Parma Summer School 2019

Risk-Benefit in Food Safety and Nutrition

SETTING THE SCENE: INTRODUCTION TO FOOD SAFETY AND RISK ASSESSMENT

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RISK ASSESSMENT

Let's start with words

EFSA Glossary

risk assessment

A specialised field of *applied* science that involves reviewing scientific data and studies in order to evaluate risks (function of probability x severity) associated with certain hazards. It involves *four steps*

hazard identification

hazard characterisation

exposure assessment

risk characterisation.

RISK ASSESSMENT

Let's go on with words from EFSA glossary

Hazard identification (*what is it?*) **the identification of an agent as capable of causing adverse health effects**

Hazard characterization (*how? at what dose levels?*) **the nature of the adverse effects; if possible, an understanding of the doses involved and related responses.**

***Output*: an estimated dose that can be assumed in food or drinking water over a lifetime without presenting an appreciable risk to health**

Exposure assessment (*how much?*) **a thorough evaluation of who or what has been exposed to a hazard and a quantification of the amounts involved.**

Risk Characterization (*1+2+3*) **the likelihood that an agent will cause harm calculated in the light of the nature of the hazard and the extent to which people, animals, plants and/or the environment are exposed**

RISK ASSESSMENT

Mind

(EFSA Scientific Opinion on Risk Assessment Terminology, 2012)

hazards occurring in the food chain unintentionally (chemical and biological contaminants), typically

- not supported by a dossier, assessment relies on available data

- flexible formulation of Question (Terms of Reference, ToRs) depending on specific needs.

- estimate of risk = probability and magnitude of the occurrence of an adverse event.

OR

RISK ASSESSMENT

...OR

-substances, products or processes intentionally added to the food chain (*from farm to fork*: GMO, plant protection products, feed additives, food additives, food contact materials, supplements, novel foods, nutrients)

- in most cases an "applicant" must provide data in accordance with sectorial legislation *(standardized sets of toxicity tests)*, and

- ToRs need to be compatible with the sectoral legislation

- assessment can be for multiple target populations (e-g., feed additives by FEEDAP Panel: *target -intended use- species, consumers, users, environment*)

- outcome concludes on the safety, i.e. adverse effects will not result from exposure to an agent under defined circumstances (use of the pesticide X on apples, not strawberries, at the dose X, not 2X)

Example of Risk Assessment

- Since the Summer School is on Risk-Benefit, let's take

A contaminant
Methylmercury

that has a major role in the risk-benefit of fish

EFSA 2012: risk for public health related to the presence of mercury and methylmercury in food

- *Hazard identification*:

An environmental product of Mercury (Hg) released into the environment by mining, smelting, industrial activities, combustion of fossil fuels, as well as natural (soil geology)

MethylHg forms in aquatic environments
biological (bacteria) and abiotic methylation of inorganic Hg
rate depends on environmental conditions
mostly sediments in fresh and ocean water but also in the water columns

Methylmercury is neurotoxic
and bioaccumulates and biomagnifies along the aquatic food chain; longlived carnivorous fish and marine mammals exhibit the highest contents.

Hazard Characterization

Rapidly and extensively absorbed in the gut,
crosses placenta, blood-brai, blood-cerebrospinal fluid barriers.

While developmental immunnotoxicity (rats), cardiovascular effects in human adults (myocardial damage, heart rate) also deserve attention
Developmental neurotoxicity (impaired neurodevelopmental scores) is the leading effect (=occurring at lowest dose)

Dose-response: *robust data from human cohorts*
A "threshold" of 11.5 mg Hg/kg in maternal hair (biomarker of maternal burden in pregnancy) is derived from the dose-response between neurodevelopmental scores in small children and hair Hg
maternal hair to maternal blood ratio is 250:1 = 46 µg/L as "threshold".
By a simple (data-supported) one-compartment toxicokinetic model, the value of 46 µg/L in maternal blood is converted to
a daily dietary mercury intake of 1.2 µg/kg b.w.

***Benchmark dose (EFSA 2017)* systematic use of all data in the dose-response curve to define a change in response (e.g., 5% increase in incidence), thus reducing uncertainties**

Hazard Characterization: the outcome

Derivation of a Tolerable Weekly Intake

Why *weekly* and not, as usual, *daily*? MetHg is stored in the body

The "threshold" daily dietary mercury intake of 1.2 µg/kg b.w
Is derived from human data, but some uncertainties must be taken into account
a *substance-specific, data-derived* uncertainty factor of 2 to account for variation in the hair to blood ratio.
a *standard factor* of 3.2 to account for interindividual variation in toxicokinetics =
total uncertainty factor of 6.4.

Tolerable weekly intake (TWI) of 1.3 µg/kg b.w.
expressed as mercury in MetHg

This TWI provides a margin of safety of about 40 compared to the BencchMark Dose 5% (BMDL05) *for the reduction in antibody response in rats.*

Exposure Assessment

Fish is the food of concern for MethylHg,
to a lesser extent also molluscs and crustaceans
Large predatory fishes are more contaminated (tuna, swordfish, pike, cod)
because they bioaccumulate (*large eats small*)
No great differences between farmed and wild fish, when farmed fish is fed with meals from small marine organisms (*also in the farm, large eats small*)

Exposure of consumers varies with geographical areas (different geology or industrial emissions) and dietary habits (Italians eat much more tuna and swordfish than Slovakians)

based on the data submitted for *total HG*,
assuming conservatively that in fish *almost 100%* is made by MetHg
and in crutaceans/molluscs *80%*
considering that samples below the *variable* LOD/LOQs (left-censored)
can contain 50%Hg of those values (approach Median-Bound, MB)
And using the EFSA Food Consumption Data Base
for different age groups ((*my diet and intake of food per kg bw at 3 years were different than today)*

Exposure Assessment: outcome

Mean exposure: from 0.06 µg/kg bw/week in *elderly (over 65)*
to 1.57 µg/kg bw/week in *toddlers (1-3 years)*

95th percentile (*in the general population, considering also non-consumers*) : from 0.14 µg/kg bw /week in *elderly*
to 5.05 µg/kg bw/week in *adolescents (10-17 years)*

high and frequent consumers of fish meat (95th of consumers only)
from 0.54 µg/kg bw/week in *elderly*
to 7.48 µg/kg bw/week in *children (up to 10 years.*

***Mind:* Data sets should give mean values and a distribution of values**

"High consumers" are very important (the highly exposed ones)
In general, considered at the 95th percentile of distribution
(EU, 5% of population, *some 9 millions people, it's a number..*)

Risk Characterization

Mean dietary exposure across age groups does not exceed the TWI with the exception of toddlers and children in some surveys.

High consumers are mostly close to or above the TWI, especially for younger age groups, in particular
"high/frequent consumers" fmay exceed the TWI by *up to six-fold.*
Unborn children are the most vulnerable group
and pregnant women can be present among high consumers.

Biomonitoring data on blood and hair:
general EU population: methylHg exposure generally < TWI.
higher concentrations in some population groups
confirming the results of risk assessment modelling

Exposure to methylmercury above the TWI is of concern
measures to reduce methylmercury exposure should consider the potential beneficial effects of fish consumption (e.g. replacing ingredients in feeds for farmed fish)

Considerations

The biology of living organisms that produce our foods

- MethylHg levels are higher in long-lived, large predatory fishes

And more from EFSA opinions

- Dioxins much higher in livers from sheep than cattle (EFSA, 2011)

These highly toxic, endocrine-disrupting and bioaccumulating combustion by-products fall down on pastures from airborme particulates and adhere to the organic fraction of soil

Sheep grazing behaviour leads to a much higher soil (hence dioxin) ingestion than cattle

- Arsenic (EFSA 2009) accumulates in

Fish and seafood where is metabolized to organic compounds (arsenobetaine, arsenosugars) with weak or very weak toxicity

Cereals and especially rice, as the highly toxic and carcinogenic inorganic As, which repreents the real concern for consumers

Considerations: the human factors

Inequality in food safety:

people of low-income groups or countries are more exposed to the hazards (money + education):

New Zealand: the working class and Maori minority (low social status + traditions) eat a lot of fish and chips made with large predatory (bioacccumulating) and cheaper fishes (e.g., sharks) = higher intake of methylmercury (Karatela et al., 2011)

Dietary habits may mitigate the adverse impact of MetHg intake

Communities consuming *large and fatty fish* are partly protected by MethylHg effects because of the maternal intake of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFAs) :

protective effect on neuro-development, especially prenatally

See the different results of the Seychelles and Faer Oer cohorts: large fish consumption, but different fish species (Seychelles: high PUFAs)

(For a thorough discussion see *EFSA 2015: benefits of fish/seafood consumption compared to the risks of methylmercury in fish/seafood*)

Can we do a similar risk assessment for nutrients?

YES

When nutrients have recognized toxicity (adverse effects at excess intakes *Dose makes the poison*)
(example: Vitamin A, teratogenic and enhancing the risk of osteoporosis, EFSA 2009)

When there are conditions of use/scenarios that need to be assessed (ToRs), in order to prevent an excess intake

Two cases of Nutritional Feed Additives (the "nutritional supplements for food-producing farm animals) assessed by the FEEDAP Panel of EFSA

General

Nutrients have a biphasic dose-response curve for adverse effects:

- **Deficiency (usually the most important concern) which mitigates as the intake increases up to reaching**
- **Sufficient intake (no adverse effects expected)**
- **Excess (biochemical indicators then frank adverse effects as the intake increases)**

The hazard characterization is often strongly dependent on age and gender (see the opinions of the EFSA NDA Panel on *Dietary Reference Values (DRVs) for Nutrients*
a summary of the DRV opinions published by EFSA in 2017

For most trace nutrients a Tolerable Upper Intake Level (UL) is set usually based on human studies
(*e.g., UL for Vitamin D, EFSA 2012; updated for infants, 2018*)

Nutrients

nutritional additives are widely used in the EU to supplement animal feeds

maximum legal limits in feeds are established for the various species; they comprise supplemental levels plus the naturally present background (total levels)

in order to prevent adverse effects for animals, consumers, or even the users or environment (Cobalt, Copper, Zinc)

Key ToR for consumer safety

the *proposed condition of use* or *the existing legal limits*

do induce a deposition in edible tissues/products (meat, liver, fat, milk, eggs, fish flesh)

so that the intake through the products from supplemented animals *plus* the already existing background intake through the diet could be *greater than the UL?*

Sometimes YES

Iodine
(EFSA FEEDAP 2005, updated in 2013)

A key endocrine active substance, ssential for thyroid function
Excess causes endocrine disruption: hyperthyroidism and increased risk of thyroid autoimmunity in humans
UL 600 µg/day (adults), 200 µg/day (1-3 yrs toddlers)

Feed supplementation especially important for dairy production and fertility
Concentrates in thyroid, specifically excreted in milk and eggs
TOR: do the maximum EU permitted levels in feeds ensure that the intake of iodine by consumers would not exceed the UL?

Substantial risk to high consumers, primarily from milk and to a minor extent from eggs, due to high and specific carry-over and consumption rates (EFSA Food Consumption Data Base)
UL for adults exceeded by a factor of 2, for toddlers by a factor of 4
EFSA recommends to reduce the maximum permitted supplementation levels in feeds for dairy ruminants and laying hens,
Maximum permitted levels are substantially higher than animal requirements, thus, a reduction would not impact on animal health

Selenomethionine from selenized yeast (EFSA FEEDAP 2011)

Se is needed to support antioxidant activity in most body systems (and for synthesis of thyroid hormones)
glutathione peroxidase activity: biomarker of bioavailable Se
- Excess: "ectodermal" toxicity (hair/nail/teeth/skin/peripheral nerves lesions), increased prothrombin time for liver effect
UL 300 µg/day (adults), 60 µg/day (1-3 yrs toddlers)

Feed supplementation with the highly bioavailable organic Se (selenomethionine) from selenzed yeast to improve Se absorption
TOR: does the use of this specific product up to the maximum EU permitted levels of total Se in feeds ensure that the UL is not exceeded?

Selenomethionine deposits in tissues (including muscle) as methionine, acting as a Se "store"
Almost double tissue deposition than inorganic Se
UL for toddlers is passed due to meat/milk/eggs and background intake of Se in vegetables (toddlers eat more food per kg weight)
EFSA recommends a specific supplementation level for organic Se (0.2 mg/kg feed) within the maxium total Se allowed in feeds (0.5 mg/kg)

A simple personal comment on these two case studies

Assessing the risk that consumer exposure will exceed the UL
while assessing the fulfilment of animal nutrition needs
(hence *both* animal health and production of foods of animal origin = *food security*)
may fit into the conceptual framework of risk-benefit assessment,
with two distinct target populations (consumers and farm animals)

And now

Four shots on

Four evolving (work still in progress)

Risk assessment topics

Shot 1

Hazard Identification

Identification of residues/metabolites of toxicological relevance

Panel on Plant Protection Products and their Residues
Guidance on the establishment of the residue definition for dietary risk assessment (EFSA 2016)

Pesticide residues often do not coincide with the parent substance

Identification of all residues resulting from abiotic (temperature, humidity) or biotic (microbial, plant metabolism) transformation

- comparable hazard (possible different potency) with the parent substance - qualitatively different profile

transformation processes might produce a high-concern metabolite (eg, genotoxic) from a low concern substance

Stepwise process of residue characterization

- Tier 1: genotoxicity potential (ability to damage DNA, no threshold identified): thorough screen *in silico,* 1) by Quantitative Structure Activity Relationship, 2) by read-across with structurally similar substances

- other high-concern hazards (developmental toxicity, endocrine disruption, etc.) QSAR still uncertain, but screen by read-across possible

IS IT SO IMPORTANT? DEFINITELY SO for PESTICIDES,

(examples from 2016 Guidance)

- azole fungicide *Epoxyconazole*: developmental toxicity and endocrine disruption (steroid synthesis inhibitor) 68 metabolites identified screened by QSAR/read across: some can be *more active* than parent and *should be tested* in vitro/in vivo

- non-genotoxic fungicide *Spiroxamine*: based on QSAR, genotoxic concerns are not excluded for 7 out of 45 metabolites belonging to *three groups* of chemical structures

(EFSA 2019) for two out of five groundwater metabolites of the herbicide *terbuthylazine*, specific toxicity should be adressed not identified as a metabolite in rast treated with terbuthylazine (= not tested in toxicity studies), nor sufficiently similar structure to tested substances (=no read-across feasible)

***Not just pesticides*: for instance not intentionally added substances (impurities, reaction/degradation products) from food contact materials**

Shot 2

Hazard Characterization

Use of Adverse Outcome Pathhways (AOP)

Panel on Plant Protection Products and their Residues Investigation into experimental toxicological properties of plant protection products having a potential link to Parkinson's disease and childhood leukaemia (EFSA 2017)

***A new tool* supported by important *international effort at OECD*, see the AOP repertory https://aopwiki.org/**

standardized, formal, transparent way to describe and report the chain of events leading from the first interaction of *any* chemical with a molecular target (molecular initiating event = MIE)

to an adverse outcome (AO: a disease, a eco/toxicological effect)

MIE and AO are sequentially linked by biologically plausible and essential key events (KEs) at subcellular, cellular, tissue level

(EFSA 2017) Parkinson disease: *two MIEs* (binding to mitochondrial complex I *and* initiation of redox cycling process)
***converge in a sequence* of KEs (mitochondrial dysfunction, impaired proteostasis, degeneration of dopaminergic neurons of the nigrostriatal pathway)**
***leading to* parkinsonian motor deficit**

Infant leukemia: (*one big hit in utero*) MIE 'in utero topoisomerase II poisoning'
leading to AO through *a single* KE 'in utero MLL chromosomal rearrangement'.

Interesting science..
but *why* risk assessors should bother about AOPs?

any **chemical triggering the upstream molecular/cellular events with sufficient intensity has the potential to perturb adversely the downstream physiological pathway =**
assess the plausibility that a chemical with a mechanism "X" is invlved in the AO "Y", e.g., (AOP 18 from AOPWiki)
PPARα activation in utero leading to impaired fertility in males

In practice
- assess the biological plausibility of epidemiological associations (pesticides associated with Parkison disease and pediatric leukemias, EFSA 2017)
- new regulatory development for pesticides and biocides (EFSA/ECHA, 2018): endocrine disruptors has to be identified based on adverse effects in vivo that are plausibly linked to endocrine mode of actions
- More in general, identify measurable *(= "thresholds" to elicit the AO sequence)* markers *in vitro/in vivo* to detect relevant mode of actions = great perspective for the development of *in vitro* testing)

Shot 3

Exposure Assessment

Characterizing uncertainties in the exposure assessment of contaminants

Guidance on Uncertainty Analysis in Scientific Assessments (EFSA, 2018)

Guidance on Communication of Uncertainty in Scientific Assessments (EFSA, 2019)

And you might wish to look also at

Mantovani A. (2018) Characterization and Management of Uncertainties in Toxicological Risk Assessment: Examples from the Opinions of the European Food Safety Authority. Methods in Molecular Biology, 1800: 219-29.

Uncertainties = gaps in knowledge and/or data sets and/or methodologies that
can exert an unwanted influence on the outcome of a risk assessment.

In principle, a certain presence of uncertainties is unavoidable, thus, transparent identification, description and weighing
Weighing = influence of a specific uncertainty
in what direction (making less or more conservative an assessment, or unknown)
and with what strength (weak, medium strong, unknown influence)

Appraisal of impact might be difficult for specific uncertainties (= direction +/-), but
- the combined effect of identified uncertainties should be evaluated

screening the assessment for uncertainties needing a detailed appraisal

Exposure: a main uncertainty for assessing contaminants on which data are not routinary collected (contrary to dioxins, aflatoxins, etc.)

The persistent perfluoalkylated PFOS and PFOA (2018)

Issues (actually shared with other contaminants)

- uneven geographical distribution of data collection (data mostly from a *few EU Countries*)

- non-standarized (= inadequately comparable) methods for sampling and/or analysis of food commodities

- analytical methods of insufficient sensitivity (often too high LOD/LOQ, and too high % of left-censored data)*

- human biomonitoring data: insufficient knowlege on the factors influencing variability within and among populations

*** studies using good sensitivity methods confirm occurrence in foods at levels close to lower bound (LB) estimates (deriving mean/median considering values < LOOD/LOQ = 0), and**
median LB data are consistent with median population blood serum levels. Thus, LB estimates lead to only a weak underestimation of risk

Shot 4

Risk Characterization

The issue of mixtures

Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA, 2019)

Risk assessment faces the challenge of real life

The preliminary step of problem formulation is important: the demarcation of the problem is more complex for mixtures

Description of the mixture sets the srage for Hazard Identification

Whole mixture approach: the whole mixture is evaluated in the same way as for a single substance (should *not vary in composition* over time! e.g., wastewater effluents)

Component-based approaches: must include the grouping of chemicals within mixture into assessment groups

Dose addition is default assumption: components are treated as if having a similar action, while potency may vary and each component contributes to the combined effect through its concentration-potency ratio

Interactions (enzyme induction, inhibition of repair, including *synergism* etc.) *might* be considered at low (i.e., below "threshold") exposures if data-supported (melamine and cyanuric acid from food contact materials form a covalent complex with much enhanced nephrotoxicity, EFSA 2010) = case-by-case extra uncertainty factor

Whole mixture approach

Mineral oil hydrocarbons in foods (EFSA 2013) from food packaging and lubricants.

***Description*: saturated (MOSH, alkanes and cycloalkanes) and aromatic (MOAH, polyaromatic hydrocarbons); mixture complexity makes it impossible to resolve MOH mixtures into individual components.**

Estimated *total* MOSH exposure: 0.03-0.3 mg/kg b.w. per day, with higher exposure in childrer. Much lower for MOAH

The No-onserved-adverse-effect-level for critical effect (liver granulomas in rats) of the *most potent* MOSH investigated used as a conservative (many uncertainties) Reference Point for MOSH exposure.

Component-based approach

Multiple pesticide residues are yearly found in over 20% of samples of fruits and vegetables in the EU.

Cumulative assessment grouping (EFSA 2013) based on I) dose addition and ii) phenomenological (*in vivo*) effects in regulatory studies, *always available* even when (often) the underlying mechanisms are not understood.

Scientifically justified, e.g.: inhibition of thyroid peroxidase, increased clearance of thyroid hormones, etc. all converge into hypothyroidism

RECENT UPDATES

The assessment of multiple pesticide residues is evolving and might provide a model for component-based approaches

cumulative assessment groups of pesticides for their effects on

the nervous system (public consultation closed on 2018)

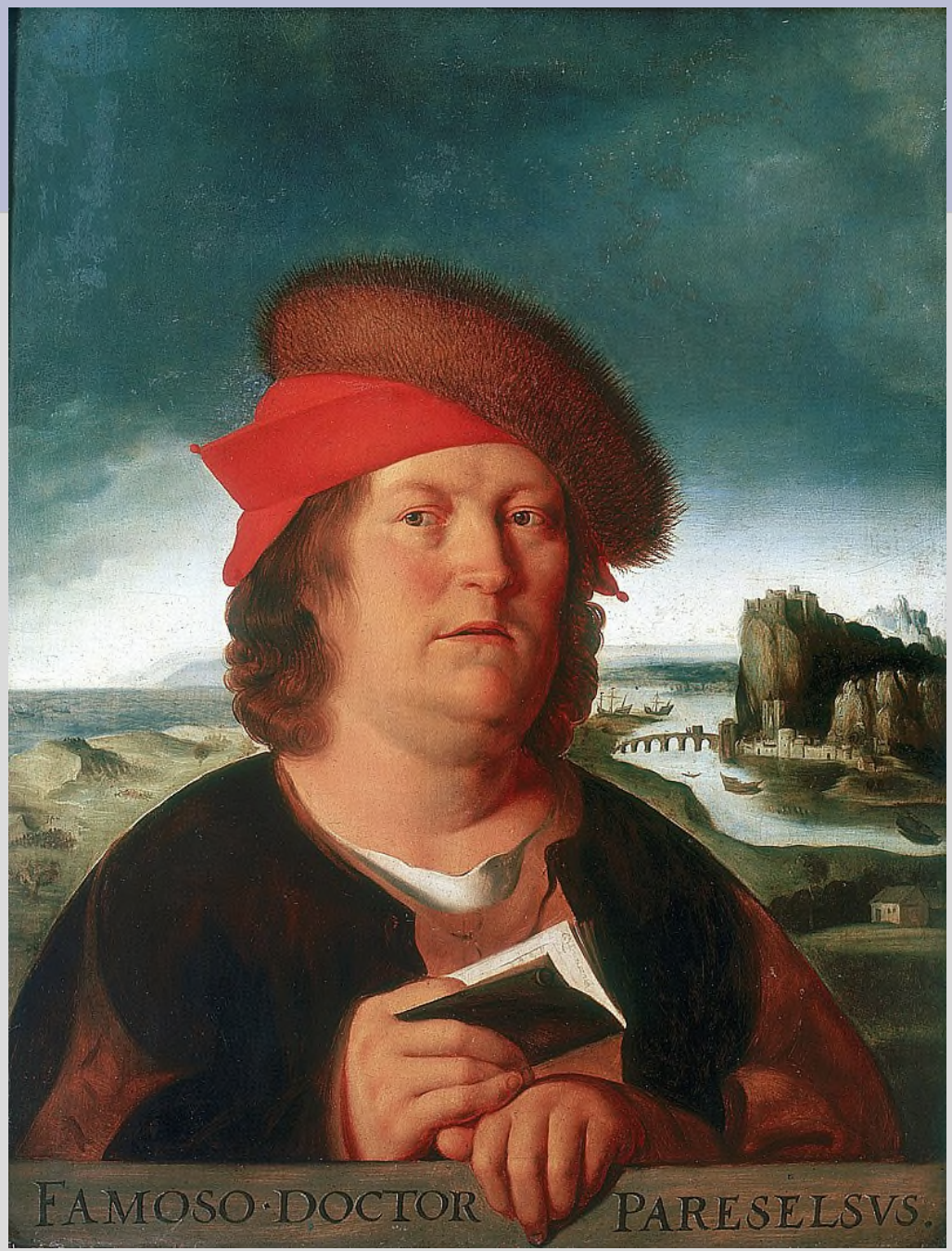
"For an efficient use of resources,...it is recommended to focus the assessment to the specific effects on the *motor division* and on brain and/or erythrocyte *acetylcholinesterase* inhibition because the highest risks are expected to be observed for these effects."

on the thyroid (public consultation closed on 2019)

"For an efficient use of resources... the assessment of the combined risks...could be focussed on hypothyroidism because the highest risks are expected to be observed for this effect."

A RIVM-EFSA project to develop the exposure part to be completed on June 2019 upon refinement with real-life aspects:

a more precise use of consumption data and of information on the effects that processing of food has on residue levels.


FAMOSO·DOCTOR PARESELSVS.

What sense would it make or what would it benefit a physician if he discovered the origin of the diseases but could not cure or alleviate them?