (Heiss *et al.*, 2007). The FMD is the consequence of a shear stress that leads to induction of endothelium derived

nitric oxide. In this way, stress applied to the system reveals a fortified physiology as a result of intake.

Also, applied *ex vivo* stress can reveal a protective response in the homeostatic range. An example is the *in vitro* challenge of blood with lipopolysaccharide (LPS) (Boots *et al.*, 2008). LPS triggers the Toll-like receptor with leads to the formation of pro-inflammatory cytokines. *In vivo* supplementation with a flavonoid can inhibit the *ex vivo* LPS response. This illustrates the anti-inflammatory action of the polyphenols.

A promising approach for the quantification of performance seems the summation of procentual effects of relevant outcomes related to endothelial function. In this way, a vascular health index can be defined in which established cardiovascular risk factors (serum lipid levels, CRP) and relevant biomarkers (GSH/GSSG, 8-iso-PGF2a, inflammatory resistance) are taken into account. This approach was recently applied in a study on the action of flavanols in vascular health (Weseler et al., 2011). The pleiotropic action of these compounds was captured defining such a vascular index. Changes in biomarkers were integrated into a vascular health index, which enabled to quantify the health benefit of monomeric and oligomeric flavanols in humans.

These fundamentals of homeostatic performance delineates and directs the fine-tuning process of future nutritional research, requiring that far more detailed methods will need to be developed than currently in use. We have given a recently published example of such a method here, but more is required, which we cannot discuss at length here at this time.

Discussion and conclusion

It seems that we are only beginning to unravel the details of the connection between food and human health, despite the knowledge of the past hundred or so years that shows an increase in public health due to, among others, improved diet. Boudreau, with reference to the famous 1937 League of Nations report (Final Report of the Mixed Committee of the League of Nations, 1937), effectively captured the time of the Great Depression in which food *security* was the dominant issue in the world, including the West: 'delegates to the League of Nations, together with League officials, launched what came to be known as the world food movement, *designed to release the economic jam by emphasizing that adequate diets were essential to human health*, ….' (Boudreau, 1947) This is not related only to food shortages as such, although these were clearly a grave issue in the 1930s. It should be remembered that in that same period the first food standards –the RDAs- for micronutrients were developed, which served as a 'goal' or 'floor' for intake below which risks of inadequacy begin to significantly increase (Leitch, 1942; Harper, 1987). As we now know, improved diets have enhanced human health.

In this contribution, we zeroed in on a few details of the connection between food and health using certain polyphenols as reference. It was shown that in order to understand the role of certain food-components in human health, searching for single and distinct (beneficial) markers, as done in pharmacology or toxicology, is a less than effective approach. Food is not medicine in terms of its direct (pharmacological) impact, and fortunately so. A different approach is required that better captures the polyvalent nature of most nutrients, the numerous small, potential beneficial, physiological changes within the defined range of biological plasticity occurring upon exposure to certain food-components such as polyphenols. Ironically, toxicology does give us tools to

better explore and quantify these small, potentially beneficial, physiological changes. The full range of these tools gives us the means to mimic the risk assessment procedure the EFSA only partially framed. But we should be mindful that 'benefit' is not the opposite of 'risk', as falsely suggested by EFSA. Etymologically, risk is a general term that captures both opportunity and danger (Althaus, 2005).

Now, it is a relative small step to construct an outline of a benefits-analysis that is *analogous* to the well-known risk assessment procedure. Defining 'risk' as capturing both opportunity and danger is in line with biphasic dose-responses that seem to underlie the connection between food and health: too little or too much of a 'good thing' is detrimental to health, whereas the optimum intake of that same 'good thing' confers benefits. As a result, the traditional risk assessment procedure can simply be partitioned into two analogous procedures: risk-'as-danger' and risk-'as benefit'.

Indeed, the latter would be quite logical to characterise as *benefit = intrinsic health capacity* × *exposure*. Such a definition does justice to probabilism that so dominates the risk-'as-danger' assessment paradigm and should be central to the benefits assessment. Ironically, despite EFSA's commonplace description of the risk assessment procedure with its well-known probability statements, for benefit, as a conservative means to protect public health, 'the manager frequently requires the evidence to be convincing.' (Scientific Opinion EFSA, 2010)

This is remarkable *and* illogical on multiple levels: (i) it essentially forgoes, in EFSA's terms, the 'mirroring' of the risk assessment paradigm, whereby; (ii) it simply and without evidential basis proffers the notion that, in order to protect public health, potential benefits from certain foods and food-components should not be rated in terms of probability but in terms of scientific absolutes, whereby; (iii) it highly overestimates the potential of science to be straightforwardly 'convincing' in its fact-finding, and inconvertibly; (iv) makes all food-advice, including coming from the EFSA itself, contentious, and finally; (v) *ad absurdum*, makes virtually all research results within nutritional science, or any other scientific field for that matter, moot. Things are aggravated even further by the limitation of scientific methodology designated as some kind of gold standard.

Here, we observe the EFSA restricts itself to those methods in pharmacology and toxicology that are regarded as best suitable to elucidate benefits from dietary patterns. In this sense, EFSA has explicitly chosen to follow a *bookkeeping* method-oriented assessment-strategy rather than a *scientific* problem-oriented one (Platt, 1964). This is dubious as the aim of science is to produce scientific knowledge and to 'gauge its adequacy by systematically pitching it against the world' (Chalmers, 1989). This is accomplishable and frequently accomplished within science but *not* with reference to some universal standard of *doing* science simply because that is impossible. Changes in elementary scientific methods and standards historically *do* occur and can be appraised from the point of view of the extent to which those changes enable the realization of the aim of science.

In nutritional science, designating the RCT with single clinical endpoints as the scientific 'gold standard' is counterproductive as it hinders methodological development essential to further our understanding of the effects of nutrition on human health. Heaney already mentioned this problem. He remarks that a 'general agreement to the effect that nutrition is important, despite the fact that the still growing number of failed trials of individual nutrients might suggest that no nutrient actually made much of a difference, a conclusion that is absurd on its face and ought to have alerted us to the possibility that there was something wrong with how we were investigating the matter. To provide the proof needed to sustain revised intake recommendations, we shall have to find a design better suited to nutrients than the randomized controlled trial as currently implemented, and we need to develop a series of global indices, nutrient by nutrient, which better capture the polyvalent nature of most nutrients. …' (Heaney, 2008). Additionally, benefits of food and food-components cannot be researched within the context of RCT as a control is lacking. Creating such a control by inducing deficiencies seems quite cumbersome and moreover unethical and, of course, potentially dangerous.

Despite the overwhelming evidence of the relation between diet and health *as a general concept*, it is an expression of the inadequacies of the nutritional sciences that the fundamental *details thereof* still elude us for the most part. This is not fault-finding as such, quite the opposite, but a pointer to the immense chemical complexity of food and the intricate 'hidden' connection between this everyday complexity and human health. Therefore, to choose between rival hypotheses about the 'hidden structure' (Godfrey-Smith, 2003) of nutrition and health should be the primary concern for the nutritional sciences and its regulatory cousing rather than fretting over the

'right kind' of method. Any health claim that is decided on with a focus on the latter loses sight on the essentiality of the former, with predictable outcomes we now see materialising in Europe. And then there is the issue that any rational regulatory approach has to decide on which level testing and public intervention is reasonable. However, the question of reasonableness is hardly ever explicitly addressed within the current precautionary 'safety assessment paradigm' (Cramer *et al.*, 1978, Hanekamp and Bast, 2006, 2007, 2008). Yet, in the face of 'the continuing challenge of iodine deficiency within the EU; the widespread anaemia in children and adult woman … the challenge of coping with escalating rates of adult chronic diseases and the huge and growing impact of the poor health of Europe's elderly' (James *et al.*, 1999), unravelling the connection between nutrition and health should have top-priority. And here is where true precaution lies.

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Figure legends

Figure 1. Representation of the risk-benefit assessment paradigm, proposed by EFSA.

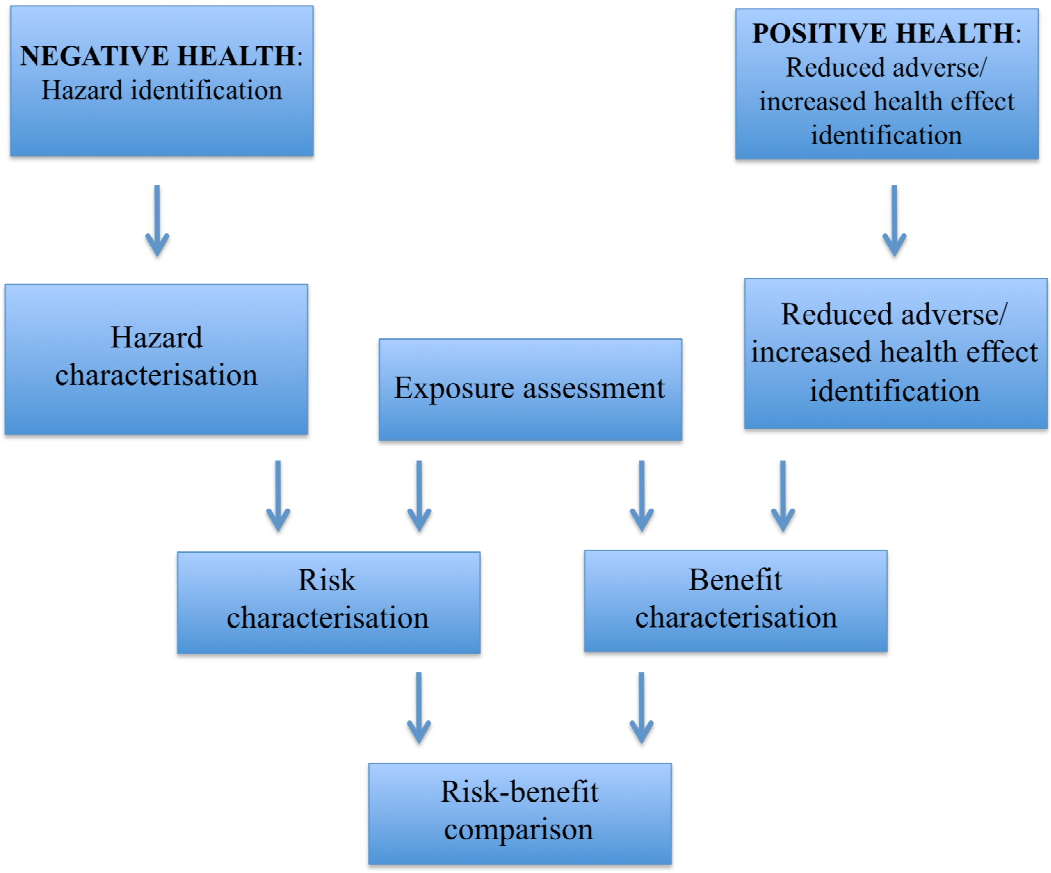

Figure 2. Molecular structure of the flavone backbone.

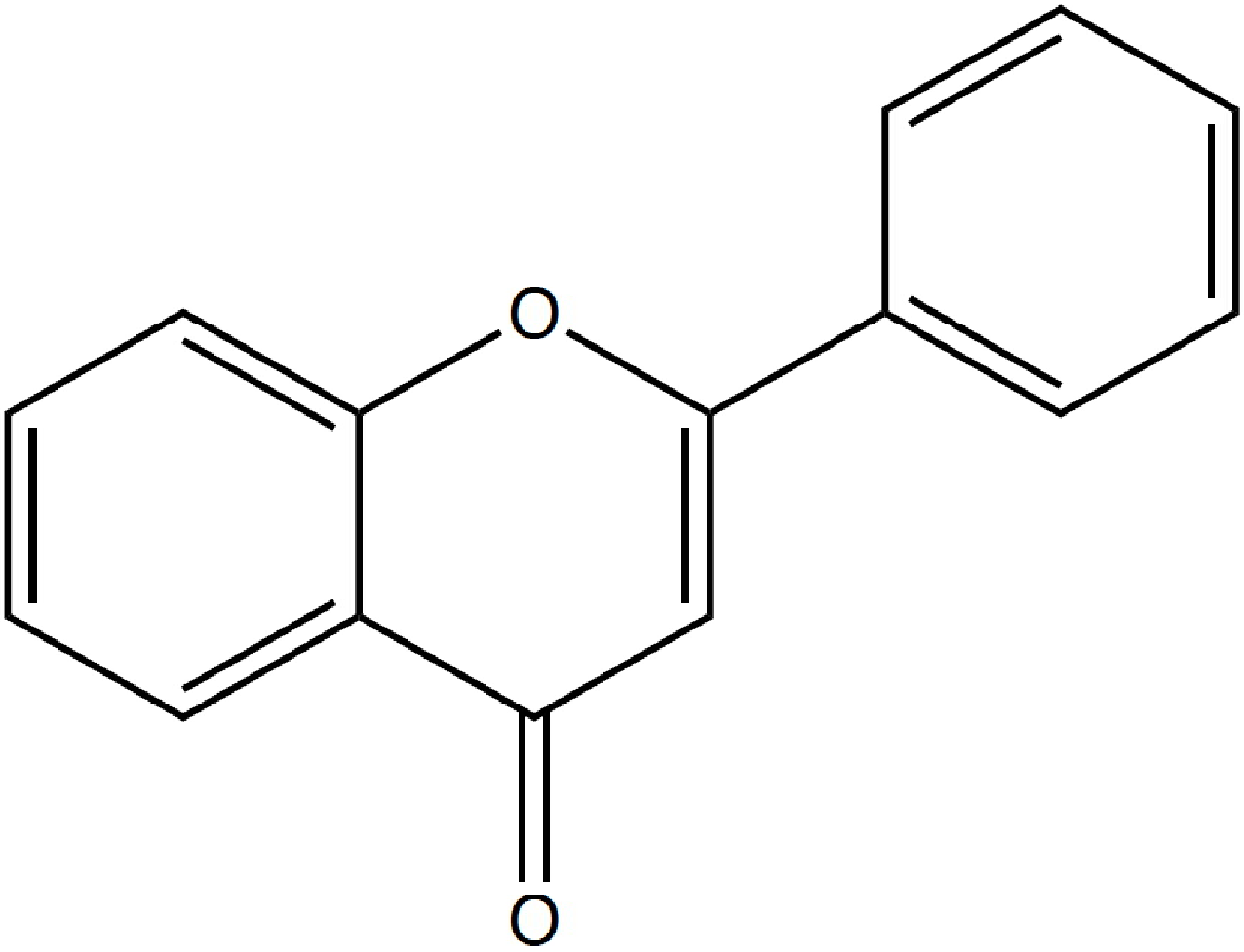

Table 1. Considerations with regard to the quantification of biological performance.

Measure condition-specific risk factors
Select a biomarker with a high (as possible) signal to noise ratio
Define a combined set of parameters that present the studied physiological response
Stress the system (*in vivo* or *ex vivo*) to investigate the enhanced protection

For dietary components the overall effect is frequently too small to observe a direct compelling physiological effect. The term 'biological performance' is introduced to describe changes within the homeostatic range. Note that a biomarker often changes when a pathogenic state is or becomes obvious.

