University of Crete Medical School
Department of Toxicology & Forensic Science

Major anthropogenic pressures from chemicals related to human and environmental health

International Conference on Biotechnology Environmental and Human Health and Sustainability
Krasnoyarsk , 1-4 October 2018

Aristides M. Tsatsakis

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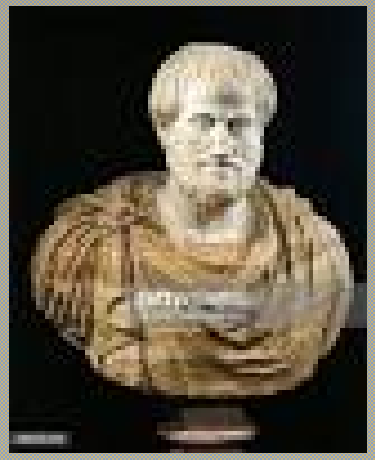
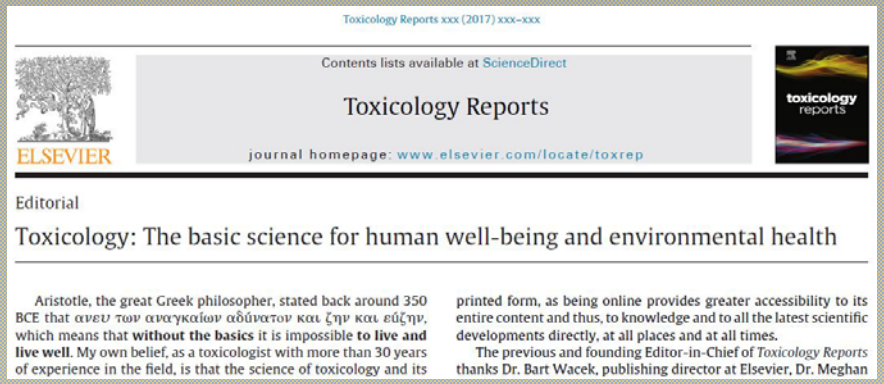
D.HonorisC. (Carol Davila), DHC (Mendeleev), DHC (FEFU),
Hon Professor (Erisman), Academician FM RAS



Aristotle



Toxicology Reports xxx (2017) xxx–xxx

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journal homepage: www.elsevier.com/locate/toxrep

Editorial

Toxicology: The basic science for human well-being and environmental health

Aristotle, the great Greek philosopher, stated back around 350 BCE that ανευ των αναγκαίων αδύνατον και ζην και εύζην, which means that **without the basics** it is impossible **to live and live well.** My own belief, as a toxicologist with more than 30 years of experience in the field, is that the science of toxicology and its printed form, as being online provides greater accessibility to its entire content and thus, to knowledge and to all the latest scientific developments directly, at all places and at all times.

The previous and founding Editor-in-Chief of *Toxicology Reports* thanks Dr. Bart Wacek, publishing director at Elsevier, Dr. Meghan

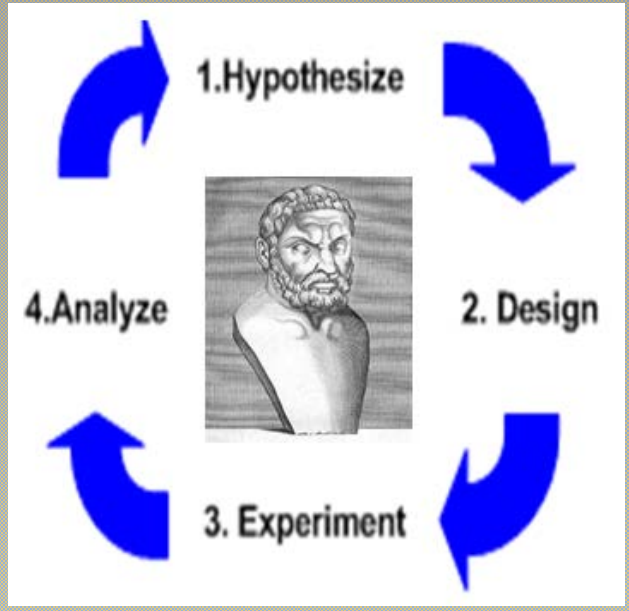
Aristotle, the great Greek philosopher ' ..... ανευ των αναγκαίων αδύνατον και ζην και εύζην,

which means that .....

without the basics it is impossible **to live and live well.**

And I too, believe that the science of toxicology and its effect to the people and the environment .. is truly such a basic, both for our existence, as well for our well being

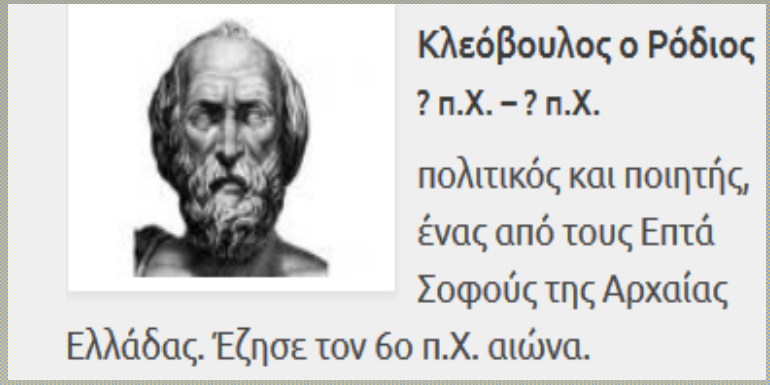
Trust the evidence…..but don't generalise into conclusions ! …..Think of personlised causality and intergarted approaches … AMT



Thales

Modern toxicology influenced by ancient philosophy

Kleovoulos Rodios



«Αρχή σοφίας, η γνώση της άγνοιας.»

Μέτρον άριστον

Η τροφή σου να είναι το φάρμακό σου και το φάρμακό σου να είναι η τροφή σου

- Ιπποκράτης

Ippocrates

Related Web sites

ISSN: 2214-7500

Toxicology Reports

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1st Russian - Hellenic Symposium with International Participation and Young Scientist' s School
BIONANOTOX 2011
BIONANOTOX 2012
Thank you for your participation.
BIONANOTOX 2013
4th International Conference
"Biomaterials and Nanobiomaterials: Recent Advances Safety-Toxicology and Ecology Issues"
Including Russian-Hellenic Workshop and School of Young Scientists
5 - 12 MAY 2013 - Heraklion Crete Greece
добро пожаловать
Καλώς ήρθατε
BIONANOTOX 2014
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BIONANOTOX 2015
6th International Conference
"Biomaterials and Nanobiomaterials: Recent Advances Safety-Toxicology and Ecology Issues"
Final Program Abstract Book
HSTOX.COM
EUROTOX
ToxPlus
ИБХФ

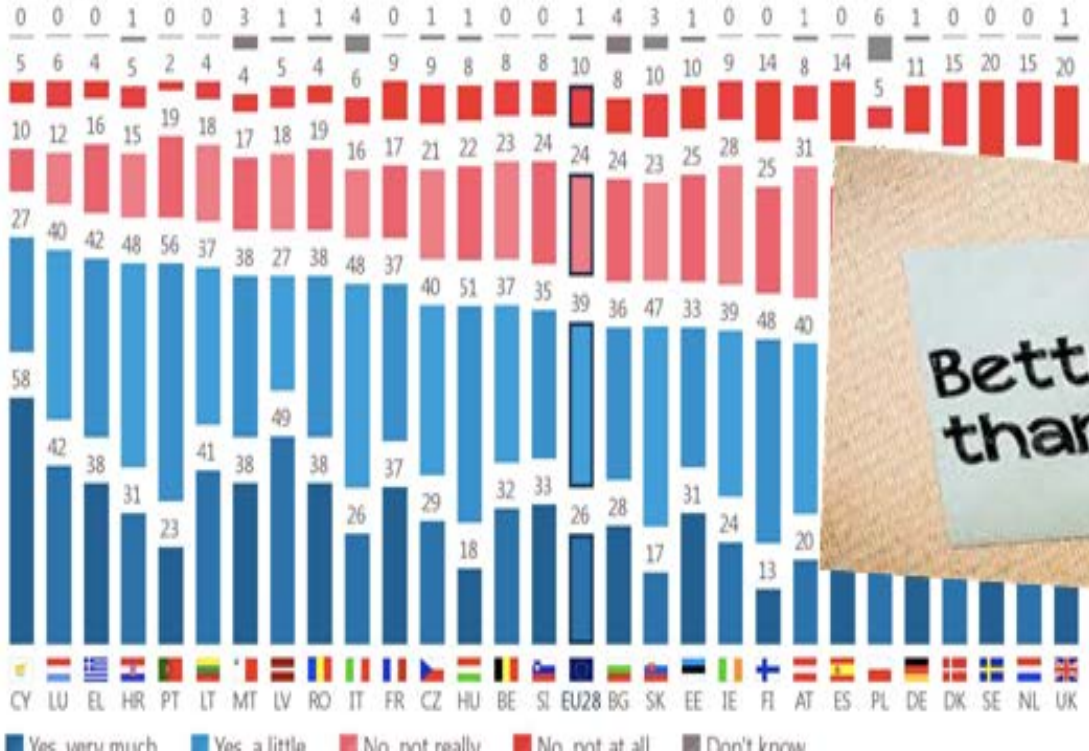
HOW EFFECTIVELY TOXICOLOGY SAVES CONSUMERS? WHY IS THIS QUESTION IMPORTANT NOWADAYS?

Concerns of increasing anthropogenic pressures to human (and environment)	Chemical mixtures (life style-new products, dietary, industry, PPPs, natural toxins), high exposure, all routes
	Electromagnetic pressure from human activities
	General pollution of seas and earth affecting consumers
	Climate changes due to anthropogenic pressures affecting consumers
New knowledge = new concerns	Endocrine disruption, low dose effects, obesity, epigenetics, etc.
Risk communication	Mainly through the media
	Consumers confusion, lack of trust

65% of EU citizens are at least a little concerned about being exposed to hazardous chemicals

26% who are 'very much' concerned.

QD1 In general, would you say that you are concerned about being exposed to hazardous chemicals in your daily life? (%)



Our job:

Be safe!

Feel safe!

Why to study Long Term Low Dose Exposure to Chemicals

Changes in Causes of Morbidity and the concern of increase of chemical exposure

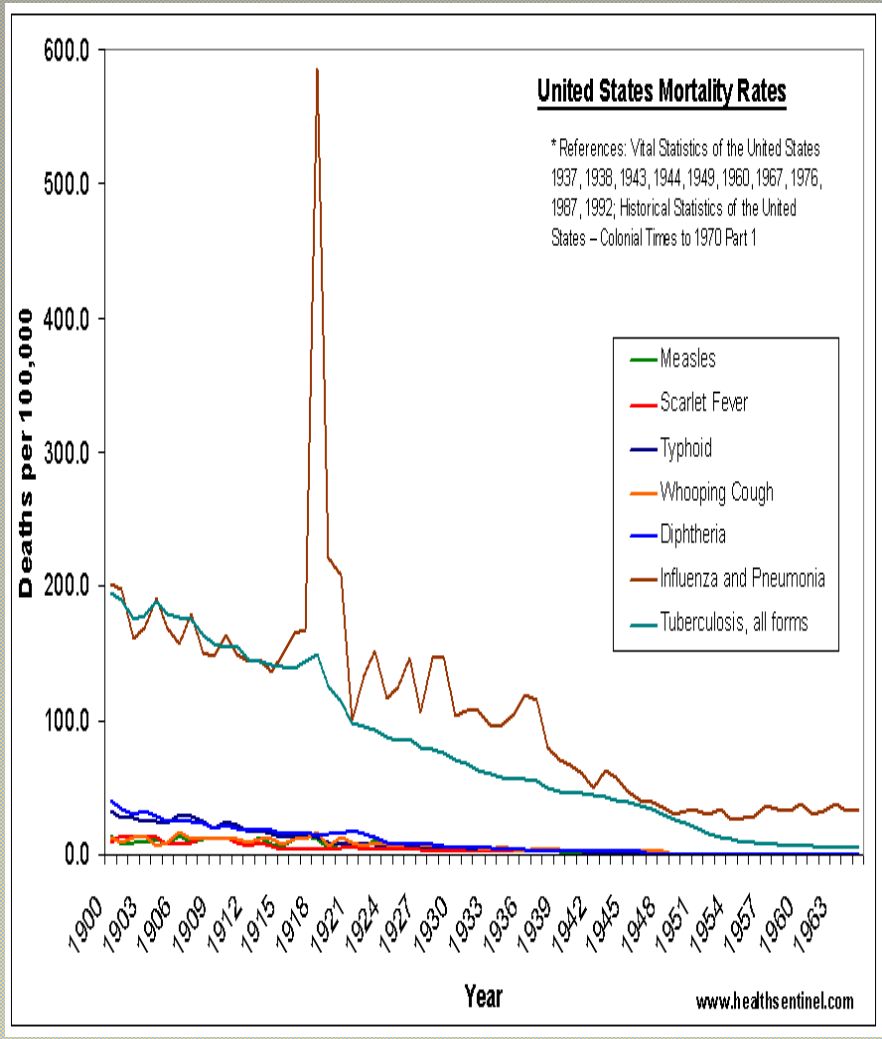
During the last 100 years changes in our planet and in life conditions and climate

have resulted in major changes in causes of morbidity and the burden of diseases

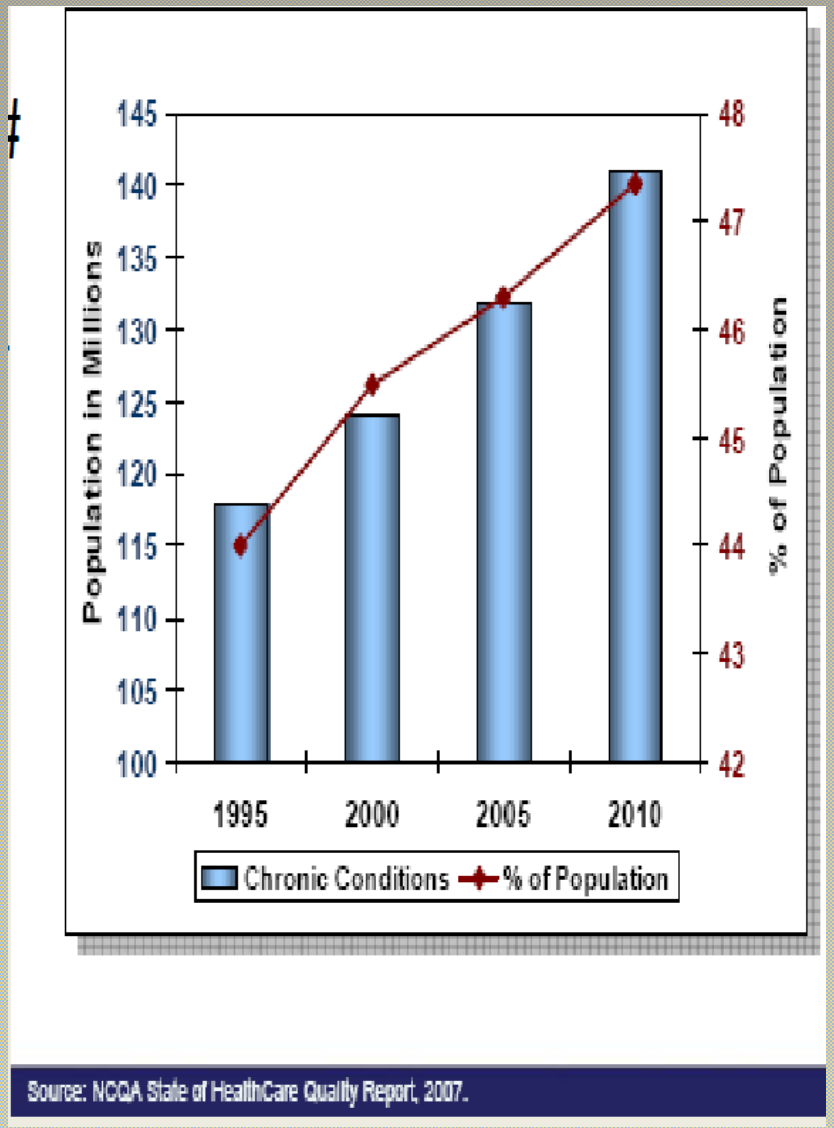
The Burden of Disease and the Changing Task of Medicine

N Engl J Med 2012; 366:2333-2338 June 21, 2012

Decrease in mortality from infectious diseases during century

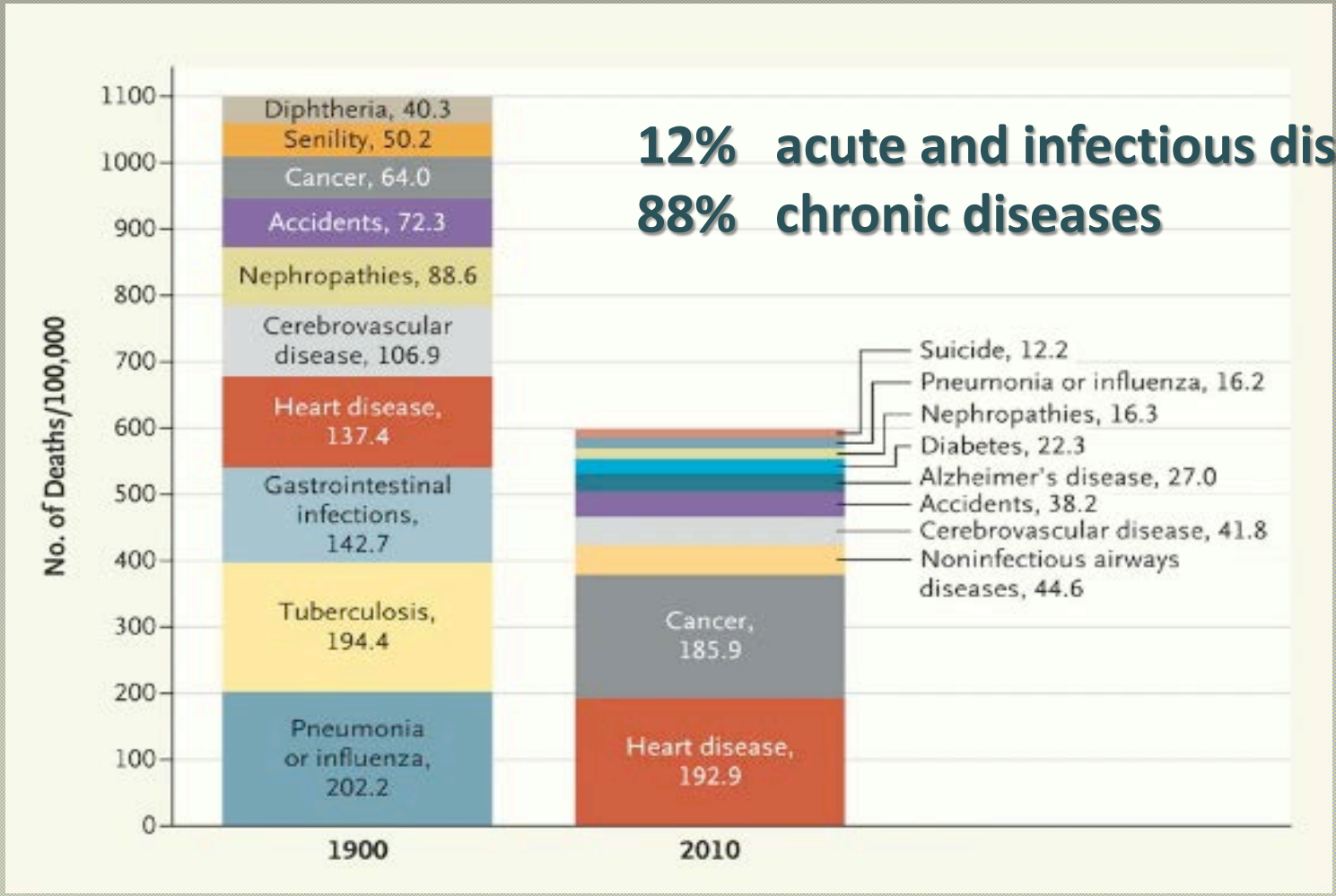


Increase of morbidity and mortality from chronic diseases during last 15 years ,1995-2010.



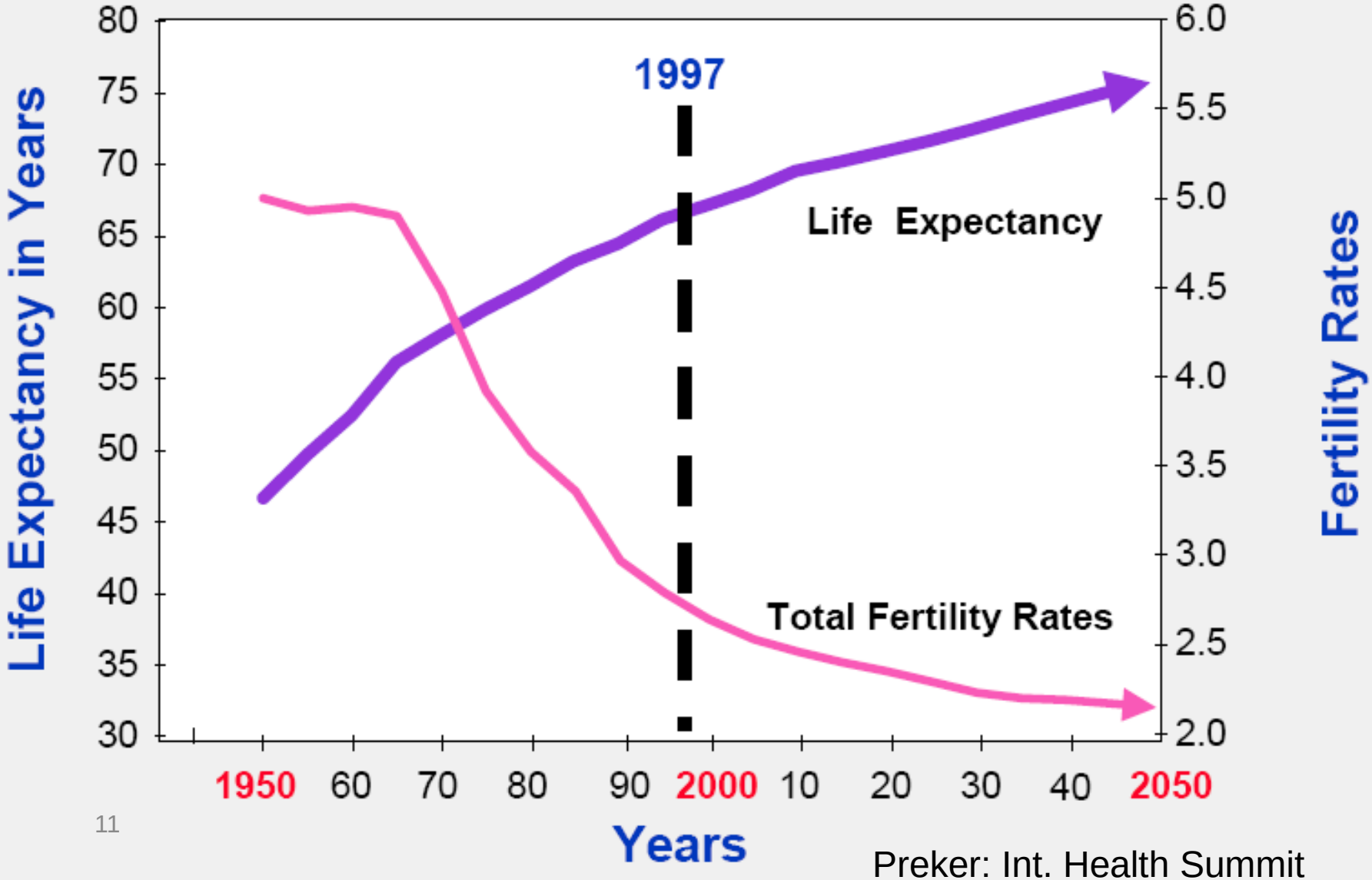
Main causes of morbidity in 1900 and 2010

60% acute and infectious diseases
40% chronic diseases

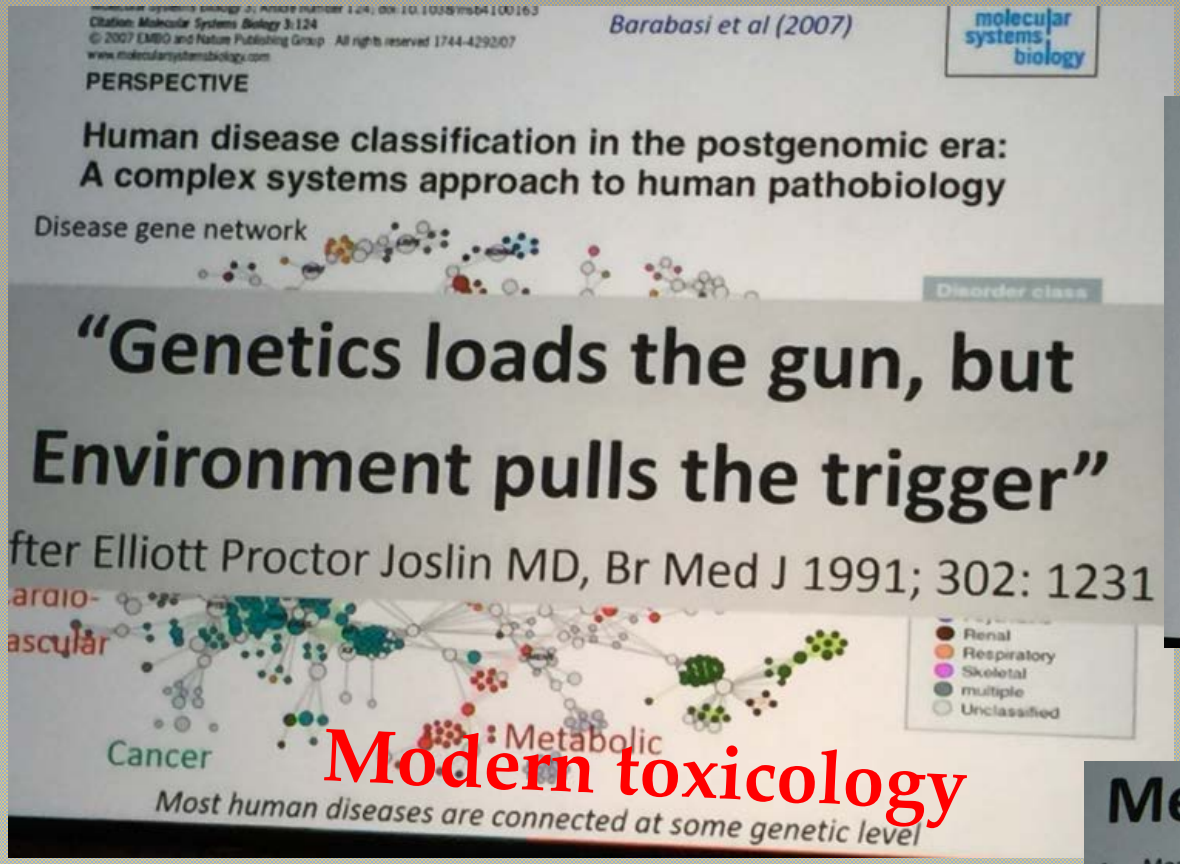


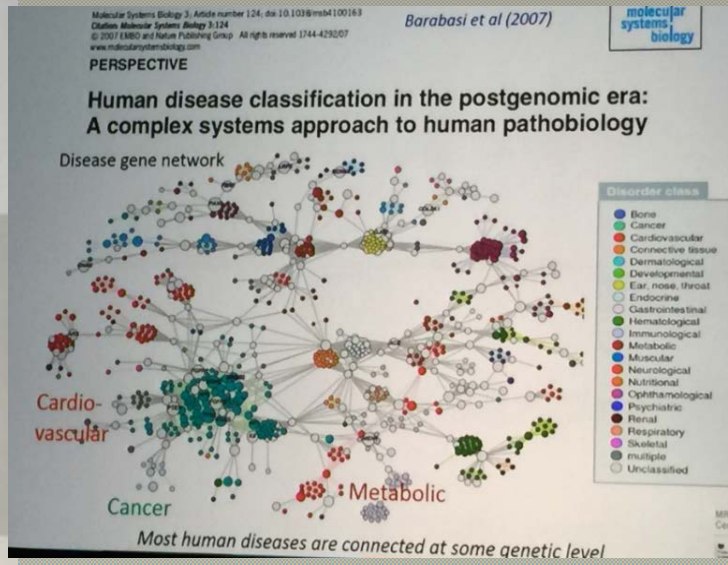
The Burden of Disease and the Changing Task of Medicine: N Engl J Med 2012

A Century of Unparalleled Improvement

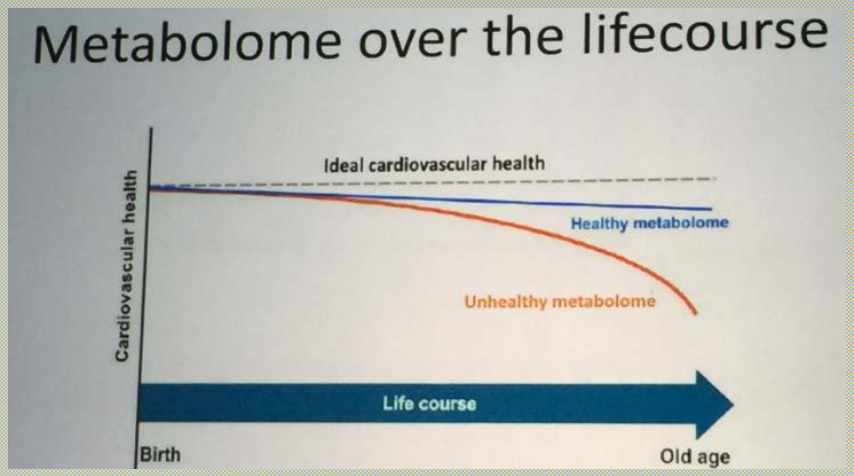


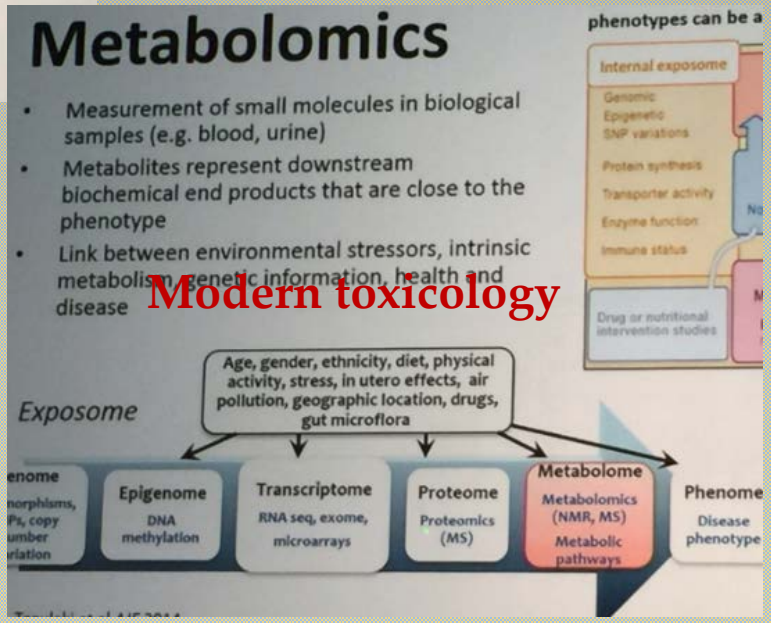
Preker: Int. Health Summit





Modern toxicology





Disease Risk Factors

20% 80%

Genes Environment

- Stephen M. Rappaport and Martyn T. Smith (2010) Environment and Disease Risks Science Vol 330
- Walter C. Willett (2002) Balancing Life-Style and Genomics Research for Disease Prevention Science 296, 695

Disease Risk Factors

Smoking

Alcohol

Exercise

Environment

Nutrition

Toxic Burden

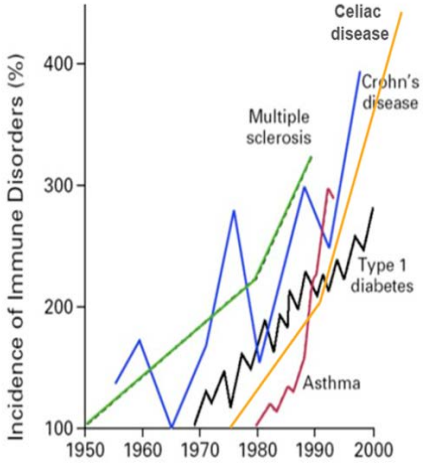
Stress

~port and Martyn T. Smith (2010) Environment and Disease Risks Science Vol 330

Precision Medicine Initiative 2015

the WHITE HOUSE
PRESIDENT BARACK OBAMA | BRIEFING ROOM

Autoimmune Diseases



*"Until now, most medical treatments have been designed for the "**average patient.**'*

*As a result of this "**one-size-fits-all**" approach, treatments can be very successful for some patients but not for others.*

*Precision Medicine, on the other hand, is an innovative approach that takes into account **individual differences in people's genes, environments, and lifestyles.**"*

https://obamawhitehouse.archives.gov/node/333101

Metabolic pressure and autoimmune diseases

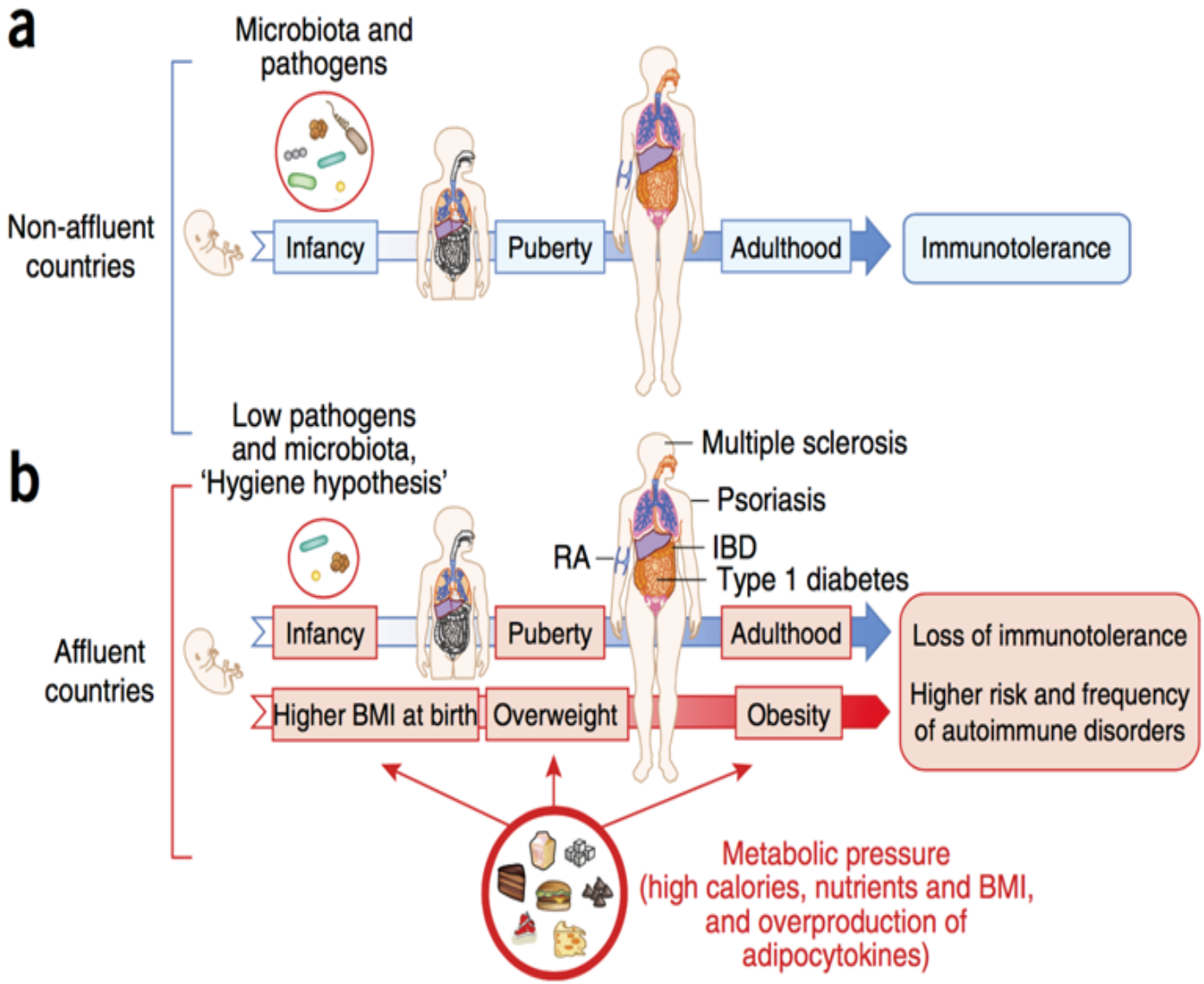
Overnutrition, the hygiene hypothesis and autoimmune disease

Changes in lifestyle have been considered a major contributor to the rise in the frequency of autoimmune disorders and allergies (by 300%) in industrialized and developing countries.

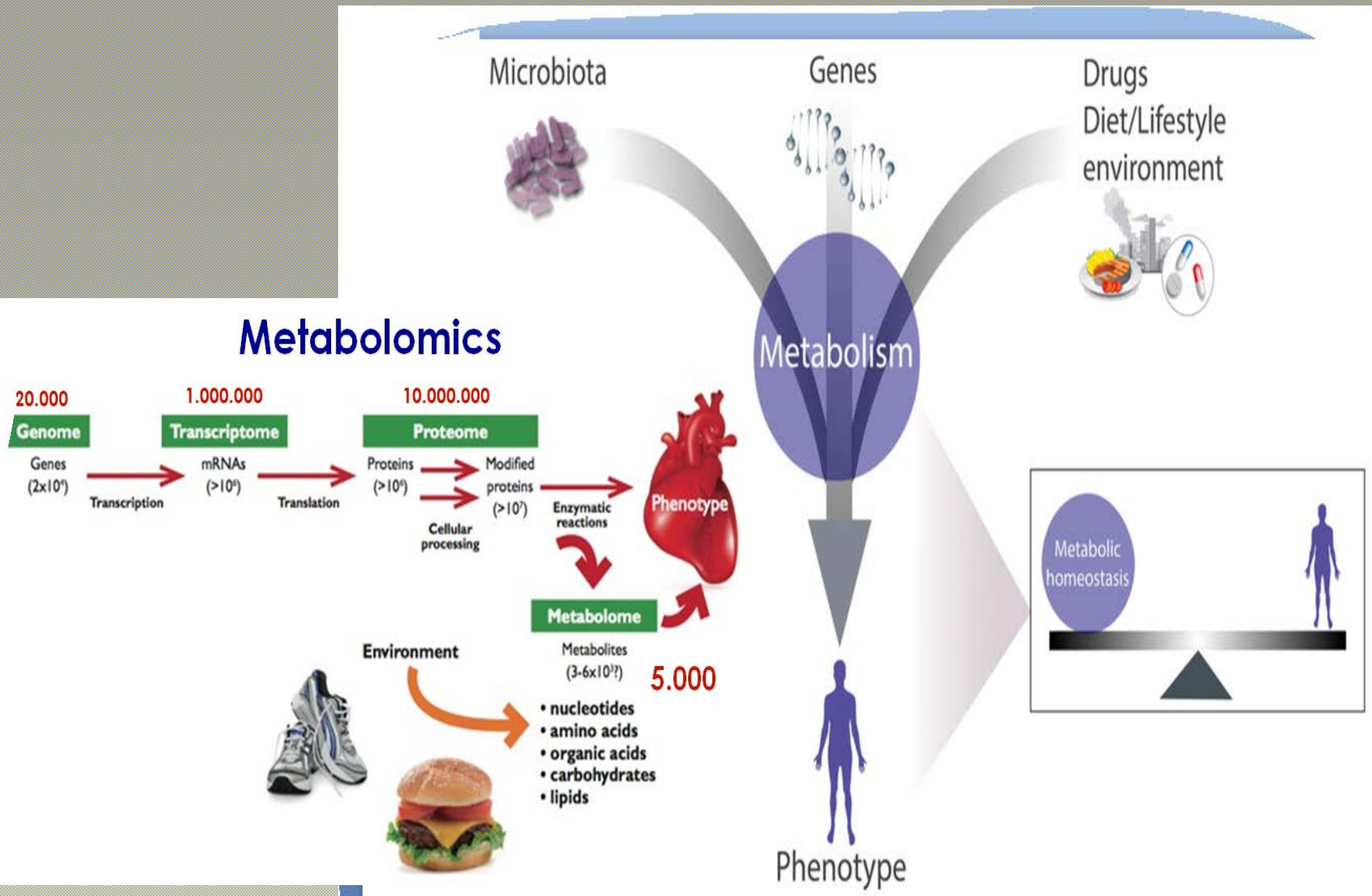
Metabolic pressure and autoimmune diseases

- Increased daily caloric intake from processed foods
- Industrialized foods "holy triad" high sugar –high fat– high salt, hyperactivate immune response (nutrient-energy-sensing pathways)
- Lack of immuno-modulating nutrients (Omega 3, vitamin C, Probiotics, vitamin D3)

1. Metabolic pressure and the breach of immunological self-tolerance Veronica De Rosa et al. Nature Immunology (2017).
2. Review The Roles of Vitamin C in Skin Health Juliet M.12 August 2017. Nutrients



Metabolic pressure and the breach of immunological self-tolerance Veronica De Rosa et al. Nature Immunology (2017).



Sharpening Precision Medicine by a Thorough Interrogation of Metabolic Individuality. K. Beebe, A.D. Kennedy / Computational and Structural Biotechnology Journal, 2016.

International Journal of
Epidemiology
Official Journal of the International Epidemiological Association

- Contribution of medical therapies in mean life length is only : 5-10%
- Contribution of hygiene in mean life length is major : 90-95%
- Hygiene as "prophylactic " preventive medicine based on public surveillance and exposure science, toxicology data and risk assessment and risk analysis
- aims to improve and maintain public health

Clin Lab. 2010. Medicine and natural science-time for a change in paradigm

Need for Changes in Scientific Model
For medical research and practice

18 Nobel Prices in basic research
8 Nobel Prices in clinical research

- Low success in chronic diseases

- due to treatment of symptoms

- and not the basic biochemical disturbances

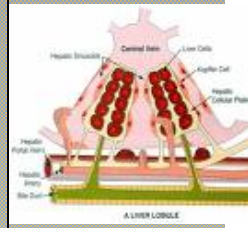
- Medical practice changes will include more knowledge from natural and molecular sciences

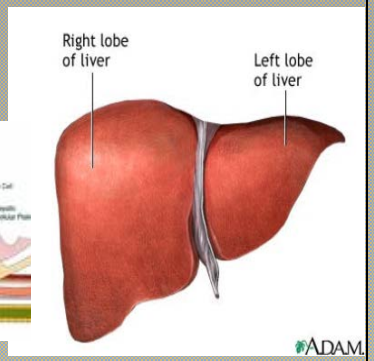
The role of medical care in contributing to health improvements within societies . Int. J. Epidemiol. (2001) 30 (6): 1260-1263.

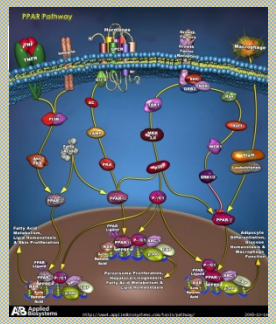
Qualitative and Quantitative Inferences Based on Mechanistic Understanding

Molecule → Cell → Tissue / Organ → Individual → Population

Parenchymal
--Diploid
--Polyploid
--Putative stem cell







NPCs
--Kupffer
--Ito
-Endothelial

Lobular and regional differences:

- **Ploidy**
- **Maturation**
- **Function**

Colorful mice, above, were used to find the weight regulation gene mahoganoid. From left, yellow and black banded, yellow, and dark reddish-brown mice.

Exposure dose/ window?

Tissue concordance across species?

Other outcomes?

Susceptible populations?

Shape/slope of population dose-response curve?

Purpose of Toxicology

Human Health

- Workers
  - Safe working environment (OEL)
- Consumers
  - Products should be safe to use as specified and under foreseeable misuse
    - ADI (food ingredients)
    - RfD (pesticides)
    - TDI (food contaminants)

Environment (ecotoxicology)

- Air, water (surface and ground), soil, bedrock
- Aquatic (fresh and salt) & terrestrial organisms (flora and fauna)

To Provide

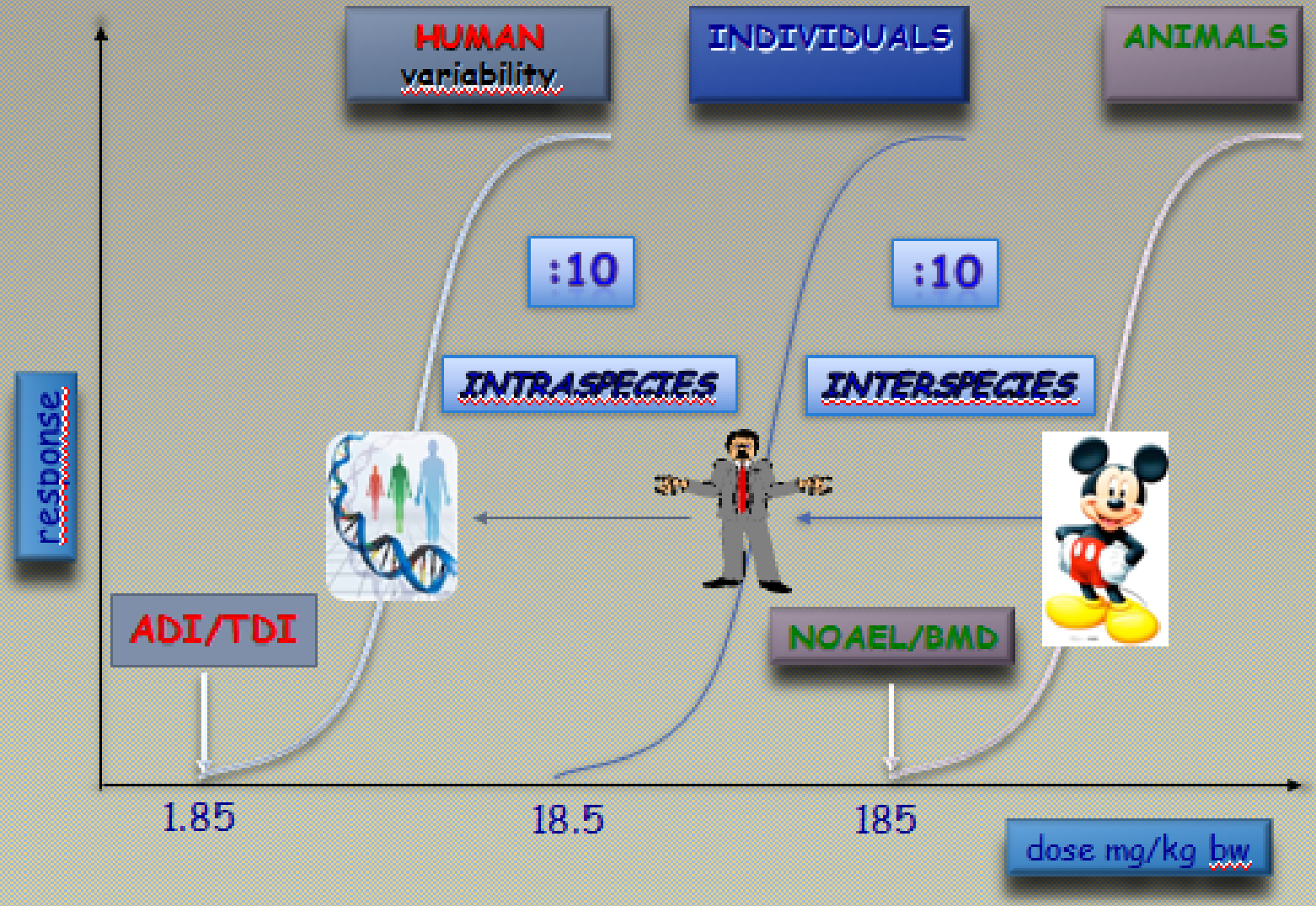
- Information to risk managers
  - About nature & severity of effects on human health and environment as it relates to specific exposures
    - Ecotoxicology often drives remediation site clean-ups
  - ~6,000,000 known chemicals
    - Approximately 100,000 currently in use worldwide, 500 new chemicals added annually
      - limited information
    - HPVC & REACH; 7th Amendment

Via Risk Assessments

- Hazard identification
- Dose-response assessment
- Exposure assessment
- Risk characterization

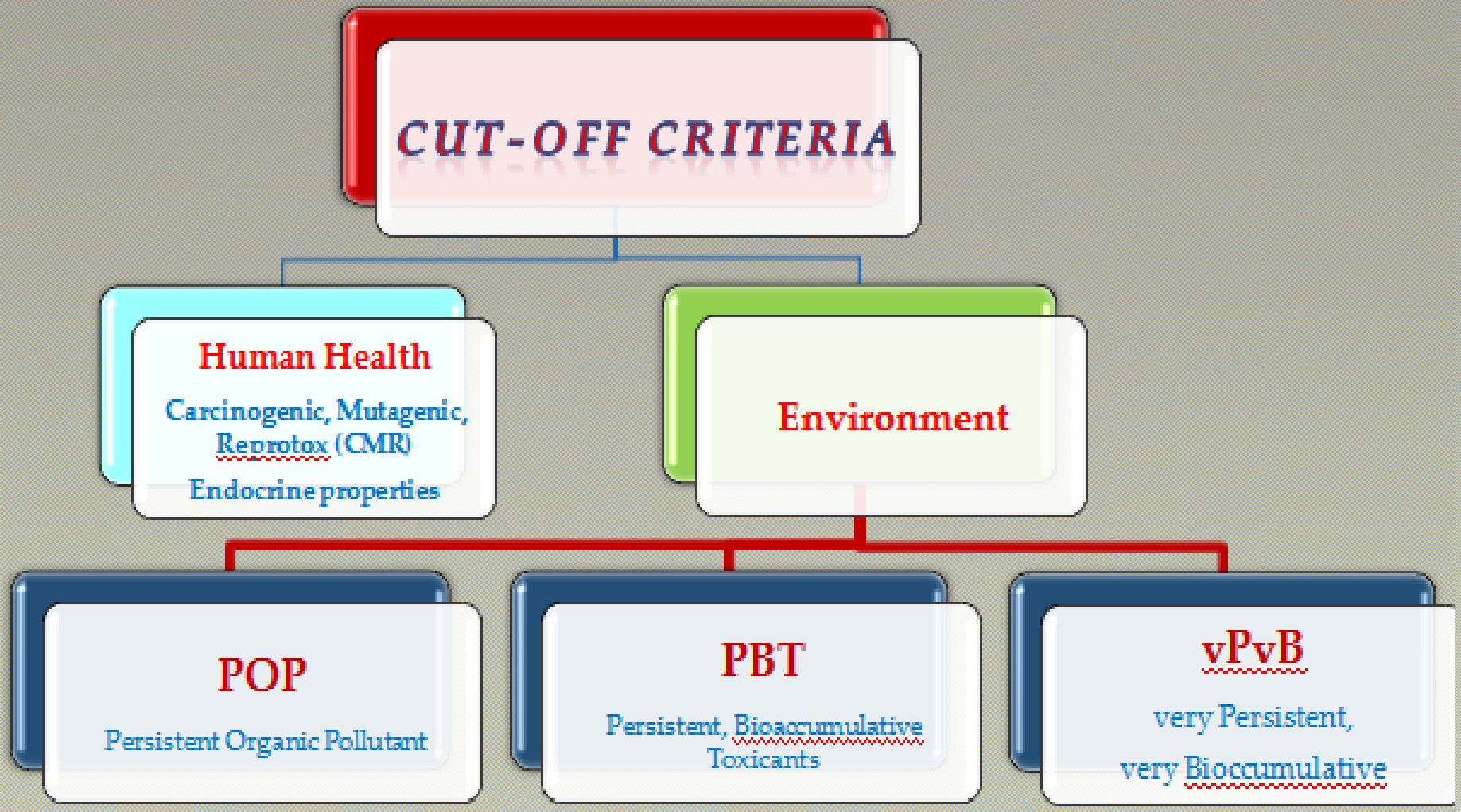
ANIMAL-BASED TOXICOLOGICAL STUDIES

(quantification of adverse health effects)

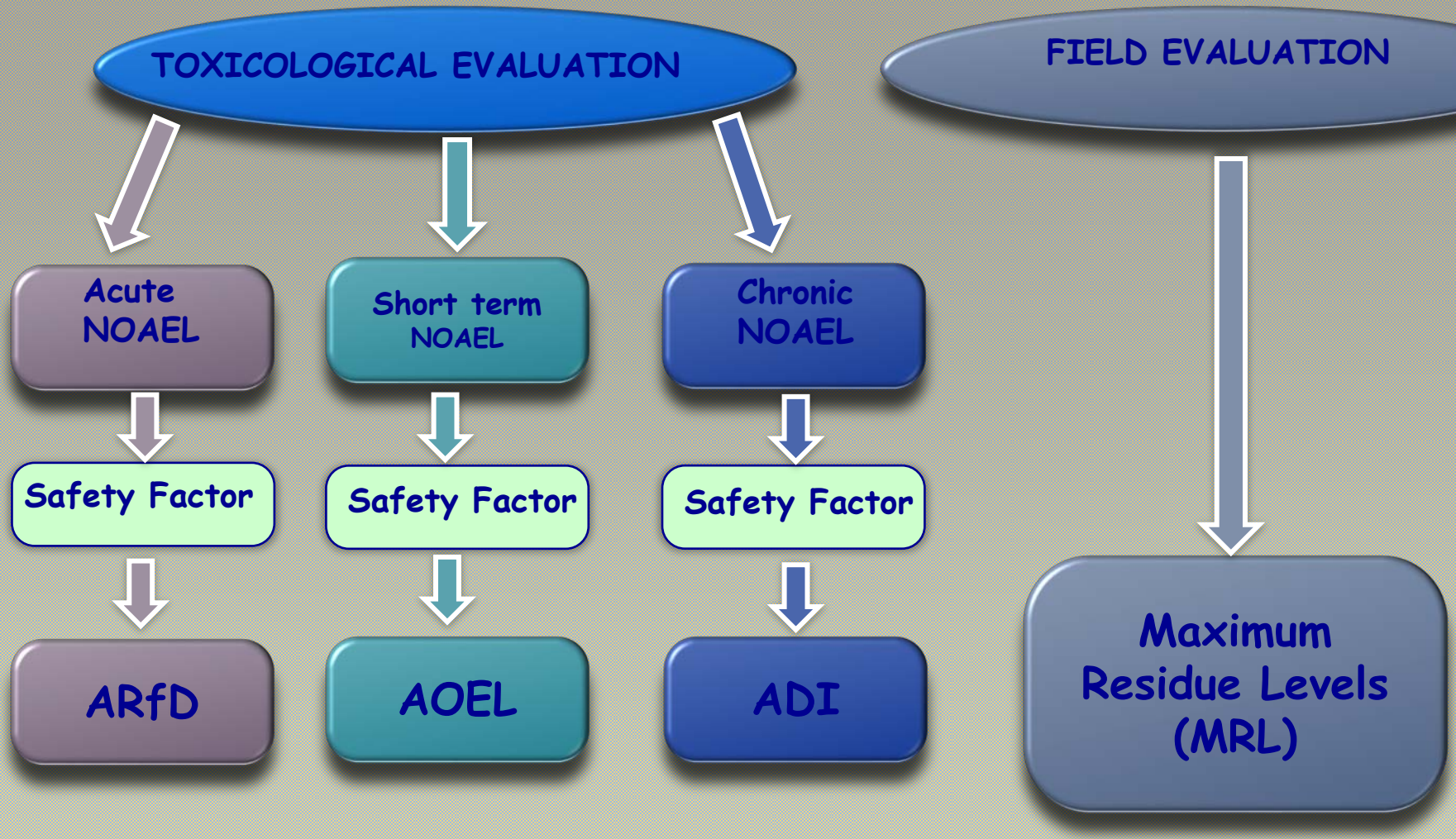


CUT-OFF AND APPROVAL CRITERIA

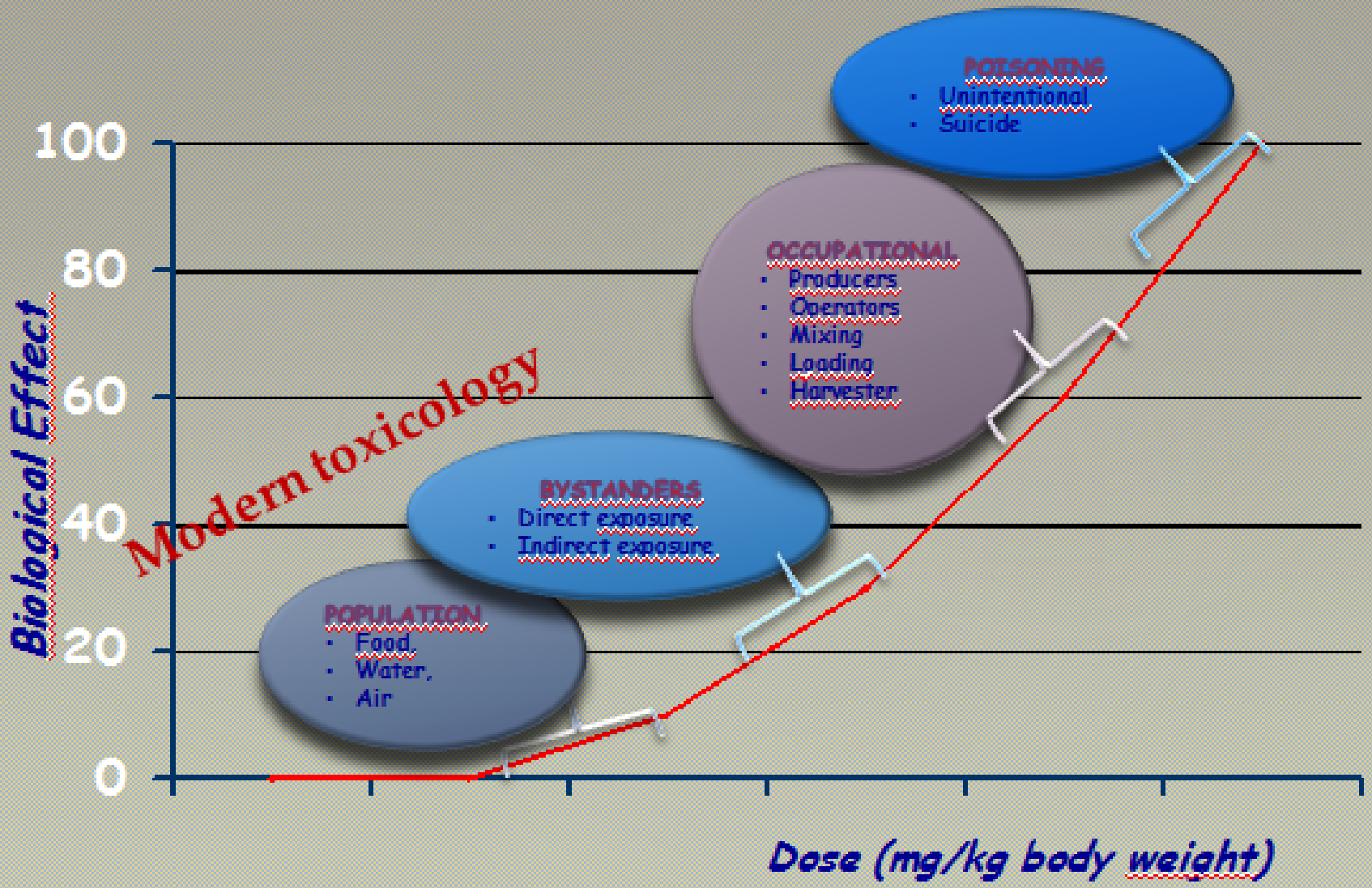
(as., safeners, synergists)



HEALTH BASED GUIDANCE

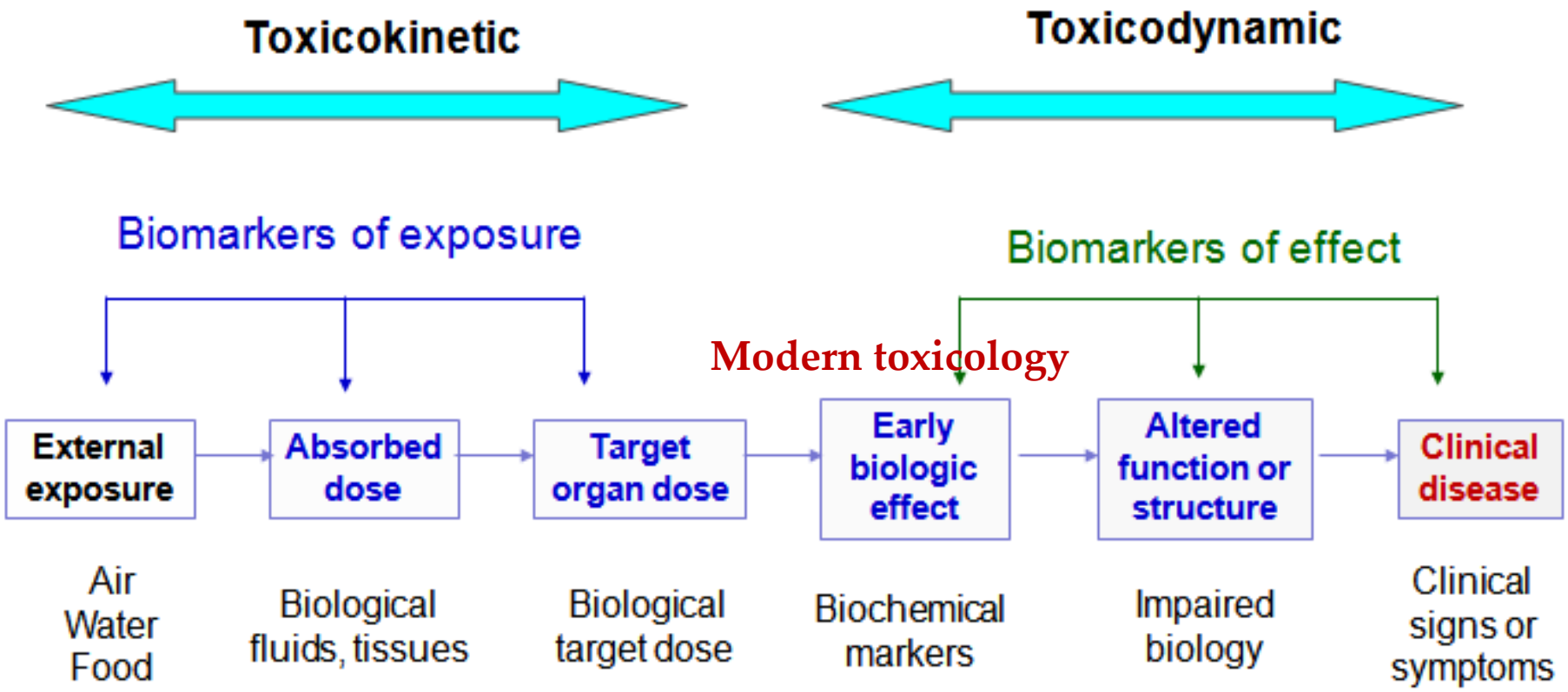


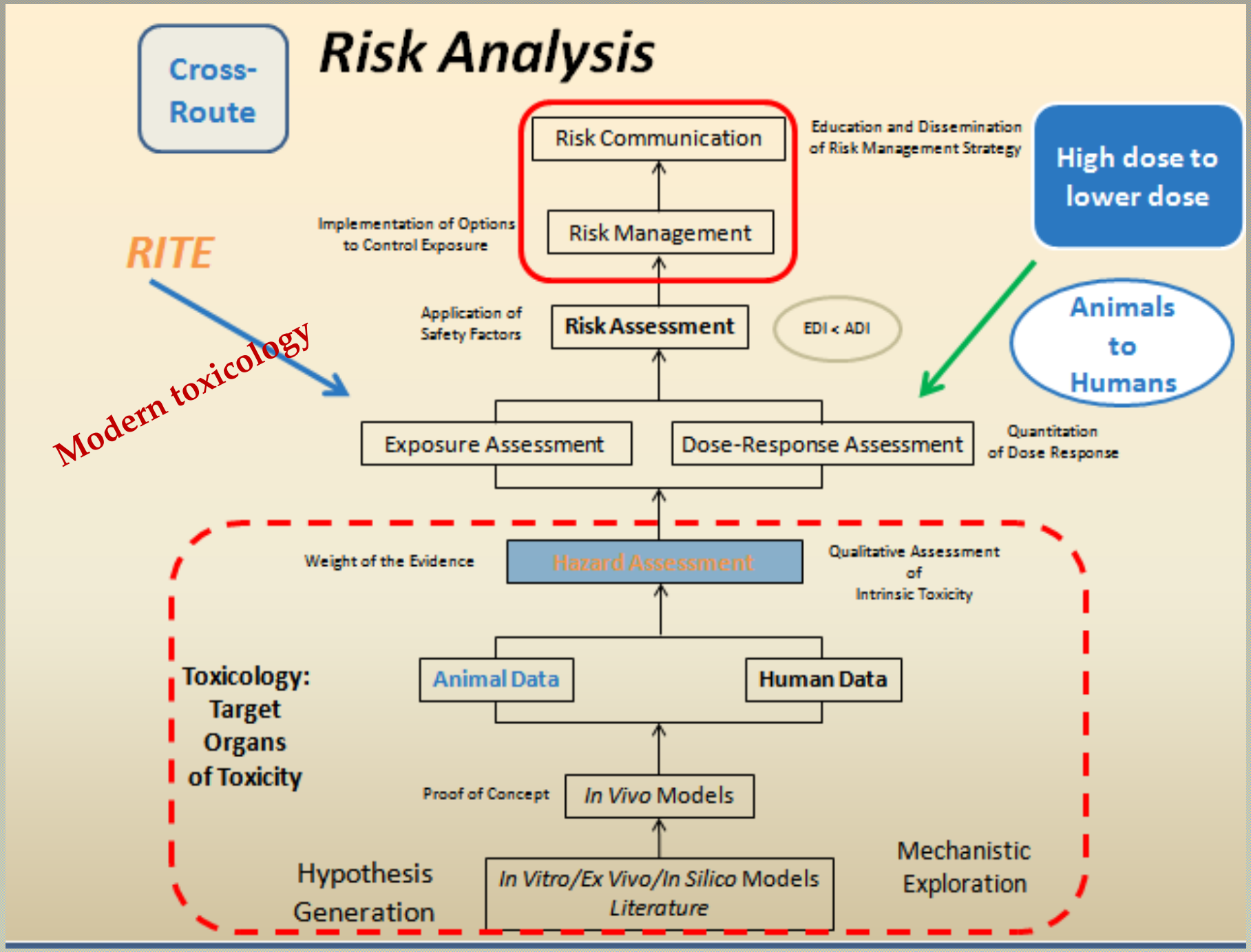
TOXICOLOGICAL ASSESSMENT EXPOSURE



Paradigm of toxicity

Continuum from exposure to disease

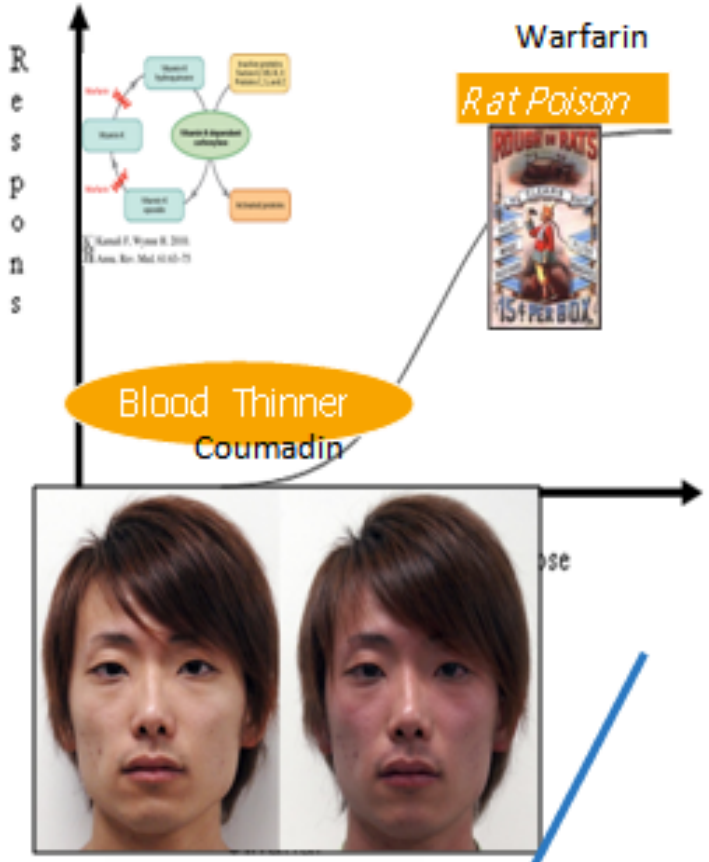



Cross-Route
Risk Analysis
Risk Communication
Education and Dissemination of Risk Management Strategy
High dose to lower dose
Implementation of Options to Control Exposure
Risk Management
RITE
Application of Safety Factors
Risk Assessment
EDI < ADI
Animals to Humans
Modern toxicology
Exposure Assessment
Dose-Response Assessment
Quantitation of Dose Response
Weight of the Evidence
Hazard Assessment
Qualitative Assessment of Intrinsic Toxicity
Toxicology: Target Organs of Toxicity
Animal Data
Human Data
Proof of Concept
In Vivo Models
Hypothesis Generation
In Vitro/Ex Vivo/In Silico Models
Literature
Mechanistic Exploration

Toxicity or hazard

Dose/timing matters

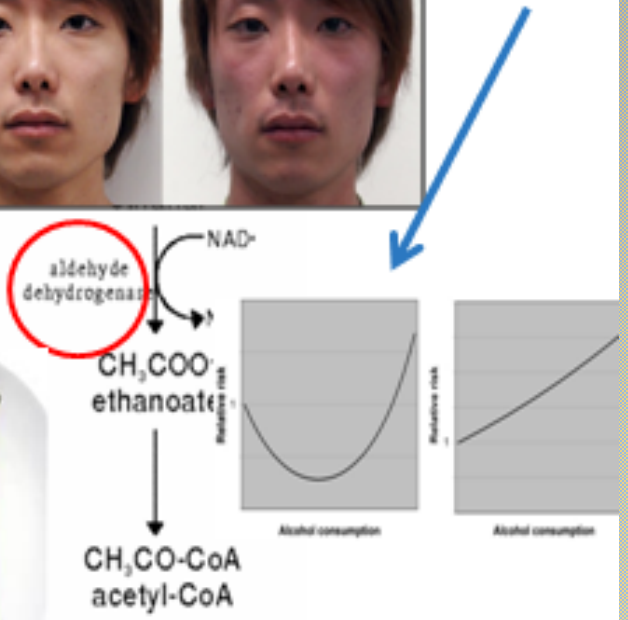
Polymorphisms
Age, gender, health, drugs



People differ

She used to drink turpentine (terebinth from *Pistacia terbinthus*?) to make her **urine smell of violets**

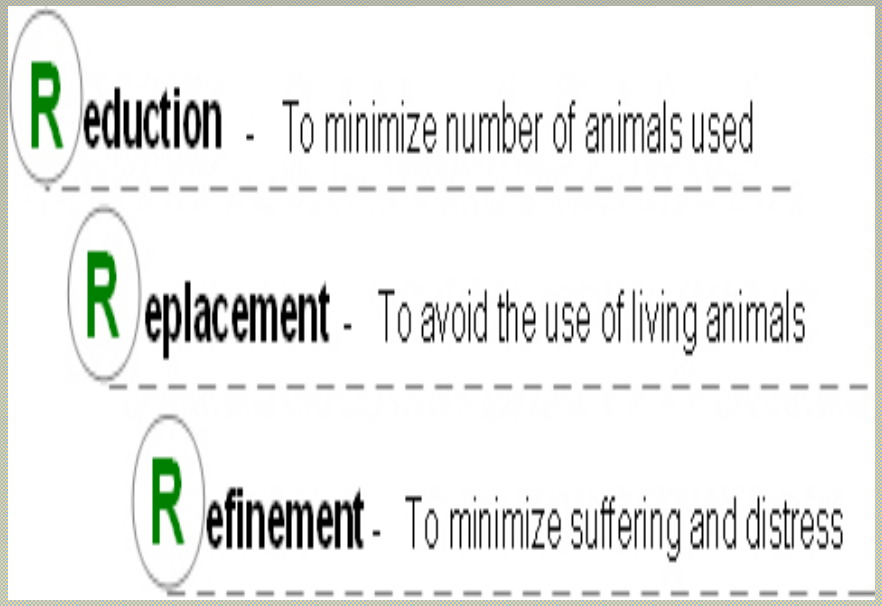
Things Change



3R and the JRC Activity

- **New integrated methods based on in-depth biological knowledge are needed. Therefore the JRC is developing and testing new animal-free methods, alternatives to animal-based tests, to be applied in an integrated safety assessment of chemicals.**
- **EURL ECVAM, the European Union Reference Laboratory for Alternatives to Animal Testing**

3R!



Examples of EU legislation that require, or strongly encourage, the replacement of animal testing

- Directive on the protection of animals used for scientific purposes (2010/63)
- Regulation on cosmetic products (1223/2009)
- REACH (2007/2006)
- Classification, Labelling and Packaging (CLP) (1272/2008)

EUROTOX

EUROTOX / SOT Debate 2013

eurotox 2013
Interlaken | Switzerland

SOT | Society of Toxicology

SOT 2013 San Antonio • Texas

SOT/EUROTOX Debate

In the Near Foreseeable Future, Much of Toxicity Testing Can Be Replaced by Computational Approaches

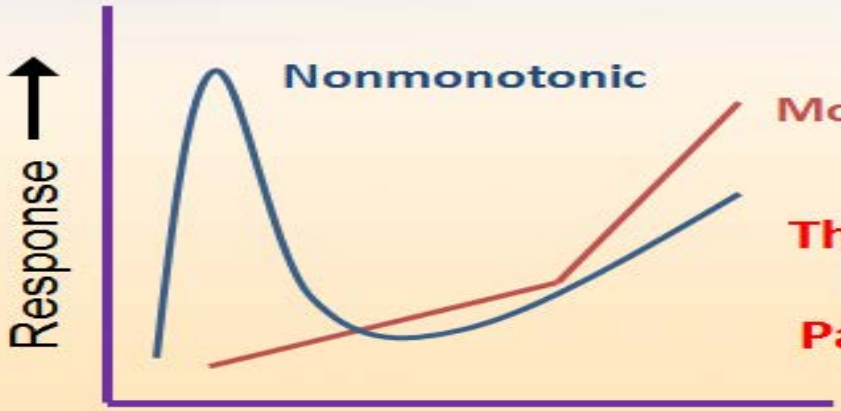
Monday, March 11, 2013

4:45 PM-6:00 PM

52nd Annual Meeting and ToxExpo

SOT / EUROTOX Debate 2014 in Phoenix and Edinburg

Are Nonmonotonic Dose-Responses at Low Dose Levels Toxicologically Relevant?

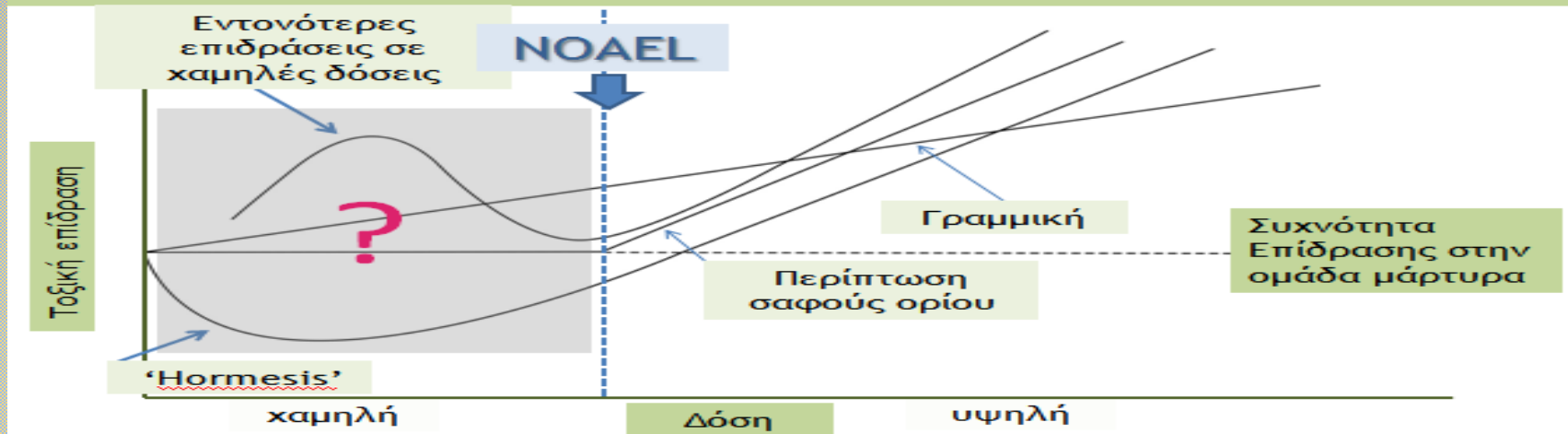


The dose makes the poison, Paracelsus .. ??

METRON ARISTON !!
Kleovoulos

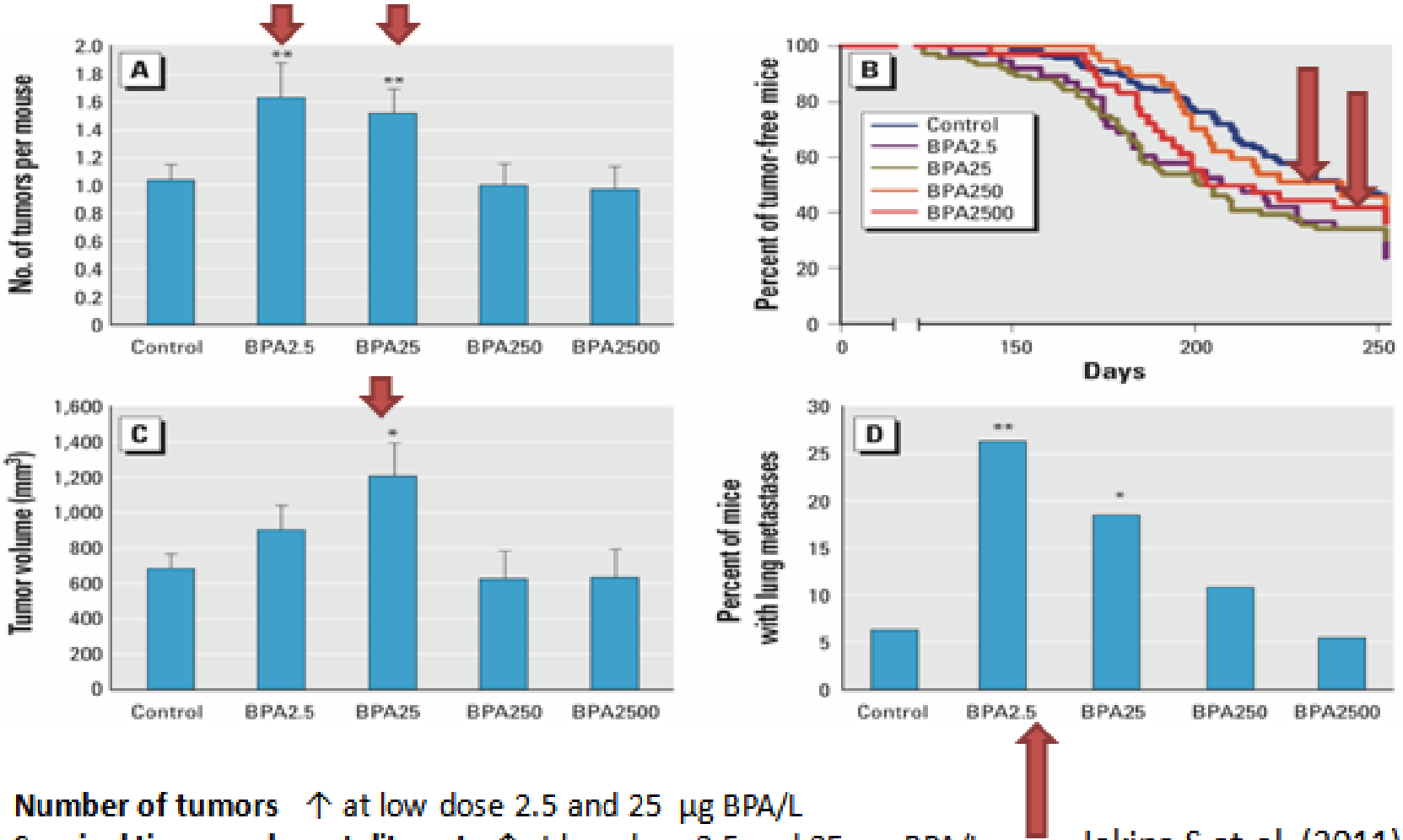
WHEN The dose makes the poison, Paracelsus .. ??

Which is the correct model?
Which is the safe dose ?



Examples of non-monotonic curves

Bisphenol A in drinking water in mice.



Number of tumors ↑ at low dose 2.5 and 25 µg BPA/L

Survival times and mortality rate ↑at low dose 2.5 and 25 µg BPA/L

Tumor volume (mm^3) ↑ at bisphenol Dose 2.5 µg BPA/L

Number of metastatic rats ↑ at bispheno dose 2.5 and 25 µg BPA/L

Jekins S et al. (2011)

Environ Health Perspectives

119:1604–1609

Examples of non-monotonic curves

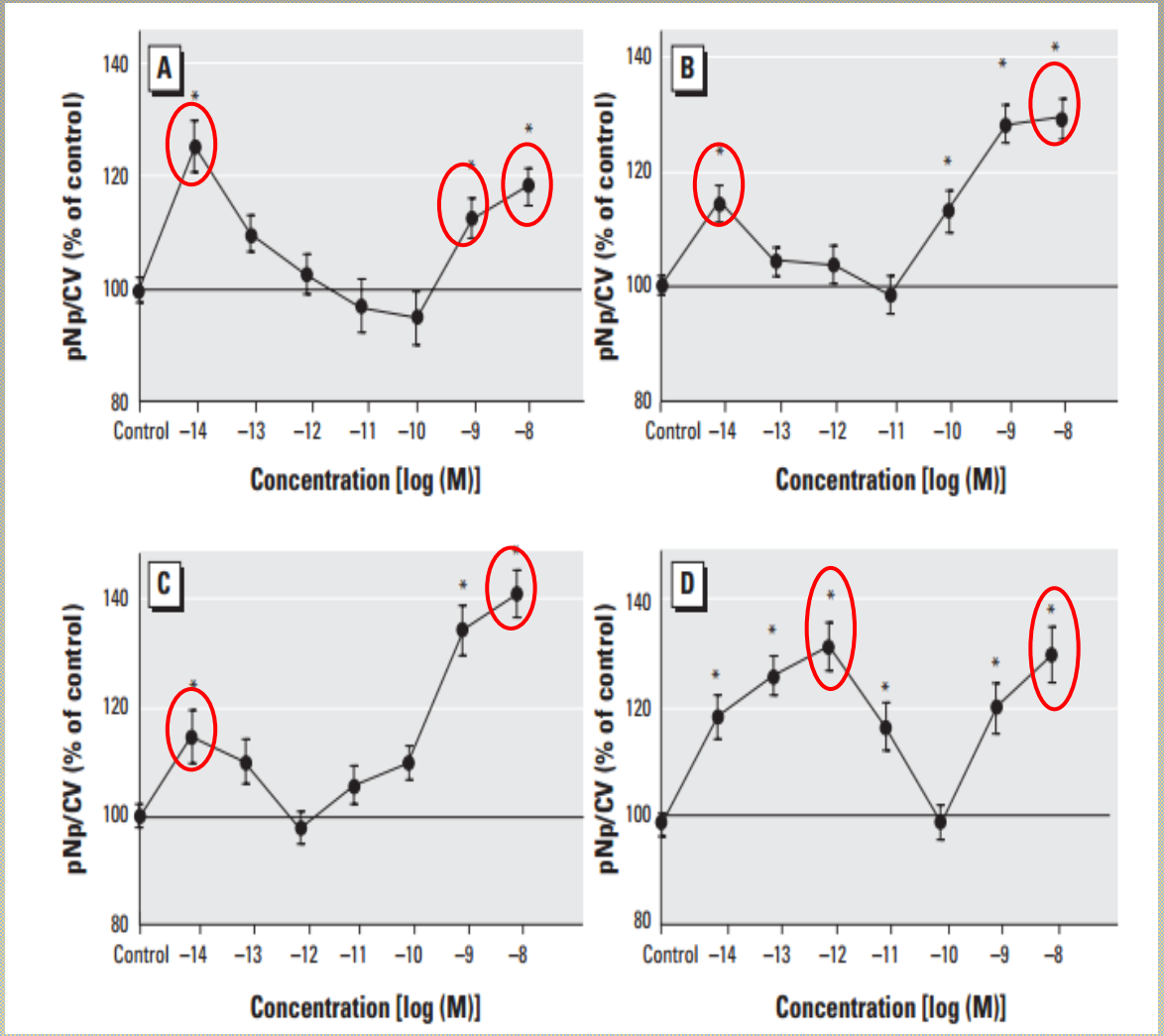
Xenoestrogens in vitro cell lines

(A)Estradiol
(B)Coumestrol
(C)p-nonylphenol
(D)endosulfan

Concentration-dependent changes in the phosphorylation status of extracellular-regulated kinases (ERK)

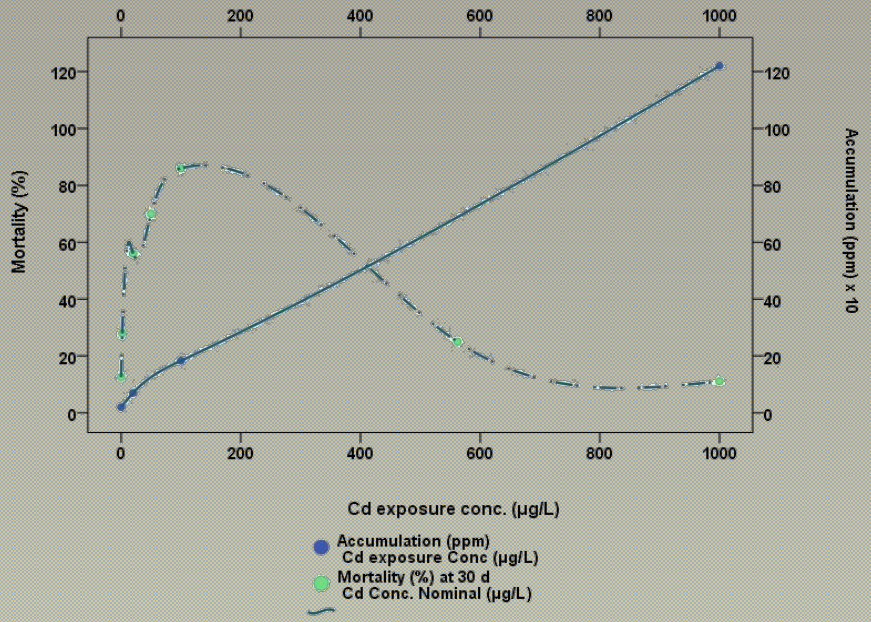
Concentrations in $\log_{10}$ scale

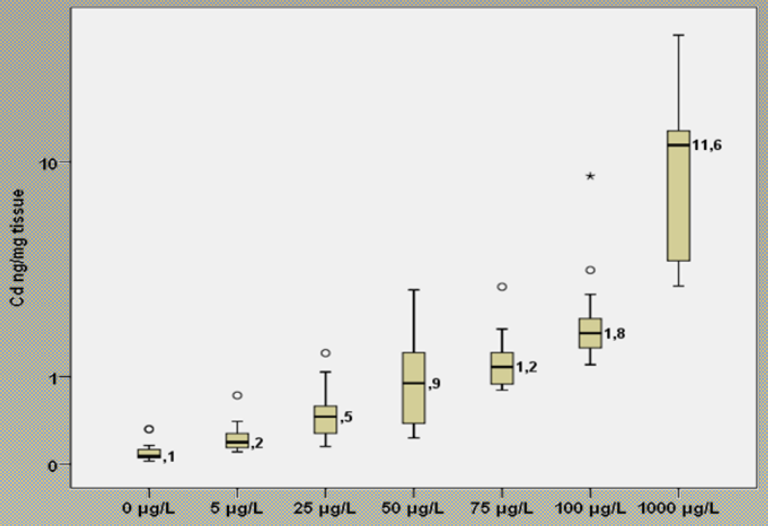
Bulayeva NN Watson CS (2004). Environmental Health Perspectives, 112(15): 1481-1487

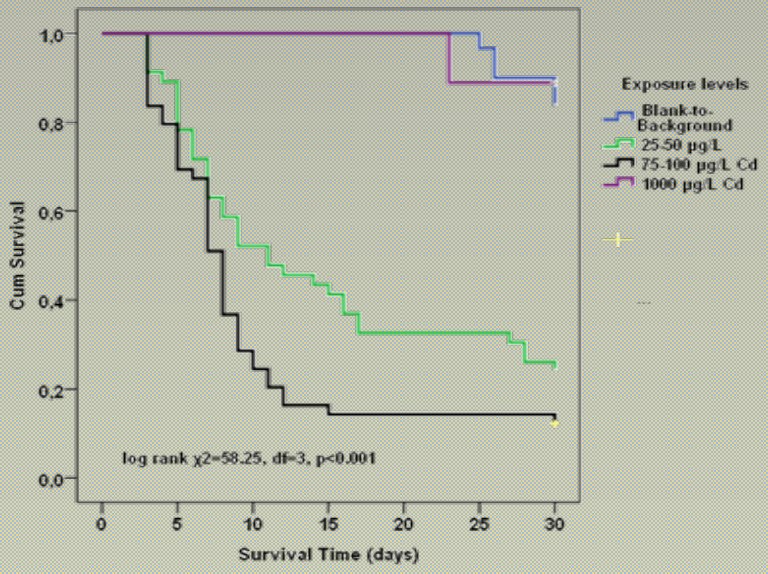


Non linear (non monotonic) response at low? doses

Mortality vs. Cd accumulation



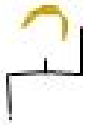




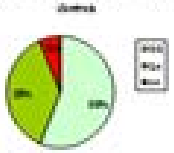
- Mortality data: this study (Renieri E.), including recent literature
  - S. Cambier *et al.*, 2010
  - L. Vergauwen *et al.* / Chemosphere (2013)
  - LA. Arini *et al.*, 2015
- Accumulation data: this study (Renieri E.)

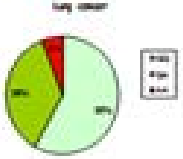
Enough, but not too much /..metron ariston, .kleovoulos

Two faces of selenium

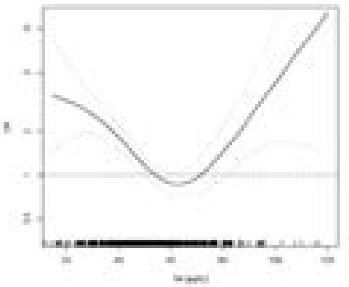


Se, *Sep15* polymorphism and lung cancer risk





Lung cancer risk associated with plasma Se concentration

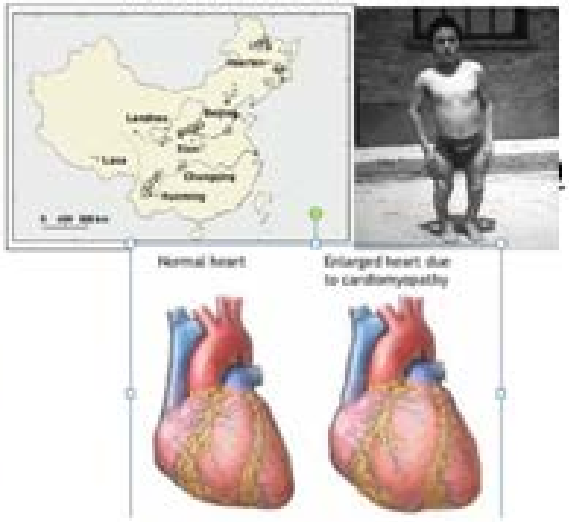


Deficiency

Poisoning

Life Window

Keshan disease



Kashin-Back

Selenosis patients

Three persons among four members of their family were selenosis patients,

During heavy prevalence, intake of Se as high as 15-38 mg were detected,

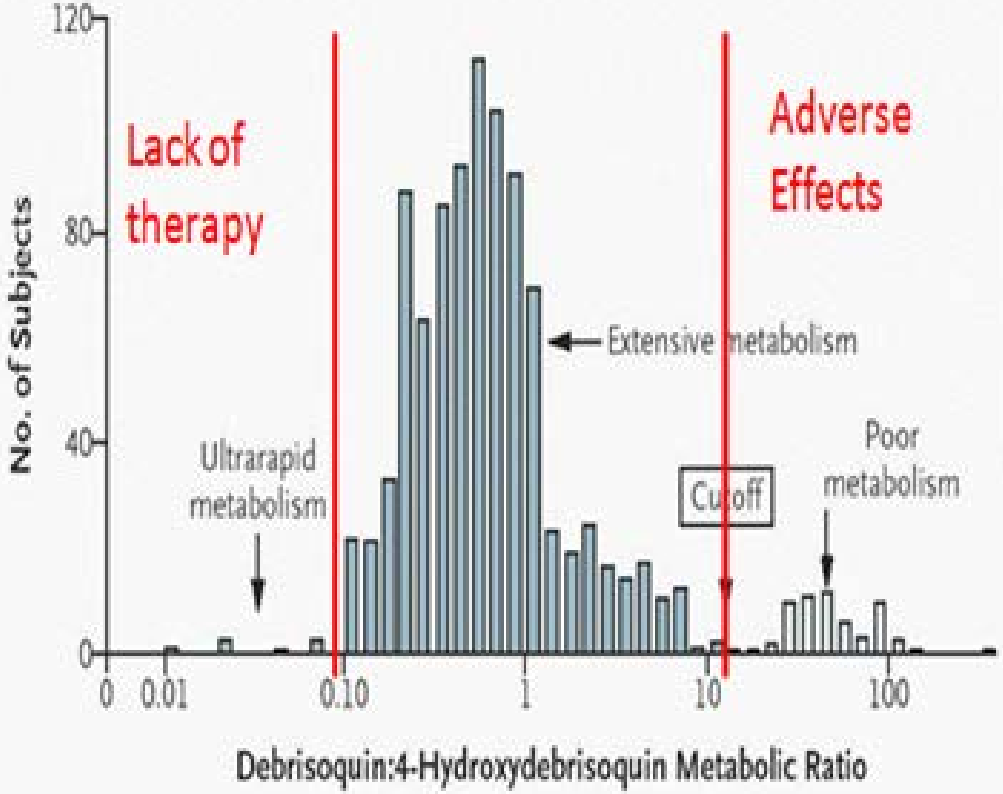
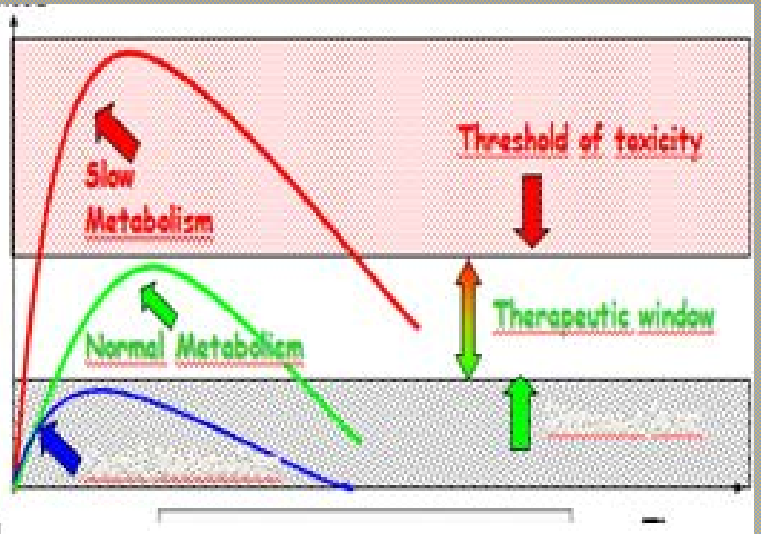
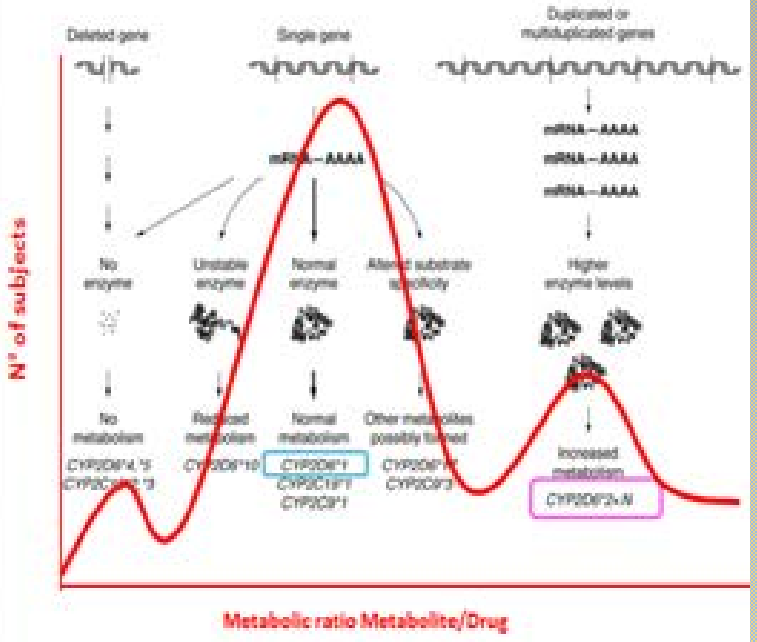


Figure 3. Pharmacogenetics of CYP2D6.

Urinary metabolic ratios of debrisoquin to its metabolite, 4-hydroxydebrisoquin, are shown for 1011 Swedish subjects. The Cutoff box indicates the cut-off point between subjects with poor metabolism as a result of decreased or absent CYP2D6 activity and subjects with extensive metabolism. Modified from Bertilsson et al.[17] with the permission of the publisher.

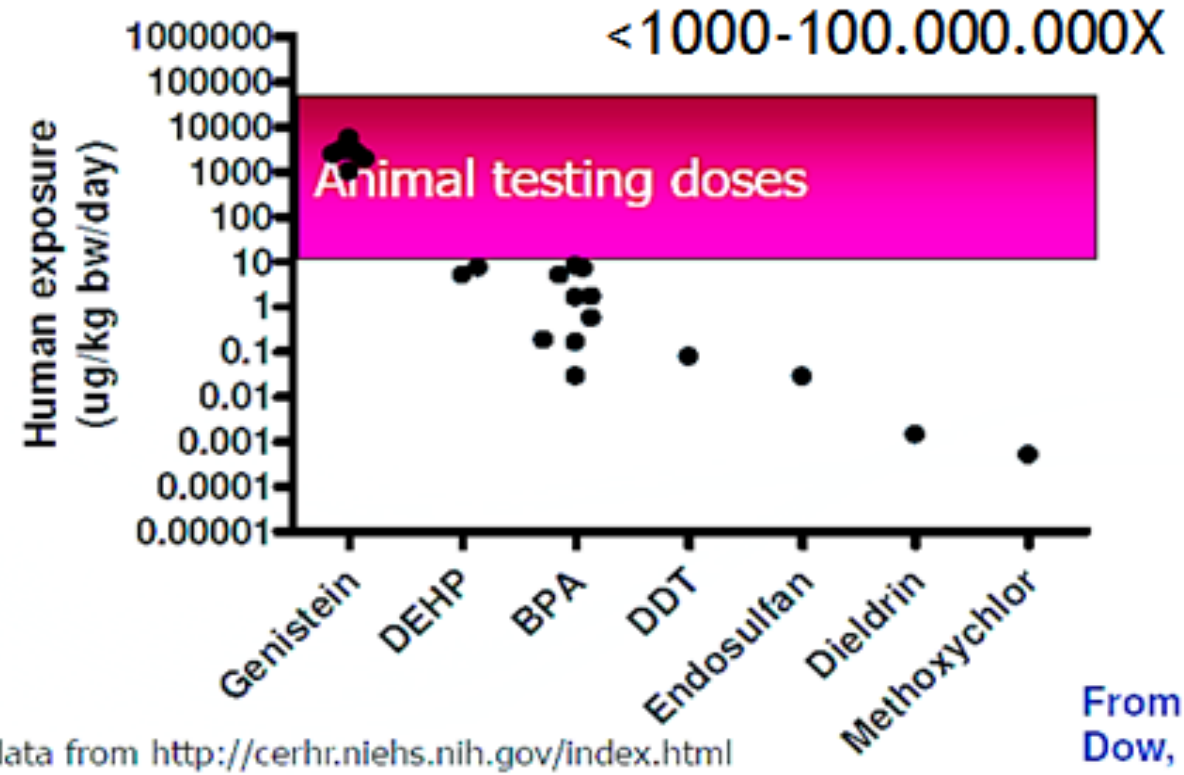


Genetic and Phenotipic differences



Presentation of studied doses in relation to levels of human exposure

human exposure vs studied doses

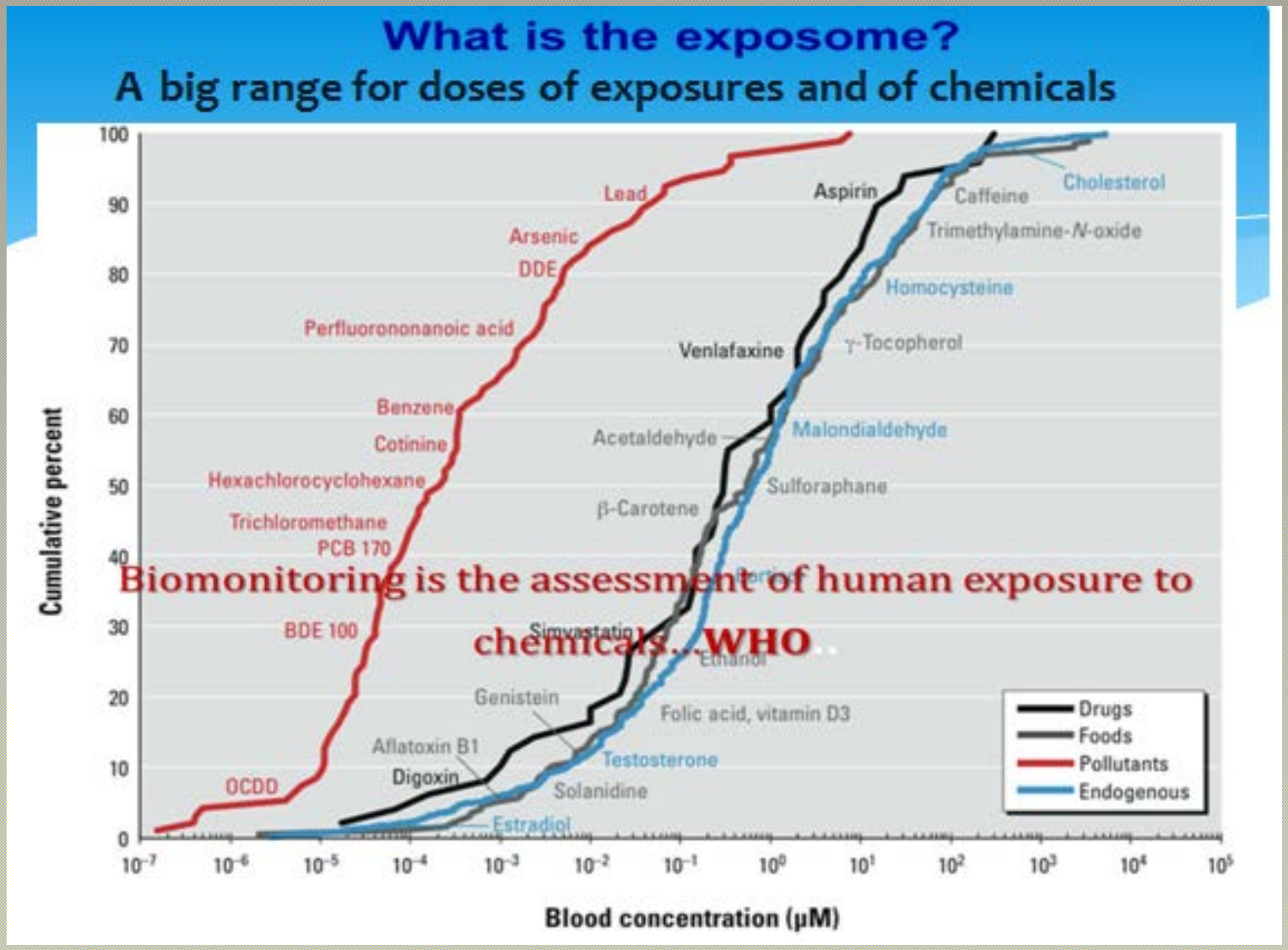


Exposure data from http://cerhr.niehs.nih.gov/index.html

From E. Carney, Dow, 2010

Thomas Hartung, The Human TOXOME Project & Endocrine Disruption Testing, JHU,USA.

Issues under consideration include high variability in levels and sensitivity and of exposure duration of the toxicological studies



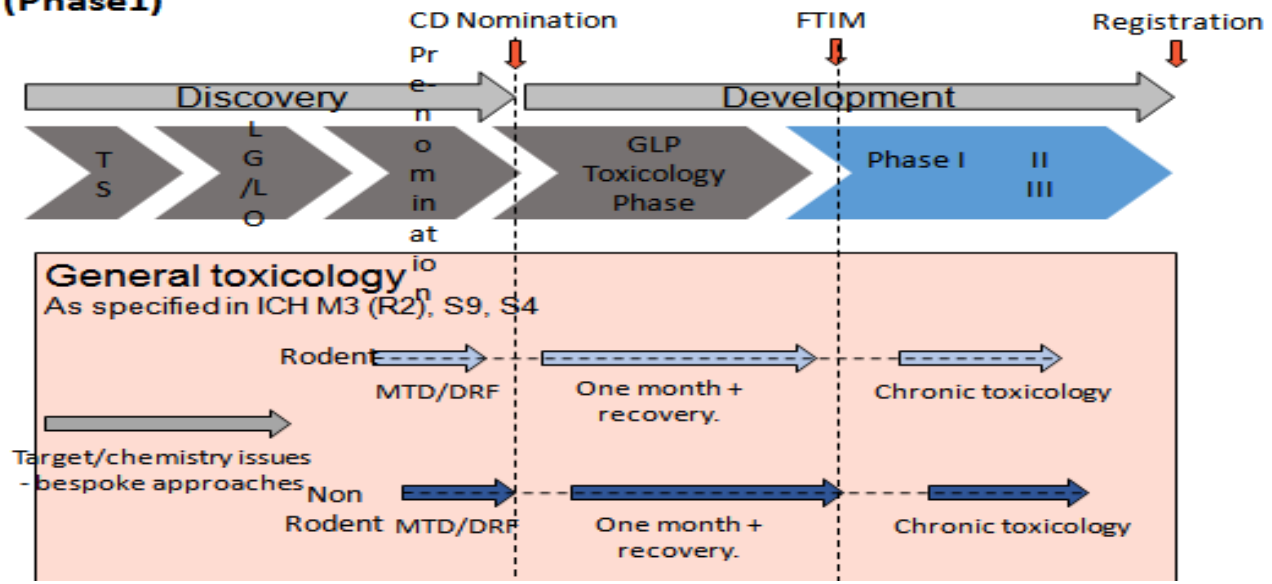
WHO , Oct. 26 2015 released review study performed by IARC

Intern Agency Research on Cancer WHO subsidiary

- Processed meat as a Group 1 Carcinogen, meaning evidence that links to cancer
- This based on meta-analysis studies evaluating 800 papers and conducted by team of 22 experts from 10 countries
- **FDA Food and Drug Administration response : Federal Government carries out its** own research through the **National Toxicology Program**
- **NTP on carcinogens will not look at processed meats as whole food items nominated for review as carcinogens**

Contraversies among regulators

Preclinical toxicology testing: a series of tests conducted prior to first time in man (FTIM) (Phase1)



SOT EUROTOX debate

Preclinical toxicity studies DO NOT predict clinical outcome.....but.....

- They play a vital role in eliminating non-viable compounds from development
- They define a no effect level (NOAEL) in support of starting dose, dose escalation and exposure limits
- They inform clinical monitoring

Epidemiological studies

Advantages

Human populations

True range of exposure
Vulnerable subgroups

Sensitive parts of lifespan
Gender-selective effects

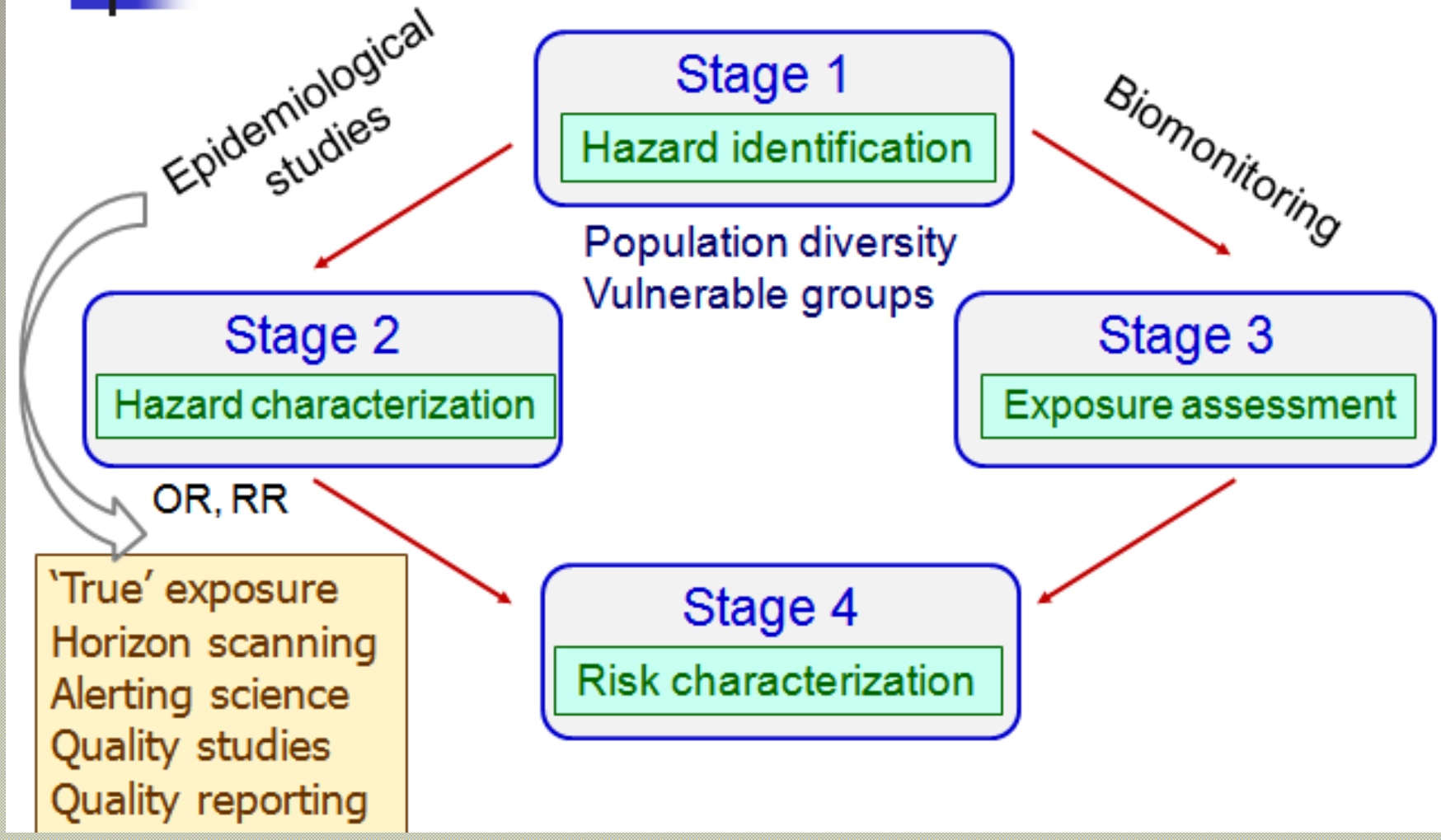
Disadvantages

Poor exposure information
No quantitative data

More than one chemical
Heterogeneity of data
Temporal concordance?
Confounders

No biological plausibility

Summary of the Risk Assessment Process

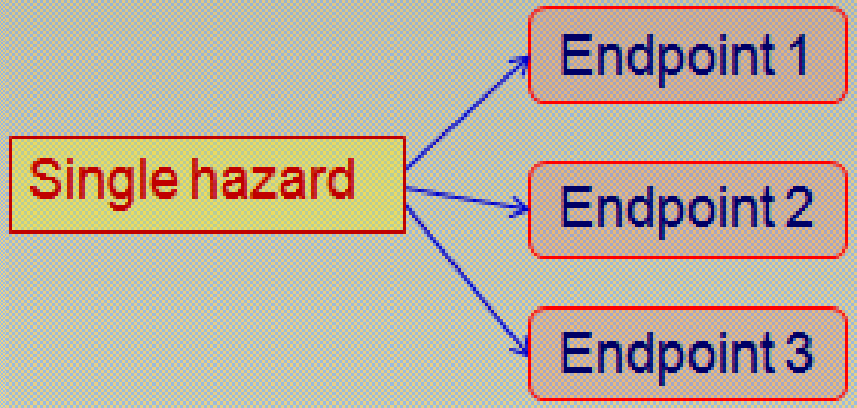


Application of epidemiological studies to pesticide risk assessment

Classical single hazard approach (*regulatory framework*)

Evaluation of one pesticide

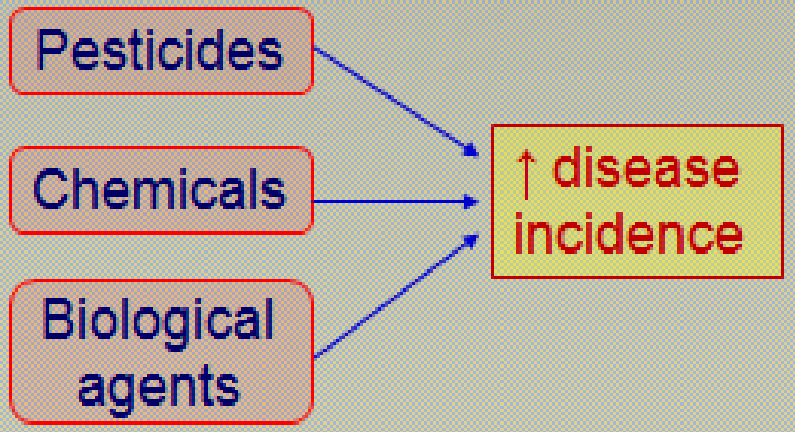
Various potential outcomes



Modern toxicology Multiple hazard *(integrated epi approach)*

Evaluation of various 'stressors'

Known (or emerging risk)



Biological plausibility for the interpretation of epi evidence

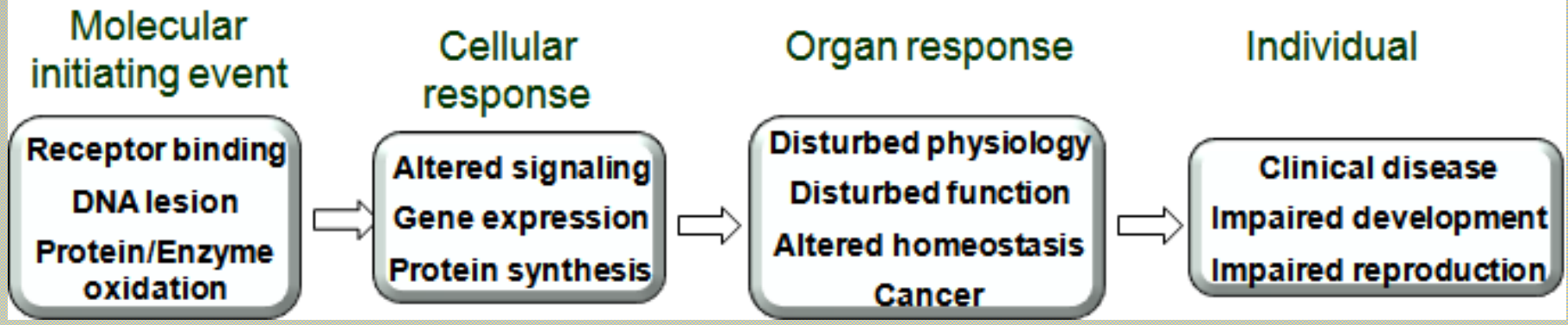
- Epidemiological studies

External exposure → Clinical disease

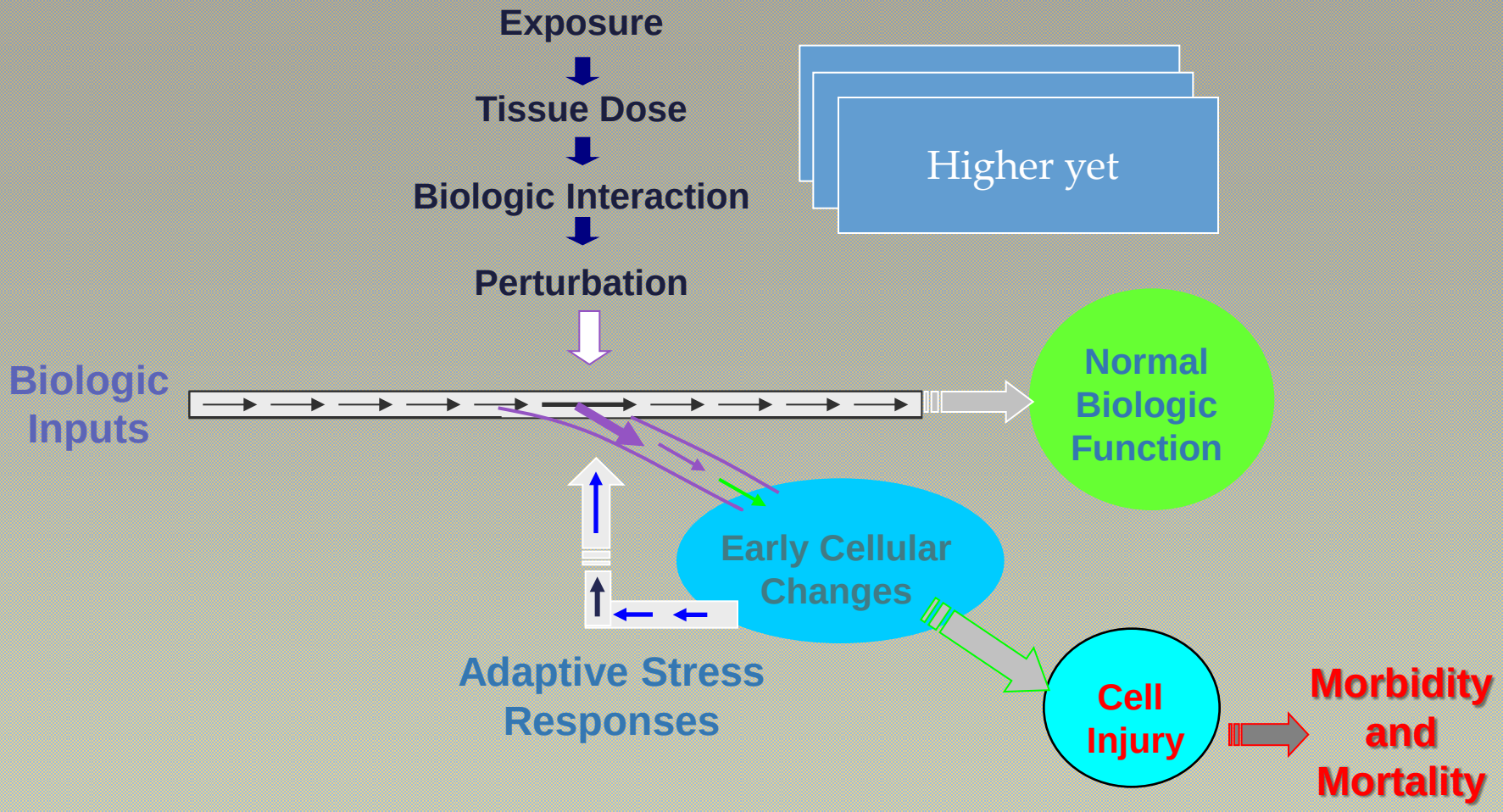
- Use of modern technologies and approaches

External exposure → Absorbed dose → Target organ dose → Early biologic effect → Altered function or structure → Clinical disease

- AOP framework may be an appropriate tool

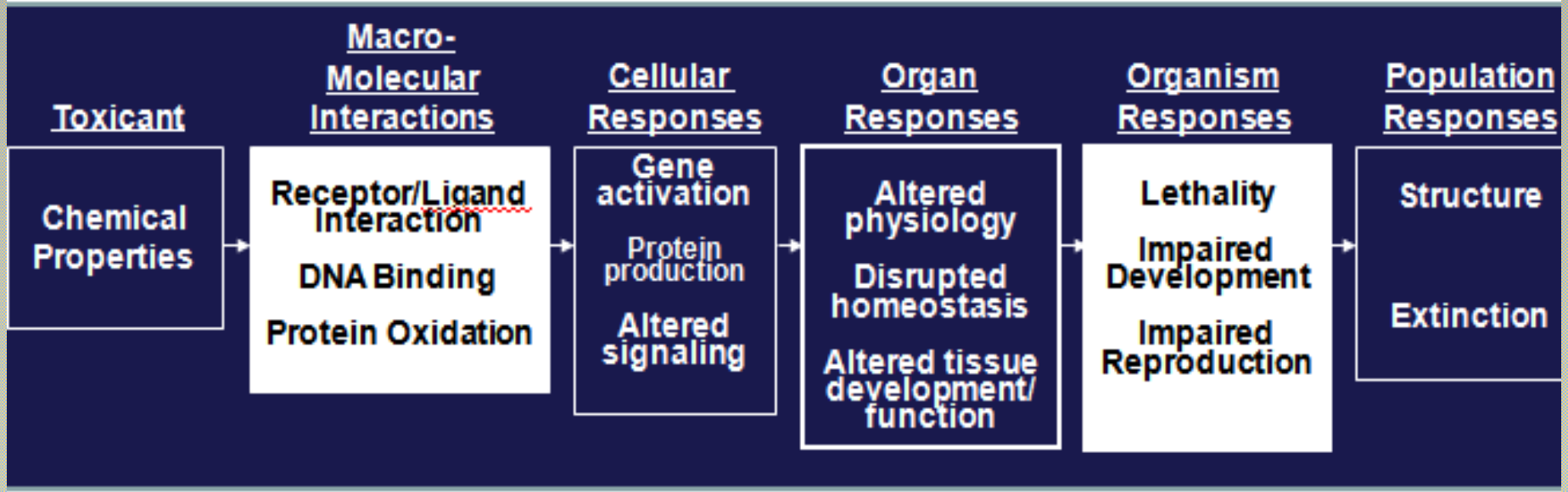


Activation of a toxicity pathway (NRC, 2007)



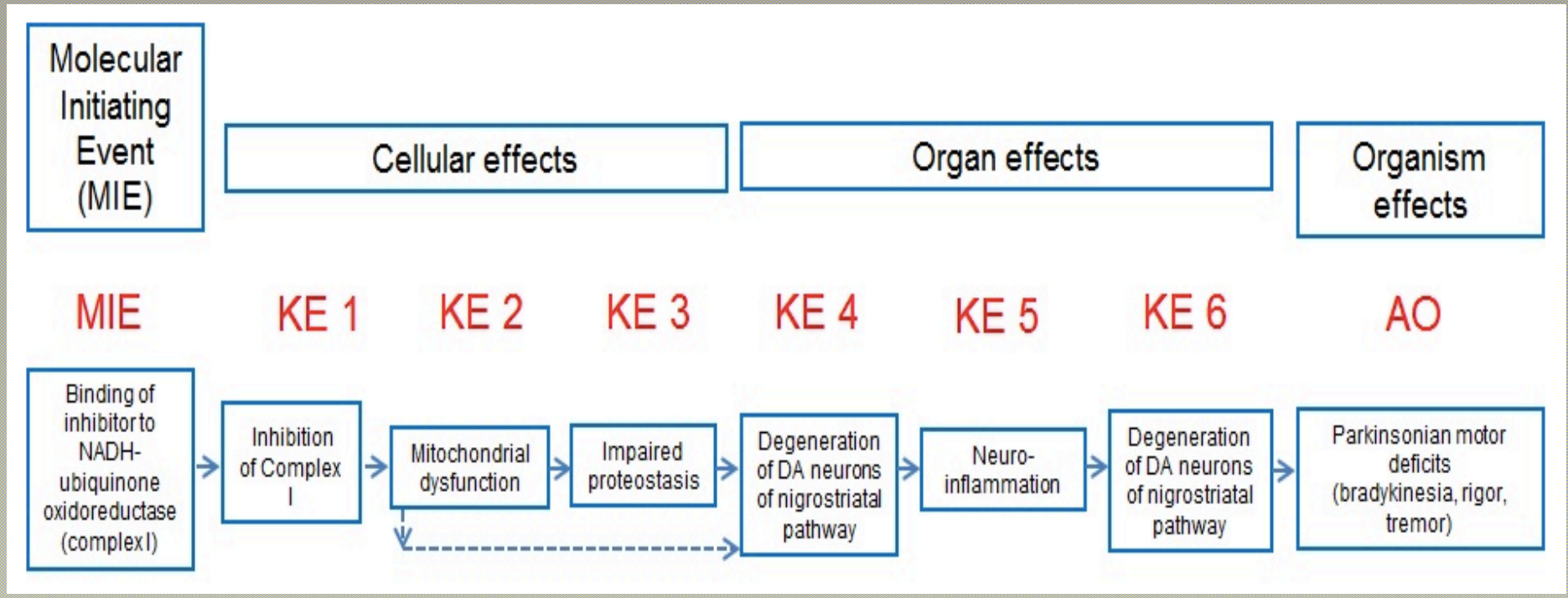
Why use AOPs?

- How do we identify chemicals that may cause adverse effects before we see impacts on human health or wildlife populations?
- We need to understand
  - HOW chemicals cause adverse outcomes and
  - Biological activities that lead to/are associated with progression toward those AOs
- Creates opportunities to use new types of data for hazard identification and/or risk-based decision-making



OECD, 2013

AOP 1: Inhibition of the mitochondrial complex I of nigra-striatal neurons leads to parkinsonian motor deficits



AOP Wiki: https://aopwiki.org/wiki/index.php/Aop:3, accessed Oct 1, 2016

Studies on Adverse Outcome pathways for many years now resulted so far only in few AOPs not without contraversies also

See EFSA recent reports on Paraquat and Parskinson Disease

University of Crete Medical School
Department of Toxicology & Forensic Science

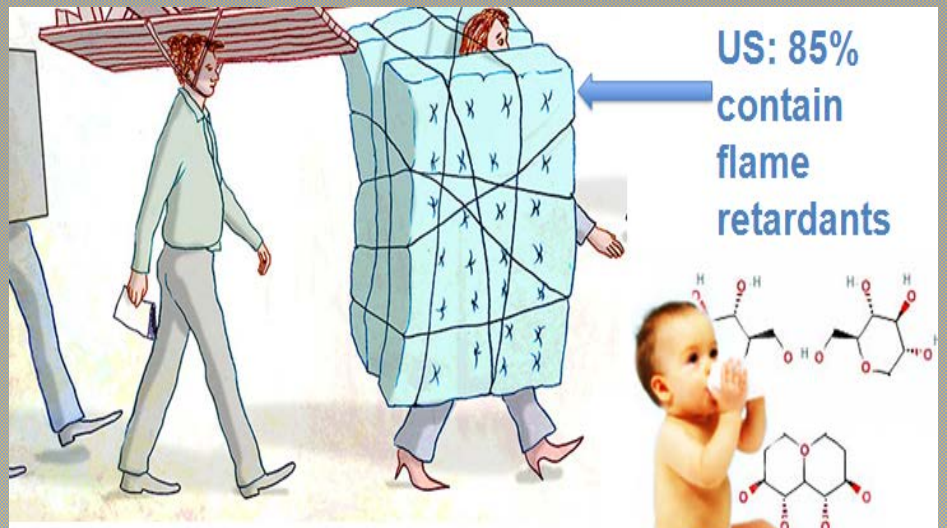
EUROTOX

EUROTOX 2015
PORTO - PORTUGAL

Prenatal long term pesticide exposure and its association with pregnancy problems and birth defects

Aristides M. Tsatsakis

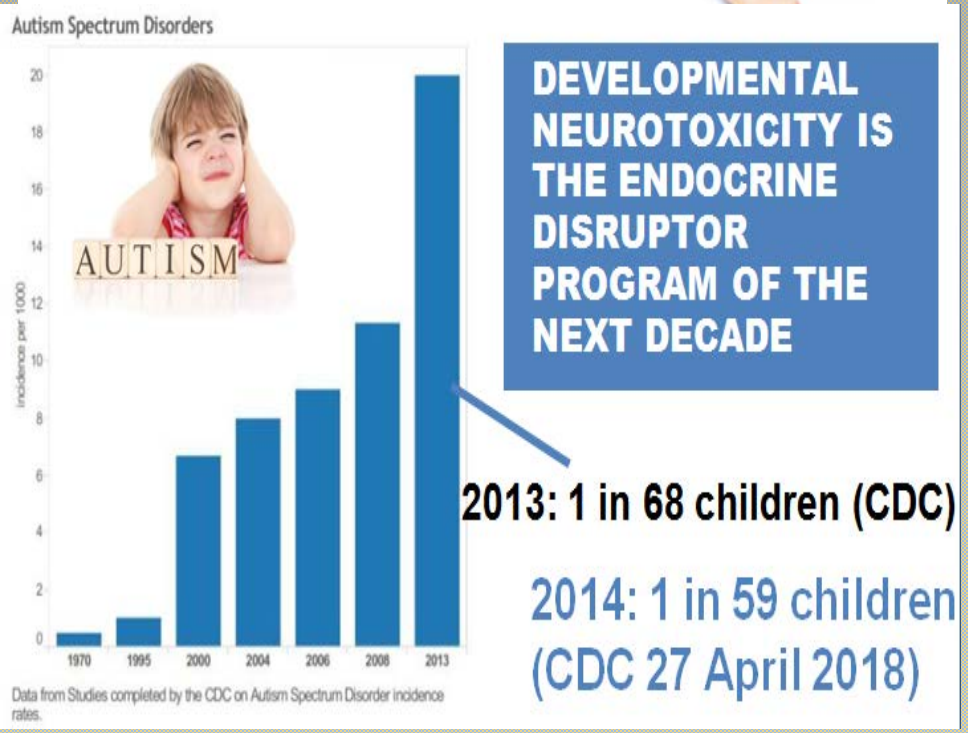
Professor and Director, MSc, PhD, DSc, ERT
Department of Toxicology & Forensic Science, Medical School, University of Crete
http://aristsatsakis.com



Are we safe?

- Simplistic identification of similar chemicals driven by data availability
- Good Read-Across Practice only emerging
- One-to-one or one-to-few read-across
- Cannot be validated

adly used in REACH!



DEVELOPMENTAL NEUROTOXICITY IS THE ENDOCRINE DISRUPTOR PROGRAM OF THE NEXT DECADE

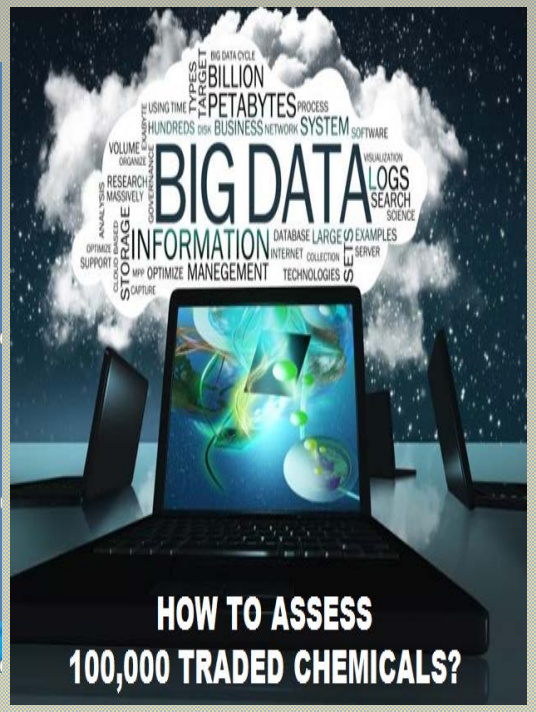
2013: 1 in 68 children (CDC)

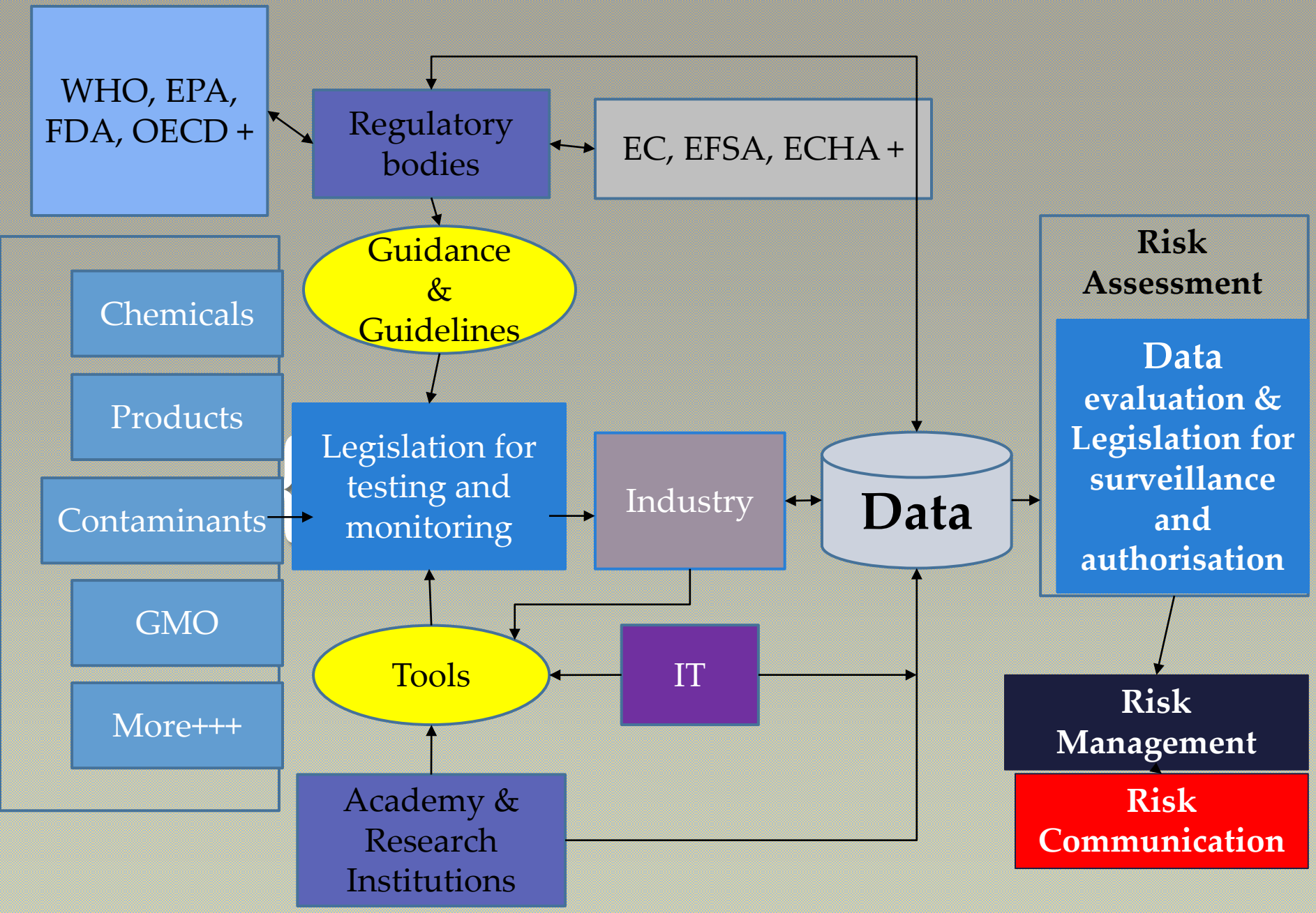
2014: 1 in 59 children (CDC 27 April 2018)

The map of the chemical universe

Similarity = proximity

ARTIFICIAL INTELLIGENCE 0,5 BILLION CALCULATIONS PER PREDICTION + CERTAINTY

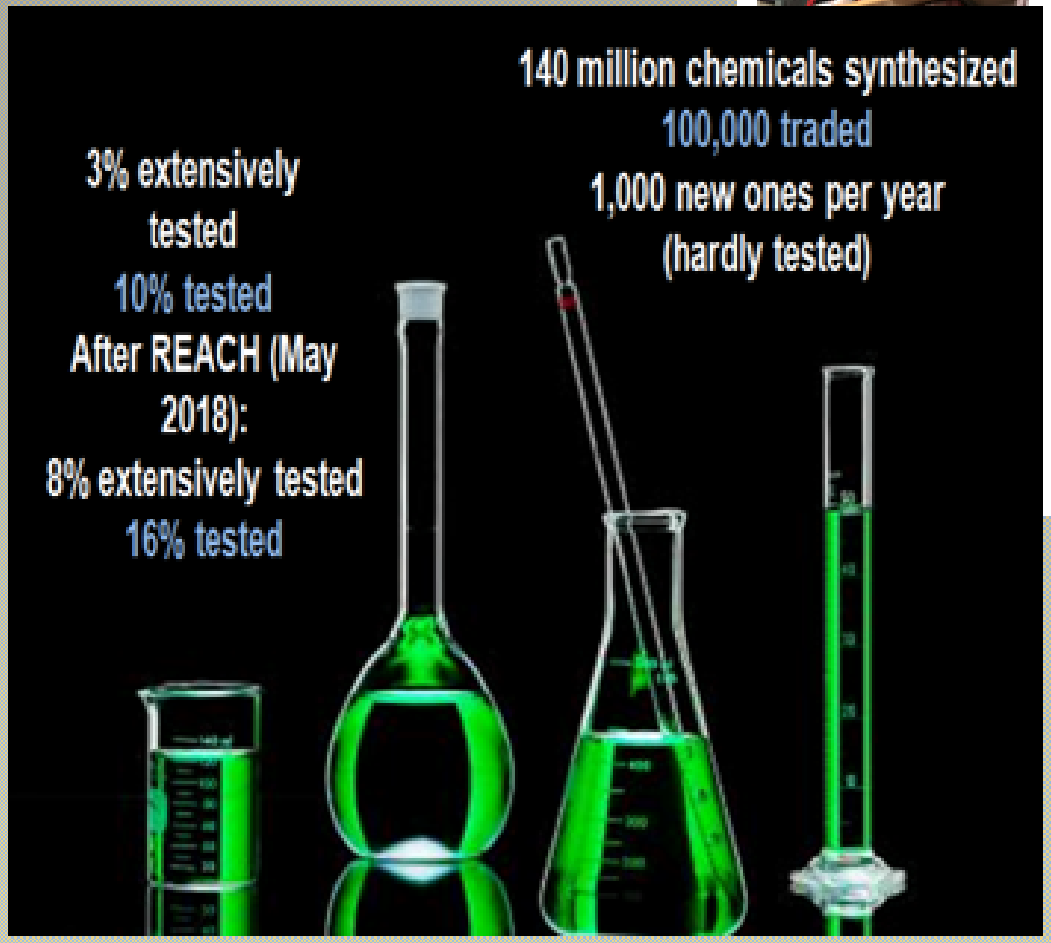



WHO, EPA, FDA, OECD +
Regulatory bodies
EC, EFSA, ECHA +
Guidance & Guidelines
Chemicals
Products
Contaminants
GMO
More+++
Legislation for testing and monitoring
Industry
Data
Risk Assessment
Data evaluation & Legislation for surveillance and authorisation
Tools
IT
Academy & Research Institutions
Risk Management
Risk Communication

Toxicology

$3 billion per year

$20 million per pesticide



Food for Thought ...

ALTEX 2018, 35:275-305

Animal Testing and its Alternatives – the Most Important Omics is Economics

Lucy Meigs [1,2], Lena Smirnova [2], Costanza Rovida [3], Marcel Leist [3] and Thomas Hartung [2,3]

Tox: $18.6 billion (14,4 *in vitro*, 4,2 *in vivo*)

40% of chemical industry is now in China
EU down from 35% to 15% in one decade

REACH 2018: only 22.000 chemicals total

7,800+ flavors in e-cigs not tested

US: 4,500 food additives with <20% public data

$1 million for a cancer study

About 5 years 20kg needed

Is Global Chemical Policy Reaching its Goals?

Benefits of an industrial chemicals management system

Increases the availability of high quality information and data on chemicals and how to use them safely

Supports the execution of the general public right to know

Improves the protection of workers and the public from chemical hazards

Improves quality of life and the environment

Supports enhanced stakeholder engagement and public participation in decision-making

saicm

UN environment

BASEL CONVENTION ROTTERDAM CONVENTION STOCKHOLM CONVENTION

Circular Economy

Strategic Approach to International Chemicals Management (SAICM)

- Adopted in 2006 in Dubai
  - supports the achievement of the 2020 goal agreed at the 2002 Johannesburg World Summit on Sustainable Development

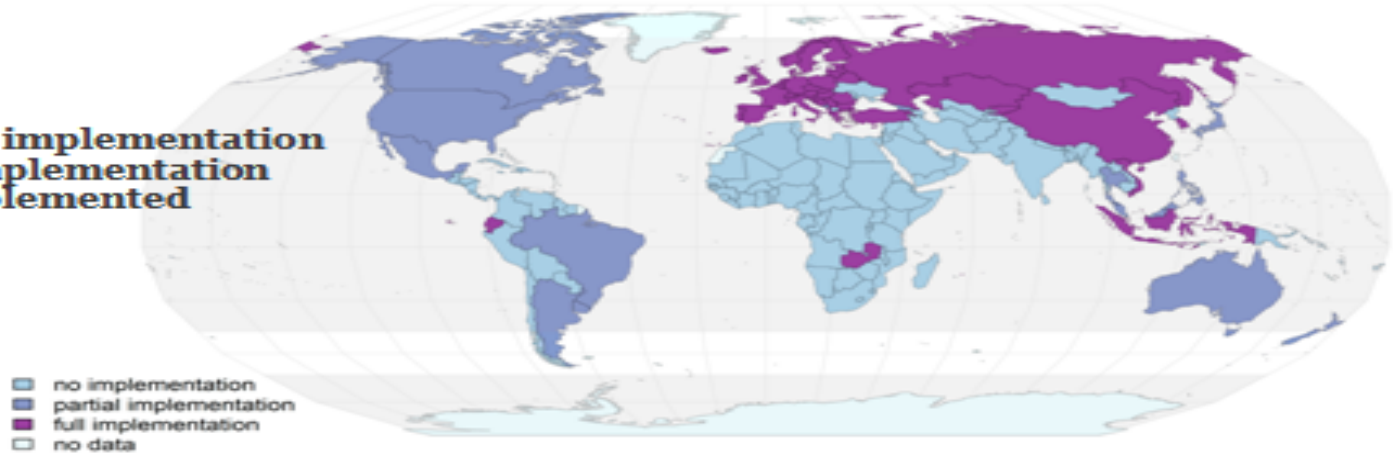
- Overall objective is the *achievement of the sound management of chemicals throughout their life cycle so that by the year 2020, chemicals are produced and used in ways that minimize significant adverse impacts on the environment and human health.*

Globally Harmonised System of Classification and Labelling

In 2002, it was agreed under the United Nations that all countries should be encouraged to implement the GHS by 2008.

As of 1 April 2017:

50 countries – full legal implementation
15 countries - partial implementation
128 countries – not implemented



Persson et al. *Sustainability* **2017**, *9*(12), 2176; https://doi.org/10.3390/su9122176

OECD

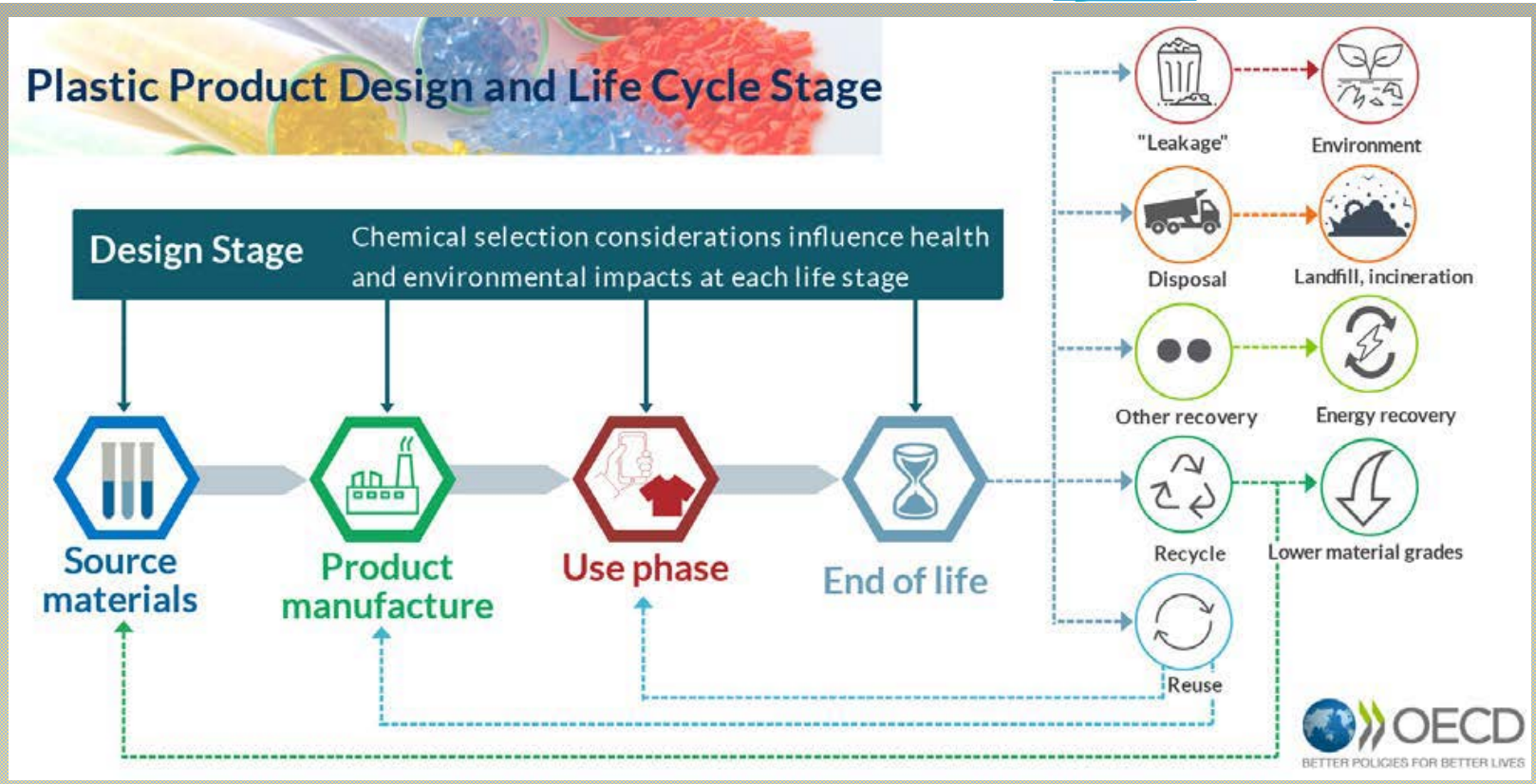
Ministry of Environment and Food of Denmark

OECD Global Forum on Environment:
Plastics in a Circular Economy

Designing sustainable plastics from a chemicals perspective

29-31 May, Copenhagen

oe.cd/plastics-forum @OECD_ENV



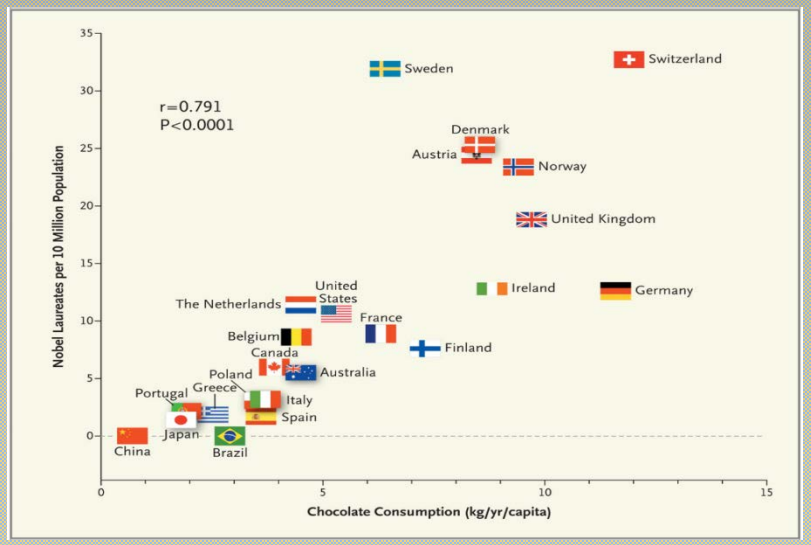
Associations related to exposures and health problems remain at the end only associations for certain cases and is not a prove for general conclusions.

Association is not Causality

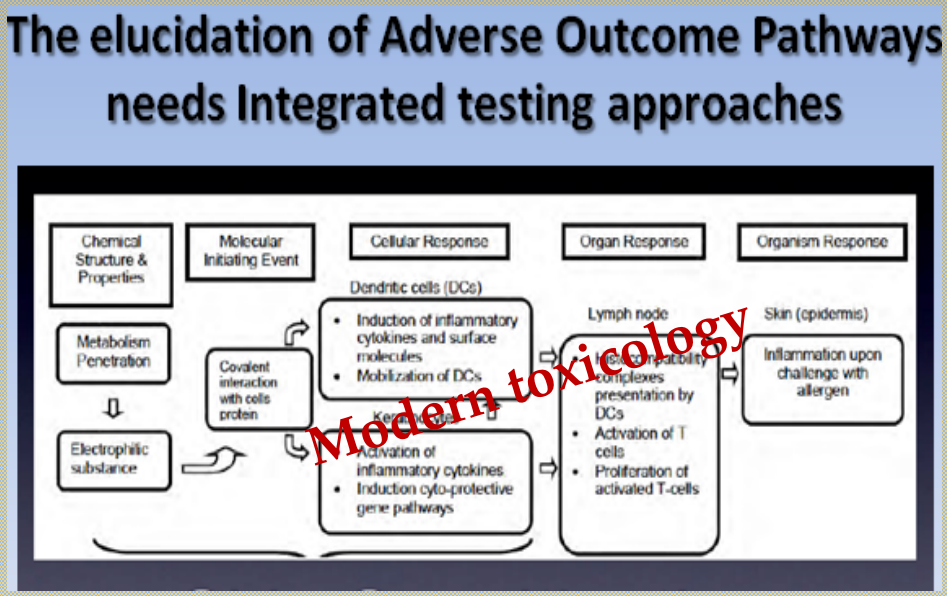
These associations are more evident in cases with strong epidemiology support and objections and restrictions are apparent .

The case of paraquat and parkinson disease in recent EFSA studies

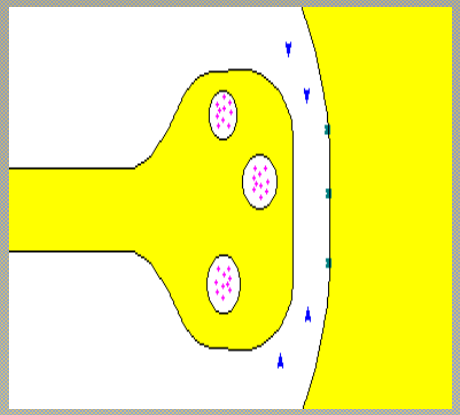
What is the value then ? Policy and Prevention and Regulatory issues, and increase of knowledge



Correlation ..Chocolate Consumption and the Number of Nobel Laureates .
Messerli (2012) New Engl J Med, 367, 1562

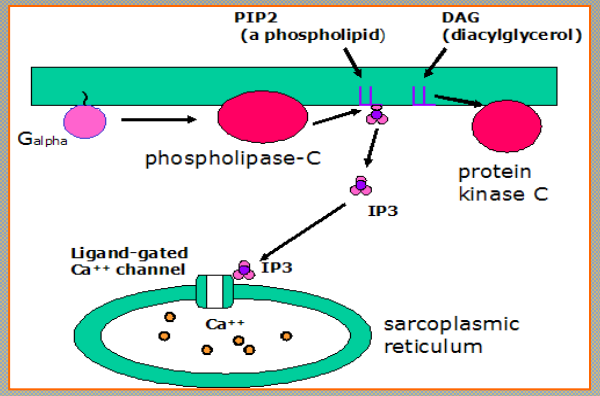


Mechanisms involved in long-term low dose exposure Paradigm for pesticides

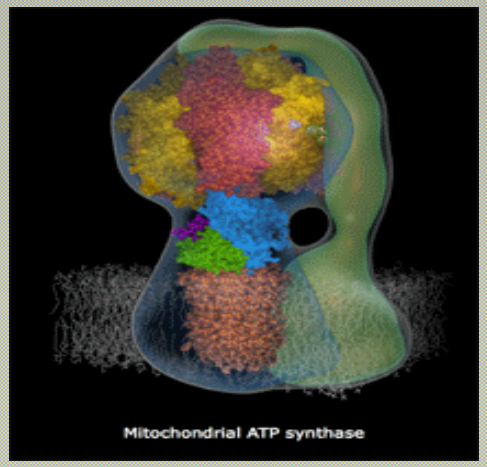


Ops, Pyrethroids and Carbamades cause cholinergic crisis

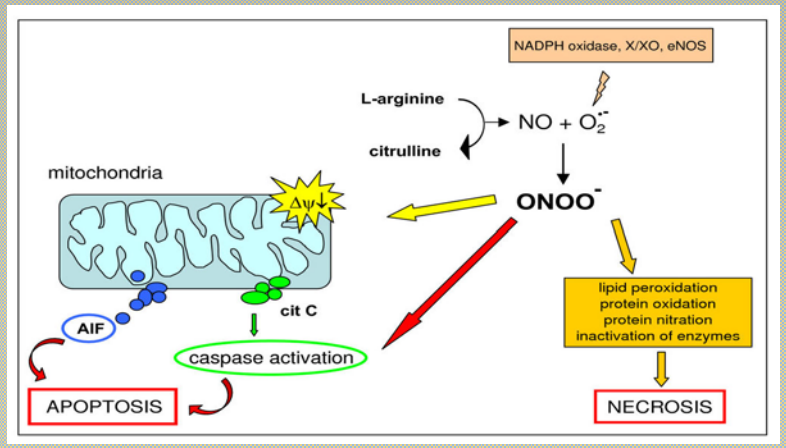
Inhibition or enhancement of enzymes, lipid peroxidation
Production of free radicals, oxidative sress
Reduction of antioxidant enzymes GHS, SOD, CAT, Gpx
Inhibition of the electron transport chain
Disturbances in transcription at the nucleus
Splitting chromatid DNA, Chromosomal damage
Apoptosis, oncogenesis, epigenetic damage, depletion of mitochondria, telomere shortening and ageing
Inhibition of protein synthesis
Disorders phosphorylation, methylation and intracellular signaling
Receptor and ion channels function's modifications



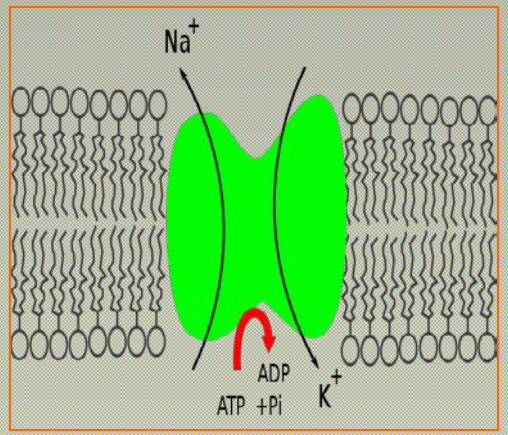
Muscarinic (Rs) secondary signaling Gps, PKC,Ca+2



Pesticides inhibit the mitochondrial energy production at mt complexes



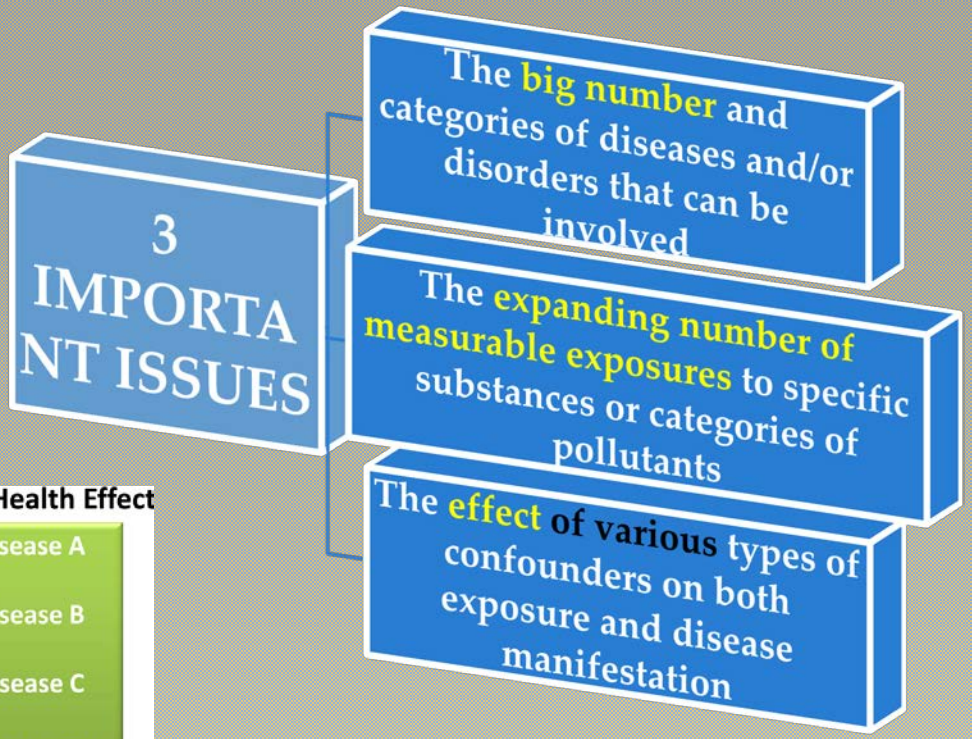
Pesticides cause oxidative stress, ROS production, apoptosis, necrosis, pimutations, ageing

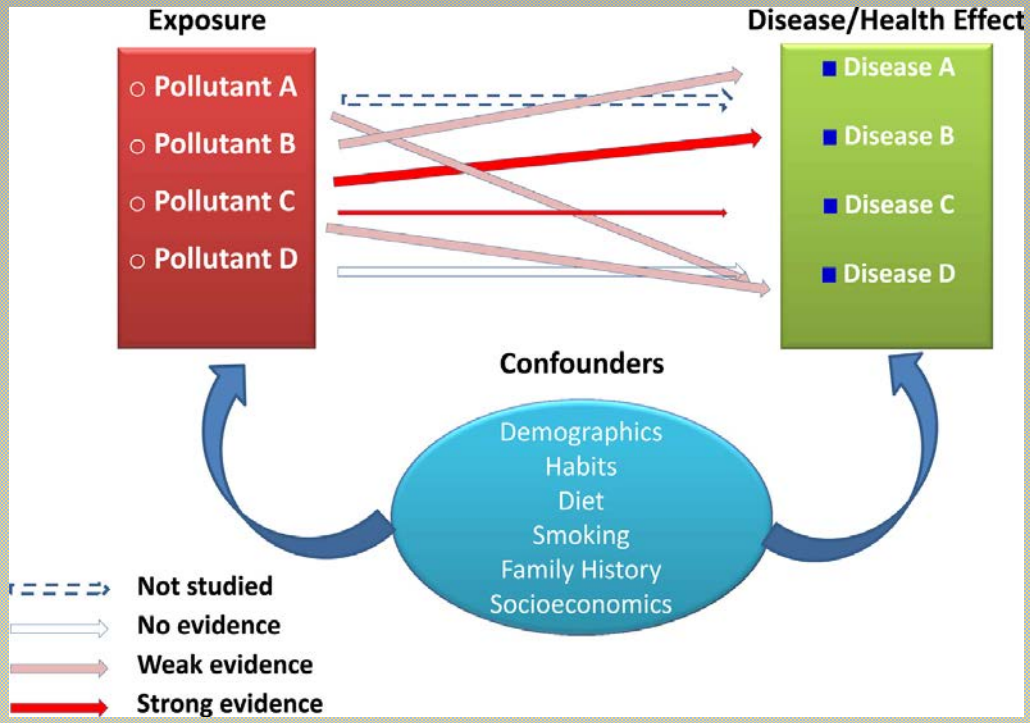


Ops and Pyrethroids inhibit Na+/K+ -ATPase

The challenge of linking diseases with exposure web of interactions

- ***The complex issue of linking exposures to diseases/health effects can be summarized underthe term "web of interactions"***

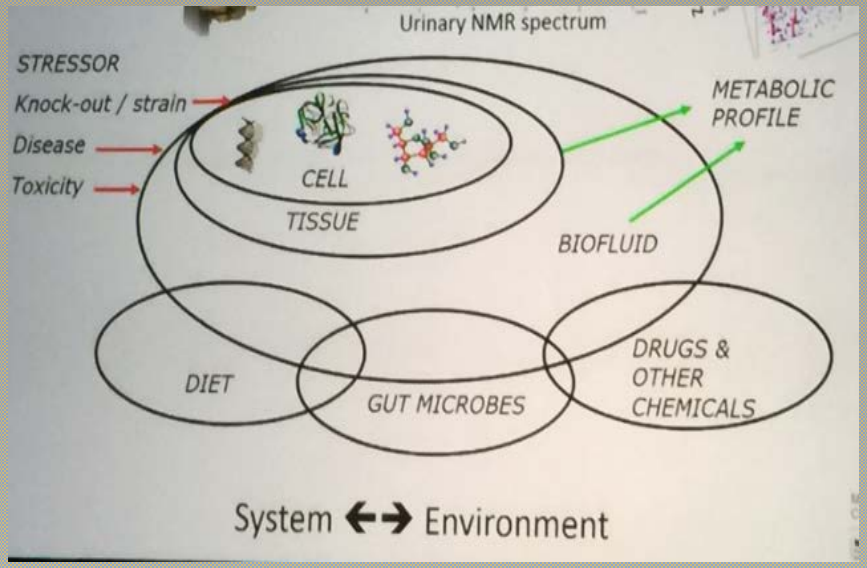




Editorial in Toxicol Letters

Environmental contaminants and target organ toxicities – new insights into old problems

Tsatsakis and Wilks



Modern toxicology

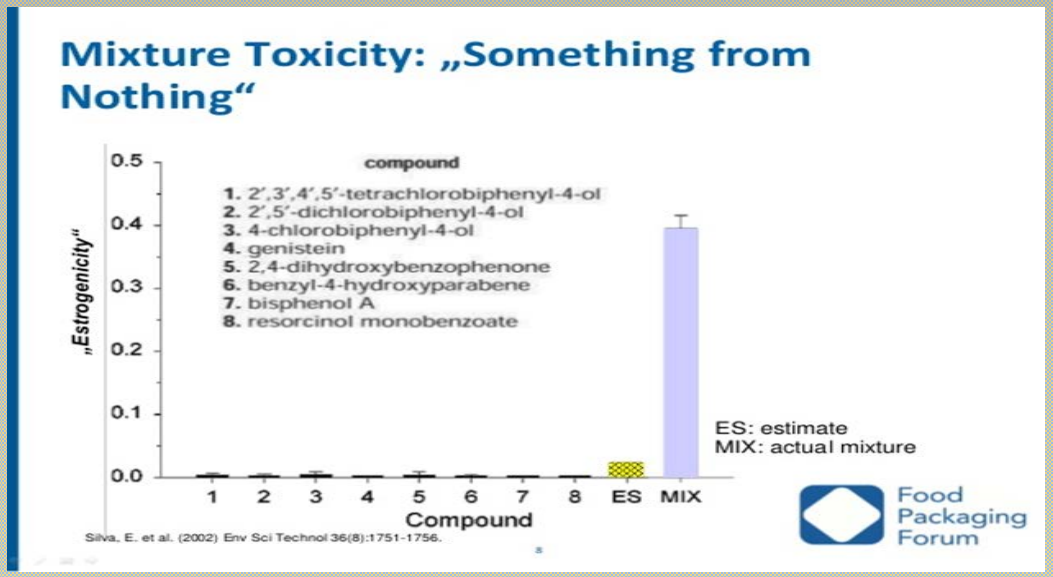
Low Combined Long Term

Health impacts from Exposures simulating real Life scenarios

Simulating real life exposures to uncover possible risks to human health: a proposed consensus for a methodological approach

- Exposure scenarios simulating real life is a complex issue since effects from multiply chemicals must be considered as a web of interactions that produce variety of mechanisms of effects and subsequently of health outcomes.
- Linear –monomodal but also nonlinear effects can be seen in the range of low and/or high concentrations of exposures

New Modern toxicology



PPPs or pesticides "any substance or mixture of substances used as insecticides, fungicides, herbicides, acaricides, rodenticides, nematicides, growth regulators, repellents, biocides to prevent, destroy or control a harmful organism ('pest') or disease, or to protect plants or plant products during production, storage and transport"
[http://ec.europa.eu/food/plant/pesticides/index_en.htm].

Food improvement agents = food additives, food enzymes and food flavouring. Additives are defined by the same EU legislation as any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value
[http://ec.europa.eu/food/safety/food_improvement_agents/additives/index_en.htm].

"Lifestyle additives" - other substances used as additives, preservatives, plasticisers, solvents, colorants that can be found in consumer related products as cosmetics, products of personal hygiene, detergents, food contact materials and medicines.

Simulating Real Life Exposures to Uncover Possible Risks to Human Health.

The present regulatory assessment disadvantage

Chronic toxicity evaluations for chemicals have been performed for a single chemical each time in order to set appropriate reference doses and regulatory limits,

Status now

such as the ADIs of additives in food and other consumer products,
the MRLs for pesticides residues in food,
the OELs to different chemicals and the DNELs (derived no-effect levels) for chemical safety assessment of chemicals under the REACH Regulation.

ECHA
EUROPEAN CHEMICALS AGENCY
BOARD OF APPEAL

REACH
Registration Evaluation Authorisation and Restriction of Chemicals

BUT

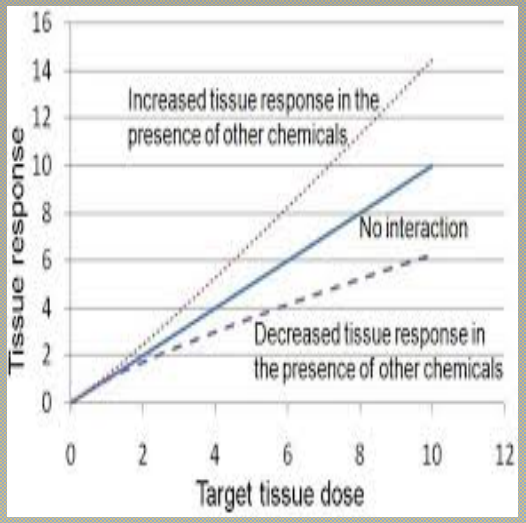
In practise the general population is never exposed to one single chemical but experiences uncontrolled multi-chemicals exposure from many different sources, at doses around or well below the regulatory limits.

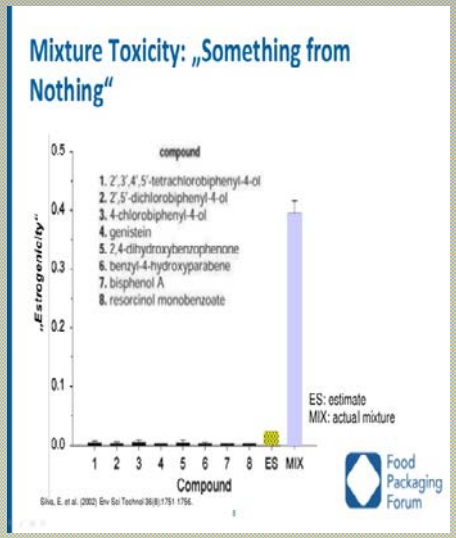
efsa
European Food Safety Authority

Limitation of toxicological studies investigating mixtures

The existing studies on mixtures disadvantages :

- **dosage schemes** proposed from the internationally recognised experimental protocols that are **too high to simulate real exposure scenarios**
- **endpoints studied - limited to** specific types of toxicity covered by the Regulations, allowing for **a gap of knowledge** for example on cardiotoxicity or neurotoxicity.
- **Long term studies** are not performed to simulate time frames for **neurodegenartive diseases**





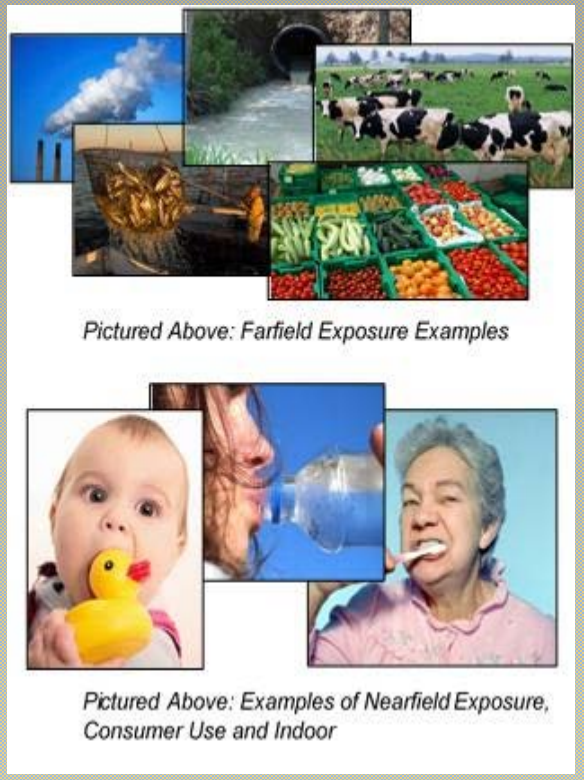
The need for a cumulative risk assessment

- Nowadays, the international scientific community as well as the international regulatory authorities **have started to realise the need for a cumulative risk assessment** and new methodologies are being developed [https://www.efsa.europa.eu/en/efsajournal/pub/3313].
- **EFSA** started to give special attention to **cumulative risks from exposure to pesticides** that produce common adverse outcomes on the same target organ/system [EFSA. **Scientific opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile.** EFSA J., 11 (2013), p. 3293].
- The lack of data from toxicological studies investigating mixtures consists one of the biggest regulatory challenges.
- **The CLP Regulation** [http://echa.europa.eu/web/guest/regulations/clp] gives the opportunity to Industry to **perform animal testing in commercial mixtures** as a last resort to prove a toxicological hazard.

Aim of the study

Our experimental methodology has the ambition to provide at one strike multi-answers to multi-questions:

- **long term toxicity study of non-commercial chemical mixtures, consisting of common everyday life chemicals (pesticides, food additives, life-style products components)**
- **at low and realistic dose levels around the regulatory limits**
- **with the simultaneous investigation of several key endpoints like genotoxicity, endocrine disruption, target organ toxicity and systemic mechanistic pathways, like oxidative stress.**



Pictured Above: Farfield Exposure Examples

Pictured Above: Examples of Nearfield Exposure, Consumer Use and Indoor

The new methodology approach high lights

Article

HET

Human and Experimental Toxicology
1–11
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DOI: 10.1177/0960327116681652
journals.sagepub.com/home/het
SAGE

Simulating real-life exposures to uncover possible risks to human health: A proposed consensus for a novel methodological approach

AM Tsatsakis[1,2,3], D Kouretas[4], MN Tzatzarakis[1], P Stivaktakis[1], K Tsarouhas[5], KS Golokhvast[2], VN Rakitskii[3], VA Tutelyan[6], AF Hernandez[7], R Rezaee[8], G Chung[9], C Fenga[10], AB Engin[11], M Neagu[12], AL Arsene[13], AO Docea[14], E Gofita[14], D Calina[15], I Taitzoglou[16], J Liesivuori[17], AW Hayes[18,19], S Gutnikov[20] and C Tsitsimpikou[21]

Food and Chemical Toxicology 96 (2016) 174–176

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Short communication

New challenges in risk assessment of chemicals when simulating real exposure scenarios; simultaneous multi-chemicals' low dose exposure

Aristidis M. Tsatsakis [a,*], Anca Oana Docea [b], Christina Tsitsimpikou [c]

[a] Department of Forensic Sciences and Toxicology, Faculty of Medicine, University of Crete, Heraklion, 71003, Greece
[b] Department of Toxicology, University of Medicine and Pharmacy, Faculty of Pharmacy, Petru Rares, 200349, Craiova, Romania
[c] Department of Hazardous Substances, Mixtures & Article, General Chemical State Laboratory of Greece, 16 An. Tsocha Str., 11521, Ampelokipi, Athens, Greece

The need for a new experimental methodology for mixture testing intending to answer to multiple questions related to health concerns after exposure to low, realistic doses raised the attention of research in the field

ELSEVIER

Toxicology Reports

Available online 20 January 2017

In Press, Corrected Proof — Note to users

toxicology reports

Open Access

Editorial

Toxicology: The basic science for human well-being and environmental health

Aristidis M. Tsatsakis, Lawrence H. Lash

Show more

http://dx.doi.org/10.1016/j.toxrep.2017.01.002

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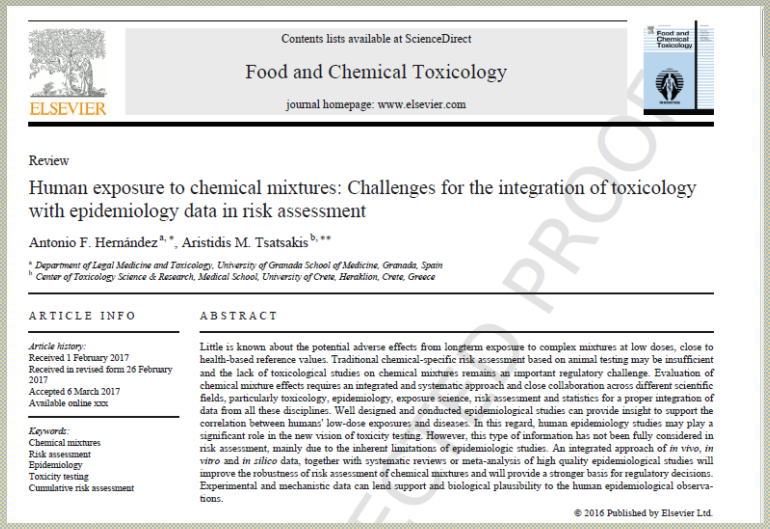

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Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com

Review

Human exposure to chemical mixtures: Challenges for the integration of toxicology with epidemiology data in risk assessment

Antonio F. Hernández [a,*], Aristidis M. Tsatsakis [b,**]

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[b] Center of Toxicology Science & Research, Medical School, University of Crete, Heraklion, Crete, Greece

ARTICLE INFO

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Received in revised form 26 February 2017
Accepted 6 March 2017
Available online xxx

Keywords:
Chemical mixtures
Risk assessment
Epidemiology
Toxicity testing
Cumulative risk assessment

ABSTRACT

Little is known about the potential adverse effects from longterm exposure to complex mixtures at low doses, close to health-based reference values. Traditional chemical-specific risk assessment based on animal testing may be insufficient and the lack of toxicological studies on chemical mixtures remains an important regulatory challenge. Evaluation of chemical mixture effects requires an integrated and systematic approach and close collaboration across different scientific fields, particularly toxicology, epidemiology, exposure science, risk assessment and statistics for a proper integration of data from all these disciplines. Well designed and conducted epidemiological studies can provide insight to support the correlation between humans' low-dose exposures and diseases. In this regard, human epidemiology studies may play a significant role in the new vision of toxicity testing. However, this type of information has not been fully considered in risk assessment, mainly due to the inherent limitations of epidemiologic studies. An integrated approach of *in vivo*, *in vitro* and *in silico* data, together with systematic reviews or meta-analysis of high quality epidemiological studies will improve the robustness of risk assessment of chemical mixtures and will provide a stronger basis for regulatory decisions. Experimental and mechanistic data can lend support and biological plausibility to the human epidemiological observations.

Long- term - low- dose exposure to pesticides mixture IN COMBINATION WITH DIETARY FOOD ADDITIVES AND LIFESTYLE ADDITIVES

ADI/5xADI/10xADI PPPs+FAs+LSAs (Methomyl+ Triadimefon+ Dimethoate+ Glyphosate+ Carbaryl+ Methyl Parathion+ Aspartame+ Benzoic Acid+ EDTA+ Ethylparaben+ Buthylparaben+ Bisphenol A)

after 9 month of exposure

Ophtalmological examination

+

Check for neurotoxic effects

3 more months of exposure

Urinalysis

Haematology and blood chemistry tests + Hormone determination in blood

Histopatological examination

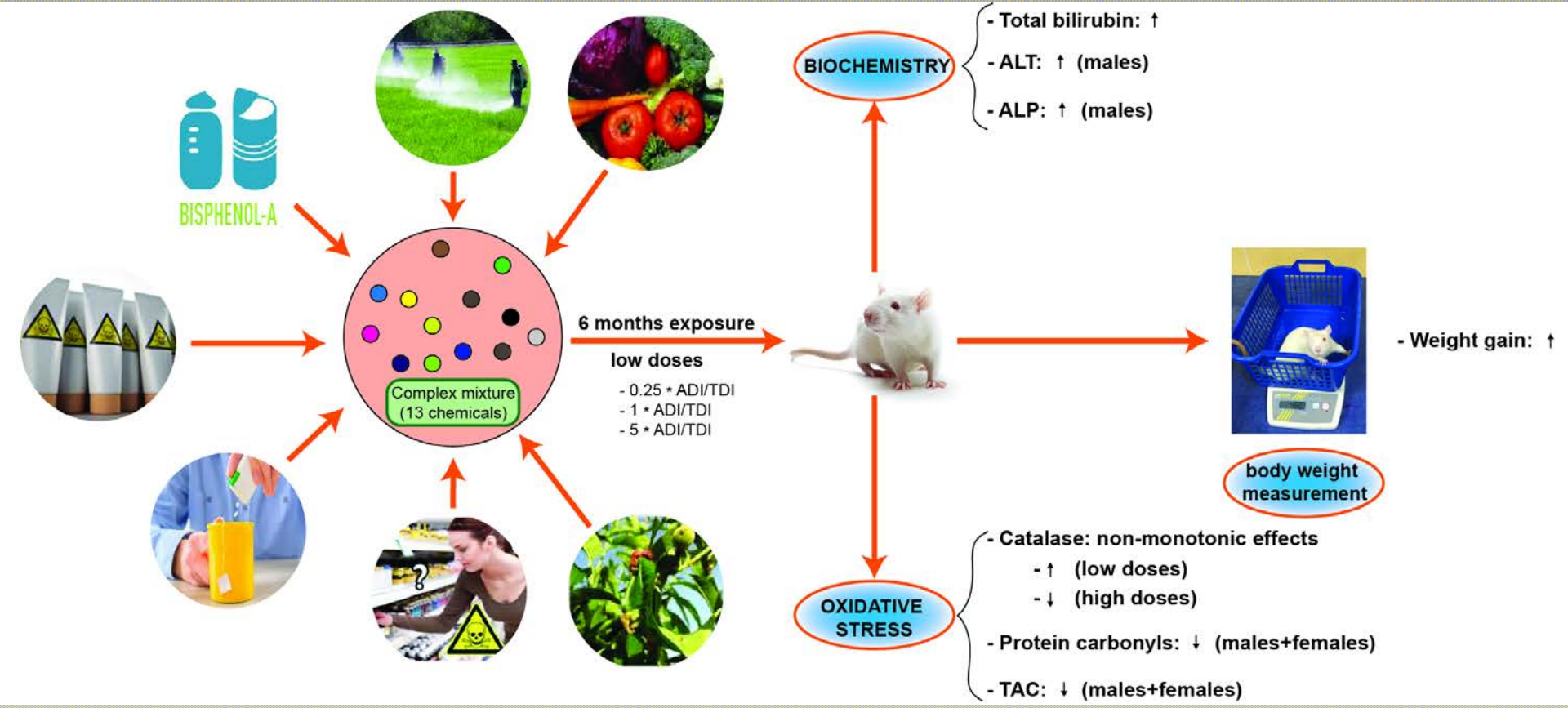
Genotoxicity test using micronucleus assay

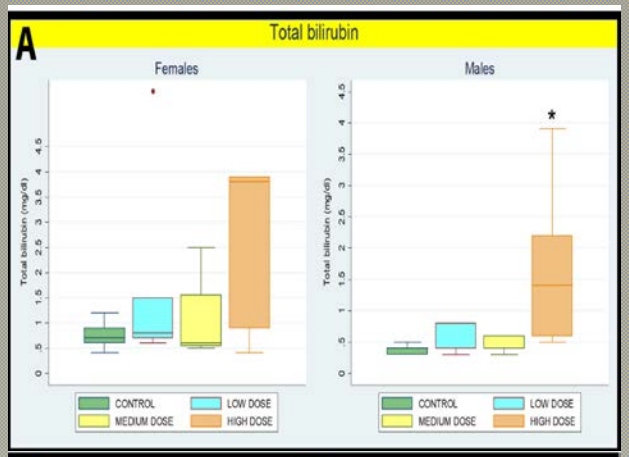
During these first 6 months of administration all the animals will be examinated once a day for signs of morbidity and mortality. Feed and water consumption will be recorded weekly.

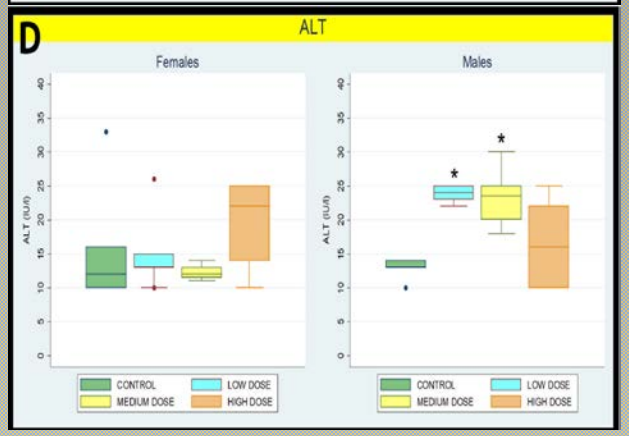
Food and Chemical Toxicology
Volume 115, May 2018, Pages 470-481

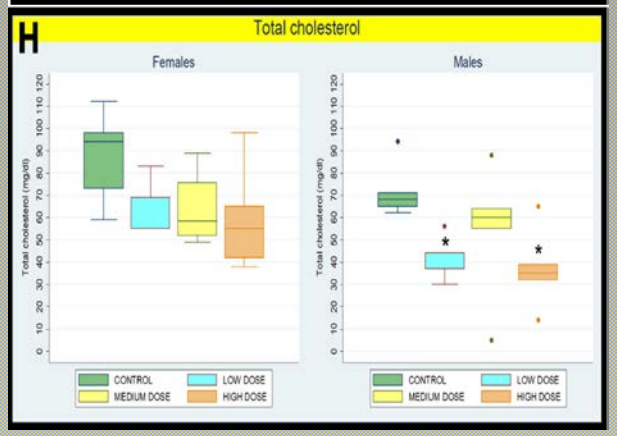
Six months exposure to a real life mixture of 13 chemicals' below individual NOAELs induced non monotonic sex-dependent biochemical and redox status changes in rats

Anca Oana Docea [a, 1], Eliza Gofita [a, 1], Marina Goumenou [b, l, 1], Daniela Calina [c, 1], Otilia Rogoveanu [d], Marius Varut [e], Cristian Olaru [f], Efthalia Kerasioti [g], Polyxeni Fountoucidou [g], Ioannis Taitzoglou [h], Ovidiu Zlatian [i], Valerii N. Rakitskii [j], Antonio F. Hernandez [k], Dimitrios Kouretas [g], Aristidis Tsatsakis [b]

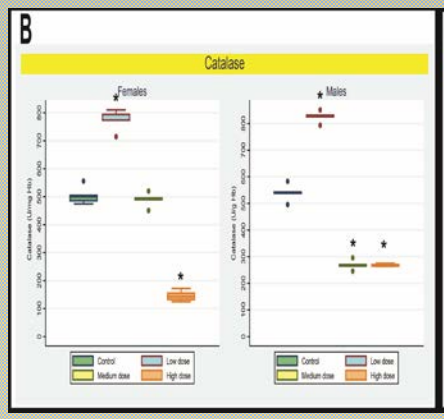


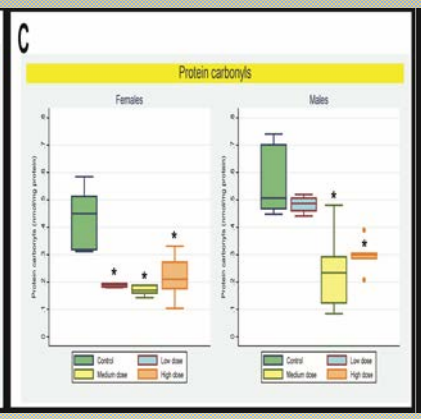


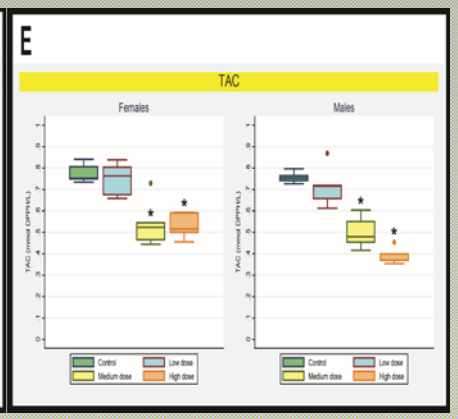




Hepatotoxic effects observed showed non-monotonic dose-response curves (Total bilirubin, ALP, ALT, tChOH).







A non-monotonic adaptive response on redox status was observed in rats exposed to low-dose chemical mixture.

Experimental location

National Institute Victor Babes, Bucuresti

Federal Research Center of Nutrition, Biotechnology and Food Safety, Moscow

Federal Scientific Center of Hygiene, F.F. Erisman, Moscow

Far Eastern Federal University, Vladivostok, Russian Federation

University of Medicine and Pharmacy, Faculty of Pharmacy, Craiova

First Experiment started in April 2016

Department of Biotechnology, Chonnam National University, Yeosu, Chonnam, Korea

University of Granada School of Medicine, Granada

University of Messina, Messina

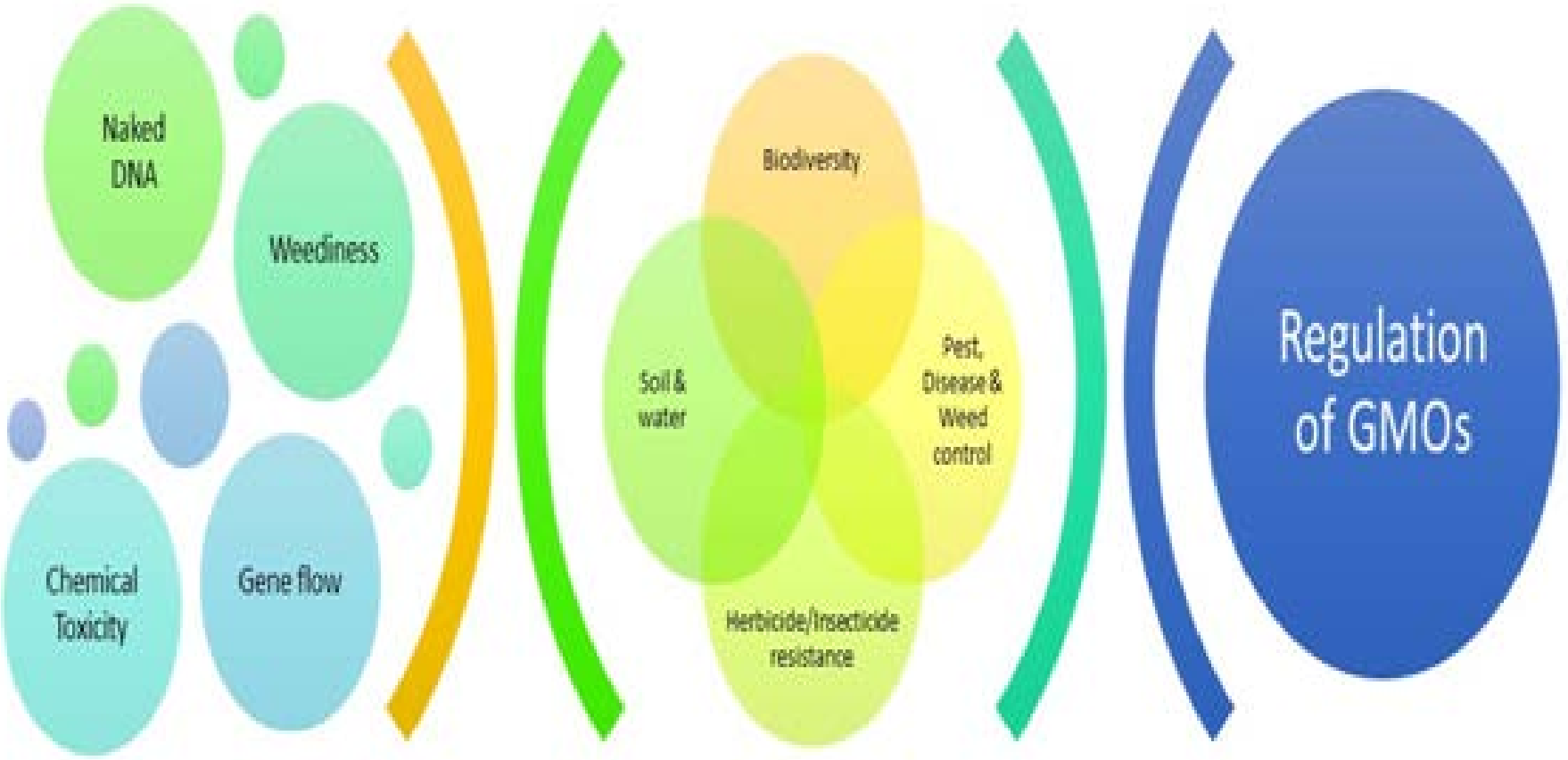
Medical School, University of Crete, Heraklion, Crete

Faculty of Pharmacy, Gazi University, Ankara

Mashhad University of Medical Sciences, Mashhad, Iran

Shandong University, Jinan, China

Environmental Implications



Direct Impacts

Indirect Impacts

Science & Politics

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Environmental impacts of genetically modified plants: A review

Aristidis M. Tsatsakis[a,b,1], Muhammad Amjad Nawaz[c,1], Demetrios Kouretas[d], Georgios Balias[e], Kai Savolainen[f], Victor A. Tutelyan[g], Kirill S. Golokhvast[b,h], Jeong Dong Lee[i], Seung Hwan Yang[c], Gyuhwa Chung[c,*]

[a] Department of Toxicology and Forensics, School of Medicine, University of Crete, Heraklion, Crete, Greece
[b] Educational Scientific Center of Nanotechnology, Far Eastern Federal University, Vladivostok 690950, Russian Federation
[c] Department of Biotechnology, Chonnam National University, Yeosu, Chonnam 59626, Republic of Korea
[d] Department of Biochemistry-Biotechnology, University of Thessaly, Larisa, Greece
[e] Harokopio University, Athens, Greece
[f] Finnish Institute of Occupational Health, POB 40 Helsinki, Finland
[g] Federal Research Centre of Nutrition, Biotechnology and Food Safety, Moscow, Russian Federation
[h] Pacific Institute of Geography, FEB RAS, Vladivostok 690041, Russian Federation
[i] School of Applied Biosciences, Kyungpook National University, Daegu 41566, Republic of Korea

ARTICLE INFO

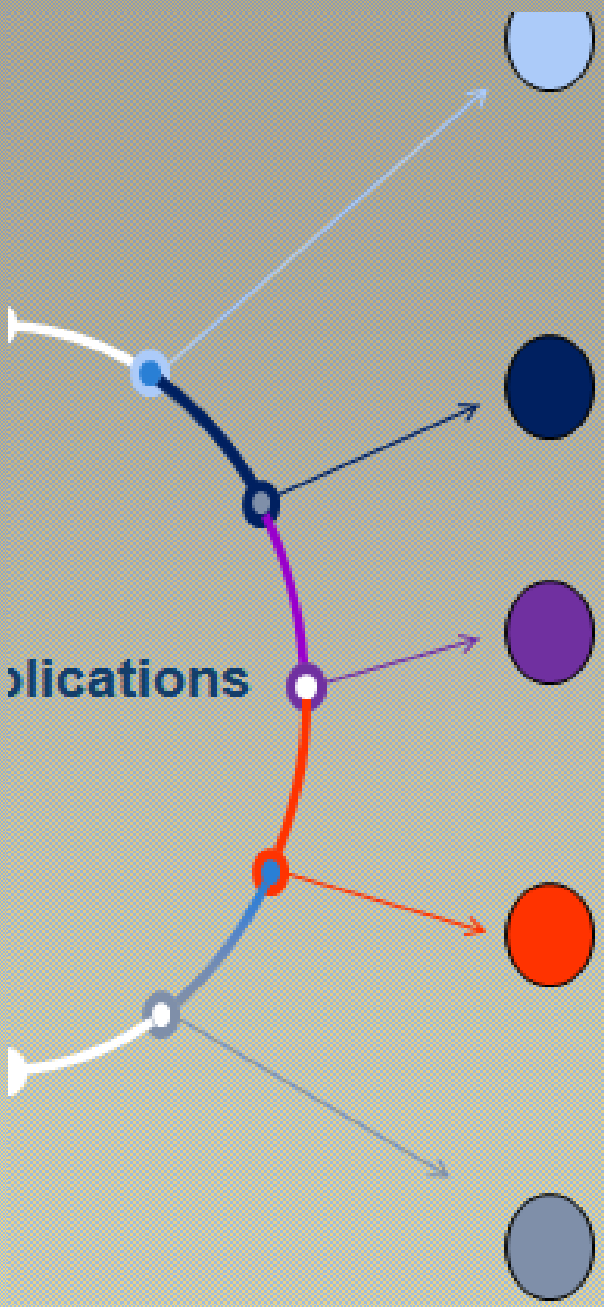
Keywords:

ABSTRACT

Powerful scientific techniques have caused dramatic expansion of genetically modified crops leading to altered

Indirect Impacts of GM

- Effect on biodiversity
- Effect on soil and water
- Reduced efficiency of pest, disease and weed control
- Evolution of insecticide and pesticide resistance
- Evolution of herbicide resistance



Gene flow

Gene flow creates reduction of differentiation between populations
Transgene x wild hybridization a possible and has been proven
Synergistic and antagonistic effects of stacked transgenes offer risks
Horizontal gene transfer can create fitness changes in the recipient organism
Weediness is potential effects of adoption of HR crops

Chemical toxicity

Natural/Novel toxins have target and/or non-target impacts on life
Herbicides have immediate or delayed adverse effects on the environmen
Bt toxins has effects tri-trophic interactions among fauna and flora

Indirect impacts of GM

Excess of Glyphosate effects soil and water
GMOs effect biodiversity
Reduced efficiency of pest, disease and weed control
Evolution of insecticide and herbicide resistance

Regulation of GMOs

EU-Comparative assessment VS US-Substantial equivalence
Authorities should consider sci. evidences as well as legitimate reasons
Chronic and sub-chronic studies have limitations

Future prospects

Evidences are not enough, case-to-case studies, large scale investigations
Complex food webs, long term exposure/full-life span for many generations

HOW EFFECTIVELY TOXICOLOGY SAVES CONSUMERS? WHY IS THIS QUESTION IMPORTANT NOWADAYS?

Concerns of increasing anthropogenic pressures to human (and environment)	Chemical mixtures (life style-new products, dietary, industry, PPPs, natural toxins), high exposure, all routes
	Electromagnetic pressure from human activities
	General pollution of seas and earth affecting consumers
	Climate changes due to anthropogenic pressures affecting consumers
New knowledge = new concerns	Endocrine disruption, low dose effects, obesity, epigenetics, etc.
Risk communication	Mainly through the media
	Consumers confusion, lack of trust

WHICH CONSUMER?

Different areas and countries means...

- Cultural differences (e.g. Asia vs Europe)
- Different regulations (e.g. Europe vs USA and among European MSs)
- Different protection goal and policies
- Different use of science (based on capacity, infrastructures, and feeling of need)

OECD

ECHA
EUROPEAN CHEMICALS AGENCY

efsa
European Food Safety Authority

European Commission

Good news!

Constant increase of global collaboration in science and regulation

ASKING WHO?

Industry

NGOs

Academy

Risk Managers

Politicians

Consumers

SOME OF THE ANSWERS..

Too much!

Too many experiments, high UFs, extreme conservatism in the exposure estimation, concerns due to risk communication from the media, etc.

Not really!

New concerns, mainly industry studies are considered, profit over science, hazards and risks overlooked, etc.

Not enough...

Poor science, old experimental protocols, many arbiter assumptions, etc.

As much as is needed / is possible...

Socioeconomic considerations, business considerations, etc.

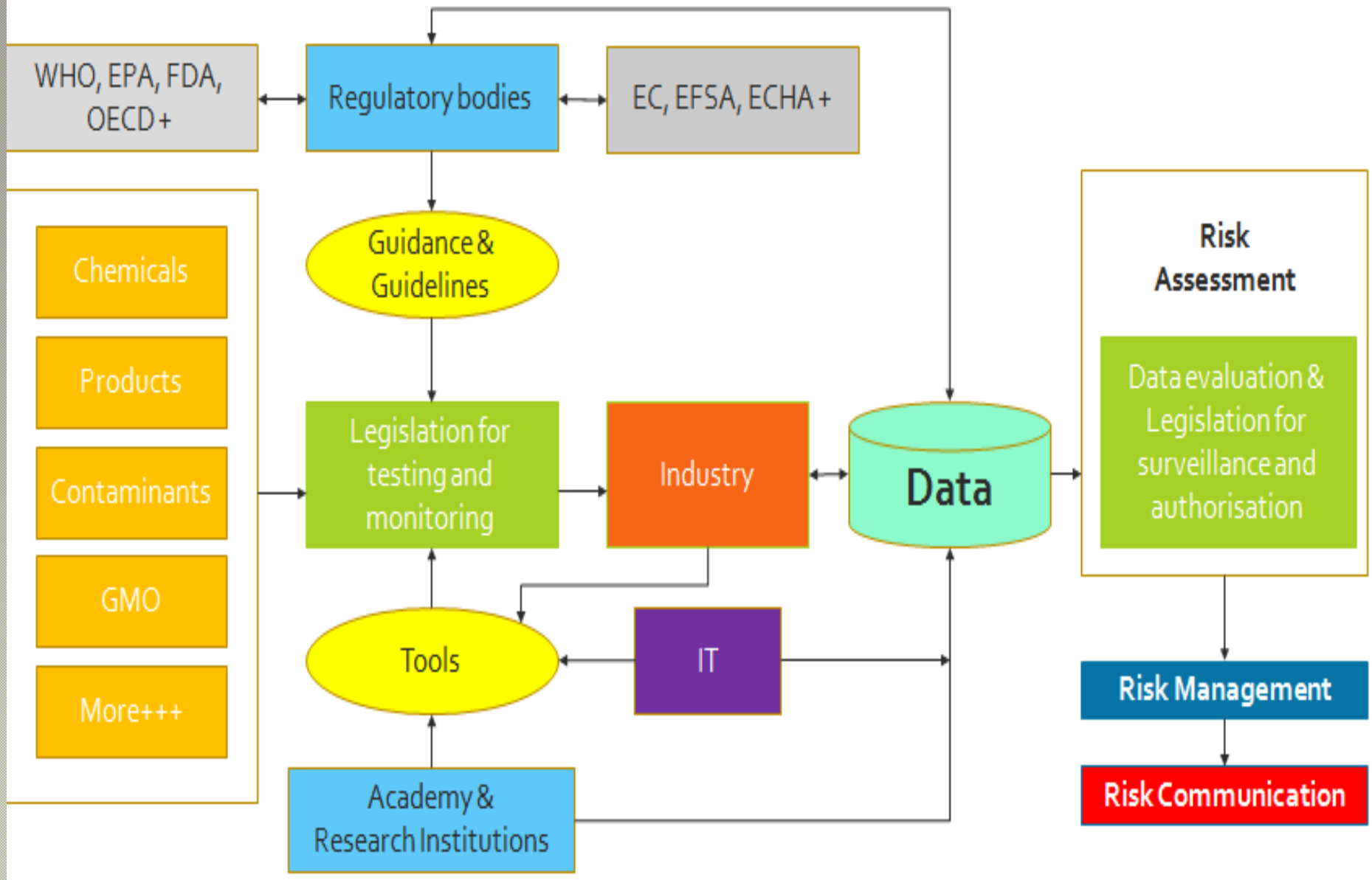
As much as society can stand...

Balance between safety and life style maintenance

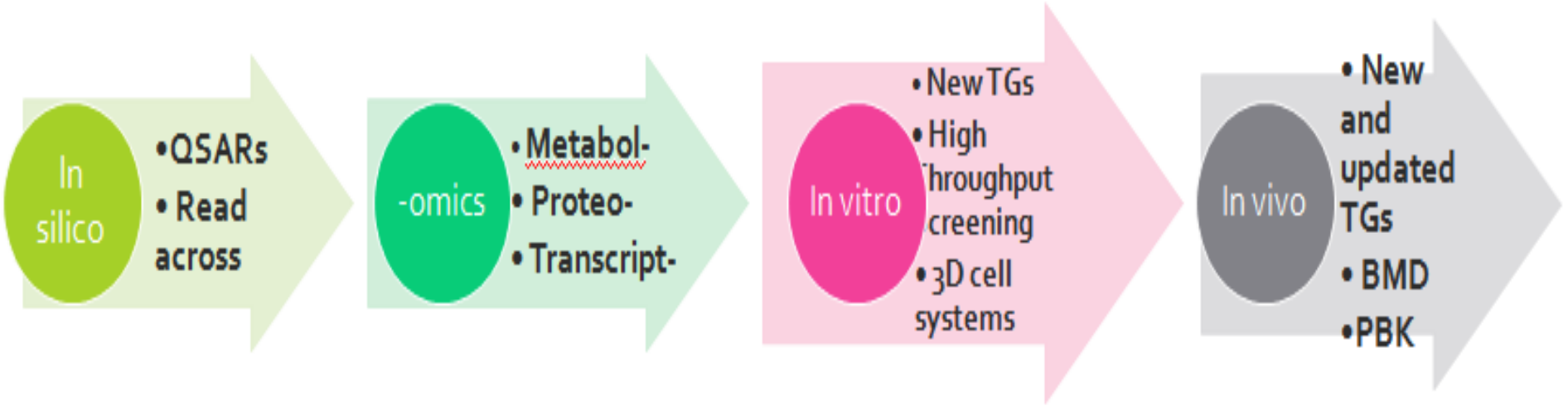
Of course not! Nobody cares!

"Did you hear the news about....!", "Don't eat...! It's full of...! I read in.... yesterday!", etc

IF I SHOULD GIVE A REPLY: MORE THAN EVER!



TOOLS



Integrated Approaches to Testing and Assessment (IATA) (e.g. AOPs/MoA)

Integrated Testing Strategies (ITS)

HOWEVER…

There are clear cases that prove that something is missing and that something can be done better

Disagreements

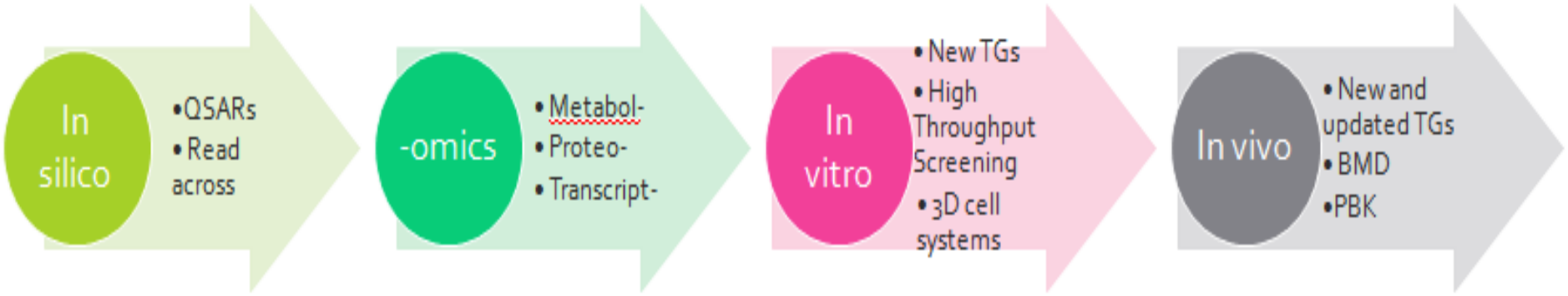
- Glyphosate: EFSA vs IARC

No or newly regulated products

- Flavourings
- Food enzymes
- Natural products

HOWEVER…

There is limited use of new tools in the regulatory context

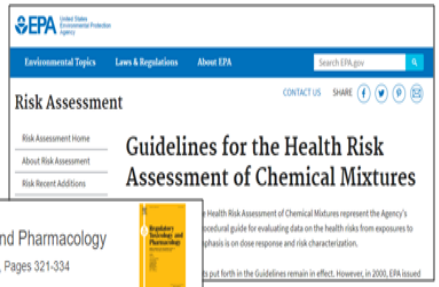


Are they used in regulatory practice?			
Limited	No	Partially	Yes
Drawbacks			
No standardised Unknown reliability	-Under development -Complexity -Specialisation	-Most protocols used are old -No clear regulatory demand	Standardised but usually only old studies are submitted. BMD and PBK in their infancy.

Integrated approaches and methodologies are under development (e.g. AOPs/MoA)

HOWEVER…

Mixtures: Progress but still no implementation



HOWEVER…

No consideration of the possibility of NMDR: EDs

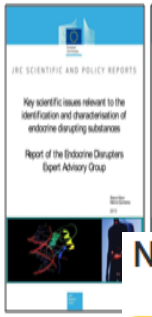
Endocrines disruption

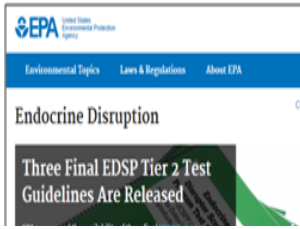
Efforts

- EC-JRC, EFSA-ECHA, EPA
- Legislation

Implementation?

- Europe: Assessment of biocides from 7 June. For pesticides, substances for which a decision is scheduled on or after 10 November 2018.
- EPA: Estrogen, androgen and thyroid hormone systems.
- **What about the rest?**





New and not so new concerns

Radiation

- EEA report for cell phones
- Phototoxicity & photomutagenicity
- Radiation + chemicals?

Epigenetics

- What we will do?

Genotoxicity

- Testing strategies

- Toxicology saves consumers better than ever
- However there is still a considerable number of known and unknown risks to be assessed
- Global collaboration is necessary
- There is no 100% safety.
- However, a desirable protection level should be set and this is a matter of will, cost, and needed time.

Trust the evidence…..but don't generalise into conclusions ! …..Think of personlised causality and intergarted approaches … AMT

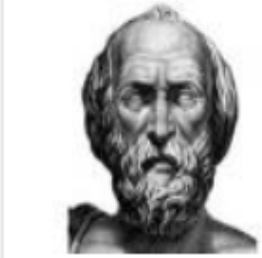
«Αρχή σοφίας, η γνώση της άγνοιας.»

Begin of Wisdom is acknowledgemen of ignorance

Pan metron ariston

Be fond of hearing rather than of talking

Be fond of learning



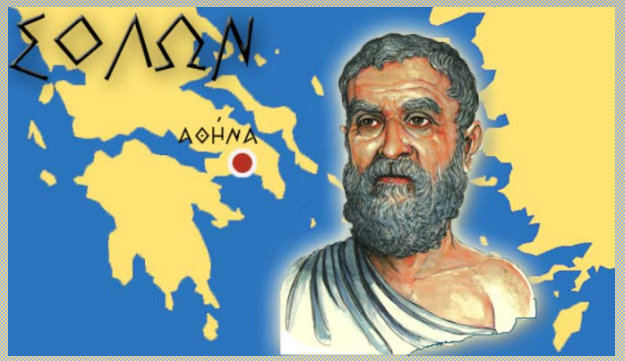
Κλεόβουλος ο Ρόδιος

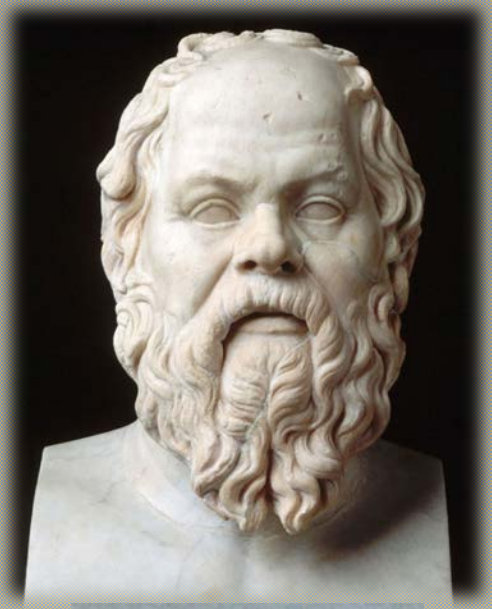
? π.Χ. – ? π.Χ.

πολιτικός και ποιητής, ένας από τους Επτά Σοφούς της Αρχαίας Ελλάδας. Έζησε τον 6ο π.Χ. αιώνα.

Γηράσκω δ' αεί πολλά διδασκόμενος

I grow old ever learning

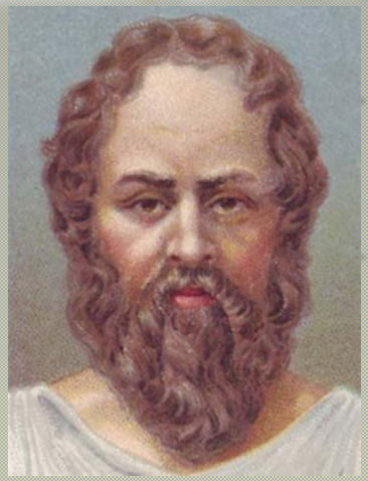




I KNOW NOTHING EXCEPT THE FACT OF MY IGNORANCE

"The only good is knowledge and the only evil is ignorance"

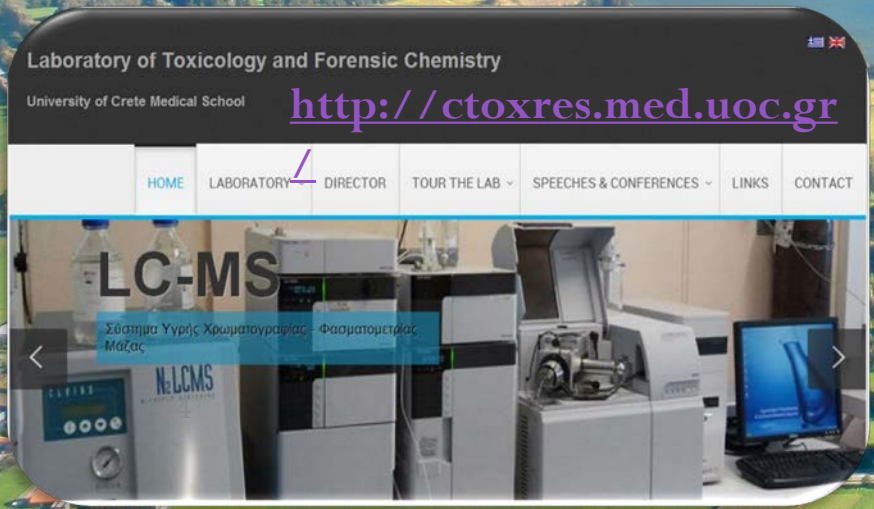
As much we increase our knowledge as more we understand that we increase the boarders with our ignorance



"I know that I know nothing"

Thank You

Support Team



Sbasibo za vnimanie

Protect Yourself !

Protect Others !

EUROTOX

iutox

Protect the Environment !