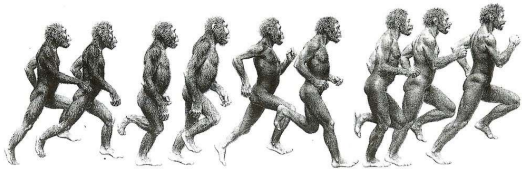
Die Evolution toxikologischer Methoden

standardisierte Studien an Tieren und Zell- und Gewebekulturen,
New Approach Methods (NAM) und Next Generation Risk Assessment (NGRA)

Robert Landsiedel

8th German Pharm-Tox Summit in Ulm
Advanced course in Toxicology „Moderne Ansätze in der Risikoabschätzung:
Weight of Evidence und Unsicherheitsanalyse und -quantifizierung"

06. März 2023



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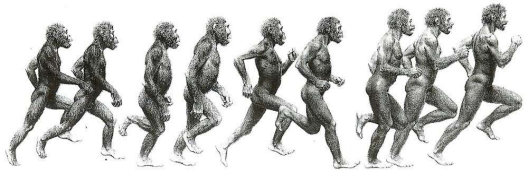
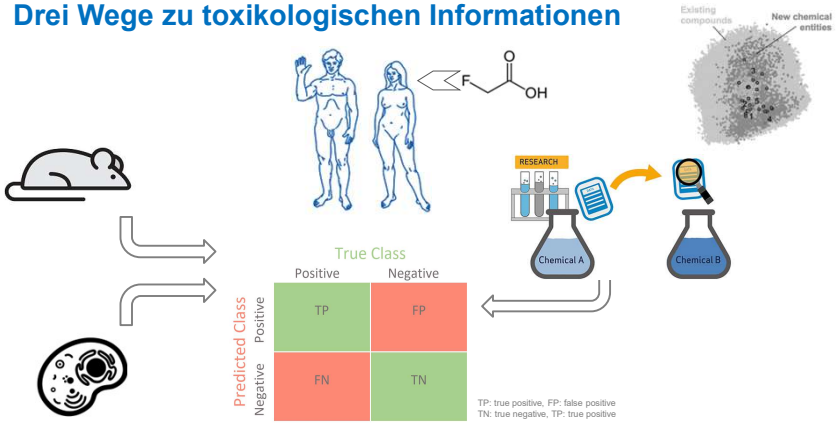


1 Tierversuche 2 Ersatzmethoden 3 NAMs 4 NGRA 5 Unsicherheiten

2

Drei Wege zu toxikologischen Informationen



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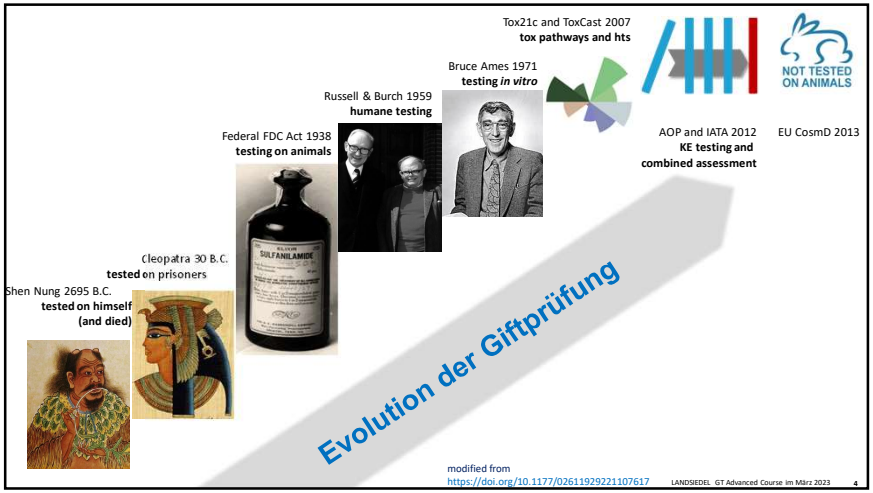

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OXFORD Toxicology Research

Toxicology Research, 2020, 9, 676–682

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Viewpoint

VIEWPOINT

A decade of toxicological trends: what the papers say

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Table 1: Search terms used to analyse trends in toxicologically relevant publications

Concept	Search term(s)
AI	'Artificial intelligence'
Gx	Genomics
Zf	Zebrafish or '*Danio rerio*'
PM	'Personalized medicine' or 'Personalized medicine' or 'Precision medicine'
Mb	Microbiome or microbiota
AOP	'Adverse outcome pathway'
MPS	'Microphysiological systems' or 'Microphysiologic system' or 'Organ on a chip'
RA	Read-across
Hor	Hormesis
3Rs	3Rs

Gx: genomics, Zf: zebrafish, Hor: hormesis, PM: personalized medicine, Mb: microbiome, RA: read-across.

Figure 1: Trends in toxicology publications from 2009 to 2019 where each of the top 10 concepts is ranked based on the number of publications from most to least per year to track changes over time.

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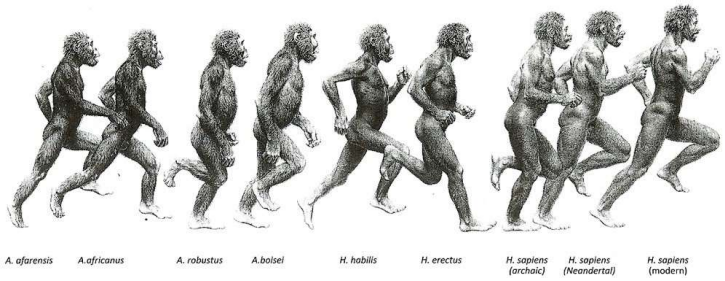
1 Tierversuche



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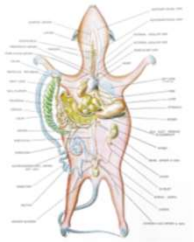
Evolution toxikologischer *in vivo* Methoden

(OECD test guideline no. 407 und 408)

1995

Liver, Kidney, Adrenal, Spleen, Heart, Testis, others optional



2008

Adrenal glands, Brain (cerebellum, mid-brain, cortex), Caecum, Cervix , Clitoral gland, Colon, Coagulation gland Duodenum Epididymides , Eyes (with optic nerve (if detectable) and Harderian gland), Femur including joint , Heart, Ileum, Jejunum, Kidneys, Liver, Lung, Lymph nodes (mandibular, mesenteric), Peyer's patches [jejunum, ileum] if detectable Pituitary gland, Preputial gland Prostate gland , Rectum, Salivary glands (mandibular, sublingual) Sciatic nerve, Seminal vesicles Skeletal muscle, Spinal cord (cervical, midthoracic, lumbar), Spleen, Sternum with bone marrow, Stomach (forestomach and glandular stomach) Testes, Thymus, Thyroid including parathyroid if detectable, Trachea Urinary bladder Uterus, Vagina, All gross lesions

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Chemikalienregulierung ist maßgeschneidert für Ergebnisse aus Tierversuchen

GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS (GHS)

Corneal opacity (CO: score 0 to 4)
Iris lesions (IR: score 0 to 2)
Conjunctiva redness (CR: score 0 to 3)
Conjunctiva chemosis (CC: score 0 to 4)

Table 3.3.1: Serious eye damage/Irreversible effects on the eye category[a, b, c]

	Criteria
Category 1: Serious eye damage/Irreversible effects on the eye	A substance that produces: (a) in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or (b) in at least 2 of 3 tested animals, a positive response of: (i) corneal opacity ≥ 3; and/or (ii) iritis > 1.5; calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material.

Table 3.3.2: Reversible effects on the eye categories[a, b, c]

	Criteria
	Substances that have the potential to induce reversible eye irritation
Category 2/2A	Substances that produce in at least 2 of 3 tested animals a positive response of: (a) corneal opacity ≥ 1; and/or (b) iritis ≥ 1; and/or (c) conjunctival redness ≥ 2; and/or (d) conjunctival oedema (chemosis) ≥ 2 calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material, and which fully reverses within an observation period of normally 21 days.
Category 2B	Within Category 2A an eye irritant is considered mildly irritating to eyes (Category 2B) when the effects listed above are fully reversible within 7 days of observation.

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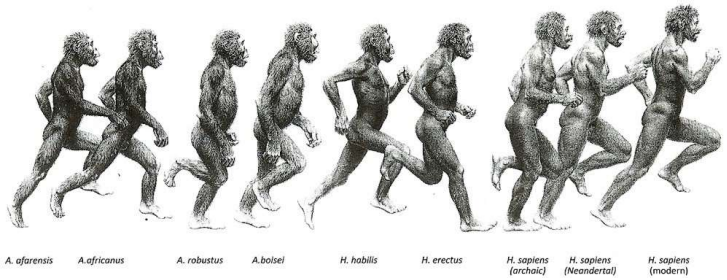
2 Ersatzmethoden, Alternativmethoden



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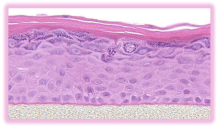
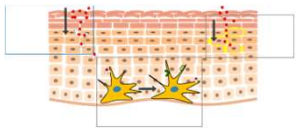
Hautreizung und Sensibilisierung der Haut





OECD TG 430, 431, 435, 439

GHS ist nicht für *in vitro* Methoden gemacht

Differenzierung von 1 und 1C is schwierig (auch *in vivo*) (und auch irrelevant)

in vitro Kriterien in GHS?
(kein Umweg über *in vivo*)

anwendbar für Formulierungen, UVCB, Polymere?

OECD TG 442C, D, E

AOP basiert (aber bilden die IATA den AOP ab?)
KE-basiert (aber sind auch alle relevanten KE erfasst?)

Anwendbar für PPP, Pflanzenextrakte, Polymere?

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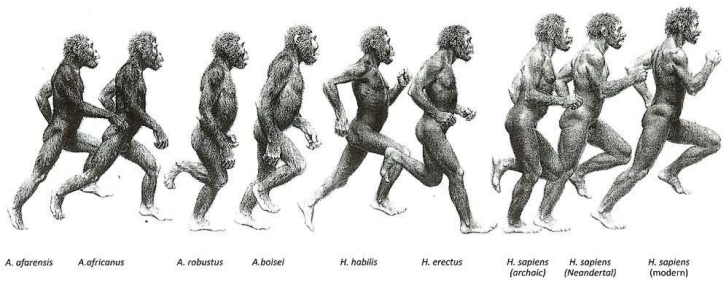
3 New Approach Methods (NAM)



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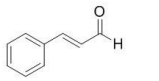
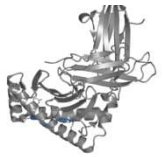
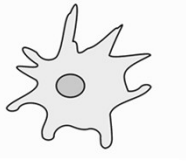
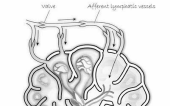
Adverse Outcome Pathways (AOP)
- die Verbindung von Key Events (KE) zu Adverse Outcomes (AO)









Molecular	Cellular	Organ	Organism
initiating event	key events	key events	adverse outcome

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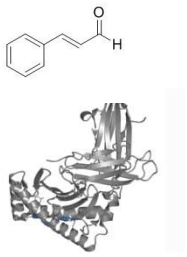
Adverse Outcome Pathways (AOP)
- die Verbindung von Key Events (KE) zu Adverse Outcomes (AO)



Molecular
initiating event

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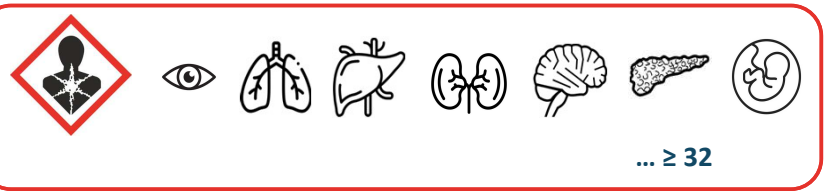
Hazard- und Risk Assessment basiert auf Adverse Outcomes



... ≥ 32

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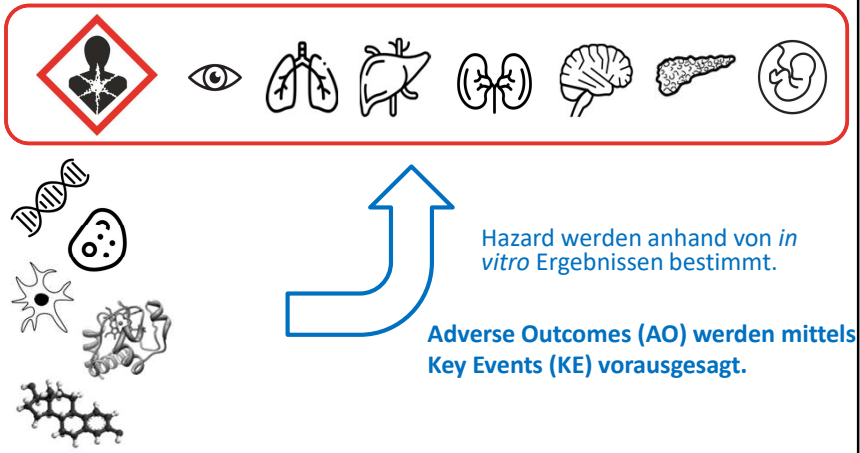

Hazard werden anhand von *in vitro* Ergebnissen bestimmt.

Adverse Outcomes (AO) werden mittels Key Events (KE) vorausgesagt.

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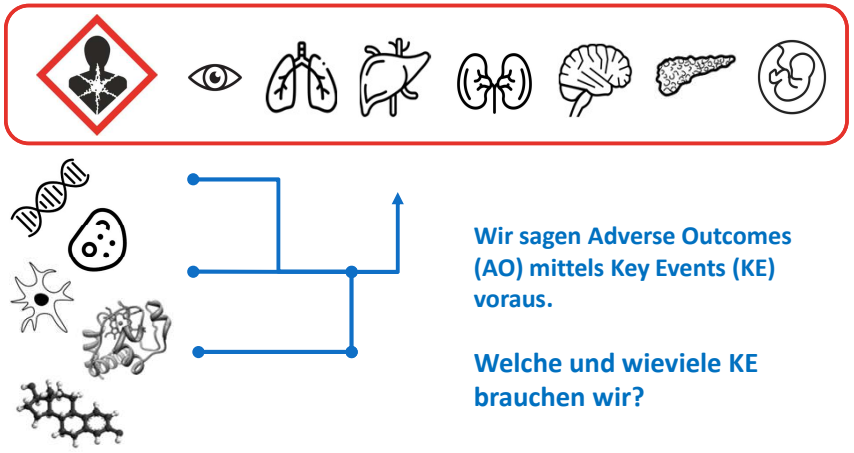

Wir sagen Adverse Outcomes (AO) mittels Key Events (KE) voraus.

Welche und wieviele KE brauchen wir?

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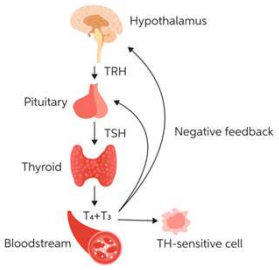
In vitro Methoden zur Erfassung der Störung der Schilddrüsenhomöostase

priorisiert von EURL ECVAM



1: Central regulation	1.a TRH; 1.b, TSH
2: TH synthesis	2.a, 2.b TPO inhibition; 2.c thyrosin iodination; 2.d iodine uptake
3: Secretion and transport	3.a TTR/TBG displacement
4: Metabolism and excretion	4.a deiodinase, 4.b glucuronidation; 4.c sulfation
5: Local cellular concentrations	5.a T3/T4 cellular uptake
6: Cellular responses	6.a TRα and TRβ reporter gene; 6.b TRβ CALUX model
7: Relevant short term assays integrating multiple MOAs	7.a Zebrafish Eleutheroembryo
8: Integrative cellular assays	8.a T-screen assay (T3 competition, proliferation); 8.b neural progenitor cells (hNPCs)

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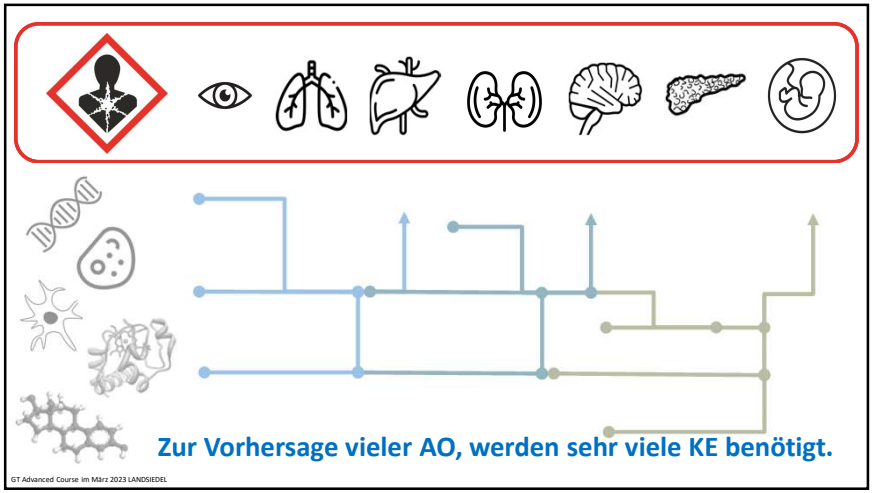

Zur Vorhersage vieler AO, werden sehr viele KE benötigt.

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Der lange Weg über die Vorhersage der Adverse Outcomes durch Key Events

32 Organe
4 KE pro Organ
5 TG pro Jahr

24 Jahre *

Speaking at an 18 May virtual forum organised by the Green Chemistry and Commerce Council (GC3),

Dr. Hansen said we're currently **40 years away** from being able to effectively predict toxicity of chemicals,

but with focused investment and regulatory needs driving the work, this **could be reduced to 20 years**.

OECD

408

adopted: 25 June 2018

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Repeated dose 90-day oral toxicity study in rodents

1)	Brain	15)	Spleen	26)	Gall bladder
2)	Spinal cord	16)	Heart	27)	Lymph nodes
3)	Pituitary	17)	Trachea	28)	peripheral Nerve
4)	Thyroid	18)	Lungs	29)	Skeletal muscle
5)	Parathyroid	19)	Aorta	30)	Skeletal bone with marrow
6)	Thymus	20)	Ovaries	31)	Skin
7)	Oesophagus	21)	Uterus	32)	Eyes
8)	Salivary glands	22)	Cervix		
9)	Stomach	23)	Vagina		
10)	small and large Intestines	24)	Coagulation glands		all gross lesions
11)	Liver	24)	Mammary gland		
12)	Pancreas	25)	Urinary bladder		
13)	Kidneys				
14)	Adrenals				

for female mice

* skin and eye assumed to be covered already

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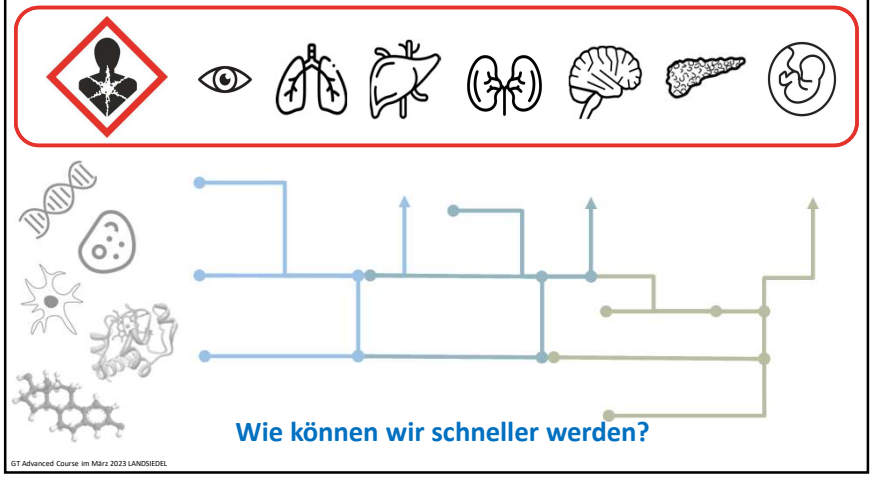

Wie können wir schneller werden?

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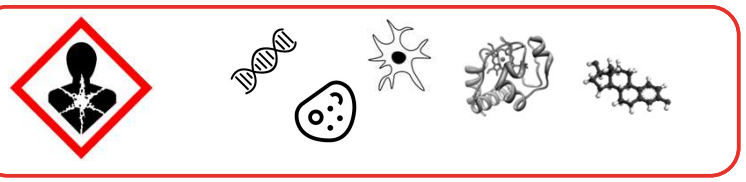

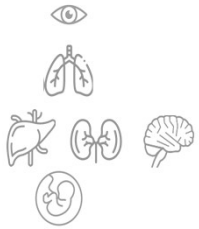

Turn it around!

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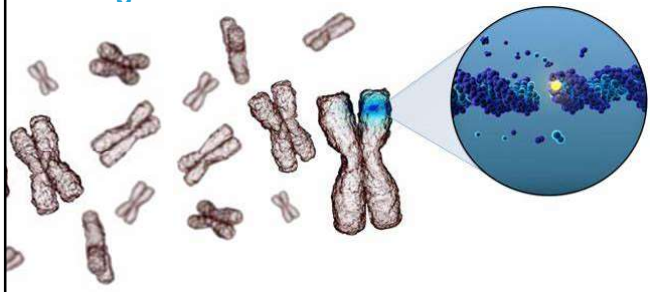
Mutagenitätstests



sehr erfolgreiche Tests
auf ein key event (KE)

15 OECD TGs (*in vitro* und *in vivo)* und *in silico* Methoden
fremdstoffmetabolisierende Enzymaktivitäten
IATA
neue Methoden und neue Bewertungsstrategien

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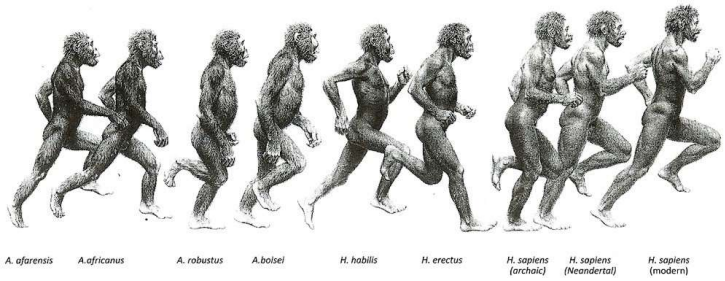
4 Next Generation Risk Assessment (NGRA)



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Next Generation of Risk Assessment (NGRA)

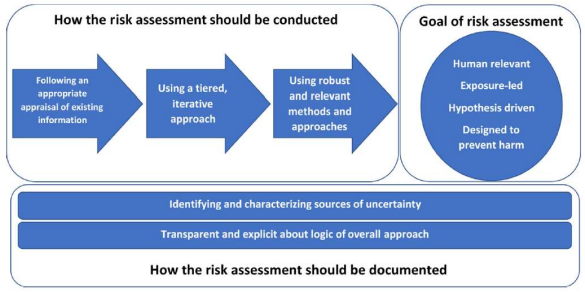

Fig. 1. Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients.

Dent *et al., 2018,* Computational Toxicology 7: 20–26

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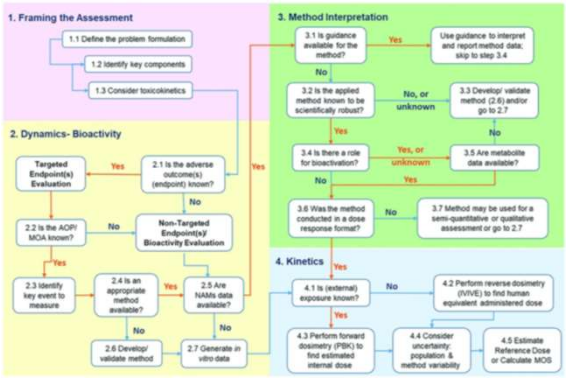
Arbeitsablauf eines NGRA



https://doi.org/10.1093/toxsci/kfad012

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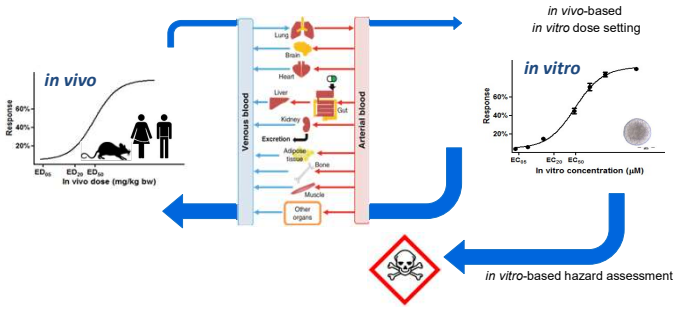
IVIVE
In vitro to in vivo extrapolation



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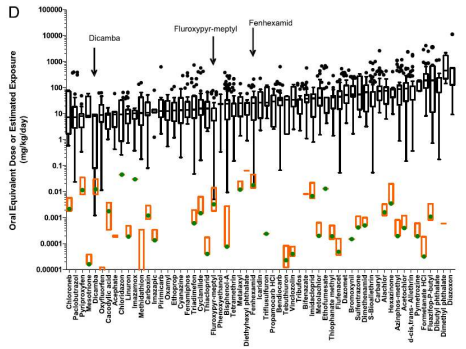
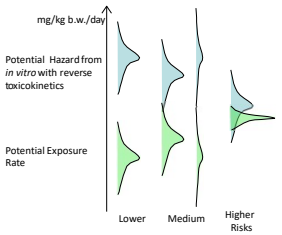
Priorisierung von Chemikalien
mit NAMs and IVIVE



High throughput *in vitro* screening can estimate doses needed to cause bioactivity
(for example, Wetmore et al., 2015)

Exposure intake rates can be inferred from biomarkers
(for example, Ring et al., 2018)



Wetmore et al. *et al.* (2012)
doi:10.1093/toxsci/kfr254

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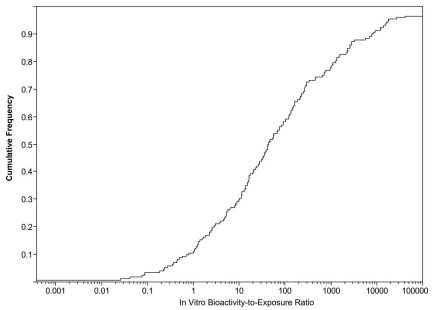
Cumulative distribution frequency of the ratio of in vitro bioactivity relative to exposure



Toxicol Sci, Volume 132, Issue 2, April 2013, Pages 327–346, https://doi.org/10.1093/toxsci/kft012

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5 Unsicherheiten

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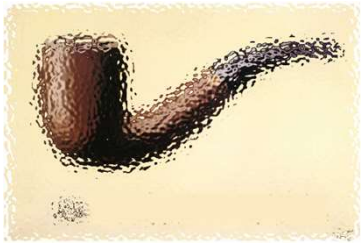
5 Unsicherheiten



All models are wrong
… some are useful.

and imprecise

(George Edward Pelham Box, Professor Emeritus of Statistics at the University of Wisconsin)

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Confidence, Competence, Reproducibility and Data Integrity

OECD GLP

confidence in the **quality**
integrity of the data
reconstruct activities performed during the conduct of … studies.

ISO/IEC 17020:2012

competence
impartiality
consistency

foster confidence in *in vitro* … methods
defined standards under which data are generated to ensure resulting data are **rigorous** and **reproducible**.

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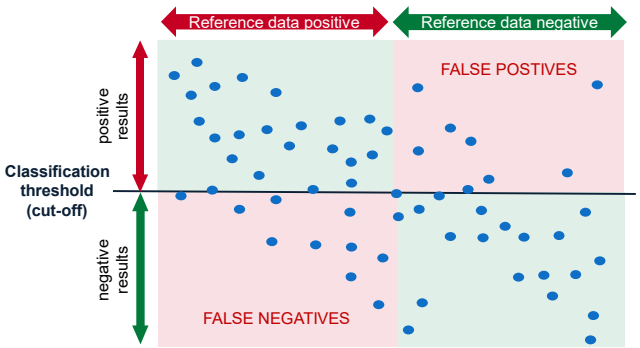
Der Graubereich an der Klassifizierungsgrenze



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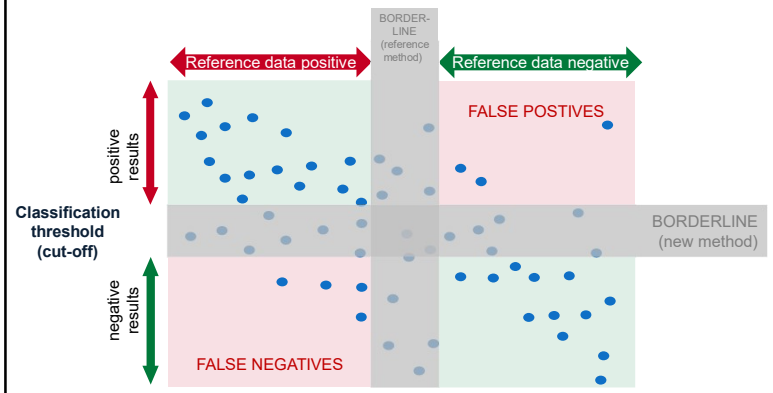
Der Graubereich an der Klassifizierungsgrenze



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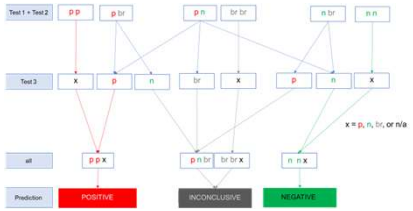
Unsicherheit durch begrenzte Präzision
Die Größe der Graubereiche und die Auswirkung auf IATA

	Endpoint	Cut-off	Borderline Range
DPRA (OECD TG 442C)	Mean peptide depletion [%]	6.38	4.95 to 8.32
	Cysteine-only depletion [%]	13.89	10.54 to 18.39
kDPRA (OECD TG 442C)	log k_{max} [$s^{-1}M^{-1}$]	-2	-1.93 to -2.07
	Cysteine-only depletion [%]	13.89	n/a
KeratinoSens™ (OECD TG 442D)	Luciferase induction (fold-change)	1.5	1.35 to 1.67
LuSens (OECD TG 442D)	Luciferase induction (fold-change)	1.5	1.28 to 1.76
h-CLAT (OECD TG 442E)	Relative fluorescence intensity CD54	200	157 to 255
	Relative fluorescence intensity CD86	150	122 to 184



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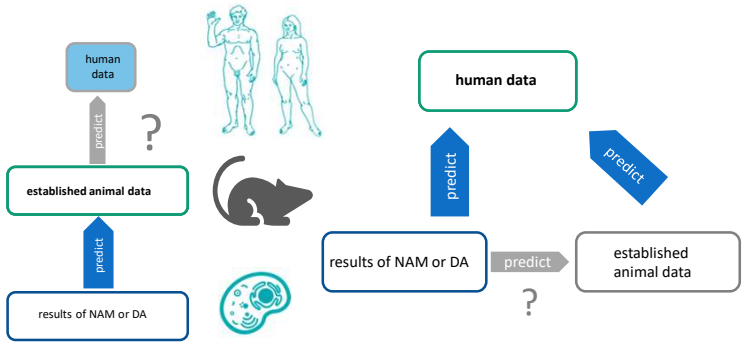
Referenzdaten



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doi:10.14573/altex.2105111

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Referenzdaten: Humandaten *versus*
murine LLNA- (links) und NAM DA- (rechts) Daten

	Sensitivity [%]	Specificity [%]	Balanced Accuracy [%]	n	Sensitivity [%]	Specificity [%]	Balanced Accuracy [%]	n
Bauch et al., 2012	96	81	**88**	50	93	95	**94**	50
Urbisch et al., 2015	91	64	**78**	111	90	90	**90**	101
Kleinstreuer et al., 2018	85	50	**68**	128	79	73	**76**	127
OECD Database	94	22	**58**	56	83	82	**83**	65
OECD database (excluding 10 borderline)	n/a	n/a	n/a	n/a	89	88	**88**	55

GT Advanced Course im März 2023 LANDSIEDEL

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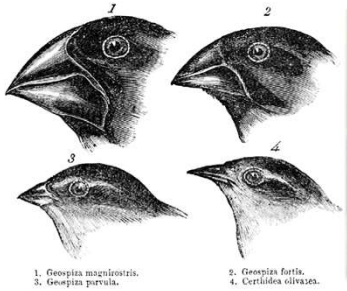
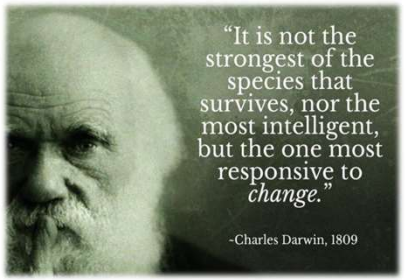
‘Evolution has no goal‘





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Was beschleunigt oder verzögert die Evolution der Toxizitätstests?

technology push

Tierschutz

reproducibility crisis

failed predictivity desasters

gutes Screening

Hazard-basierte Bewertungen

Wunsch nach Eindeutigkeit

Validierung

zögerliche Translation

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