GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

20 YEARS

BfR
German Federal Institute for Risk Assessment

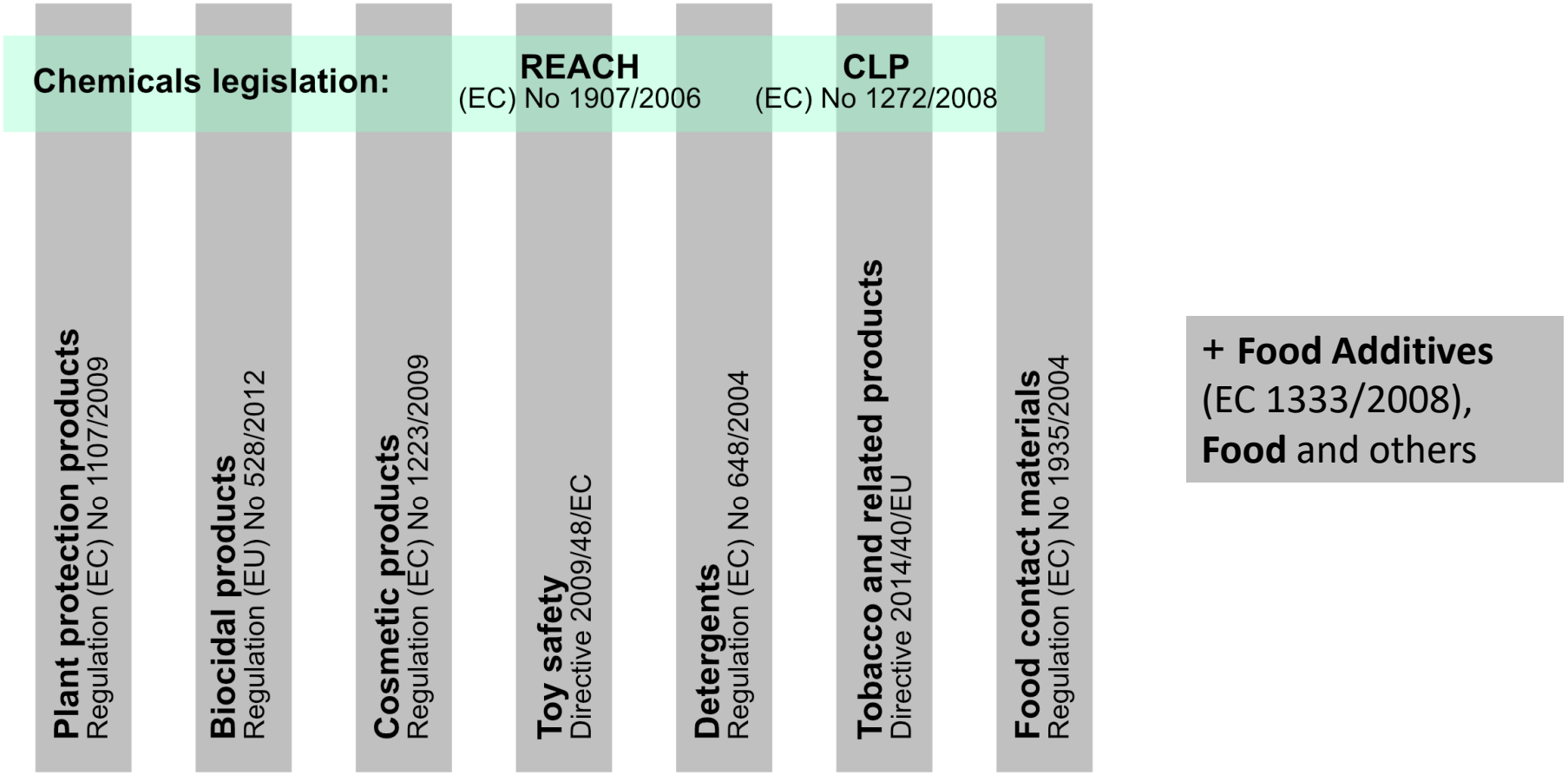
Mixture risk assessment – 20 years of toxicology and still no quick fix. Can NAMs finally provide a solution?

Dr. Tewes Tralau

Regulatory *Status Quo*: Where do we stand?

≥ 350.000 chemicals marketed worldwide, with ~ 20-30 k registered under REACH

⇒ Toxicological assessments, data requirements and regulatory measures usually are subject to separate legal “silos“.



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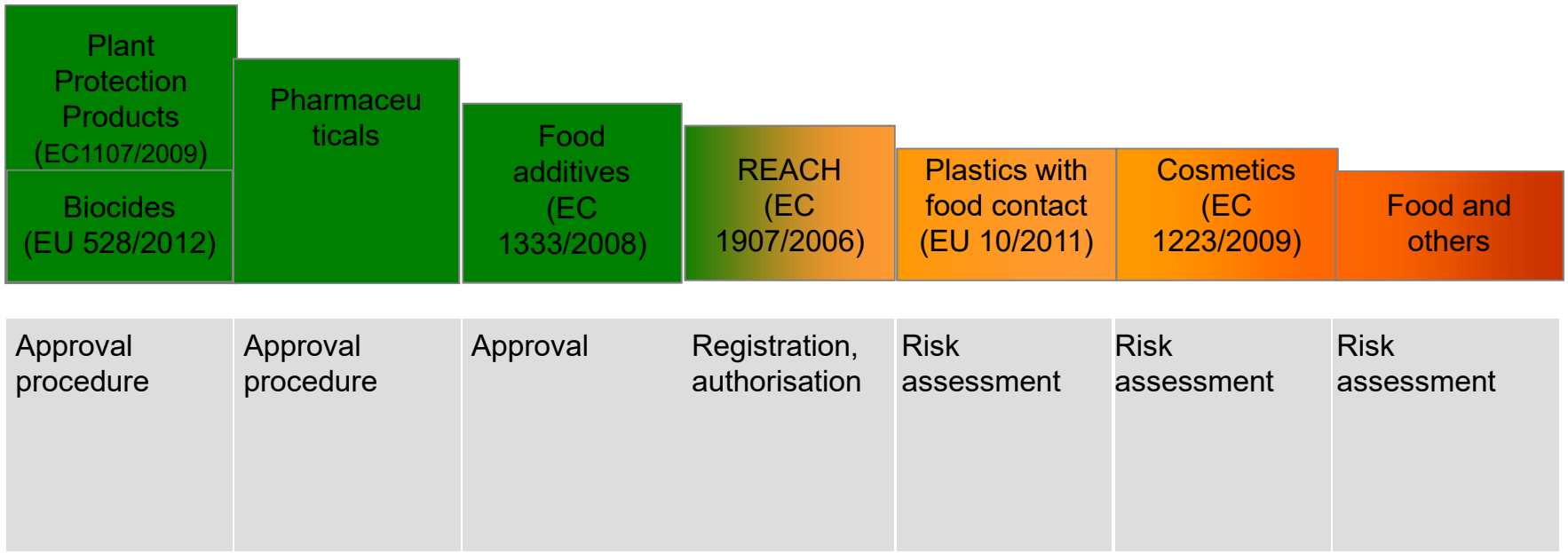

⇒ In terms of legal procedures separation into silos warrants a reliable and sound regulation. However, this comes at the price of some regulatory gap because exposures across frameworks are only accommodated to a (very) limited extent as are possible mixture effects.

Sketch courtesy of Prof. Dr. B. Schäfer & Dr. P. Marx-Stölting

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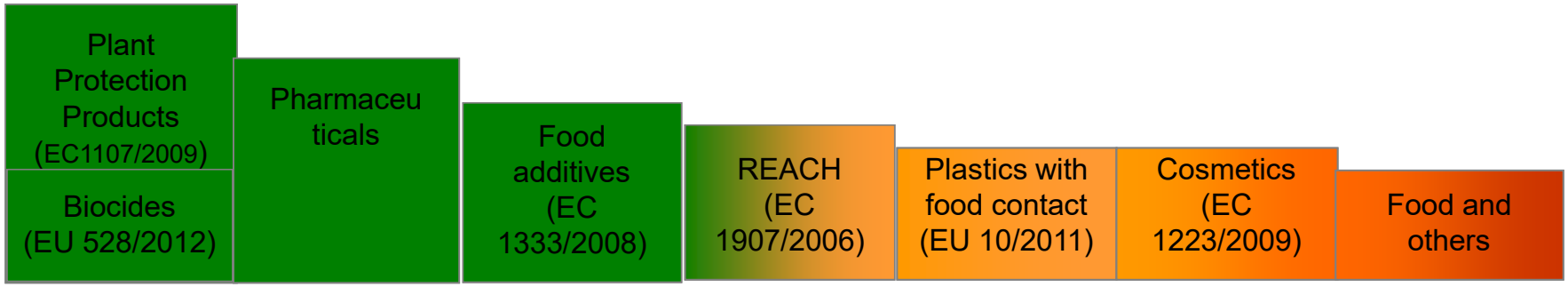

Approval procedure	Approval procedure	Approval	Registration, authorisation	Risk assessment	Risk assessment	Risk assessment

Assessment of potential mixture effects?

(✓)	(✓)	×	×	(✓)	×	×

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Plant Protection Products (EC1107/2009) / Biocides (EU 528/2012)	Pharmaceuticals	Food additives (EC 1333/2008)	REACH (EC 1907/2006)	Plastics with food contact (EU 10/2011)	Cosmetics (EC 1223/2009)	Food and others
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Except for food contact materials assessments remain restricted to their respective silos.

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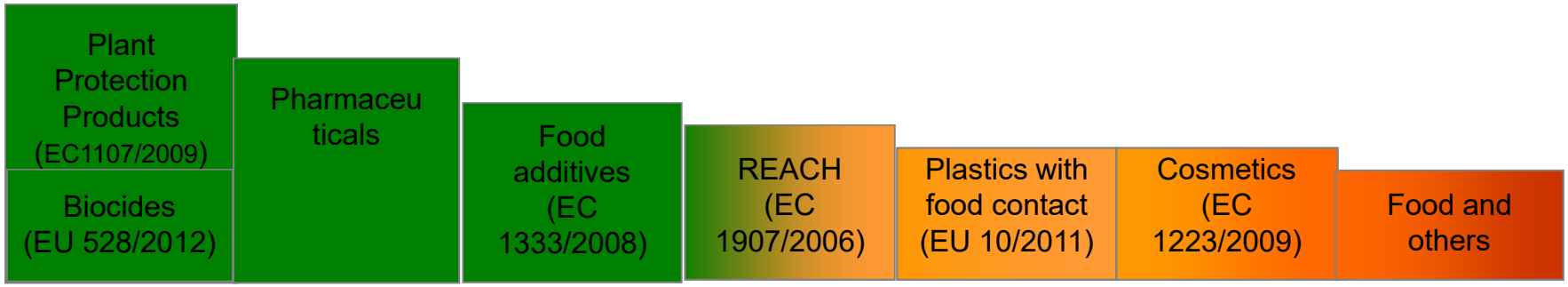


Assessment of potential mixture effects?

(✓)	(✓)	×	×	(✓) *	×	×

Except for food contact materials assessments remain restricted to their respective silos.

⇒ a) Is this a problem?
b) If so, how big/pressing is it?
c) Can we adequately deal with it?

Is it a problem?

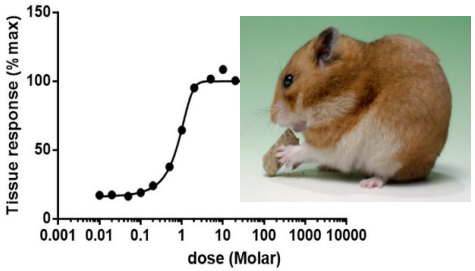
- The possibility of mixture effects has toxicologically been known for some time now.

- For such effects to occur **several pre-conditions have to be met**. That is, **co-exposure to the substances in question in relevant doses** (exceeding the potential for detoxification) and **action on pathways affecting the same endpoint**. Depending on the underlying mechanism and the pathways affected effects can either be additive, antagonistic or synergistic.

- With regard to human health **current data do not indicate mixture toxicity to be an imminent health concern**. Neither with regard to chemicals, nor to food.

- Uncertainties remain to a certain extent for chronic endpoints as well as for prolonged exposures. While there is, again, no significant indication for a general concern, there is **also not enough data to conclude the risk to be negligible in all cases**.

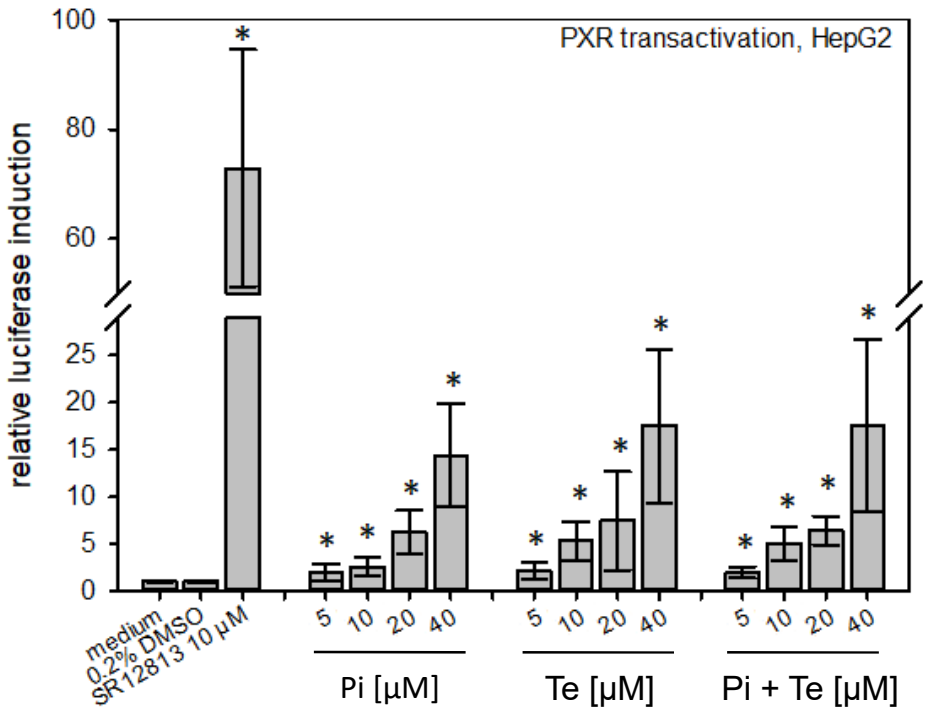
⇒ Thus there **is some need to evaluate more closely for which cases mixture toxicity might be a problem** and how to address this.



How big of a problem is it?

- For mixture toxicity to occur substances need to affect the same toxicological endpoint. This can either occur by a similar mode of action (MoA) or by a dissimilar MoA. In the first case effects will usually be additive, in the second they can also be synergistic.

- Most known mixture effects are additive (example below) or antagonistic. Synergism occurs less frequently and is usually restricted to a fold-range of 2 ≤ 100.



Triazole fungicides (propiconazole (Pi); tebuconazole (Te) tested for individual and combined effects on liver toxicity-related endpoints.

Both substances are PXR agonists, with the mixture suggesting additive effects on PXR activation.

Shown is dose-dependent induction of a luciferase reporter based on a fusion protein of GAL4 with the ligand binding domain of human PXR. n
Data shown are from 3 independent experiments with $p < 0.05$ indicated by asterisks.

Knebel C. *et al.* (2018), Toxicol. Sci. 163 (1):170–81.

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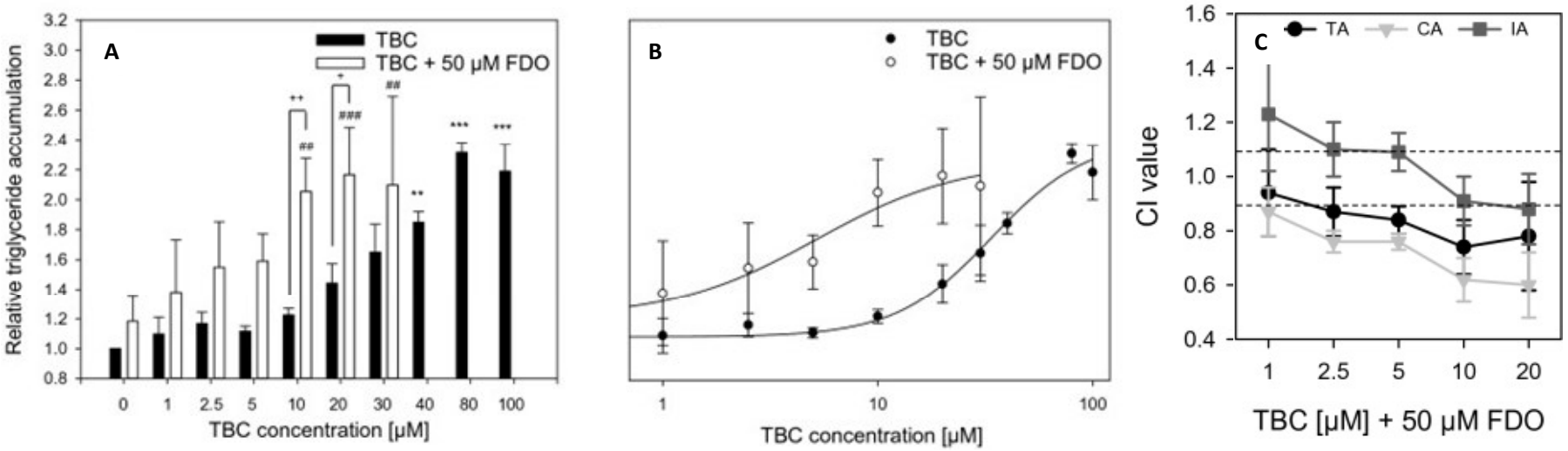


Triglyceride accumulation in HepaRG cells induced by tebuconazole (TBC) in absence or presence of 50 µM fludioxonil (FDO). Shown is the relative accumulation of intracellular triglycerides as determined with AdipoRed after 72 h of incubation (**A**; $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$), the corresponding dose response curves as fitted for calculation of the EC_{50} (**B**) and the combination indices (CI) calculated based on the assumptions of technical additivity (TA), concentration additivity (CA) or independent action (IA), respectively (**C**). CI < 0.9 indicates synergism, CI ≈ 1 dose addition, and CI > 1.1 antagonism.

Lasch A. *et al.* (2021), Arch. Toxicol. 95 (4):1397-411.

How big of a problem is it?

Some additional facts to consider:

a) Additivity

For additive mixtures **most** of the observed **mixture effects are subject to so-called "drivers".** That is, the overall effect is "driven" by a limited number of identifiable substances.

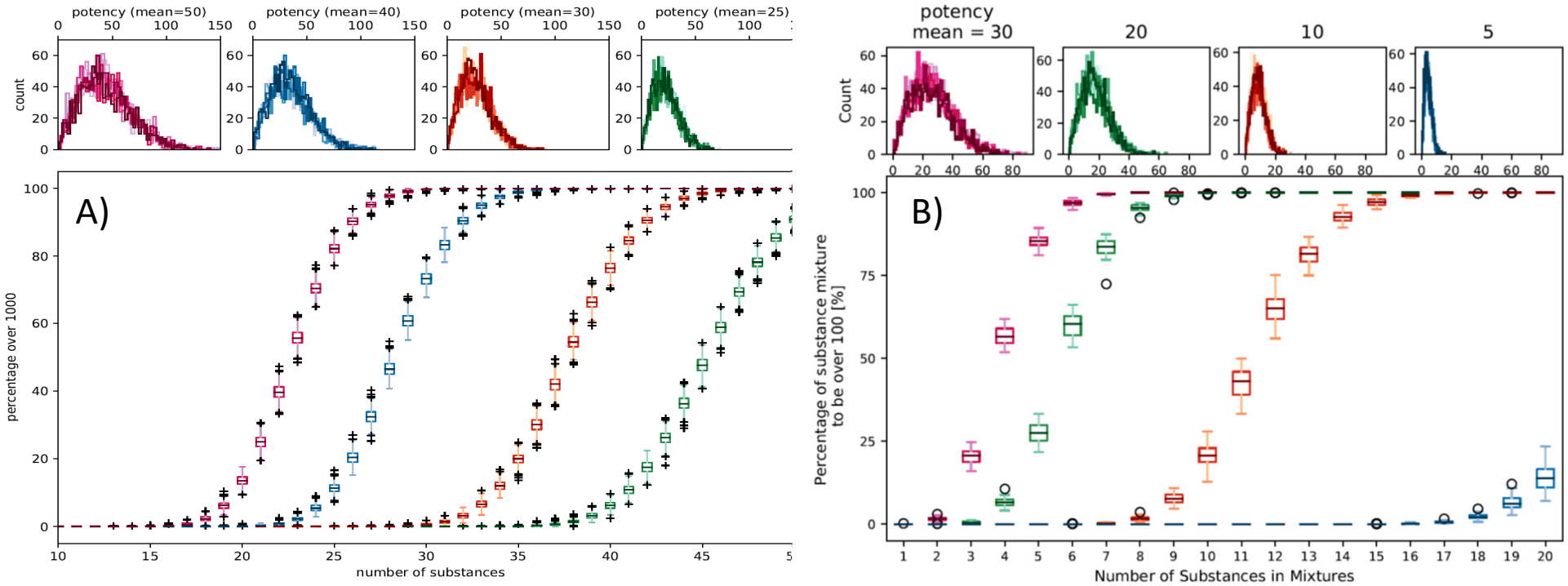
b) Synergism

Quoted examples for synergism often refer to concentrations close to the no observed adverse effect level (NOAEL). However, the NOAEL usually relates to systemic effects rather than underlying biomarkers. This increases the risk of overestimation and false allocation due to the so called „gate-keeper effect".

Moreover, the more constituents a mixture harbours, the less relevant synergism becomes as more sensitive endpoints and/or additive "drivers" start to dominate.

⇒ ***In most cases of established mixture toxicity the assumption of additivity has hence proven to be sufficiently conservative.*** *The established safety factors for species transferability add to this by providing additional mitigation.*

How big of a problem is it?



Simulation of effect occurrence for n substances along Weibull distributed potency ranges from 1 to 100. Upper panels show exemplary Weibull distributions for potency distributions as indicated using a form factor of 2 and n = 1000, respectively. The lower panel shows the percentage of incidences for which the corresponding cumulative potency of a mixture of n substances would exceed safety factors of 1000 (A) or 100 (B), respectively. Plotted are 500 drawings of 1000 random distributions each.

Factor 1000: 30 % effect-probability requires 21 - 36 substances with a potency range of 30 - 50.
Factor 100: 30 % effect-probability requires 3 – 11 substances with a potency range of 10 - 30.

⇒ ***Mind – most potencies tend to be ≤ 10! Together with the need for concomitant exposure the probability of occurrence for such effects thus would be deemed small.***

Mixture toxicity - how to deal with it?

While the problem of mixture toxicity as such is most likely limited in extent and remit there is a need addressing this issue in the face of exposure to an ever increasing number of chemicals.

- With mixture toxicity being subject to the preconditions of sufficiently high co-exposure and action on the same endpoint **the main objective should be the identification of cases where current assessment practices fail to notice critical co-exposures, hitherto unrecognised effect interactions and effect drivers.**

⇒ Given that unintentional exposure occurs across regulatory silos, **silo-specific solutions are not fit for the job** as they will increase regulatory inconsistencies and conceptual incompatibilities. For example, out of 428 active substances listed for plant protection in 2016, 38 and 55 were also registered for biocidal use or under REACH, respectively.

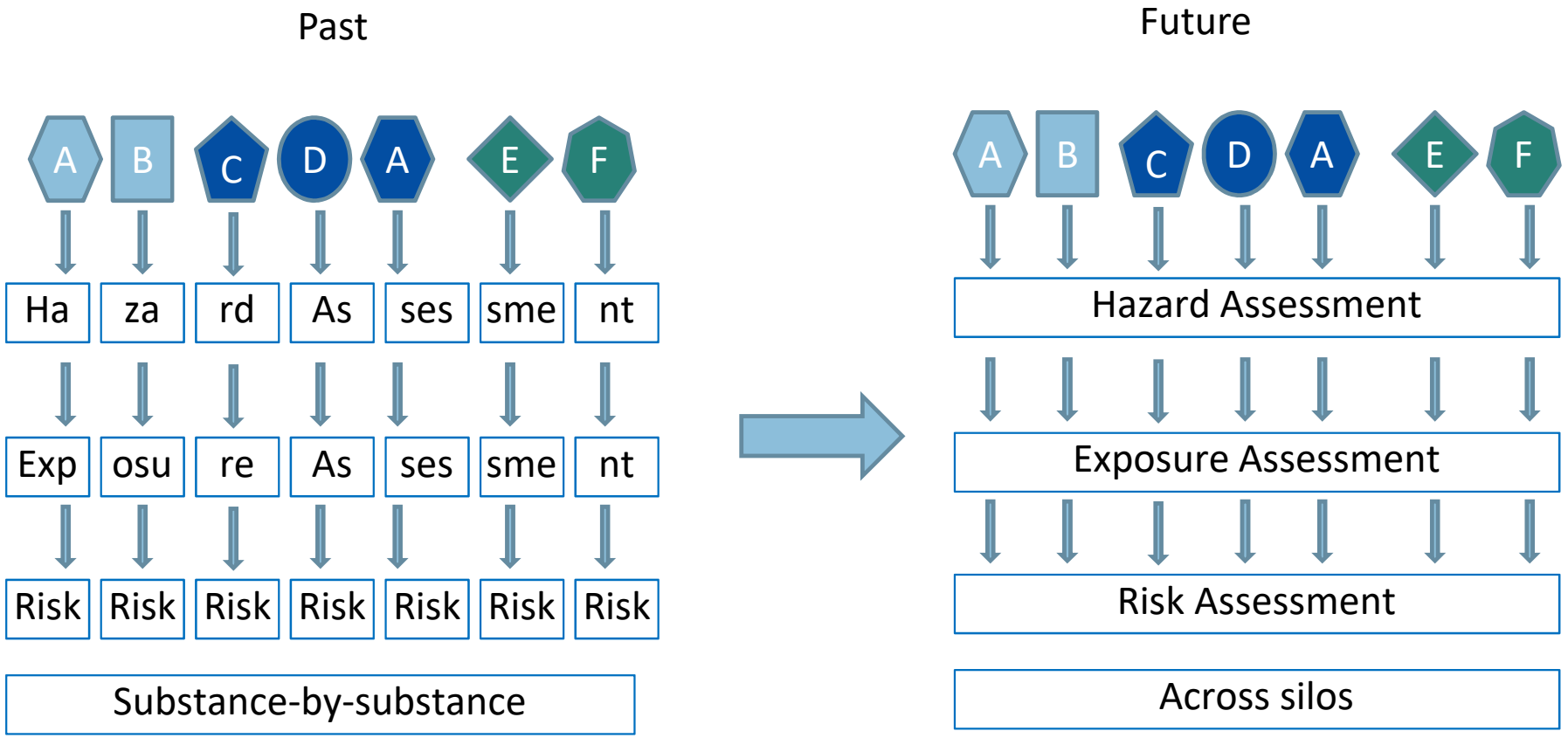
⇒ **Generic solutions are subject to the same limitations.** Tentatively easy to implement they too are subject silo-“blindness“ and hence issues of compatibility. What is more, they tend to manifest the silo-specific *status quo,* effectively impeding any future adjustments across regulations.

⇒ **Need for data driven approaches**

I; based on exposure;

II; and the potential of increased hazard due to combination effects.

The aim: “one substance – one assessment”



⇒ **Available options differ significantly for data rich or data poor substances. For the first mixture assessments are already performed, for the latter this is more challenging.**

Sketch courtesy of Dr. P. Marx-Stölting

Exposure – to asses or not to asses

Data rich compounds

Comprehensive data on hazard

Comprehensive data on exposure

E.g., active substances in plant protection products or biocides, high production volume chemicals under REACH

-> proceed with CAGs (cumulative assessment groups)

Data poor compounds

Limited or no data on hazard

Limited or no data on exposure

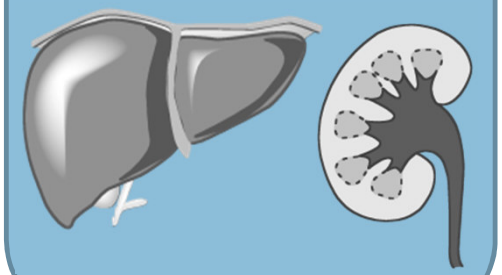
E.g., many contaminants low production volume chemicals under REACH

-> proceed with NAMs (new approach methodologies

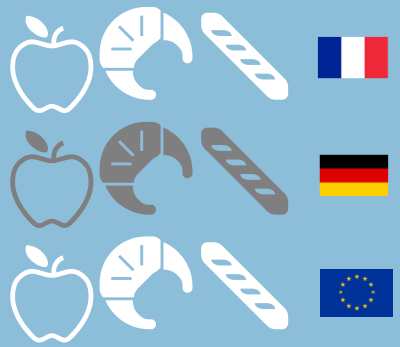
Sketch courtesy of Dr. P. Marx-Stölting

Data rich substances – assessment options in the land of plenty

Hazard:
Target organs and effects known, sort accordingly



Exposure 1:
Monitoring data (food)

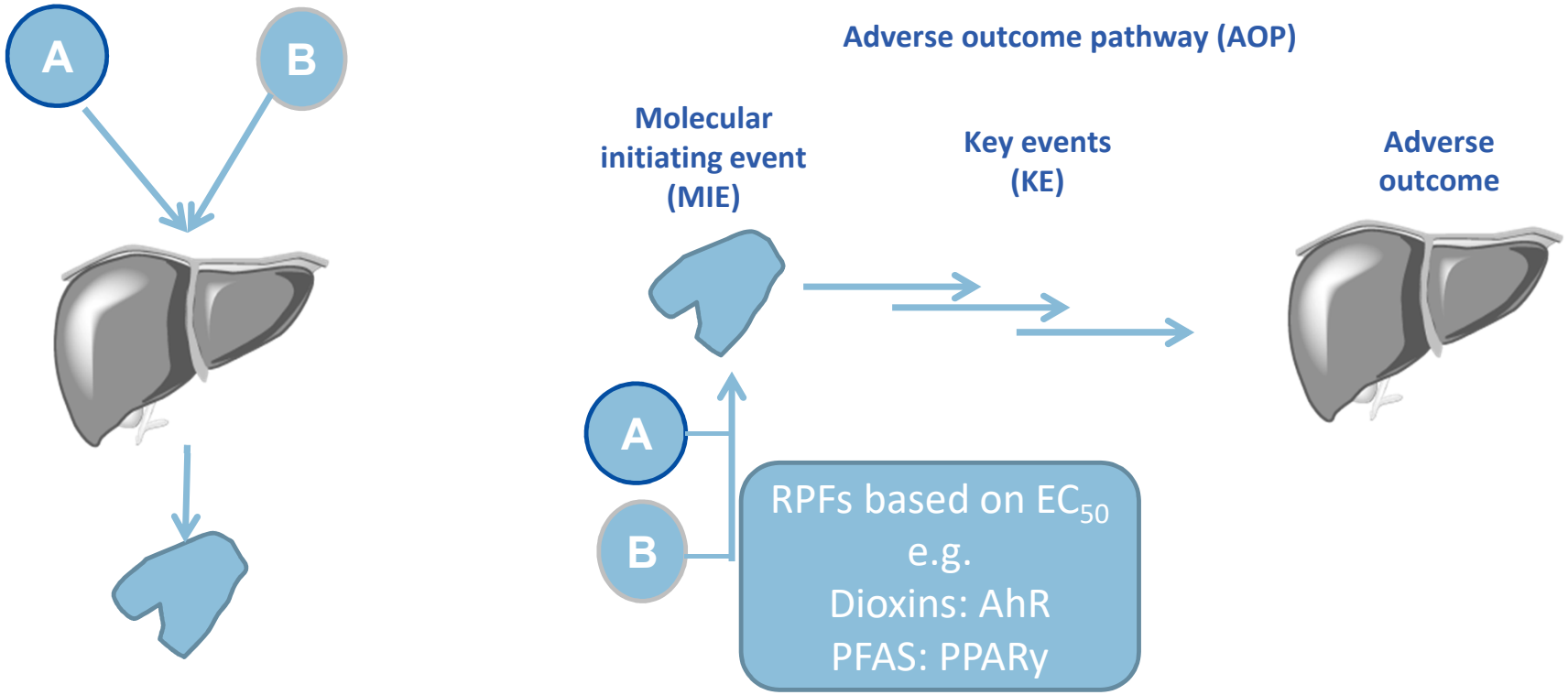


Exposure 2:
Food baskets for different groups of the population

- Group substances according to toxicology (targets/kinetics)
- Cumulative Assessment Groups (CAGs or CKGs)
- Calculate combined exposure based on data
- Perform additive assessment

Sketch courtesy of Dr. P. Marx-Stölting

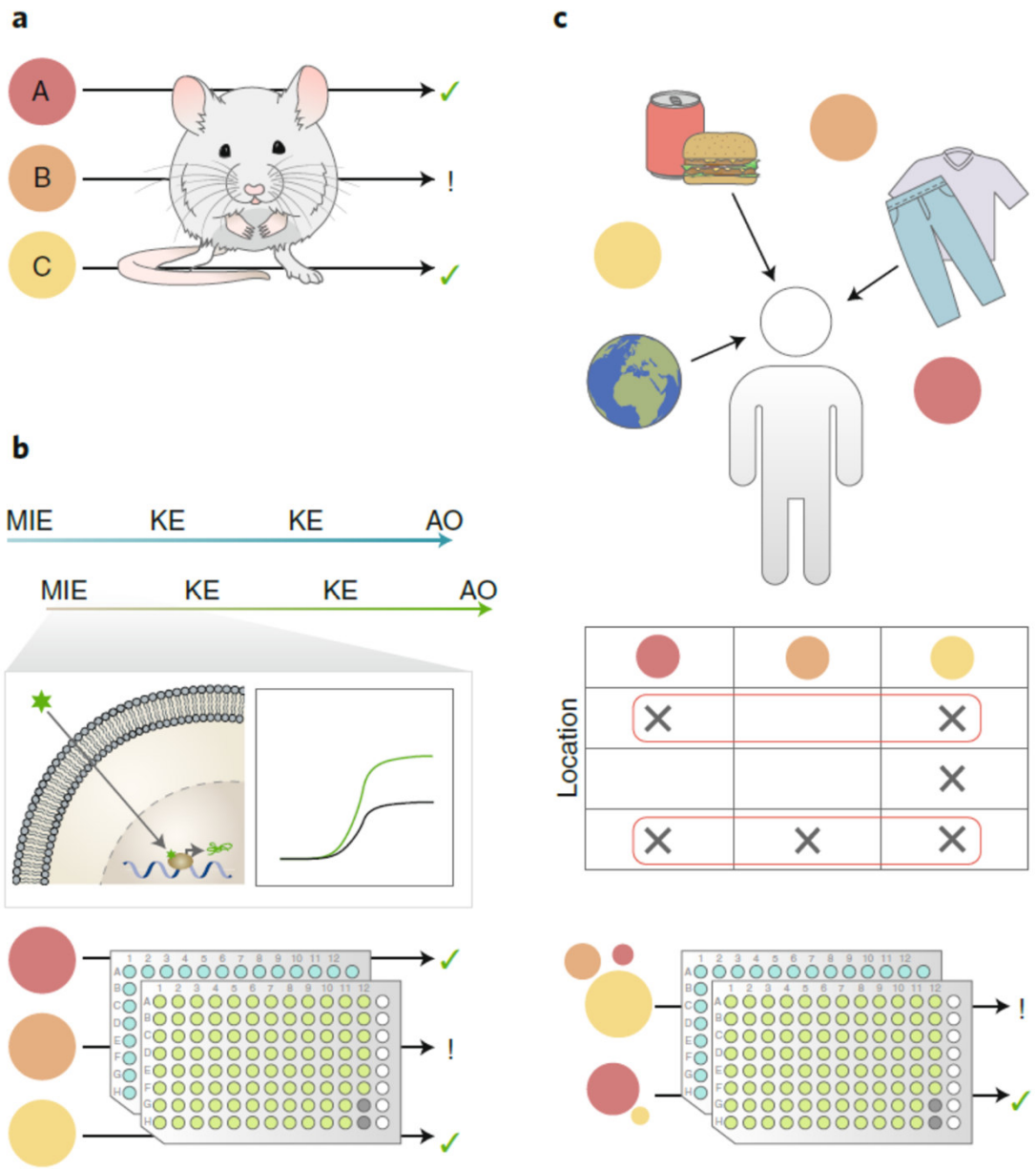
Data poor substances – little do we know, yet still something



- Group substances according to structure (Dioxins, PFAS)
- Derive relative potency factors for relevant MIE in relevant AOPs
- Calculate combined hazard based on RPFs
- Successfully applied for some contaminants (dioxins, PCBs)

Sketch courtesy of Dr. P. Marx-Stölting

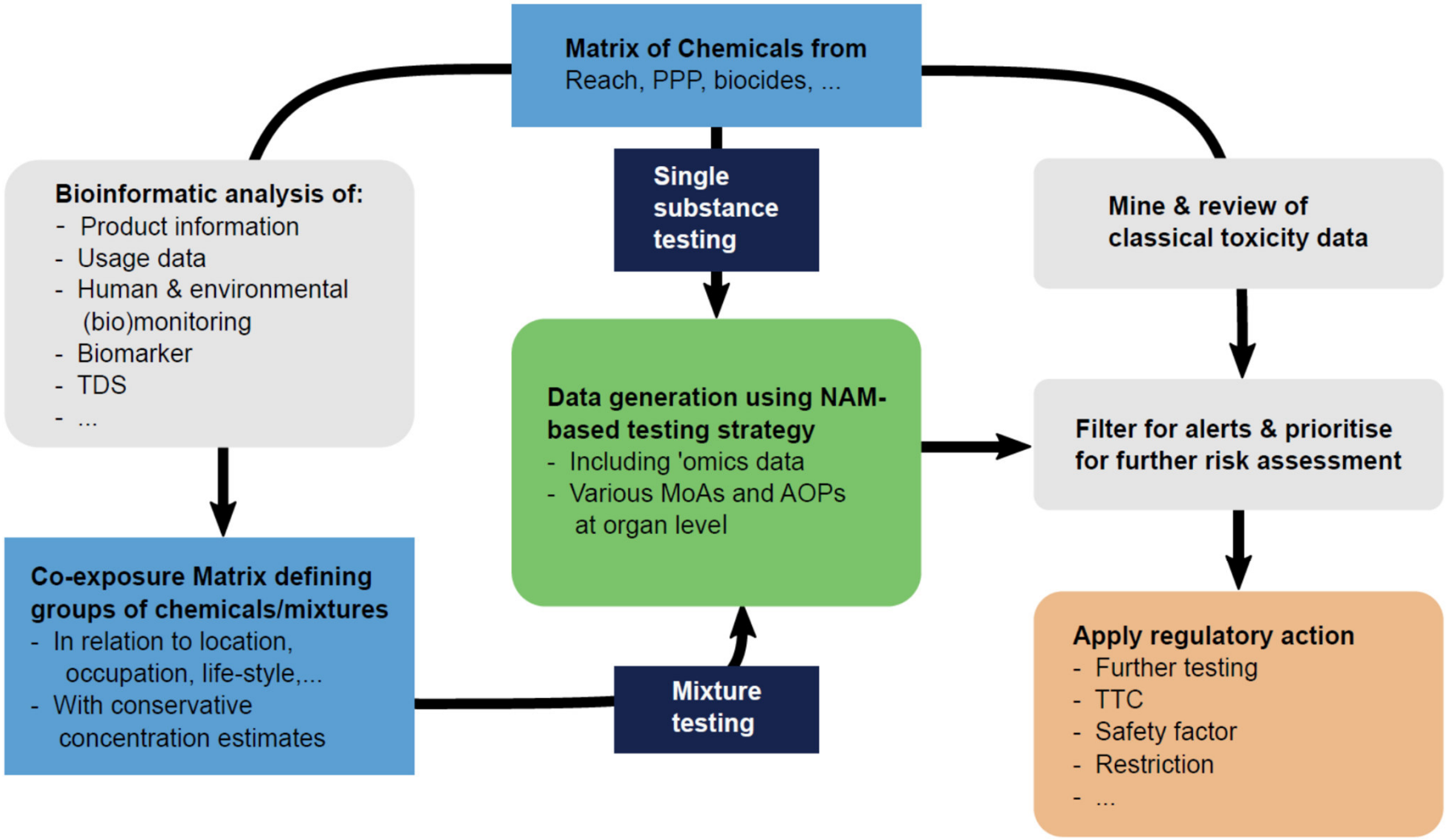
From poor to less - the need to separate the good from the bad and the ugly



Risk assessment approaches. **a**, In the classical approach, risk assessment relies on testing of substances (red, orange and yellow circles) in animal tests, from which it is possible to conclude whether a risk has been identified (exclamation mark) or not (green tick), but not the mode of action of the substance. **b**, New assessment methods (NAMs) are based on adverse outcome pathways (AOPs) for which molecular initiation events (MIE), key events (KE) and adverse outcomes (AO) are defined. These methods rely on *in vitro* assays, which can often be used in a high-throughput fashion and convey information about the mode of action. Especially for MIE and KE, these *in vitro* assays often rely on reporter cell lines, in which an easily detectable reporter gene is expressed upon substance stimulus. **c**, To analyse possible mixtures of substances, our proposed approach of experimental regulatory toxicology combines parts of the exposome concept with the NAMs. From usage patterns, product data and surveys and analytics (top), it would be possible to create a correlation matrix in which likely co-exposures can be estimated (middle – indicated by red boxes for mixtures in two different locations). These possible mixtures could then be tested in established NAMs (bottom).

Tralau T. *et al.* (2021), Nat. Food 2 (7):463-8.

The need to separate the good from the bad and the ugly



Starting point is a matrix of chemicals populated with substances registered under REACH, plant protection products and biocides. From various resources information about possible co-exposure will be gathered in order to define groups of chemicals likely occurring together. Thus established mixtures and their respective single substances will be tested using a NAM-based testing strategy. Data generated by this approach will be complemented with classical toxicity data such that further regulatory actions can be applied. Abbreviations: REACH – Registration, Evaluation, Authorisation and Restriction of Chemicals; PPP – Plant Protection Products; NAM – New Approach Method(ologie)s; MoA – Mode of Action; AOP – Adverse Outcome Pathway; TDS – Total Diet Study; TTC – Threshold of Toxicological Concern.

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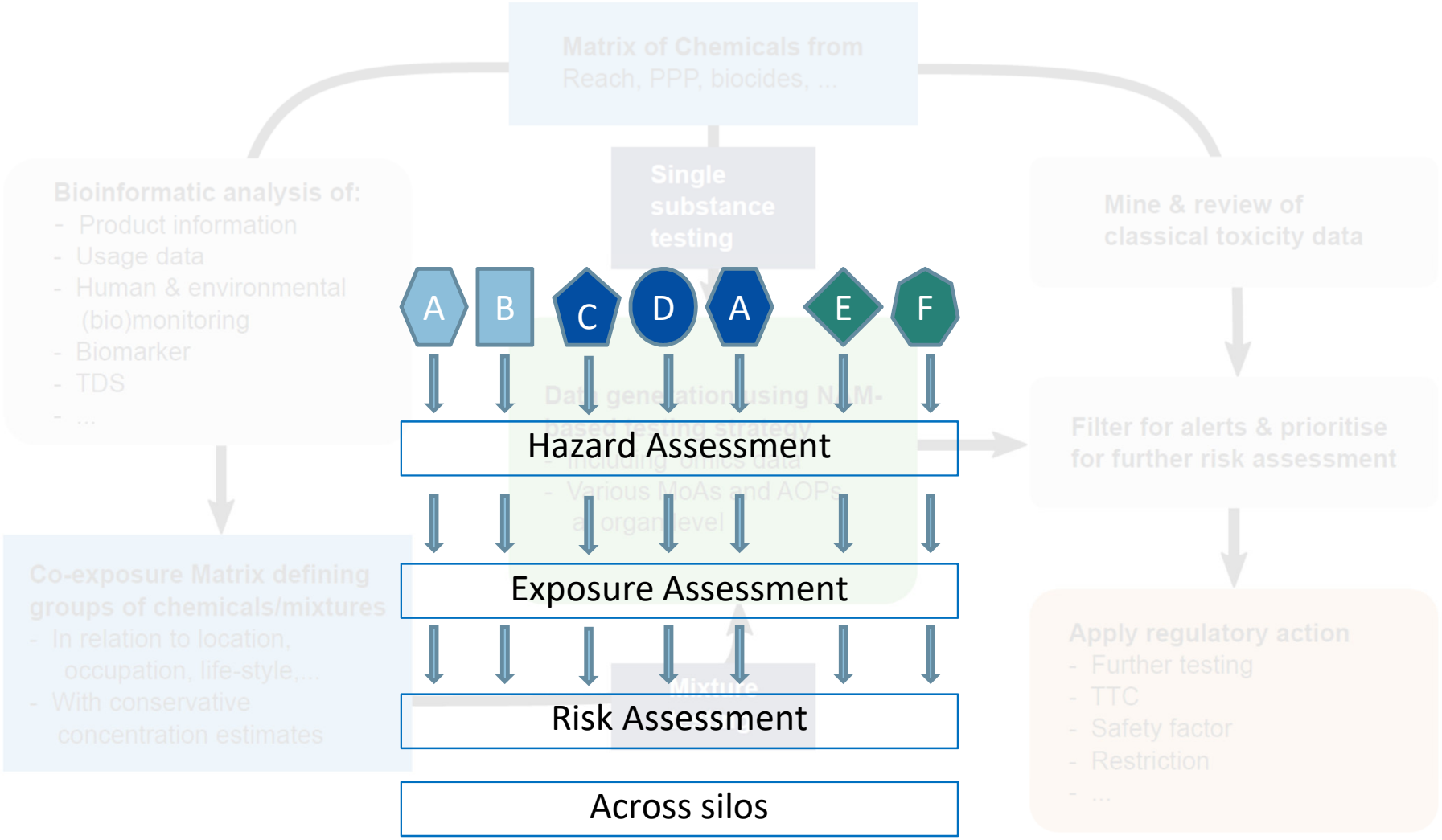

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