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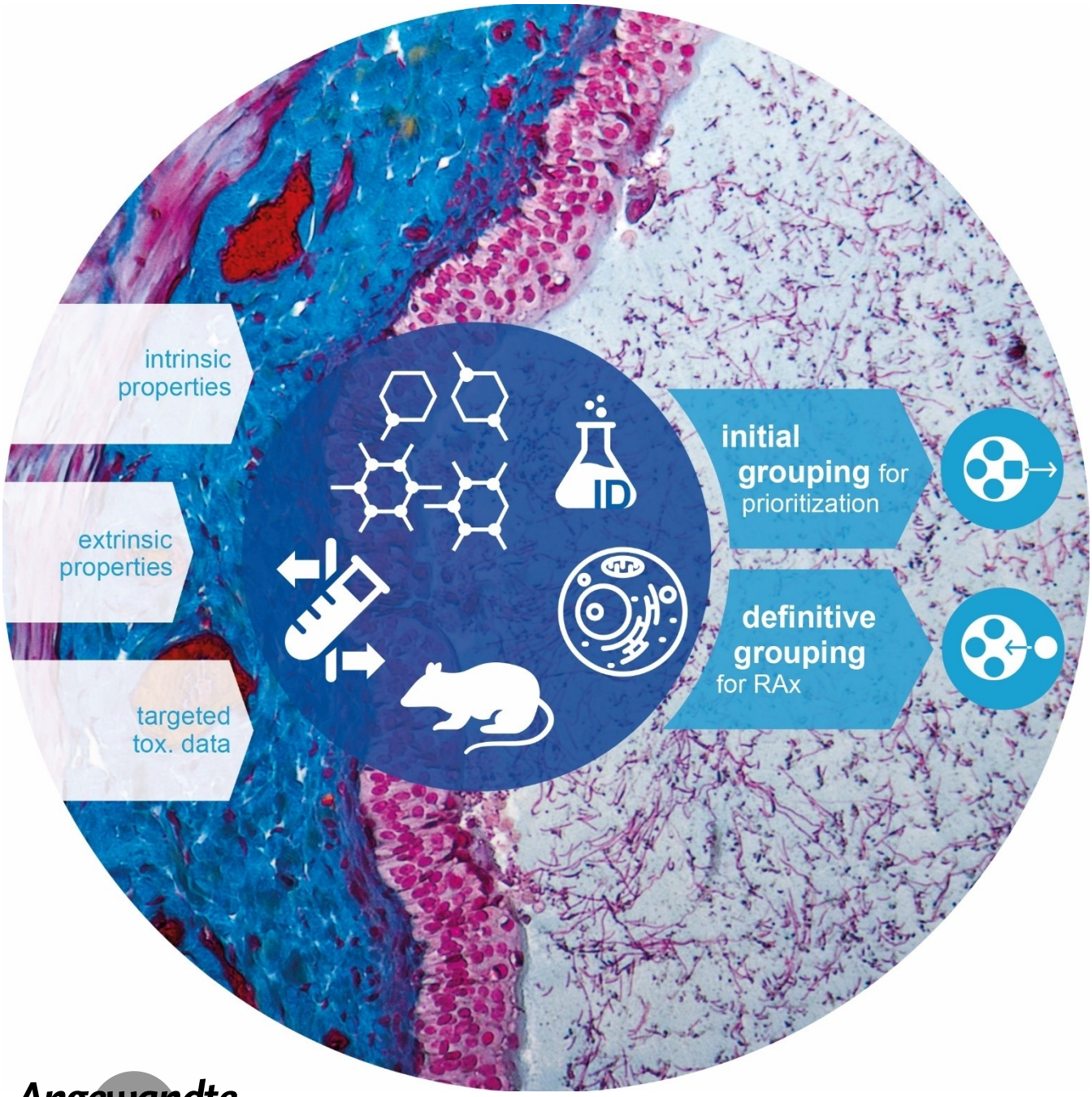
Toxicology

Lessons Learned from the Grouping of Chemicals to Assess Risks to Human Health

Wendel Wohlleben, Annette Mehling, and Robert Landsiedel**



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Abstract: In analogy to the periodic system that groups elements by their similarity in structure and chemical properties, the hazard of chemicals can be assessed in groups having similar structures and similar toxicological properties. Here we review case studies of chemical grouping strategies that supported the assessment of hazard, exposure, and risk to human health. By the EU-REACH and the US-TSCA New Chemicals Program, structural similarity is commonly used as the basis for grouping, but that criterion is not always adequate and sufficient. Based on the lessons learned, we derive ten principles for grouping, including: transparency of the purpose, criteria, and boundaries of the group; adequacy of methods used to justify the group; and inclusion or exclusion of substances in the group by toxicological properties. These principles apply to initial grouping to prioritize further actions as well as to definitive grouping to generate data for risk assessment. Both can expedite effective risk management.

1. Introduction

The concept of grouping enables predictions of features common to all members of this group, based on their similarity or other features used as grouping criteria. The term "grouping" describes the system for, or a process of, classifying objects (or living beings) with common features into groups. Grouping can serve various purposes depending on the common aspects to be predicted. Chemical substances can be grouped for similar physical or chemical properties (Figure 1), similar use, or similar toxicity (Figure 2). The purpose will determine the grouping criteria and the required similarity. The motivation of this review is the increasing but diverging use of grouping to efficiently assess hazards of chemical substances under the EU's registration, evaluation, authorization, and restriction (REACH) legislation and in the "generic approach to risk management" (GARM). The European Chemicals Strategy for Sustainability (CSS) proposes to extend the scope of GARM. The present Review is also intended to touch upon relevant regulatory topics via the perspective that grouping is at the very core of the chemical discipline: the periodic system of elements, which structures all elements in groups, and many of the principles of grouping can be introduced using this example. Dmitrij Ivanovič Mendeleev famously recognized the similarity of elements: "I wish to establish some sort of system not guided by chance but by some sort of definite and exact principle." [1]

To define a group, a fit for purpose hypothesis is needed along with the justification why this is the case based on available data and which can be refined by additional data. The similarity and systematic scaling of chemical properties found in the periodic table would constitute the "grouping hypothesis" (Figure 4), the experimental work by Robert Bunsen, Stanislao Cannizzaro, and many more provided the "justification". The successful predictions made by Mendeleev were largely based on atomic weight and the behavior of the elements in chemical reactions. These properties were only later rationalized by the occupancy of electron orbitals and later explained by quantum theory—"the justification". The periodic system (Figure 1) displays descriptors for each element, such as electronegativity, which have proven to be useful to predict the behavior of the element in a complex chemical reaction. In the terminology of grouping concepts, the electron configuration would be considered to be an "intrinsic property" but the redox behavior an "extrinsic property", because this property also depends on the reaction partner.

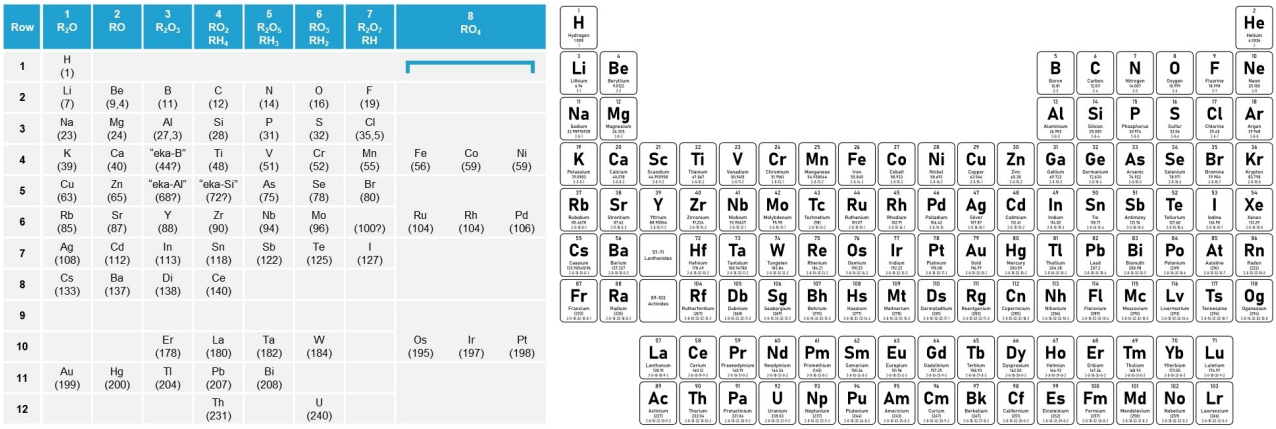
In toxicology, grouping describes the process of clustering substances with toxicological properties that are likely to be similar or to follow a regular pattern as a result of structural similarity into a "group" or "category".[2] According to the current regulatory practice in Europe (in particular within the EU under the REACH legislation), but also in the U.S. under the TSCA New Chemicals Program,[3] structural similarity is a prerequisite for grouping, but in general, any relevant descriptor, property, or behavior of a chemical substance can serve as a criterion to group for common toxicity (e.g. reactivity of alkylchloroformates, see Table 1). The commonly shared toxicity can be a single toxic effect or multiple toxic effects which are qualitatively (demonstrating similar attributes and/or effects) and/or quantitatively (measuring the magnitude of these attributes or effects) similar.[4] Regulatory principles identify groups of substances expected to have similar toxic effects. This is analogous to how Mendeleev grouped elements in the periodic system. These defined groups are used to fulfil toxicological data requirements and assessments of those chemical substances in the group for which insufficient data is available.

[*] Dr. W. Wohlleben
Department of Analytical and Material Science, BASF SE
67056 Ludwigshafen am Rhein (Germany)
E-mail: wendel.wohlleben@basf.com

Dr. A. Mehling
Dept. of Advanced Formulation and Performance Technology
BASF Personal Care and Nutrition GmbH
40589 Duesseldorf (Germany)

Dr. W. Wohlleben, Dr. R. Landsiedel
Department of Experimental Toxicology and Ecology, BASF SE
67056 Ludwigshafen am Rhein (Germany)
E-mail: robert.landsiedel@basf.com

Dr. R. Landsiedel
Free University of Berlin, Biology, Chemistry and Pharmacy—Pharmacology and Toxicology
14195 Berlin (Germany)
E-mail: robert.landsiedel@fu-berlin.de



Row	1 R_2O	2 RO	3 R_2O_3	4 RO_2 RH_4	5 R_2O_5 RH_3	6 RO_3 RH_2	7 R_2O_7 RH	8 RO_4		
1	H (1)									
2	Li (7)	Be (9,4)	B (11)	C (12)	N (14)	O (16)	F (19)			
3	Na (23)	Mg (24)	Al (27,3)	Si (28)	P (31)	S (32)	Cl (35,5)			
4	K (39)	Ca (40)	"eka-B" (44?)	Ti (48)	V (51)	Cr (52)	Mn (55)	Fe (56)	Co (59)	Ni (59)
5	Cu (63)	Zn (65)	"eka-Al" (68?)	"eka-Si" (72?)	As (75)	Se (78)	Br (80)			
6	Rb (85)	Sr (87)	Y (88)	Zr (90)	Nb (94)	Mo (96)	(100?)	Ru (104)	Rh (104)	Pd (106)
7	Ag (108)	Cd (112)	In (113)	Sn (118)	Sb (122)	Te (125)	I (127)			
8	Cs (133)	Ba (137)	Di (138)	Ce (140)						
9										
10			Er (178)	La (180)	Ta (182)	W (184)		Os (195)	Ir (197)	Pt (198)
11	Au (199)	Hg (200)	Tl (204)	Pb (207)	Bi (208)					
12				Th (231)		U (240)				

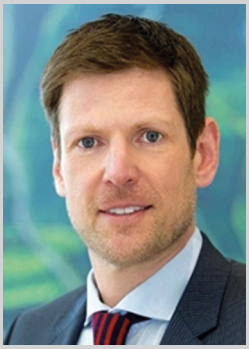
Figure 1. Periodic system of elements. a) As published by Mendeleev in 1871[1]—with predictions of up till then unknown elements by grouping and extrapolation, and b) current version.

Acquiring toxicological information on every chemical substance is resource-intensive, can involve undue animal testing, and may indeed not be necessary. Substances that are similar with respect to functional groups, or common breakdown products, or other characteristics (Figure 2), will most likely cause similar toxicity. Consequently, grouping is a prominent tool for chemical risk assessment, and it was used long before modern chemical regulations were implemented. The decisive questions have always been the same: What defines similarity and how similar is similar enough?

Even if grouping is less demanding than experimental assessment of individual substances, it still requires information on the intended use, good knowledge of the intrinsic and extrinsic substance properties and their relevance to the predicted hazard, and knowledge of the limitations of the test methods providing the data to justify grouping (Figure 2). Over the years some pitfalls have been encountered, especially when the selected similarity criteria was not sufficient to predict the toxicity endpoint; notably a basic structural similarity alone was not always sufficient. Dissimilarity of biological, including toxicological, effects despite structural similarity is allegorically called an "activity cliff".[5] Examples are stereoselective biological effects,[6] e.g. the two positional isomers of acetylaminofluorene, where the 2-isomer was found to be a genotoxic carcinogen while the 4-isomer was not,[7] or *n*-hexane causing neurotoxicity, in contrast to the higher or lower homologues.[8]

Wendel Wohlleben studied at the University Heidelberg and École Normale Supérieure, Paris, then obtained a PhD in physics at the University of Munich. He is now senior principal scientist at BASF, developing analytical methods on particles and polymers, specifically as relevant for regulatory assessment. He was affiliated as a postdoc to University Marburg, and as a visiting scientist to the Weizmann Institute, Rehovot, and to the Harvard TH Chan School of Public Health, Boston.

Annette Mehling is a trained biologist and received her PhD in microbiology. After her postdoc in immunology at the department of dermatology in Muenster (Germany), she started work at Cognis, later BASF, in 2001, where she has been a senior scientist in the area of skin sciences and toxicology. Over the years she has been involved in activities to promote the use of non-animal testing in the area of skin sensitization and irritation.



Robert Landsiedel holds a doctoral degree in chemistry and a habilitation in pharmacology and toxicology. He has been working for BASF in several positions, including assignments in North Carolina, USA, and Tokyo, Japan. He is now vice president of special toxicology, performing toxicological studies and developing new methods at BASF. He is also teaching toxicology at the Free University of Berlin and is the president-elect of the German Toxicology Society.

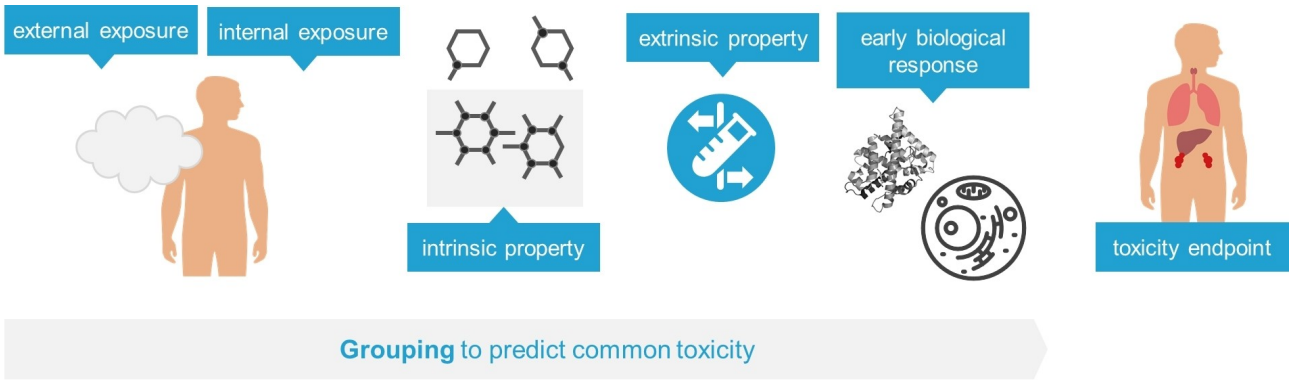


Figure 2. Generic introduction to properties and interactions of substances leading to toxicity. Grouping is using criteria describing the chemical substances in question to define a group with one or more common features, leading to common toxicity endpoints. In this example, the chemical structure, an intrinsic property, is used to define a group with a common toxicity endpoint, e.g. liver tumors. The link between chemical structure and toxicity endpoint is, however, a process progressing from extrinsic properties (e.g. lipophilicity, log K_{ow}) over exposure to the resulting molecular interaction with critical constituents of the human body, e.g. receptor binding. This can cause early biological responses, e.g. altered protein expression. This can progress to adverse outcomes, toxicity endpoints, e.g. liver tumors. This scheme represents an adverse outcome pathway (AOP). Any step (for AOP termed key event, KE) of this scheme can be used as grouping criterion, not only intrinsic properties, but also extrinsic properties, exposure, interactions with constituents of the body, early biological responses, and adverse outcomes. Applicable regulations and guidances select only some of these criteria, as demonstrated in Sections 2 and 3. In a scientific perspective, any of the steps can be the common feature to be predicted by the grouping. Grouping will be more reliable if the link between the grouping criteria and the targeted common feature is tight; this will often be the case if these are nearby in this scheme (e.g. early biological response and toxic endpoint) and if the penetrance of the criterion towards the common feature is high (e.g. little regulation and few intermediate key events). Usually already or readily available properties are used as criteria for grouping; there may, however, be a trade-off with availability and relevance of the grouping criteria. The grouping can be limited to qualitatively predict toxicity or include quantitative measures (e.g. receptor affinity or potency of the toxic effect by a chemical substance, cf. ref. [151]).

Table 1: Grouping according to OECD: Examples of similarities presented in the years 2002 to 2010, adapted from "Grouping of Chemicals: Chemical Categories and Read-Across—OECD". Further examples that helped to shape the REACH Guidance on Chemical Categories and Read Across, including inorganic metal compounds, polyols, and petroleum substances, were compiled by Worth and Patlewicz in 2007.[26]

Similarity Examples of substance groups	Common part of the molecule	Variable part of the molecule	Common physical-chemical properties, mode of action or precursors or breakdown products
Benzyl derivative group of 10 substances	benzene ring bonded directly to an oxygenated functional group	aldehydes and carboxylic esters	readily hydrolyzed or readily oxidized to benzoic acid
Mononitroanilines	nitro, amino	isomeric forms	methemoglobin formation
Ethylene glycol group of 5 substances	two terminal hydroxyl groups	number of oxyacetylene units	differ by increasing molecular weight
Long chain alcohol group of 30 substances (C6–C22 primary aliphatic alcohols)	primary hydroxyl	alkyl chain lengths	oxidation to aldehyde and carboxyl acid
Alkyl amine oxide group of 15 substances	amine oxide	alkyl chain lengths and branching	surfactants with a polar "head" and inert, hydrophobic "tail"
Alkylchloroformate group of 30 substances	chloroformate	alkyl chain lengths	reactivity

Depending on the purpose of grouping, i.e., *which* behavior is relevant, one substance can belong to different groups. This entails that different similarity criteria or thresholds may be relevant for the assessment of that specific group. The purpose of grouping may consist in the prediction of the hazard in one toxicological endpoint or in several endpoints, or it may regard the exposure in the intended or unintended uses. In analogy, all materials with zero band gap—especially metals—are conductors of electrons, although the numerical values of conductivity differ 100-fold between manganese and silver. The separate groups of semiconductors and insulators have values that are at least 10^6-fold lower. Yet, for the selection of an appropriate material for a certain purpose, conductivity may be less relevant than other properties, such as magnetism (e.g. computer storage devices) or ductility (e.g. metal-forming processes), and hence diverse groups and grouping criteria must be considered for the intended purpose.

Chemical regulations aim to ensure effective protection of human health and the environment. To this aim, under-

standing the relevant behavior, the interaction of the substance in biological compartments (Figure 2, Figure 3), is key. This requires toxicological information to facilitate risk assessments to identify and risk management to reduce risks to the level that the society is ready to accept. A hazard describes a substance's potential to cause harm. Toxicological information describes the type of potential harm ("endpoints", e.g. skin irritation, specific organ toxicity, or cancer) and the dose–response relationship (potency). A vulnerable individual will only be harmed by a substance if exposed to sufficient quantities of this substance (exposure).[9] Risk describes the probability of this to happen, taking hazard and exposure into account. In established risk assessment, both hazard and exposure are each assessed prior to risk management measures being recommended and/or ultimately restricting the use of substances in specific exposure scenarios or banning them altogether. Moreover, care needs to be taken, that the data being used to predict the outcomes is sufficiently robust for the endpoint being assessed. The outcome of such grouping is used for regulatory decisions to protect the health of humans and the environment—our most valuable assets. In the following sections, we discuss critically the scientific lessons learned from toxicological grouping approaches that were relevant to different global regulations, without the aim of reviewing all global grouping regulations. We include the most recent approaches and describe how future groupings and assessments of toxicity could be conducted based on these learnings. We will then conclude with the key components that make grouping for CSS an efficient, effective, and acceptable tool in the safe use of chemicals.

2. Grouping for Generation of Hazard Information

2.1. Classical Examples

Toxic effects of groups of substances were recognized early on and have been used for risk management of these groups of substances for years (Table 1). This includes substances of natural origin such as pyrrolizidine alkaloids (a group of more than 600 substances which are produced by plants and therefore found in food) and aflatoxins (a group of more than 20 difurocoumarine substances which are produced by filamentous fungi of the *Aspergillus* genus—a common mold) with many, but not all being toxic and/or carcinogenic to the liver,[10] and fibrous silicate minerals ("asbestos"), as well as anthropogenic substances whether accidentally or intentionally produced, such as polycyclic aromatic hydrocarbons (PAHs) or halogenated dibenzofurans and -pyrenes (often just termed "dioxins"). A short summary of these examples highlights the different basis, methods, and aims (or purposes) of groupings (Figure 3).

"The evil effects of asbestos dust" were recognized in 1898 by the factory inspector Lucy Deane.[11] In 1984, the World Health Organization (WHO) grouped asbestos and other fibers by common properties of physical structure (fixed cutoffs of more than 5 μm in length, less than 3 μm in width, and an aspect ratio above 3:1) and of chemical interaction in the biological system (biopersistence, without clear cutoff) to identify the "WHO fibers" for which the hazard assessment can be performed jointly.[12] In the EU, "the manufacture, placing on the market and use of these fibres and of articles and mixtures containing these fibres added intentionally is prohibited."[13] Interestingly, the scientific formulation of the "fiber paradigm", was later refined to *remove* subgroups of fibers from the ban, by identifying threshold behaviors in biopersistence, which were also measurable in screening techniques, thereby

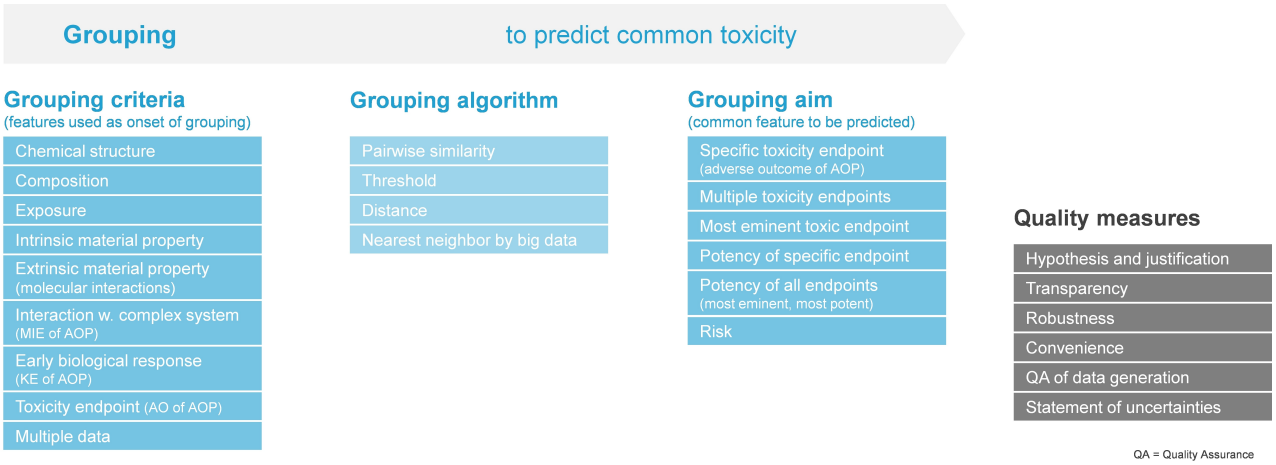


Figure 3. Terminology of grouping concepts. According to the OECD: "A chemical category is a group of chemicals whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern, usually as a result of structural similarity." As evaluations are based on the intrinsic and inherent properties of the substance, these approaches therefore allow primarily hazard-based assessments, but also exposure or risk can be the aims of the grouping. Both exposure and hazard need to be given to constitute a risk, but hazard is inherent to the chemical structure, whereas exposure depends on the actual use of the chemical.

helping to guide the design of alternatives to them.[14] Most recently, it was recognized that the tendency to form fibrils upon release of dusts requires *addition* of fibers to the group of hazardous fibers.[15] At the same time, the regulatorily approved criterion of mechanical rigidity led to *removal* of other fibers from the group, because "low diameter MWCNT (<30 nm) are not subject to the proposed classification, as it is assumed that due to a more tangled morphology, the fibre pathogenicity paradigm does not apply".[15,16] The example highlights the science-based refinement from a one-sided criterion (threshold) to a two-sided criterion with an upper and lower range of a property required for group members.

Polycyclic aromatic hydrocarbons, PAHs, were first recognized as carcinogenic by Sir Percival Pott in the 18th century.[17] At that time, they were only known as constituents of soot and pitch, and were later identified as a group of chemicals having fused aromatic rings.[18] PAHs are carcinogenic[10c] when metabolic activation leads to the formation of DNA-binding and hence mutagenic epoxides.[19] The carcinogenic potency of a PAH is linked to these properties, i.e. the propensity to be metabolized to reactive epoxides and to form mutagenic DNA adducts, and PAHs are grouped for their carcinogenic potential using the formation of genotoxic metabolites as a grouping criterion. This does not mean other mechanisms of genotoxicity other than epoxide formation (e.g. the formation of benzylic sulfates,[20]) or other mechanisms of carcinogenicity (receptor-mediated, see below) do not exist, but other grouping criteria may then be necessary.

Halogenated biphenyls [i.e. polychlorinated biphenyls (PCBs)] were recognized as toxic after ingestion of contaminated rice oil in the 1960s [油症 (Yusho) literally "oil symptoms"[21]]. PCBs were synthesized for their chemical stability and high dielectric constant and were used in various applications such as transformer oils and in heating systems. Leaks led to contamination. Halogenated dibenzofurans and -pyrenes (i.e. chlorinated dibenzodioxins, PCDDs) are byproducts of intentionally produced substances, such as halogenated biphenyls and phenoxy acid herbicides [e.g. the herbicide Agent Orange was contaminated with 2 ppm of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) leading to various adverse health effects].[22] 209 PCBs and 75 PCDDs form groups of congeners. Some of these halogenated substances are carcinogenic through activation of the aromatic hydrocarbon receptor (AhR) which mediates transcriptional responses.[23] The ability to bind to and activate the AhR is therefore used to define the group consisting of subgroups of halogenated biphenyls, halogenated dibenzofurans and -pyrene congeners exhibiting this biochemical ability. The affinity to the AhR is also used to rank potency of different substances within this group or within mixtures, to define toxic equivalency (TEQ[24]). The numerical toxic equivalency factor (TEF) is the ratio of the toxicity of one of the compounds in this category to the toxicity of the two most toxic compounds in the category, represented here by the most potent substance, TCDD.[25]

TEQs are an example of a grouping criterion via a common mode of action. TEFs are used to quantify the effect and rank substances from a group based on the mode of action. Substances can also be excluded from groups based on properties different from the grouping criterion: Azo dyes can be enzymatically reduced to release aromatic amines which are carcinogenic as they form electrophilic, DNA-binding metabolites after metabolic activation of the amino moiety. This would form the group of carcinogenic Azo dyes. A way to avoid this is to ensure that all aromatic amine metabolites are water soluble, e.g. by sulfonic acid moieties at the aromatic rings on both sides of the diazenyl group; this will increase clearance of the metabolites from the body. Azorubine (disodium 4-hydroxy-3-[(*E*)-(4-sulfonato-1-naphthyl)diazenyl]naphthalin-1-sulfonat) is such a sulfonated azo dye which has not shown evidence of mutagenicity or carcinogenicity and is authorized for use in certain foods (E 122, FD&C Red No. 10). The constituent 2-naphthylamine, without the sulfonic acid moiety, is, however, a known human bladder carcinogen.

Per- and polyfluoroalkyl substances (PFAS) and bisphenols, e.g. Bisphenol A (BPA), have long been the focus of regulatory interest to better protect both the environment and human health. Perfluoroalkyl and polyfluoroalkyl substances are a class of synthetic chemicals that have been used for decades. Due to their stability, PFAS were or are widely used in different industrial and consumer applications, e.g. textile impregnation, construction, electronics, Teflon, firefighting foams, and materials used in aerospace applications.[27] Sometimes referred to as "forever chemicals", the moniker is derived from the ability of PFAS to persist in the environment for years or even decades and accumulate in organisms (bioaccumulation). Initially PFAS were considered inert in terms of adverse health effects, but evidence has increased over the years that kidney and liver toxicity, reproductive and developmental endpoints, and alteration of thyroid hormone synthesis and signalling may be an issue; 45 PFAS had evidence across animal and epidemiology data streams.[28] The OECD has identified more than four thousand PFAS[29] that share a common structural feature, a perfluorinated methyl or methylene group. This number has increased dramatically depending on how PFAs are defined and/or categorized, and over 12000 are now listed in the EPA CompTox Chemicals Dashboard.[30] The OECD proposed a grouping approach (termed "PFAS categorization") in 2018. Since then, more grouping schemes to devise regulatory restrictions have been introduced.[31] Instead of deriving similarity from one source chemical, most PFAS approaches use structural descriptors to *build up* categories and subcategories, delimited by chain length as non-polymers, short-chain or long-chain polymers, or by chemical structures such as perfluoroalkyl acids (PFAAs), perfluoroalkyl carboxylic acids (PFCAs), perfluorooctane sulfonates (PFOS) etc. The Royal Society of Chemistry (RSC, UK) has argued that group-based risk assessment prevents regrettable substitution, and proposed a risk-based framework based on a decision-tree approach around PFAS use and scientific evidence of hazard, resulting in five large groups that differentiate

between phase-out and safe-to-use.[32] The most precautionary grouping suggests phasing out all PFAS based on their high persistence alone (the so-called "P-sufficient" approach). The EU is currently discussing restricting PFAS to essential uses only. The least precautionary grouping approach advocates subgrouping PFAS.[33] The U.S. EPA aims via the National PFAS Testing Strategy to categorize PFAS based on similar features. Initially this will rely on structural characteristics, but refinements will include insights from data generated from in vitro high-throughput toxicity (HTT) testing. These categories will help inform potential candidates for further in vivo testing to support hazard assessments.[25a,34] The RSC, EU, and EPA thus proposed three very different approaches to risk management via grouping and similarity, using different criteria including exposure, structure, fate, and hazard (Figure 2). The complexity of defining subgroups of PFAS and possible health hazards was the focus of a publication of an expert panel, in which it was stated that "it is inappropriate to assume equal toxicity/potency across the diverse class of PFAS" and that persistence alone is insufficient for grouping for the purpose of human health risks. They also stated that overgeneralized statements should be avoided and clear differentiation should be made as to which specific substance or subgroup those statements pertain to; broad definitions should only be used as a starting point.

Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl)propane 4,4′-isopropylidenediphenol) is another example of a substance that has received significant attention for many years. BPA has been widely used in the manufacture of plastics, in particular polycarbonate plastics, and epoxyresins since the 1960s. BPA was associated with estrogenic activity, leading to endocrine disrupting effects early on. BPA was hence replaced with BPS—initially thought to be a weak endocrine disruptor (ED). Indeed, BPS did have a lower binding capacity to endocrine receptors (ERs) but a higher affinity to bind to other receptors thereby being an example of a "regrettable substitution".[35]

The groups of PAHs, halogenated biphenyls, dibenzofurans and dioxins, PFAS, and asbestos fibers are used for monitoring exposure[36] and managing risks.[37] They are also used as criteria to design substances with similar technical functionality but with less or no toxicity, thus catering to one of the key ambitions of the CSS, safety (and sustainability) by design (SSbD) concept[38] and avoiding regrettable substitutions.[39,40]

2.2. Read-Across Principles

The most prevalent aim of toxicological grouping is to provide toxicological data for substances which have not been or only insufficiently tested for the specific hazard endpoint of interest (Figure 3). Read-across and grouping, sometimes just termed "read-across" (RAx),[26] is a widely used technique to predict toxicological endpoints for one substance based on information available from other similar substances. According to the OECD, "[···] In the read-across approach, endpoint information for one chemical (the source chemical) is used to predict the same endpoint for another chemical (the target chemical), which is considered to be "similar" in some way (usually based on structural similarity or on the basis of the same mode or mechanisms of action). In principle, read-across can be used to assess physicochemical properties, toxicity, environmental fate and ecotoxicity. For any of these endpoints, it may be performed in a qualitative or quantitative manner [···]".[41] RAx has been used by authorities such as the OECD and the U.S. Environmental Protection Agency (EPA) for more than twenty years[42] and is still one of the most used methods to fill in data gaps in REACH dossiers[43] and to reduce animal testing.

Grouping and read-across has evolved from ad hoc procedures to comprehensive assessments using standardized tools[44] including computational methods using large databases.[45] As a result, RAx can be an efficient approach to hazard assessments as it is significantly less time- and resource-consuming than generating new experimental data for each single substance and reduces the number of animal studies needed. RAx is intentionally endpoint specific. Hence a substance can belong in different groups that were formed for the purpose of RAx of different endpoints. Groups that are formed for the RAx of multiple endpoints conversely include fewer substances than groups specifically formed for one toxic endpoint. Obviously, there is a trade-off between the number of substances in a group and the number of groups needed to address several endpoints. Hence, some guidances advise forming specific subsets more relevant for specific endpoints within a broader group (e.g. Chapter 2.3.2 in ref. [46]of); this has recently been applied to the large group of PFAS (see Section 2.1).

The key premise made in RAx is that substances with a high structural similarity will have similar toxic effects. From a historical perspective, RAx is conducted based on one of two approaches, namely the analogue or category approach (Figure 4 and Figure 5).[44d] The analogue approach refers to grouping based on a limited number of substances and when the target and source chemicals share a known common mode (and/or mechanism) of action.[44d] The category approach is based on grouping chemicals into categories based on their physical-chemical, toxicological, and ecotoxicological properties with the assumption that they are likely to be similar or to follow a regular pattern with respect to the toxicological endpoint being assessed.

Over the years, some key issues have evolved, e.g. once again defining what similarity actually is, identifying and reducing uncertainties, the justification of the RAx being made as well as harmonization across legislations. Since RAx is also based on relevant grouping criteria, these criteria also need to be harmonized for regulatory acceptance.

Ball and co-workers[47] elucidated some aspects of discordant regulatory acceptance using dipropylene glycol methyl ether acetate (DPMA) as a case study for mammalian genotoxicity, subchronic repeated dose toxicity, and developmental toxicity. At the time, the RAx approach was accepted under the OECD high production volume (HPV) program but not under REACH. According to the REACH

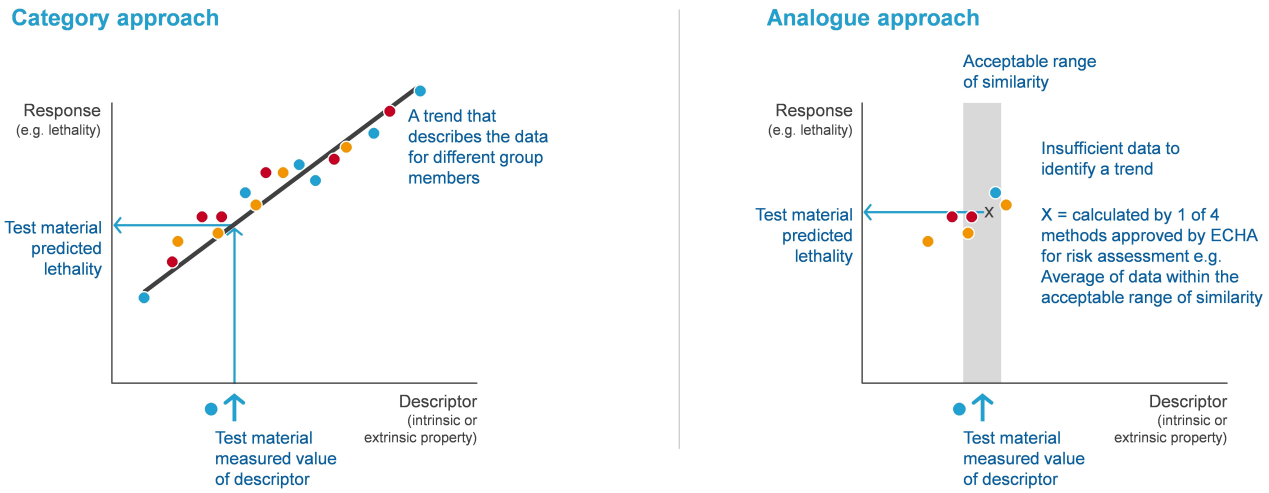


Figure 4. Category approach and analogue approach. Redrawn after ref. [49]. Note that "category approach" is a defined term resembling but not being identical to quantitative structure activity relationships (QSARs).[42]

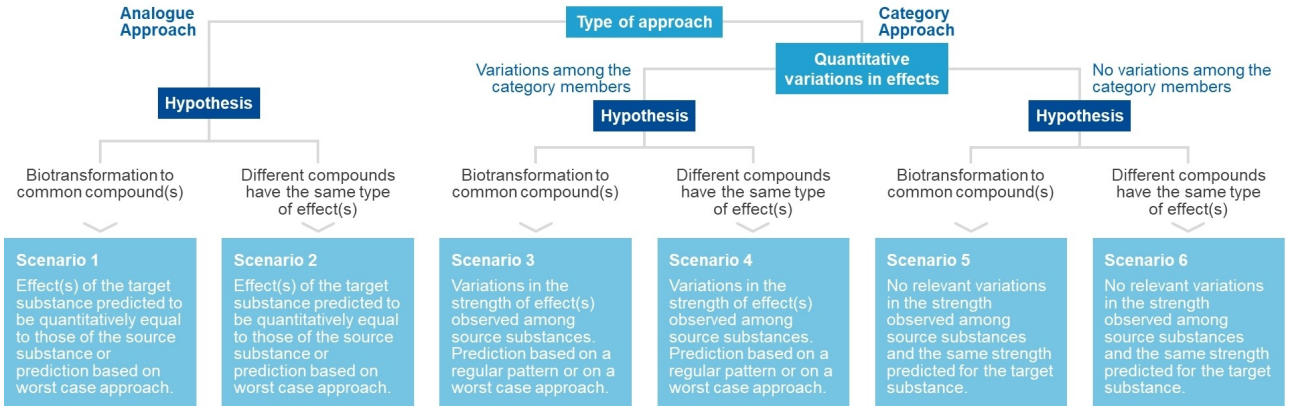


Figure 5. Scenarios of Read-across justifications according to the Read-across assessment framework of ECHA (RAAF), redrawn from ref. [2].

legislation "Application of the group concept requires that physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s) within the group by interpolation to other substances in the group (read-across approach)" indicating interpolation is favored, whereas the OECD HPV program supported both extrapolation and interpolation. An additional uncertainty addressed was the level of data needed and the acceptance of data where there was an absence of toxicity. In this respect, the questions were raised "How much uncertainty is too much? Are there differences in 'acceptable uncertainty' depending on the endpoint, or whether the read-across predicts the presence or absence of toxicity?" This would need to be addressed to avoid unnecessary animal testing and to increase acceptance of the RAx with an appropriate share of higher tier animal studies: In many cases, 10 % of substances with higher tier data might not provide enough data and 100 % of substances with higher tier data may be unnecessarily high. Indeed, a fixed percentage of higher tier studies may not be ideal for all groupings, rather, the demand for higher tier studies could be adjusted to the specific need of the very grouping. Patlewicz and co-workers addressed these issues in a broader scope as a "food for thought" article.[48] They further pointed to possibilities to improve RAx by possibly incorporating mechanistic information, adverse outcome pathways (AOP), and integrated approaches to testing and assessment (IATA). In a recent study, the approaches and tools used for decision making and needs of different regulatory bodies were elucidated.[42] Among the four agencies surveyed, decisions were generally based on hazard assessments. The evaluation processes were also studied and, interestingly, the types of approaches (primarily based on read-across) and decisions were found to vary even within one agency. This possibly reflects an affinity to different tools (e.g. OECD QSAR Toolbox), how routinely they are used, profiling methodologies, etc. Furthermore, the scientific justification by the applicant of one substance looked at was insufficient when presenting the analogs; different starting points for defining categories led to inconsistent results. A common concern was also how to characterize or describe the scientific confidence needed to make a prediction, the level

of uncertainty that was acceptable, the completeness of reporting needed, and variability of in vivo and/or reference data, and access to sufficiently curated toxicological data of high quality. One must also respect that the numerical value of the similarity depends on the algorithm for the distance between two chemicals in the multidimensional space that describes them, and accordingly the limit of similarity for an acceptable RAx must be defined for the specific distance metric.[49]

Over the years much effort has been put into building confidence in RAx approaches. With the application range of computational toxicology (Section 2.3) and data sciences constantly expanding, computational methodologies, such as quantitative structure–activity relationships (QSARs), are moving to the center stage with the models, underpinning algorithms, and data bases rapidly evolving.

Frameworks and good practices are being developed and adopted, as summarized excellently by Patlewicz and co-workers.[42,50] QSAR is most useful and meaningful if a high number of good-quality experimental data exist, the mechanism leading to the toxic effect is well understood, and there is a good correlation with molecular descriptors.[51] Thus many physico-chemical properties can be well predicted by QSAR. For human toxicology, toxic effects with less complexity are better predicted than those with higher complexity: results of bacterial gene mutation tests (close to the molecular event of covalent binding of the test substance to the DNA) or skin sensitization tests (binding of the test substance to skin proteins). Recently, the first OECD guideline[52] included QSAR predictions in one of two defined approaches (DA) to combine methods to assess skin sensitization.[53] According to ECHA, QSAR results of more complex toxic effects, like repeated-dose specific target organ toxicity or reproductive toxicity, should be limited to supporting information for regulatory purposes.[51]

The OECD and others are also in the process of evaluating New Approach Methodologies (NAMs;[54] sometimes termed non-animal methods).[55] In a recent study by Ball and co-workers,[56] a framework for incorporating NAMs into hazard and exposure assessments to meet the requirements of REACH was proposed. A clear consensus of the industry and other stakeholders involved was that the way chemical safety assessment is done needs to change from the system that was developed in the 1980s, and a paradigm shift is needed—not in the least because science, methodologies, and the understanding thereof have vastly changed since that time.[57] In 2017, ECHA published its Read-Across Assessment Framework (RAAF) which structures the scientific evaluation of grouping and read-across approaches under REACH (Figure 6).[44c] Although several frameworks and workflows are used in the regulatory context, e.g. for grouping (described in ref. [58] and compared in [45a]), this is probably the first guidance of its kind published by a regulatory authority. The RAAF provides a framework and guidance for justification of scientific aspects of a proposed read-across case, thereby allowing ECHA and member states to evaluate in a consistent way. It also aims at giving registrants guidance on reporting, improving their dossiers to satisfy the requisite

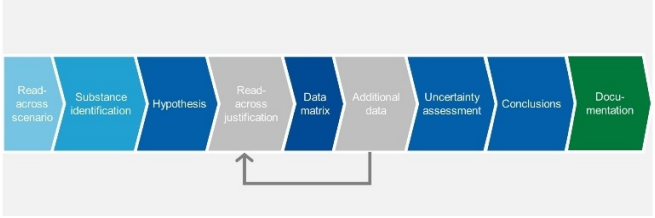


Figure 6. Read-across according to the Read across assessment framework of ECHA (RAAF).[2]

information requirements. It is structured to highlight different scenarios each comprised of different assessment elements addressing aspects deemed to be essential to ascertain the validity of the RAx.[59] It is also stressed that each RAx case is unique and that the document should be a "living framework". As is often the case when approaches are more widely implemented, difficulties and issues can become evident which need further scrutiny or guidance. As the RAAF is also a "living framework" further knowledge can fine-tune assessments and enhance predictions. ECHA later published examples of how RAAF assessments can fail.[60] Reasons can range from insufficient information and characterization of the target and source substances, missing read-across hypotheses, and absence of supporting data for substantiating hypotheses to category definitions (Table 2).

Current RAx primarily focuses on mono-constituent substances; some guidance is available for other substance types, such as UVCBs.[44c] Research is also looking into these substance classes. Integration of biological methodologies to further substantiate RAx is also being used to a greater degree to discern biological patterns.[56,61] The data taken from e.g. NAMs, along with the expansion of in silico tools and machine learning will further reduce uncertainty and improve accuracy.

2.3. Big Data Approaches

The present section explores how big data approaches can support the formulation of a grouping hypothesis and

Table 2: Frequent deficiencies of Read Across (RAx) justifications as identified by ECHA (adopted from ref. [60]).

Read Across justification	Reported deficiencies
Identification source and target substance	Insufficient characterization (identifiers, structure, composition)
Toxicological data of source substance	Data are not reliable or cannot be assessed regarding their reliability
Data matrix of target substances	Inconsistent data, different effects with different substances
Formation of common metabolite or break-down product	Insufficient data, conversion incomplete or not rapid enough, no data on toxicity of the product
Common mechanism of toxicity	No data on mechanism of target substance, clearly different effects or mechanisms

justification of inclusion or exclusion of chemicals from a group. Big data in toxicology may not have reached the dimension of other areas yet, but large collections of toxicological data have been assembled over the years by traditional toxicological studies using laboratory animals and by human biomonitoring data. In addition, new in vitro methods generate high-throughput bioactivity data, together with omics technologies, for elucidation of mechanisms and support of safety assessment.[62]

Since 2007, when the U.S. National Research Council published the report "Toxicity Testing in the 21st century" (TT21C), which called for a paradigm shift from toxicity testing from high-dose animal studies to toxicity testing by in vitro methods, big data approaches have evolved into important tools.[63] The intent of TT21C was to shift toxicology testing from merely identifying safe concentration levels towards a more mechanistic understanding of the adverse effects on the molecular level. Identification of a specific *mode of action* (MOA) constitutes a grouping hypothesis: all chemicals that share a common mode of action are candidates for grouping.[64] Yet, challenges are encountered in the determination of potency, which is needed for proper hazard and risk assessment.[65] Some candidate group members may be excluded due to another MOA that may be more "fit for purpose" for an endpoint. In addition to MOAs,[66] different terminologies for the mechanistic understanding were developed, including the TT21C concept of *toxicity pathway*,[63] the OECD-driven *adverse outcome pathway* (AOP),[67] and the ALTEX-driven concept of *pathway of toxicity*.[68] Each concept can be translated into a grouping hypothesis.[64,69] For reasons of simplicity, the focus in the following will be on MOA and the intent is not to exclude any alternative concept.

NAMs, e.g. cell-culture testing, are evolving into important sources for data generation, but often have limited application ranges, e.g. aqueous culture media pose challenges to the sample preparation of lipophilic or particulate substances. When *establishing* a grouping hypothesis, big data approaches from biological methodologies may need to resort to some data generated by animal testing. In some cases, combinations of "Omics" (e.g. transcriptomics or metabolomics) from in vivo testing and in vitro testing are needed to validate and establish the relevant AOPs.[70] Pairwise comparisons of the in vivo metabolomic profiles of more than 500 substances demonstrated, e.g. that two acetylaminofluorene positional isomers that are also structurally very similar are quite dissimilar in the metabolomics and consequently of low toxicological similarity.[71] In another case, the RAx of two homologues—2-aminoethanol and 3-aminopropanol—was supported by metabolome data by adding confidence in mechanistic similarity.[72] The big data approach thus supported inclusion and exclusion from groups by biological similarity and not just structural similarity.

For the *justification* aspect, the grouping hypothesis was established using animal data, thus making use of the whole-organism complexity, but the same molecular markers used in any of the big data approaches were then derived from in vitro high-throughput screening (HTS) testing to justify the addition of a new chemical to that group.[73] However, although HTS significantly reduces animal testing and increases toxicological data points for assessment, it has some limitations, which need to be considered: HTS is technologically demanding and not widely available (yet). This makes it difficult to generate the multidimensional dataset needed to allow comparisons of a new chemical with the existing databases and to ultimately justify RAx. In addition, the lack of standardization hampers data acquisition and reporting, for both in vitro and in vivo approaches.[74] On the upside, HTS can reliably be used to assign a substance to a group if a MOA can be identified for that chemical, e.g. a specific ligand–receptor interaction constituting a molecular initiating event (MIE) and leading to a shared toxicological concern.[67c] In order to focus testing on the MIE, connectivity mapping[75] or other approaches can be used to tailor in vitro assays to a particular chemical class, and although the applicability domain may be reduced, increased efficiency or accuracy can be attained. One example would be endocrine activity, in which HTS in vitro testing with connectivity mapping resulted in groups of chemicals that were each similar in terms of their behavior as agonists or antagonists to specific receptors.[69] Since cellular pathways do not act in the same manner in all cells, a minimum requirement for grouping purposes is that the justification of similarity is derived from the same assay with same cell line and receptors for all compounds to be assessed on the mechanistic level.[69]

Big data approaches furthermore enable the generation of data covering more diverse chemical compositions. Luechtefeld and co-workers approached the question of how many chemicals need to be known to assess by grouping and read-across all other chemicals.[45b] The specific example performed a pairwise comparison of each of 33383 substances with no data to each of 1387 chemicals with known data from Annex VI of the REACH legislation. An automated nearest neighbor prediction (also termed RASAR for read-across-based structure–activity relationship[45b]) thus identified for a specific CLP data gap the closest neighbor that was negative and the closest neighbor that was positive in this classification. A random selection from the list of 1387 chemicals indicated that less than 25 % of the 33383 chemicals remain unassigned, with 600 chemicals available for read-across (Figure 7). The present example used only structural similarity, and adjusted the required similarity to the level of reproducibility of the in vivo OECD test guidelines.[45b] The read-across of CLP classifications is highly relevant to the Green Deal's GARM, without further differentiation currently.[76] However, Luechtefeld and co-workers also demonstrated that the balanced accuracy of the structure-based approach can be improved from 70–80 % to 80–95 % when additional toxicological information is incorporated in a "data fusion RASAR". In their initial approach, the additional information consisted of CLP data from other health hazards, but potential HTS testing approaches would also be feasible.

In another implementation, Shah et al. developed the freely available Generalized Read-Across (GenRA) software package that infers missing data by interpolation from

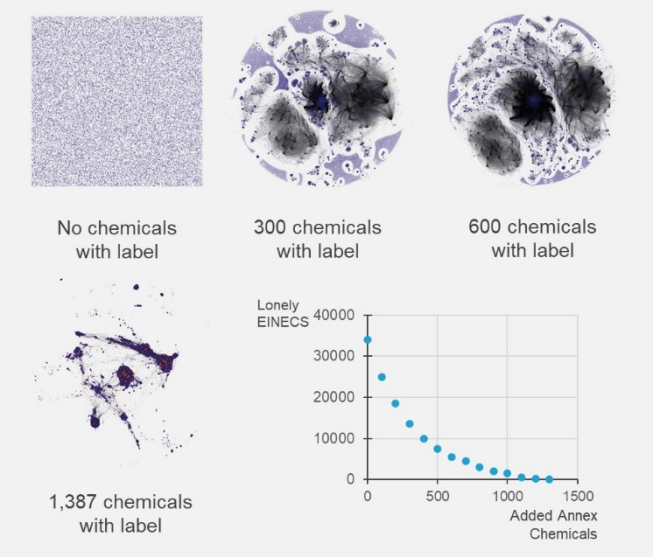


Figure 7. Read-across (RAx) of CLP classification: Two substance lists of 33 383 substances (European Inventory of Existing Commercial Chemical Substances [EINECS]), representing here chemicals with no data, and 1387 chemicals (Annex VI of the CLP legislation) are used, representing chemicals with labels. EINECS compounds are represented in blue and ANNEX VI Table 3.1 compounds are in red. At the start, none of the 33 383 has neighbors with data. When an increasing number of chemicals are chosen from the 1387-chemical list, more and more chemicals find neighbors indicated by the contraction of dots linked by Jaccard similarities. The number of neighbors is symbolized by the size of red dots. Edges represent similarities between EINECS compounds and Annex compounds. Reproduced from ref. [45b] under Creative Commons BY-NC, © The Author(s) 2018. Published by Oxford University Press on behalf of the Society of Toxicology.

the nearest neighbors. Interestingly, the determination of the nearest neighbors can be based either on distances in chemical structure descriptors or by distance in extrinsic properties, such as experimentally measured bioactivity data or both.[45c] The GenRA can then predict either categorical data such as CLP data or continuous endpoints such as LD_{50} toxicity. Also the ChemBioSim software package combines intrinsic descriptors of structural similarity with extrinsic descriptors of bioactivity to predict in vivo genotoxic (MNT), hepatic (DILI), and cardiological (DICC) issues.[77]

The diverse class of petroleum substances was successfully grouped by high-content imaging and high-throughput transcriptomic analysis of cells exposed to solubilized extracts. Grimm et al. observed a high degree of correlation between ToxCast bioactivity profiles and physicochemical properties, as well as improved groupings when intrinsic structure and extrinsic biological activity were combined.[78]

Challenges and opportunities in big data approaches were reviewed by Richarz and are relevant to both the formulation of a hypothesis and the justification of inclusion or exclusion.[62a] Building models on a broader data basis increases the applicability domain of the hypothesis[79] and also generates the read-across source data that is essential as demonstrated in Figure 7. However, data comparability (Figure 8)[62a] may be the most critical challenge to the use of big data approaches for grouping. Making sense of the multidimensional HTS data requires extensive data interpretation and expertise in computational toxicology;[80] often reduction e.g. via visualization, principle component analyses, or wherever possible AOPs, for a better understanding of the complex data involved is needed. The challenges of variability (of the assay) are exacerbated by variability of the test item, where Richarz mentions nanomaterials, which are inherently polydisperse in particle size and other properties, but the same applies to polymers, which are by definition polydisperse in molar mass.

This may present a considerable challenge with the upcoming registration of large numbers of polymers for REACH.[76] Approaches to the grouping of nanomaterials are reviewed in Section 2.4. Richarz also noted that HTS-based NAMs could be validated directly by epidemiology (without conventional animal testing). This would address

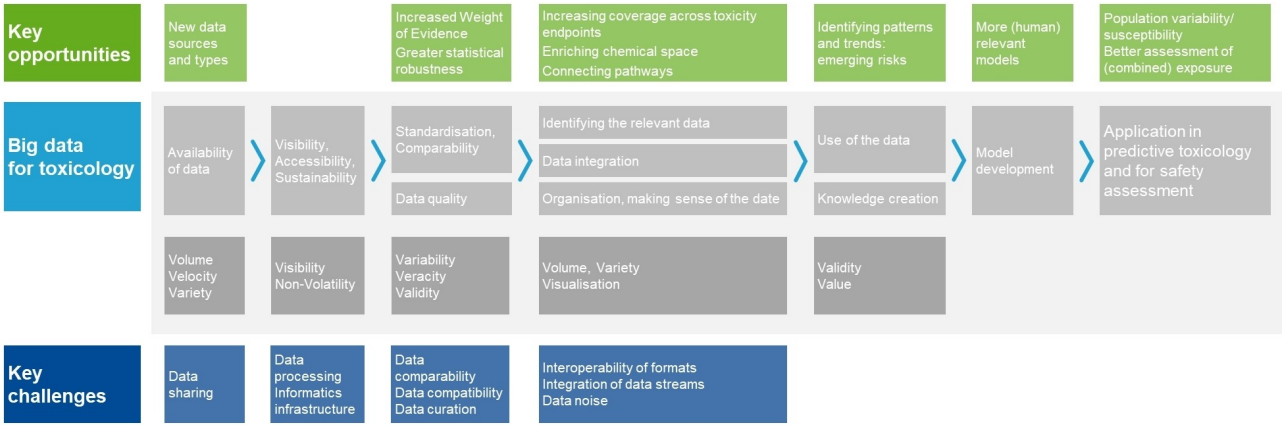


Figure 8. Challenges and opportunities of big data approaches, redrawn after Richarz.[62] Specifically for grouping, data comparability is the most critical challenge, and the expansion of the chemical space is the most valuable opportunity. Additionally, wherever big data helps to identify a pattern, it can motivate a hypothesis of grouping, and the increased weight of evidence and greater statistical robustness can strengthen the justification of grouping.

an interesting way forward as it places emphasis on risk assessment instead of hazard assessment, because both exposure of the individual as well as the internal exposure of target sites could be used as steps of the validation process[62a]

2.4. Two Ongoing Challenges: Nanomaterials and Polymers

Nanomaterials challenge chemical regulations—and grouping approaches— owing to their existence in several forms of the same substance that differ at least in their size, with each form additionally being polydisperse (having a distribution) in size. In the EU, nanomaterials are defined for regulatory purposes as the form of a substance that contains more than 50 % of the particles in the size range 1 to 100 nm.[81] The consideration of the physical structure, in addition to the chemical structure, adds a further layer of complexity due to the numerous descriptors of the nanomaterial that potentially justify a grouping hypotheses: Just as examples, the band gap of semiconductor nanomaterials and more specifically the electrochemical potential of the conduction band may be linked to induction of biological oxidative damage,[82] the size may be linked to systemic uptake,[83] and the covalently bound surface treatment, and the adsorption to proteins may be linked to cellular recognition and endocytosis.[84] Two decades of intense global research gravitated around various specific aspects:[85] Was the chemical substance of the essence or were the physical size, shape, crystallinity more important?[86] What was the correlation with in vivo endpoints and ultimately by the predictivity for adverse effects on human health?[87] Could alternative methods that use in vitro screenings and "functional assays" that measure the *interaction* of the nanomaterial be used for justification of grouping?[88] Decision trees were among the OECD approaches to the grouping of nanomaterials.[89] A decision tree reduces ambiguity and reduces the number of potentially relevant grouping criteria. The revised REACH regulation (2020) placed the focus on decision-making based on the chemical substance, particle size, shape, and surface treatment as the properties that define the boundaries of a registration dossier.[81] The ECHA guidance prioritized primarily descriptors of behavior and interaction to justify grouping, such as the propensity to aerosolize by occupational handling (dustiness), or the solubility and dissolution rate, biological reactivity, dispersion stability, and more.[90] No guidance was provided on valid and accepted methods nor on decision criteria. For registration of sets of similar nanoforms, ECETOC NanoApp filled this gap by providing a freely available digital tool to guide registrants to methods, and to process resulting data by comparing the similarity of the different nanoforms in a simple x-fold algorithm with predefined quantitative decision criteria (Figure 9). Considering the inherent polydispersity of nanoforms with distributions in their defining properties, the NanoApp allows a simplified Tier 1 (Figure 9) decision when the distributions overlap, e.g. when the mean size differs less than 1.5-fold, but the typical polydispersity is about 50 %, any clear

Figure 9. Data to substantiate similarity of nanoforms, as proposed by ECETOC.[91a] For each of the properties listed above, potential tests and quantitative thresholds for sufficient similarity were specified.

distinction between the different nanoforms is difficult.[91] For less similar nanoforms, Tier 2 would require the measurement and pairwise comparison of extrinsic properties and in vitro assays (Figure 9). But also in Tier 2, criteria of acceptable similarity are predefined in the NanoApp and come to grouping decisions without expert judgement.

The GRACIOUS grouping framework went a step further to justify endpoint-specific grouping and provided a logical and science-evidenced approach to group similar nanoforms, allowing read-across of hazard information from source nanoforms (or non-nanoforms) with adequate hazard data to target nanoforms that lack such data.[92] The GRACIOUS framework guidance[93] and digital blueprint[94] provided support to the user to generate a grouping hypothesis that encompasses the relevant physicochemical characteristics, route of exposure, and hazard endpoints. Integrated Approaches to Testing and Assessment (IATAs) are then used to gather the existing information needed to justify the grouping hypothesis, and to guide the generation of new data to fill data gaps. The IATAs consist of decision trees, possible tiered testing strategies (e.g.[95]), and guidance on standardized methods. If sufficiently similar, the data can then be used to support grouping and RAx. A re-analysis of the most frequently used decision criteria in all IATAs confirmed the prioritization of properties of the NanoApp as well.[96] However, only recently it was discussed and demonstrated how important it is to crop data to the biologically relevant range before applying similarity analysis (Figure 10).[49] Although developed on descriptors of nanomaterials, e.g. dissolution rate or surface reactivity, this concept is transferable to other grouping aims, where the pairwise distance of two chemicals should be set to zero if both have values beyond the biologically relevant range, which can be defined by controls or representative test materials (RTMs).[94] Examples include very slowly dissolving materials and very low toxicity materials, such as the PSLT category.[97] Nanoform grouping across substances was not specifically addressed by the current ECHA guidance,[90,98] but grouping across substances is explicitly considered by the REACH legal text.[81] The comparison and potential grouping across substances was explored by NIOSH.[87] Health Canada developed a prioritization of

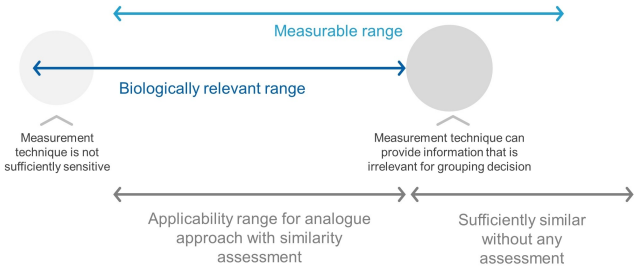


Figure 10. Limitations to the application range. The range of descriptor values that is measurable and the biologically (or environmentally) relevant range may not map onto each other perfectly. Understanding of this relationship helps to inform data ranges suitable for similarity assessment of "where they go" and "what they do" for both analogue approaches and for category approaches. Similarity assessment is possible and required only for an analogue approach within the overlap of measurable and biologically (environmentally) relevant ranges—this overlap defines the application range. Ideally, representative test materials (RTMs) for the upper and lower limits are included in the measurement of a candidate NF group. Redrawn after ref. [49].

nanomaterials that first grouped by substance, and then assessed exposure, intended use, properties, and toxicity, hence a balanced mix of descriptors relevant to risk assessment.[99] Regarding the relevant properties, the concept referred to the intrinsic and extrinsic properties selected in the ECETOC grouping scheme, which is consistent with the more recent NanoApp (Figure 9).[100]

Also polymers are by definition polydisperse, and many of them will have at least partially overlapping molar mass distributions. Only some polymers exist as solid and insoluble particles,[101] and may then fulfill the criteria of the ECHA restriction of intentionally produced primary microplastics.[102] Other polymers are not microplastics but may still change their form during different lifecycle stages, e.g. by dissolution, swelling, or degradation. But in either case, polymers are generally not present as monoconstituent substances, but as complex polymer products consisting of the polymeric substance (polymeric macromolecules), intentionally added substances (IAS; e.g. stabilizers), and non-intentionally added substances (NIAS; e.g. impurities).[103] Since the exemption of polymers from REACH registration will be gradually revoked, the number of polymers that will require assessment was estimated to exceed 100 000,[104] and here risk assessment by grouping will be even more crucial for polymers than for any other class of substances. Only the OECD concept of "polymers of low concern" (PLC) constitutes an established group of polymers; PLC are delimited by one-sided cut-off criteria on molar mass (no bioavailable components), reactive groups (none), charge (no cationicity), and biodegradability (none, hence no need to assess degradation products),[105] and may contain most polyolefins and other plastics with low NIAS concerns. Further proposals on a general approach towards polymer grouping have been made.[95] This include examples on polymer classes such as polyether polyols that may form a category with systematic scaling of properties.[106] However, for assessing polymers at all, there is no consensus on the properties to be assessed[104] and method applicability.[107]

3. Grouping beyond the Generation of Hazard Information

3.1. Toxicological Threshold of Concern (TTC)

The threshold of toxicological concern (TTC) is considered to be a pragmatic risk-based approach that has gained traction over the past decades.[108] It is used, e.g. for safety assessments for which there may be limited toxicity data available. The TTC concept relies on establishing a human exposure threshold for substances, below which there is a very low probability of harming human health. Threshold values are based on toxicological information, endpoint, and potency of a large set of substances. This mandates that these databases are curated to contain adequate and relevant data of high quality.

The TTC was originally developed for substances found in low levels in consumer foodstuffs. The European Food Safety Authority (EFSA) adopted a guidance document in 2019 describing the use of the TTC concept in food safety assessments.[108b] Cramer and co-workers[109] classified organic chemicals into one of three classes (Table 3): Later, these three classes were amended by two groups: substances with structural alerts for genotoxicity[110] and neurotoxicity due to inhibition of choline esterases.[111] As such, the TTC can be

Table 3: Grouping of substances for assigning thresholds of toxicological concern (TTC).

	Description	TTC ($\mu g\,kg^{-1}$ b.w./day)
Cramer Class I	Substances of simple chemical structure with known metabolic pathways and innocuous end products which suggest a low order of oral toxicity.	30
Cramer Class II	Substances that are intermediate. They possess structures that are less innocuous than those in Class I but they do not contain structural features that are suggestive of toxicity like those in Class 3.	9
Cramer Class III	Substances with chemical structures that permit no strong initial impression of safety and may even suggest a significant toxicity.	1.5
Substances with structural alerts for …	Genotoxicity	0.0025
	Cholinesterase inhibition	0.30

considered a type of grouping albeit not based on the typical structural similarities found in other types of grouping approaches.

The no observed adverse effect level (NOAEL) is a scalar descriptor of potency; it describes the greatest concentration or dose of a substance at which no adverse effects are observed in a given experimental model or a monitored human population. Munro and co-workers[112] assigned 613 organic chemicals to the Cramer classes and used 2941 NOAELs of non-cancer effects found in various experimental studies with these substances to propose TTC values for the Cramer classes. As a rule, the 5th percentile of the most conservative NOAELs was selected, derived from tests using the most sensitive species, sex, and endpoint. In addition, a factor of 0.01 (a so-called "safety factor" of 100 to account for inter- and intraspecies variability in toxicokinetics and toxicodynamics) was applied to establish the final TTC. The databases are constantly being updated which expands the chemical space and allows the inclusion of additional substances[113] i.a. for PFAS (Section 2.1)[114] and with emphasis on specific toxicity endpoints.[110,115]

The original TTC was established for life-long oral exposure. It has now been expanded to other exposure scenarios: TTCs have been defined for occupational inhalation exposure[116] and for cosmetics, which are topically applied.[108a,117] However, far fewer dermal repeated-dose studies have been conducted than oral. Therefore, no TTC values based on the dermal route can be calculated, making the oral TTC values still applicable. Dermal TTC were, however, established for skin sensitization.[118]

The TTC is used in regulations of pharmaceuticals (genotoxic impurities)[119] and food (flavoring and packaging[120]). TTC as a pragmatic filter to deprioritize testing needs for the REACH legislation has been proposed but is not fully incorporated in its implementation.[121] A similar—however, more limited—concept of grouping substances with a one-sided assessment is the PSLT concept (poorly soluble particles of low toxicity).[122]

3.2. Grouping for Risk Management: Exposure and Control Banding

Occupational Exposure Banding (OEB) is a tiered strategy used in a workplace setting with the aim of informing the selection of appropriate protective measures to ensure worker safety (Table 4). OEB and control banding differ in that control banding directly links the hazard to specific exposure-based control measures. In contrast to other grouping concepts, which use similarity of physical or chemical properties of chemical substances to form groups for a joint risk-assessment, OEB primarily uses hazard information to inform risk management. It groups primarily air-borne substances, for which no occupational exposure limits (OELs) have been defined, into one of five distinct groups (here called "bands"). This is achieved by taking combinations of hazard-based information derived e.g. from safety data sheets and linking it to exposure-based information from other substances with a similar hazard profile and classifying according to the severity of potential health hazards associated with worker exposure to that substance (Table 4). Since it is hazard based, OEB is also known as "hazard banding". Combined with data available on the toxicity of the substance, a tiered approach can be used to assign the substance to a range of concentrations (bands, Table 4) thereby informing chemical risk management decisions (Figure 11). A broad range of hazard endpoints are assessed and the endpoint exhibiting the most severe effects is used to assign the substance to an OEB. The hazard phrases (H350 etc.) also reflect CLP classification, as used in Sections 2.3 and 3.3. OEBs are not meant to replace Occupation Exposure Limits (OELs) which are based on detailed and specific risk assessments.[123–125] OEBs are meant to derive exposure limits and with it risk mitigation measures that ensure worker health and thereby provide an interim solution until an OEL can be provided.[126]

3.3. Emerging Examples of Grouping for Risk Assessment and Restriction

The capacities for toxicological research and risk assessment are limited. ECHA uses grouping for prioritization of substances which require further regulatory action. Initial,

Table 4: NIOSH Occupational Exposure Banding and COSHH/HSE group of hazards that have adequate control (step 1).[105]

Occupational Exposure Band	A	B	C	D	E
Particulate matter/dust [mg/m³] NIOSH	>10	>1 to 10	>0.1 to 1	>0.01 to 0.1	≤0.01
Particulate matter/dust [mg/m³] COSHH	>1 to 10	>0.1 to 1	>0.01 to 0.1	<0.01	–
Gas/vapor [ppm] NIOSH	>100	>10 to 100	>1 to 10	>0.1 to 1	≤0.1
Gas/vapor [ppm] COSHH	>50 to 500	>5 to 50	>0.5 to 5	<0.5	–
Allocation of H-phrases (step 1 of COSHH)	H304, H315, H319, H336, EU66	H302, H312, H332, H371	H301, H311, H314, H317, H318, H331, H335, H370, H373, EU71	H300, H310, H330, H351, H360, H361, H362, H372	H334, H340, H341, H350, EU70

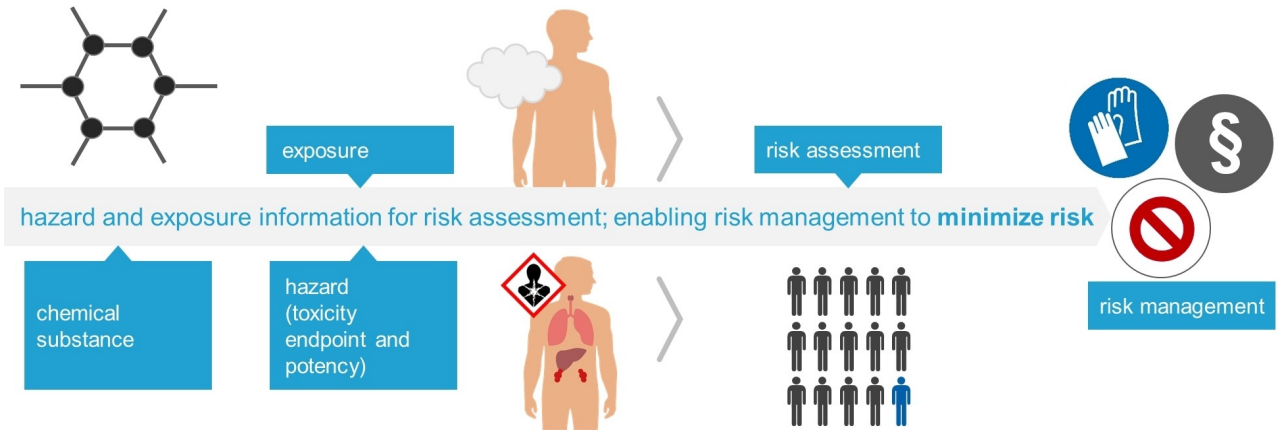


Figure 11. Information on hazard (type of toxic effect and its potency) and exposure (external and/or internal exposure) is used to assess the probability and magnitude of potential harm to human health (risk assessment). This information is used to devise protective measures (Risk Management, such as warnings, personal protective measures or restriction and bans) to minimize the risk to an accepted level.

putative group members are identified by chemical similarity and profilers of the toxicological endpoint to be addressed; then the grouping is manually refined based on further information and assessment.[127] ECHA's Integrated Regulatory Strategy aims to speed up data generation, identification of groups of substances of concern, and regulatory action. It uses initial grouping to clarify which substances are a high priority for further regulatory risk management or data generation, and which are currently a low priority.[128] The number of chemicals screened has increased from around 200 per year to 1900 by initial grouping according to informal estimates.[129] The "restriction roadmap" of the EU defines groups of substances, e.g. PFAS (Section 2.3), medium-chain chlorinated paraffins, substances containing PAH, bisphenols (Section 2.3), pyrazoles, *ortho*-phthalates, acrylates, and methacrylates. This grouping is intended as an interim approach to prioritize restrictions until a GARM is fully implemented.[130]

The EU-funded project HBM4EU identified groups of priority substances for human biomonitoring.[131] These were identified in three subsequent prioritization processes. The selection criteria are complex and are not based entirely on scientific evidence but also include support of EU policy making. Criteria include concerns to human health, evidence of human exposure, public concern, potential for innovation of regulatory risk assessment, and open policy questions as well as financial and technical feasibility of monitoring.[132] The decision process involves surveys and expert judgement. The individual substances within a group are divided into four categories mainly based on the availability of human biomonitoring data, its regulatory status, hazard information, and the availability of analytical methods for biomarker analysis.[133] The rationale for grouping substances can include: the use of common analytical methods for detection; substances put to common uses; and/or substances that exhibit similar toxicological profiles.[132] Each group identified by this procedure reflects several of the criteria; one common criterion is predominant for some groups: There are groups of structurally related substances (such "Aniline family", PAHs, or PFAs) with presumed common toxicity mechanisms but also groups of structurally unrelated groups of substances with common applications (such as flame retardants, UV-filters, or pesticides), common physical properties (such as aprotic solvents) or common origin (such as mycotoxins). Grouping is not the focus of the HBM4EU project, but it is an example of a complex grouping process using various criteria to prioritize substances.

Humans are constantly co-exposed, intentionally or unintentionally, to a multitude of substances. For pesticides, active substances with common target organ toxicity and toxicity mechanisms are summarized in so-called cumulative assessment groups (CAG). Bräuning and co-workers[134] proposed to additionally consider toxicokinetic effects for the grouping of substances to predict mixture toxicity. The proposed common kinetics groups (CKG) are defined using inhibition and induction of xenobiotic-metabolizing enzymes and transporter proteins as criteria.

The EFSA drafted a guidance document on criteria for grouping substances into groups for risk assessment of combined exposure.[135] The grouping procedure uses a hierarchical approach and encompasses common AOPs and common target organ toxicity. AOP information is considered to be the gold standard criteria to form assessment groups. Whereas toxicokinetic information should not be used in isolation for defining assessment groups, the combination of toxicokinetic and toxicodynamic aspects could provide a robust basis for grouping. Since there are many possible combinations of substances, assessment groups can be prioritized using a risk-based (i.e., hazard and exposure) or a solely exposure-driven approach.

3.4. Chemicals Strategy for Sustainability (CSS), Generic Approach to Risk Assessment (GARM) by Classification and Labelling of Products (CLP)

Over the past two decades, the European Union has been trailblazing chemicals legislations with regulations such as

Registration, Evaluation, Authorization and Restriction of Chemicals (REACH[136]), and most recently the European Green Deal policy priority (EU GD[76]). The "Chemical Strategy for Sustainability: Towards a Toxic-free Environment" (CSS) has taken the regulatory policy to a new level.[137] The CSS is an element of the "zero pollution plan"[138] and is a key component of the EU Green Deal. It encompasses, amongst other aspects, banning harmful substances from consumer products where possible, taking the effects of chemical mixtures better into account, and establishing a less complex " one substance one assessment" process for assessing hazards and/or risks of substances. Currently, possible revisions to the REACH legislation to accommodate the new requisites posed by the EU GD are being impact-assessed by the Commission with a view to making a legislative proposal by the end of 2022.[139]

Risk management to mitigate risks is one of the ultimate goals of REACH and CSS. Grouping offers a way to swiftly achieve this.[140] It informs the ECHA Integrated Regulatory Strategy (Section 3.3) and provides a basis for possible group restrictions. These restrictions can be implemented by the generic approach to risk management (GARM; often used synonymously with generic risk approach, GRA). GARM uses hazard classes to derive risk management measures. The European Chemical Industry Council (Cefic) describes the GARM as "[…] an automatic trigger of predetermined risk management measures (e.g. packaging requirements, restrictions, bans, etc.) based on the hazardous properties of the chemical and generic considerations of their exposure […]" whereby "[…] specific risk assessments consider the hazard, the use of the substances and related specific exposure scenarios for humans and the environment, and risk management measures are triggered based on their outcomes […]".[141]

Within risk assessments, the hazard and the risk (exposure to the hazard) are considered (Figure 11). Although the hazard (intrinsic property of the substance) remains the same, risk can vary considerably, depending on the potency (, i.e., how much of the substance is required to produce an effect), how, where, how much of the substance one is exposed to and how much of the substance is actually absorbed and how fast it is eliminated from the body. GARM is an relatively undemanding process, yet the risk management is based on hazard and disregards other relevant information to assess the risk to be managed.

Any simplifying approach—grouping of substances, classifying to hazard classes, or generic approaches to risk management—offers the benefits of practicality and convenience and bears the risk of unwarranted assessments. This has been critically discussed: The German Federal Institute for Risk Assessment (BfR) mentioned in a recent commentary "[…] the more 'pragmatic' but at the same time less scientifically sound such proposals are, the more can they be expected to create conceptual incompatibilities and severe problems for downstream regulation later on […]".[142] And van Dijk and co-workers concluded "Politicians require the simplification and standardization of risk assessments, but at the same time, it is essential that the use and utility of novel scientific findings are increased […]. There is an urgent need to strengthen the utility of science for policy and to improve the science–policy interface. […]".[143]

4. Summary and Outlook

4.1. Lessons learned from existing grouping approaches

Most grouping approaches generate missing data on hazard by acting as a prediction tool. The principle is similar to that of the periodic table, which enabled the prediction of hitherto unknown elements and their chemical properties based on the electron structure, whereby this underlying principle was only clarified in retrospect—the periodic table was assembled by observation of physical and chemical properties only. Sound grouping can initially be empirical and does not always need to deliberate the underlying principle. PAHs were initially grouped as carcinogens by the same heuristics. Nonetheless, the grouping of PAHs, grouping of polyhalogenated aromatic hydrocarbons, but also grouping of all asbestos fibers was only fully efficacious once the common mechanism of the group's specific toxicity was established (Section 1, Section 2.1). This enabled quantitative ranking of the substances *within* a group and the search for substances at the verges or even *beyond* the defined boundaries of the group, resulting in both guidance for product development and relevant regulations.

Establishing the mechanism of toxicity—and with it the grouping criteria—, polyhalogenated aromatic hydrocarbons (PAHs) and asbestos fibers required decades of research. These timeframes are not practical for the grouping of the many different substances needed for regulation and risk management. Science and computational tools are now readily at hand to do so, but questions remain: How much evidence is needed, how comprehensive must a grouping justification be, how detailed shall this be prescribed in rules, and what are the demands on the science needed to back predictions? Grouping and RAx are widely used methods to fulfil information requirements of REACH. Their application, however, was found to be deficient in numerous cases (Table 2). Several of the early groupings within the OECD program (Table 1) would now not be fully acceptable for REACH according to the RAAF. While the RAAF ensures a consistent assessment of RAx, it is also highly demanding and requires a high level of certainty. Fit for purpose groupings, which are deficient according to the RAAF, can still be valid and useful for many purposes—including some aims of REACH.

RAAF and other grouping guidances describe a limited set of grouping criteria and above all, with structural similarity being indispensable. This can err on two sides: similar structures can cause dissimilar biological responses (cf. activity cliffs, example of isomers of acetylaminofluorene in Section 1) and structurally dissimilar substances can cause the same biological response (cf. promiscuity of the AhR, Section 1). An earlier review of lessons learned from RAx of chronic health endpoints also concluded that similarity in chemistry was often not enough justification, and instead toxicokinetic and/or toxicodynamic similarity (especially

similarity of metabolism) was essential and, if not known, a driver of the overall uncertainty.[144] Modern grouping concepts for nanomaterials use different criteria, including external properties (functionality) of the substances. These are arranged in tiered approaches. This allows for using the data best fitting the substances under scrutiny and the toxicity to be predicted. OECD guidance on grouping is currently being updated to reflect experience gained, and by including modern approaches to nanomaterials (Section 2.4).[46,144]

Among the data to be included in the construction of grouping justifications are so-called "big data" (Section 2.3). These include use of data-rich methods (including data generated on biological effects using Omics) for the substance itself as well as use of the wealth of existing data from other substances. Good knowledge of the applicability and limitations of the test methods is therefore also key. The most convincing grouping justifications are indeed those that combine distinct intrinsic and extrinsic properties with big data (Figure 3). This requires even more complex evaluations of large data quantities, tiered approaches, and decision trees—all of high quality. The main challenge will then most likely be the high level of expertise needed to understand and interpret the data. Oversimplification could then well lead to less accurate predictions and/or less acceptance.

Decision trees with pre-defined quantitative decision criteria are well described for the new grouping approaches for nanomaterials (Section 2.4): the properties of a substance to be used as a decision criterion, the numerical threshold for the decision and the method to obtain the data for this decision. This is largely also the case for traditional well-elaborated groupings, such as those for PCBs or those related to the fiber paradigm (Section 1). Others, such as PSLT (Section 2.5) are established concepts, but the exact criteria are still under discussion. Guidances on general grouping of chemicals (Section 2.2) primarily provide just that—a guidance—rather than decision trees with exact decision criteria. Thus, those grouping decisions are ultimately expert-driven rather than data-driven and qualitative in nature.[45a] Grouping without data-based decisions according to defined rules and criteria can lead to debatable decisions—as highlighted by some of the examples in Section 2.1.

The data to support and justify grouping can also be questionable if provided by methods with limited accuracy. Grouping concepts have not yet systematically implemented the assessment of uncertainty, although several proposals exist e.g.[45a,145,146]. Estimating and reporting uncertainties of a grouping and its justification allows judging its accuracy and reliability. This entails caution when grouping substances with properties just above or below the thresholds of the decision criteria. Yet, the confidence in the decisions made should also lead to them being accepted, if generated using the proposed criteria— without additional precautionary principles then being implemented.

Toxic effects themselves are not an intrinsic property of a substance, but the result of its interaction with biological material, i.e., an extrinsic property (Figure 2, Figure 11). Extrinsic properties which are closely related to the toxic effect and the use provide good justifications for a grouping, as they require good knowledge of the relation of substances' external properties and their toxicity. The current research on describing and characterizing AOPs with key events (KE) and KE relationships (KER) is a step in the right direction. Ideally, NAMs addressing KEs should be used wherever possible, as these NAMs can be fit to generate data to support and justify grouping. At the same time, grouping and RAx are methods that also generate toxicological data and hence are also regarded as NAMs. Indeed, the use of NAMs, grouping, and next generation risk assessment (NGRA) can be synergistic and hybrid approaches are emerging (examples: nanomaterials,[147] for cosmetics,[148] PFAS,[28] and ongoing research projects[149]). The draft EFSA guidance sees AOP-based grouping as the "gold standard", but allows other criteria, such as a common toxicological effect, both for group inclusion and exclusion.[150] Quantitative considerations would require quantitative AOPs (qAOPs), which is another focus of current research,[151] that also needs to take internal doses and biokinetics into account.[152] The true impact of biokinetics is still an open question.

Current grouping concepts to generate hazard information consider quantitative aspects when using category approaches if there is a trend, i.e. a regular pattern of change in a property among the substances of the category. Analogue approaches demand a high degree of similarity of target and source substances regarding quality (e.g. type of toxic endpoint) and quantity (e.g. potency) of effects, each with different decisions to be made. When the distinct information on hazard is not needed, other grouping approaches may be more suitable and possibly more accurate for the "purpose"; for example, even the simple classification of any substance with a pH of >11 as being corrosive can be fit for purpose where exposure to skin or eyes is anticipated, although the criterion is actually a one-sided grouping based on an external property only.

Historically, hazard information derived from animal studies was the tool for risk assessments used to ultimately protect human health by risk management measures. "REACH aims to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances."[153] NAMs do not provide the same hazard information as animal studies currently used in regulations although some can lead to the same classification if developed to do so. Yet, the historically used methodologies may not be fit for purpose in the future. NAMs and/or grouping provide information that could and should be directly used for risk assessments or to prioritize substances for individual assessment. Several applications of grouping do exactly this: OEB uses grouping to devise risk management measures limiting occupational exposure. HBM4EU, EFSA, and ECHA use grouping to prioritize substances for human biomonitoring, mixture assessment, and further regulatory actions, respectively (Section 3). In contrast, the proposed GARM, which is part of the CSS, will automatically restrict or ban substances based on predefined hazards

alone. Categorizing large groups of substances with initial concerns based on broad criteria is an efficient way to prioritize substances for future assessments, or as preliminary action (Section 3.1). However, the categorizing (or grouping) may then not be sufficient to actually justify the restrictions as they do not take exposure or potency into account which are major aspects of risk and risk management, i.e. may not be fit for purpose in this context. For that, detailed assessments of the very specific substance or group, and/or more scrupulous grouping schemes may well be needed. A further aspect to consider: RAx and the increased use of AOPs are used for various legislations, be it biocides, food, and feed, ingredients used in cosmetic applications, pharmaceuticals, or other chemicals, and not only in Europe —but mutual acceptance is not a given. Thought also needs to be given how to remedy this, e.g. by cross-sector and cross-regional cooperations as are currently ongoing.

Grouping of substances for RAx principally focusses on *including* specific substances which have not been tested for specific toxic effects with the aim to obtain information regarding this toxicity endpoint for that substance (Section 2.2). To achieve this, rules and criteria must be defined to demonstrate that the substance falls within the predefined boundaries of the group. In contrast, grouping approaches for prioritization and defining initial concerns follow another principle—defining wide-ranging criteria to include a substance to a group. Here rules and definite criteria are needed to then *exclude* a substance from a group. A process to determine whether or not the properties of chemicals within a category fall within an appropriate domain from which a reliable read-across prediction can be made was introduced by Pestana et al.[154] Prioritization has been used in the past to find less hazardous substances with the same or similar technical applicability, for example synthetic vitreous fibers instead of asbestos (Section 2.1), with this principle being a central element of CSS: finding the "right" chemicals by SSbD.[155] Unfortunately, regrettable substitutions do occur if evaluations are not done meticulously and with use of good science-backed knowledge;[79] one example is the substitution of dichloromethane (a suspected carcinogen, category 2) as a brake cleaner by the neurotoxic *n*-hexane. The substitution of *n*-hexane by *n*-heptane, which does not cause this specific neurotoxicity, is an example of a successful substitution (see "activity cliffs" in Section 2). To reach the aim of CSS, to better protect citizens and the environment, and boost innovation for safe and sustainable chemicals, de-grouping substances may be as important as grouping substances.

4.2. Future Grouping Approaches Require Key Components

Since the very beginning of grouping substances, "what is similarity?" has been a key question. Grouping uses similarity of known properties of substances ("source" criteria) to predict previously unknown properties or toxicities of a substance in question (target). We have reviewed and discussed the similarity of substances in terms of their impact on human health. Here, grouping is a tool to aid risk assessment and ultimately minimize risks to human health. As such, a change of thinking to grouping away from hazard towards risk may be needed (Figure 11). This may in turn necessitate shifting from focusing on qualitative data to assessing more quantitative type data. Grouping is also key when more efficient approaches are needed—be it for constraints of time and resources or to reduce animal testing. Acceptance of grouping for regulatory purposes helps reduce animal testing but requires clear grouping criteria, which in turn requires data targeting and/or complying to these criteria. Unaligned timing and data requirements can create uncertainty for regulatory decision-makers and the public, as well as the industries developing, producing, and using substances (e.g. Bisphenol A, Section 2.1.[156] It is therefore critical that grouping is based on solid scientific standards, is evidence-based, and the principles applied coherently across legislations without losing focus on the purpose and justification of the grouping.[155,157] In the past, grouping was primarily based on qualitative approaches using structural similarities to predict hazards. In meantime, valuable experience has been gained, and the scope of grouping, criteria and justification tools have further developed.: grouping can cover both hazard and risk including quantitative assessments and grouping can also incorporate multiple data including big data on biological interactions. This makes grouping a more powerful tool; at the same time, this requires structured and well-defined procedures. As stated by Mendeleev "I wish to establish some sort of system not guided by chance but by some sort of definite and exact principle." Relevant and clear guiding principles will, indeed, be needed for grouping in the regulatory context. Based on the lessons learned, certain standards need to be clarified and wherever possible harmonized (Figure 12). These principles do not establish a new framework, but amend and differentiate current practices:

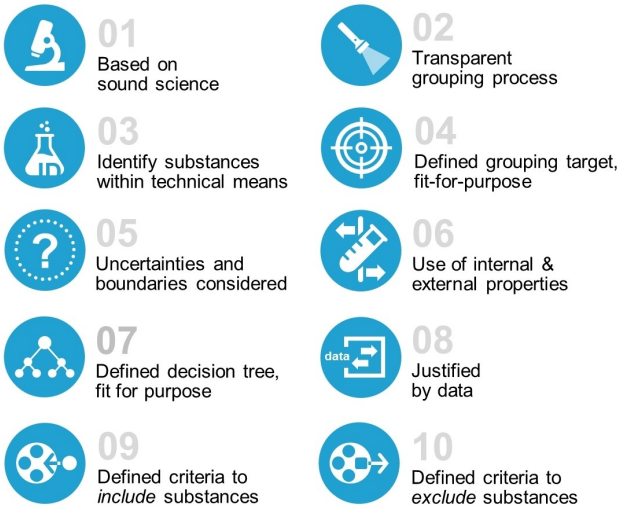


Figure 12. Ten principles of grouping, based on the lessons learned on several substance classes.

(1) Grouping should be based on solid **scientific standards** and should be applied **coherently** across legislations.
(2) The grouping process should be **transparent** and comprehensible for all stakeholders experienced in grouping and the methodologies involved.
(3) The **substances** in a group should be **identified** within technical means.
(4) The **target of grouping** should be clearly defined: a) for all endpoints or a specific hazard or risk assessment, b) qualitatively or quantitatively, c) to generate definitive data or to identify initial concerns.
(5) The boundaries and the **uncertainty** associated with the grouping should be quantified and described for the methods providing the data towards the decision criteria, the decision thresholds of the criteria as well as the overall grouping decision, inclusion, or exclusion of a substance.
(6) Grouping **criteria** should consider a) substances' **intrinsic and** b) **extrinsic properties**, including c) interaction with biological systems; this can amend and, in some cases, overrule structural similarity.
(7) Grouping should use a **pre-defined "fit for purpose" decision tree** including decision criteria and decision thresholds, wherever possible. Intended use and exposure primarily define the "fit for purpose".
(8) Grouping should be **justified by scientific data**. The **relevance** of the grouping criteria for a certain purpose should be described as well.
(9) The **data required** to justify the assignment of a substance to a group should be defined including the method to generate these data.
(10) Likewise, data required to **exempt substances** based on a) the grouping criteria or based on b) other relevant properties should be defined.

Several of our principles reflect current regulatory practice, and some are already addressed in more recently issued guidances, such as the consideration of uncertainty budgets in numerical values.[158] Some other of the above principles are rarely implemented but would be highly beneficial, such as the coherence across legislations, the use of external properties,[90] the contextual information on methods, the relevance of the intended use. The last principle—the data-based exemption from grouping—is a reaction to the "restriction roadmap" in the EU.[128] One must consider that traditional grouping in toxicology aims at filling data gaps by RAx, hence generating the information needed to e.g. fulfill information requirements needed for REACH registrations. In this, it replaces a specific experimental study—in most cases an animal study. Results of this grouping can then be used for informing decision making for risk management. Consequently, accurate and meticulous grouping procedures are necessary, as described in guidance documents, e.g. the ECHA RAAF.

A profoundly different application of grouping is the identification of initial concerns, as laid out, e.g. by the "restriction roadmap" in the EU.[128] Also, the identification of initial concerns has long been used for StageGate project decisions during product development in industry, in other words SSbD decisions. This grouping applies less strict criteria and less precise procedures for the benefit of fast and efficient grouping, which in turn requires less data. This "initial grouping"—based on structure alone or screening assays for a larger number of chemicals—targets prioritization for further actions whereas the "grouping for RAx" generates definitive (toxicological) data. Any candidate substance identified as having no adverse effect in screening will undergo further, more defined and relevant testing during product development and also to fulfill regulatory demands before entering the market. False negatives are tolerable; they will be rectified later. False positives will exclude a substance from further development and stop what could have been a beneficial substance. Hence, screening assays just as "initial grouping" should be optimized for few false positives, whereas definite studies and "grouping for RAx" should avoid false negatives. This resonates with the recommendations (9) and (10) and stresses their importance.

Grouping has been most successful when a well-defined property was used as the source criterion which is stringently linked to an adverse effect (Section 2.1). More knowledge on the association of substance's properties with adverse effects on human health and the environment has been gained, thereby providing a sounder basis to select the most relevant data. Moreover, initial groupings for regulatory purposes can still evolve and mature into tiered testing and grouping strategies, thus paralleling the screening strategies for product development. Both aim for production and use of substances that are safe and sustainable. Grouping will help to achieve this more efficiently and reliably. This could accelerate the innovative capacity for production and use of chemicals that are safe for humans and the environment—one of the most prominent goals of the CSS—by safe and sustainable-by-design concepts.

As Mendeleev stated: "It is the function of science to discover the existence of a general reign of order in nature and to find the causes governing this order." The periodic table of elements represents an early yet very successful grouping, which is still relevant for modern chemistry, and future holds much in store in terms of grouping.

Abbreviations

AhR	Aromatic hydrocarbon receptor
AO	Adverse outcome
AOP	Adverse outcome pathway
BfR	German Federal Institute for Risk Assessment
BPA and BPS	Bisphenol A and S, respectively
CAG	cumulative assessment groups
Cefic	European chemical industry council
CKG	Common kinetics groups
CLP	Classification and labelling of products
CMR	Carcinogenic, mutagenic, toxic for reproduction
CRO	Contract research institute

CSS	European Chemical Strategy for Sustainability: Towards a Toxic Free Environment
DMPA	Dipropylene glycol methyl ether acetate
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Authority
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
EU GD	European Green Deal
GLP	Good laboratory practice
GARM	Generic approach to risk management
HBM4EU	An European human biomonitoring project
HPV	High production volume
HTS	High throughput screening
HTT	High-throughput toxicity (testing)
IATA	Integrated approach to testing and assessment
KE	Key Event
MIE	Molecular initiating event
MOA	Mode of action
NAM	New approach methodology (also non-animal method)
NF	Nanoform
NGRA	Next generation risk assessment
NIOSH	U.S. National Institute for Occupational Safety and Health
NOAEL	No observed adverse effect level
OEB	Occupational exposure banding
OECD	Organisation for Economic Co-operation and Development
OEL	Occupational exposure limit
OSOA	One substance one assessment
PAH	Polycyclic aromatic hydrocarbons
PBT	Persistent, bioaccumulative and toxic
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-*p*-dioxin
PFAS	Per- and polyfluoroalklyl substances
PSLT	Poorly soluble particles of low toxicity
qAOP	quantitative Adverse outcome pathway
QSAR	quantitative structure activity relationships
RAAF	Read across assessment framework
RASAR	Read-across-based structure/activity relationship
RAx	Read across
REACH	Registration, evaluation, authorisation and restriction of chemicals
SSA	Specific surface area
SVHC	Substance of very high concern
TCDD	Tetrachlorodibenzo-*p*-dioxin
TEQ	Toxic equivalency factors
TT21C	Toxicity testing in the 21st century
TTC	Threshold of toxicological concern
UVCB	Substance of unknown or variable composition, complex reaction products or biological material
vPvB	Very persistent and very bioaccumulative
WHO	World Health Organization

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Conflict of Interest

The authors declare no conflict of interest.

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[1] D. Mendeleev, *rearranged by Marshall, James L Marshall; Marshall, Virginia R Marshall (2007) "Rediscovery of the elements: The Periodic Table" The Hexagon:23–29. periodic table.pdf (unt.edu) retrieved 16 April 2022, Vol. Suppl. 8, 133–229; Berichte, 1871, 4, 342–352*, Ami. Chem. Phann, **1871**.
[2] ECHA, *Read-Across Assessment Framework (RAAF)* **2017**, 614e5d61-891d–4154-8a47-87efebd1851a (europa.eu).
[3] EPA, *TSCA New Chemicals Program (NCP) Chemical Categories*, can be found under www.epa.gov/sites/default/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf **2010**.
[4] I. Linkov, D. Loney, S. Cormier, F. K. Satterstrom, T. Bridges, *Sci. Total Environ.* **2009**, *407*, 5199–5205.
[5] G. M. Maggiora, *J. Chem. Inf. Model.* **2006**, *46*, 1535–1535.
[6] a) M. Hagen, U. Pabel, R. Landsiedel, I. Bartsch, C. N. Falany, H. Glatt, *Chem.-Biol. Interact.* **1998**, *109*, 249–253; b) M. G. Nilos, J. Gan, D. Schlenk, *General, Applied and Systems Toxicology*, Wiley, Hoboken, **2009**.
[7] J. H. Weisburger, E. K. Weisburger, H. P. Morris, *J. Am. Chem. Soc.* **1952**, *74*, 4540–4543.
[8] Y. Takeuchi, Y. Ono, N. Hisanaga, J. Kitoh, Y. Sugiura, *Clin. Toxicol.* **1981**, *18*, 1395–1402.
[9] T. Paracelsus, *Werke. Bd.* **1965**, *2*, 510.
[10] a) B. Stegelmeier, J. Edgar, S. Colegate, D. Gardner, T. Schoch, R. Coulombe, R. Molyneux, *J. Nat. Toxins* **1999**, *8*, 95–116; b) X. Yan, H. Kang, J. Feng, Y. Yang, K. Tang, R. Zhu, L. Yang, Z. Wang, Z. Cao, *Int. J. Mol. Sci.* **2016**, *17*, 318; c) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Human, Vol. 32*, IARC, New York, **1983**.
[11] L. Deane, *HM Chief Inspector of Factories and Workers, 1899, Annual Report for 1898* **1898**, 171–172.
[12] WHO, in *Proceedings of a WHO/IARC Conference*, **1984**.
[13] ECHA, *Annex XVII to REACH restriction on the manufacture, placing on the market and use of certain dangerous substances, mixtures and articles*, can be found under https://echa.europa.eu/documents/10162/3bfef8a3-8c97-4d85-ae0b-ac6827de49a9 **2022**.
[14] a) IARC, *Man-Made Vitreous Fibres*, World Health Organization, **2002**; b) R. A. Baan, Y. Grosse, *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2004**, *553*, 43–58.
[15] D. Broßell, A. Meyer-Plath, K. Kämpf, S. Plitzko, W. Wohlleben, B. Stahlmecke, M. Wiemann, A. Haase, in *Workshop summary and conclusions, Wright Stephanie, Graham Uschi M., Moskal Arkadiusz Adres publikacji w Repozytorium URL/Publication address in Repository*, **2020**.
[16] a) ECHA, *Highlights from March RAC and SEAC meetings*, can be found under https://echa.europa.eu/de/-/highlights-

from-march-rac-and-seac-meetings **2022**; b) ECHA, *CLH report: Multi-Walled Carbon Tubes (synthetic graphite in tubular shape) with a geometric tube diameter range ≥30 nm to <3 μm and a length ≥5 μm and aspect ratio ≥3:1, including Multi-Walled Carbon Nanotubes, MWC(N)T*, can be found under https://echa.europa.eu/documents/10162/2842450/clh_rep_Multi-Walled+Carbon+Nanotubes+%28MWCNT%29_en_SPS-019094-21_19588_en.pdf/e55d14a1-40d0-2e39-5613-0577ceab3a10 **2021**.

[17] J. R. Brown, J. L. Thornton, *Br. J. Ind. Med.* **1957**, *14*, 68.

[18] E. Kennaway, I. Hieger, *Br. Med. J.* **1930**, *1*, 1044.

[19] H. V. Gelboin, *Physiol. Rev.* **1980**, *60*, 1107–1166.

[20] H. Glatt, *FASEB J.* **1997**, *11*, 314–321.

[21] Y. Masuda, *Dioxins and health*, Springer, Heidelberg, **1994**, pp. 633–659.

[22] a) C. Rappe, *Ecol. Bull.* **1978**, 28–30; b) A. L. Young, J. A. Calcagni, C. E. Thalken, J. W. Tremblay, *The toxicology, environmental fate, and human risk of herbicide orange and its associated dioxin*, **1978**.

[23] J. McKinney, P. Singh, *Chem.-Biol. Interact.* **1981**, *33*, 271–283.

[24] S. H. Safe, *J. Anim. Sci.* **1998**, *76*, 134–141.

[25] a) EPA, *National PFAS Testing Strategy: Identification of Candidate Per and Poly fluoroalkyl Substances (PFAS) for Testing*, can be found under https://www.epa.gov/system/files/documents/2021-10/pfas-natl-test-strategy.pdf **2021**; b) EPA, *Dioxin and Dioxin-Like Compounds Toxic Equivalency Information*, can be found under https://www.govinfo.gov/content/pkg/FR-2007-05-10/pdf/E7-9015.pdf **2007**.

[26] EU, *A Compendium of Case Studies that helped to shape the REACH guidance on chemical categories and read across*, can be found under https://op.europa.eu/en/publication-detail/-/publication/3339157f-7e08-4ba1-9b9b-238b42a7f402 **2007**, *22481*.

[27] J. Glüge, M. Scheringer, I. T. Cousins, J. C. DeWitt, G. Goldenman, D. Herzke, R. Lohmann, C. A. Ng, X. Trier, Z. Wang, *Environ. Sci.: Processes Impacts* **2020**, *22*, 2345–2373.

[28] L. M. Carlson, M. Angrish, A. V. Shirke, E. G. Radke, B. Schulz, A. Kraft, R. Judson, G. Patlewicz, R. Blain, C. Lin, *Environ. Health Perspect.* **2022**, *130*, 056001.

[29] OECD, *Toward a new comprehensive global database of Per- and Polyfluoroalkyl Substances (PFASs): Summary report on updating the OECD 2007 list of Per-and Polyfluoroalkyl Substances (PFASs)*, can be found under https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-JM-MONO%282018%297&doclanguage=en **2018**.

[30] a) EPA, *PFAS Master List of PFAS Substances*, can be found under https://comptox.epa.gov/dashboard/chemical-lists/PFASMASTER **2022**; b) Z. Wang, A. M. Buser, I. T. Cousins, S. Demattio, W. Drost, O. Johansson, K. Ohno, G. Patlewicz, A. M. Richard, G. W. Walker, *Environ. Sci. Technol.* **2021**, *55*, 15575–15578.

[31] GoC, *Technical consultation: proposed subgrouping of bisphenol A (BPA) structural analogues and functional alternatives*, **2020**.

[32] RSC, *Risk-based regulation for per- and poly-fluoroalkyl substances (PFAS)*, can be found under https://www.rsc.org/globalassets/22-new-perspectives/sustainability/a-chemicals-strategy-for-a-sustainable-chemicals-revolution/pfas-policy-position-dec-2021.pdf, **2021**.

[33] I. T. Cousins, J. C. DeWitt, J. Glüge, G. Goldenman, D. Herzke, R. Lohmann, M. Miller, C. A. Ng, M. Scheringer, L. Vierke, *Environ. Sci.: Processes Impacts* **2020**, *22*, 1444–1460.

[34] G. Patlewicz, A. M. Richard, A. J. Williams, C. M. Grulke, R. Sams, J. Lambert, P. D. Noyes, M. J. DeVito, R. N. Hines, M. Strynar, *Environ. Health Perspect.* **2019**, *127*, 014501.

[35] a) EU, *Workshop on the reform of the REACH authorization and restriction system*, can be found under https://ec.europa.eu/growth/system/files/2021-11/Background%20paper%20authorisation%20and%20restrictions%20workshop%2012%20Nov_FINAL.docx.pdf **2021**; b) E. Grignard, S. Lapenna, S. Bremer, *Toxicol. In Vitro* **2012**, *26*, 727–731.

[36] EU, *Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin—like PCBs and non-dioxin—like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014 (Text with EEA relevance)* **2017**.

[37] EU, *Commission Regulation (EU) No 1275/2013 of 6 December 2013 Amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as Regards Maximum Levels for Arsenic, Cadmium, Lead, Nitrites, Volatile Mustard Oil and Harmful Botanical Impurities* **2013**, *328*, 86–92.

[38] a) WCED, *Report of the World Commission on Environment and Development: Our Common Future*, can be found under https://sustainabledevelopment.un.org/content/documents/5987our-common-future.pdf **1987**; b) EC, *Safe and Sustainable by Design chemicals and materials*, can be found under https://op.europa.eu/en/publication-detail/-/publication/eb0a62f3-031b-11ed-acce-01aa75ed71a1/language-en **2022**.

[39] P. Harremoës, D. Gee, M. MacGarvin, A. Stirling, J. Keys, B. Wynne, S. G. Vaz, *Late lessons from early warnings: the precautionary principle 1896–2000*, Citeseer, **2001**.

[40] J. B. Zimmerman, P. T. Anastas, *Science* **2015**, *347*, 1198–1199.

[41] OECD, *Grouping of Chemicals: Chemical Categories and Read-Across*, can be found under https://www.oecd.org/chemicalsafety/risk-assessment/groupingofchemicalschemicalcategoriesandread-across.htm, **2014**.

[42] G. Patlewicz, L. E. Lizarraga, D. Rua, D. G. Allen, A. B. Daniel, S. C. Fitzpatrick, N. Garcia-Reyero, J. Gordon, P. Hakkinen, A. S. Howard, *Regul. Toxicol. Pharmacol.* **2019**, *106*, 197–209.

[43] a) ECHA, *The use of alternatives to testing on animals for the REACH Regulation : Third report under Article 117(3) of the REACH Regulation*, doi/10.2823/023078, **2017**; b) ECHA, *Non-animal approaches : current status of regulatory applicability under the REACH, CLP and biocidal products regulations*, doi/10.2823/000784, **2017**; c) ECHA, *The use of alternatives to testing on animals for the REACH Regulation*, doi/10.2823/092305, **2021**.

[44] a) W. Teubner, R. Landsiedel, *ATLA Altern. Lab. Anim.* **2015**, *43*, P67–P71; b) N. Ball, M. T. Cronin, J. Shen, K. Blackburn, E. D. Booth, M. Bouhifd, E. Donley, L. Egnash, C. Hastings, D. R. Juberg, *ALTEX Altern. Anim. Exp.* **2016**, *33*, 149; c) ECHA, *Read—Across Assessment Framework (RAAF)*, ECHA Helsinki, Finland, **2017**; d) OECD, *Guidance on Grouping of Chemicals, Second Edition*, doi:https://doi.org/10.1787/9789264274679-en, **2017**.

[45] a) G. Patlewicz, G. Helman, P. Pradeep, I. Shah, *Comput. Toxicol.* **2017**, *3*; b) T. Luechtefeld, D. Marsh, C. Rowlands, T. Hartung, *Toxicol. Sci.* **2018**, *165*, 198–212; c) I. Shah, T. Tate, G. Patlewicz, *Bioinformatics* **2021**, *37*, 3380–3381.

[46] OECD, *Guidance on grouping of chemicals, second edition. In Series on Testing & Assessment No. 194*, can be found under https://www.oecd-ilibrary.org/docserver/9789264274679-en.pdf?expires=1655799026&id=id&accname=guest&checksum=4C1A7D18633819414F17F9216B25ED83 **2014**.

[47] N. Ball, M. Bartels, R. Budinsky, J. Klapacz, S. Hays, C. Kirman, G. Patlewicz, *Regul. Toxicol. Pharmacol.* **2014**, *68*, 212–221.

[48] G. Patlewicz, N. Ball, R. Becker, *ALTEX Altern. Anim. Exp.* **2014**, *31*, 387–396.

[49] N. Jeliazkova, E. Bleeker, R. Cross, A. Haase, G. Janer, W. Peijnenburg, M. Pink, H. Rauscher, C. Svendsen, G. Tsiliki, A. Zabeo, D. Hristozov, V. Stone, W. Wohlleben, *NanoImpact* **2022**, *25*, 100366.

[50] a) G. Patlewicz, M. T. Cronin, G. Helman, J. C. Lambert, L. E. Lizarraga, I. Shah, *Comput. Toxicol.* **2018**, *6*, 39–54; b) G. Patlewicz, *Front. Toxicol.* **2020**, *2*, 2.

[51] ECHA, *QSARs and their assessment under dossier evaluation*, can be found under https://echa.europa.eu/-/qsars-and-their-assessment-under-dossier-evaluation **2021**.

[52] OECD, *Guideline No. 497—Defined Approaches for Skin Sensitisation*, can be found under https://www.oecd-ilibrary.org/docserver/b92879a4-en.pdf?expires=1664447851&id=id&accname=guest&checksum=BF82AB84A5361218E264F4204ADAE0B8 **2021**.

[53] a) D. Urbisch, A. Mehling, K. Guth, T. Ramirez, N. Honarvar, S. Kolle, R. Landsiedel, J. Jaworska, P. S. Kern, F. Gerberick, *Regul. Toxicol. Pharmacol.* **2015**, *71*, 337–351; b) O. Takenouchi, S. Fukui, K. Okamoto, S. Kurotani, N. Imai, M. Fujishiro, D. Kyotani, Y. Kato, T. Kasahara, M. Fujita, *J. Appl. Toxicol.* **2015**, *35*, 1318–1332.

[54] C. Rovida, T. Barton-Maclaren, E. Benfenati, F. Caloni, C. Chandrasekera, D. R. Dietrich, J. Kisitu, M. Leist, G. Pallocca, T. Hartung, *ALTEX Altern. Anim. Exp.* **2020**, *37*, 579–606.

[55] OECD, *Report on considerations from case study on Integrated Approaches for Testing and Assessment (IATA)*, can be found under https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-CBC-MONO(2021)36%20&doclanguage=en **2021**.

[56] N. Ball, R. Bars, P. A. Botham, A. Cuciureanu, M. T. Cronin, J. E. Doe, T. Dudzina, T. W. Gant, M. Leist, B. van Ravenzwaay, *Arch. Toxicol.* **2022**, *96*, 743–766.

[57] C. Rovida, S. E. Escher, M. Herzler, S. H. Bennekou, H. Kamp, D. E. Kroese, L. Maslankiewicz, M. J. Moné, G. Patlewicz, N. Sipes, *ALTEX Altern. Anim. Exp.* **2021**, *38*, 140–150.

[58] S. Wu, K. Blackburn, J. Amburgey, J. Jaworska, T. Federle, *Regul. Toxicol. Pharmacol.* **2010**, *56*, 67–81.

[59] B. Marks, www.wca-environment.com, **2017**.

[60] REACH, *Dossier Evaluation Process-principles applied by ECHA in reviewing read-across and category approaches*, echa.europa.eu, **2020**.

[61] a) J. S. House, F. A. Grimm, W. D. Klaren, A. Dalzell, S. Kuchi, S.-D. Zhang, K. Lenz, P. J. Boogard, H. B. Ketelslegers, T. W. Gant, *ALTEX Altern. Anim. Exp.* **2022**, *39*, 388–404; b) N. Ball, J. Madden, A. Paini, M. Mathea, A. D. Palmer, S. Sperber, T. Hartung, B. van Ravenzwaay, *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2020**, *853*, 503172.

[62] a) A.-N. Richarz, *Big data in predictive toxicology: challenges, opportunities and perspectives*, can be found under *10.1039/9781782623656-00001*, **2019**; b) V. Vitcheva, *Big Data in Predictive Toxicology*, Royal Society of Chemistry, London, **2019**, pp. 38–68.

[63] NRC, *Toxicity testing in the 21st century: a vision and a strategy*, National Academies Press, Washington, **2007**.

[64] S. Enoch, D. Roberts, *Approaches for grouping chemicals into categories*, Royal Society of Chemistry, London, **2013**.

[65] P. E. Goodrum, J. K. Anderson, A. L. Luz, G. K. Ansell, *Toxicol. Sci.* **2021**, *179*, 262–278.

[66] A. R. Boobis, S. M. Cohen, V. Dellarco, D. McGregor, M. Meek, C. Vickers, D. Willcocks, W. Farland, *Crit. Rev. Toxicol.* **2006**, *36*, 781–792.

[67] a) G. T. Ankley, R. S. Bennett, R. J. Erickson, D. J. Hoff, M. W. Hornung, R. D. Johnson, D. R. Mount, J. W. Nichols, C. L. Russom, P. K. Schmieder, J. A. Serrrano, J. E. Tietge, D. L. Villeneuve, *Environ. Toxicol. Chem.* **2010**, *29*, 730–741; b) OECD, *Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA)*, doi:https://doi.org/10.1787/44bb06c1-en, **2017**; c) M. Vinken, *Toxicology* **2013**, *312*, 158–165.

[68] T. Hartung, *ALTEX Altern. Anim. Exp.* **2010**, *27*, 253–263.

[69] K. N. De Abrew, R. M. Kainkaryam, Y. K. Shan, G. J. Overmann, R. S. Settivari, X. Wang, J. Xu, R. L. Adams, J. P. Tiesman, E. W. Carney, *Toxicol. Sci.* **2016**, *151*, 447–461.

[70] a) J. D. Ede, V. Lobaskin, U. Vogel, I. Lynch, S. Halappanavar, S. H. Doak, M. G. Roberts, J. A. Shatkin, *Nanomaterials* **2020**, *10*, 1229; b) S. Christiansen, M. Axelstad, M. Scholze, H. K. Johansson, U. Hass, K. Mandrup, H. L. Frandsen, H. Frederiksen, L. K. Isling, J. Boberg, *Environ. Int.* **2020**, *142*, 105870.

[71] B. Van Ravenzwaay, M. Herold, H. Kamp, M. Kapp, E. Fabian, R. Looser, G. Krennrich, W. Mellert, A. Prokoudine, V. Strauss, *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2012**, *746*, 144–150.

[72] S. Sperber, M. Wahl, F. Berger, H. Kamp, O. Lemke, V. Starck, T. Walk, M. Spitzer, B. Ravenzwaay, *Regul. Toxicol. Pharmacol.* **2019**, *108*, 104442.

[73] a) E. Teixidó, T. Kießling, N. Klüver, S. Scholz, *Arch. Toxicol.* **2022**, 1–17; b) K. Jagiello, S. Halappanavar, A. Rybińska-Fryca, A. Willliams, U. Vogel, T. Puzyn, *Small* **2021**, *17*, 2003465.

[74] M. R. Viant, T. Ebbels, R. D. Beger, D. R. Ekman, D. J. Epps, H. Kamp, P. E. Leonards, G. D. Loizou, J. I. MacRae, B. Van Ravenzwaay, *Nat. Commun.* **2019**, *10*, 3041.

[75] J. Lamb, E. D. Crawford, D. Peck, J. W. Modell, I. C. Blat, M. J. Wrobel, J. Lerner, J.-P. Brunet, A. Subramanian, K. N. Ross, *Science* **2006**, *313*, 1929–1935.

[76] EU, *The European green deal. COM (2019) 640 final* **2019**.

[77] M. Garcia de Lomana, A. Morger, U. Norinder, R. Buesen, R. Landsiedel, A. Volkamer, J. Kirchmair, M. Mathea, *J. Chem. Inf. Model.* **2021**, *61*, 3255–3272.

[78] F. A. Grimm, Y. Iwata, O. Sirenko, G. A. Chappell, F. A. Wright, D. M. Reif, J. Braisted, D. L. Gerhold, J. M. Yeakley, P. Shepard, *Green Chem.* **2016**, *18*, 4407–4419.

[79] A. Maertens, E. Golden, T. Hartung, *ACS Sustainable Chem. Eng.* **2021**, *9*, 7749–7758.

[80] R. N. Ram, D. Gadaleta, T. E. H. Allen, *Comput. Toxicol.* **2022**, *23*, 100232.

[81] EU, *Commission Regulation (EU) 2018/1881 of 3 December 2018 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annexes I, III,VI, VII, VIII, IX, X, XI, and XII to address nanoforms of substances* **2018**, http://data.europa.eu/eli/reg/2018/1881/oj.

[82] a) H. Zhang, Z. Ji, T. Xia, H. Meng, C. Low-Kam, R. Liu, S. Pokhrel, S. Lin, X. Wang, Y. P. Liao, M. Wang, L. Li, R. Rallo, R. Damoiseaux, D. Telesca, L. M+¤dler, Y. Cohen, J. I. Zink, A. E. Nel, *ACS Nano* **2012**, *6*, 4349–4368; b) A. Bahl, B. Hellack, M. Wiemann, A. Giusti, K. Werle, A. Haase, W. Wohlleben, *NanoImpact* **2020**, *19*, 100234–100234; c) E. Burello, A. P. Worth, *Nanotoxicology* **2011**, *5*, 228–235.

[83] H. S. Choi, Y. Ashitate, J. H. Lee, S. H. Kim, A. Matsui, N. Insin, M. G. Bawendi, M. Semmler-Behnke, J. V. Frangioni, A. Tsuda, *Nat. Biotechnol.* **2010**, *28*, 1300–1303.

[84] a) A. Tsuda, N. V. Konduru, *NanoImpact* **2016**, *2*, 38–44; b) V. Castagnola, J. Cookman, J. M. de Araujo, E. Polo, Q. Cai, C. P. Silveira, Z. Krpetic, Y. Yan, L. Boselli, K. A. Dawson, *Nanoscale Horiz.* **2017**, *2*, 187–198; c) M. P. Monopoli, C. Aberg, A. Salvati, K. A. Dawson, *Nat. Nanotechnol.* **2012**, *7*, 779–786.

[85] a) A. G. Oomen, K. G. Steinhäuser, E. A. J. Bleeker, F. van Broekhuizen, A. Sips, S. Dekkers, S. W. P. Wijnhoven, P. G. Sayre, *NanoImpact* **2018**, *9*, 1–13; b) K. G. Steinhäuser, P. G. Sayre, *NanoImpact* **2017**, *7*, 66–74; c) A. Baun, P. Sayre, K. G. Steinhäuser, J. Rose, *NanoImpact* **2017**, *8*, 1–10.

[86] E. Kuempel, V. Castranova, C. Geraci, P. Schulte, *J. Nanopart. Res.* **2012**, *14*, 1029.

[87] N. M. Drew, E. D. Kuempel, Y. Pei, F. Yang, *Regul. Toxicol. Pharmacol.* **2017**, *89*, 253–267.

[88] a) J. H. E. Arts, M.-A. Irfan, A. M. Keene, R. Kreiling, D. Lyon, M. Maier, K. Michel, N. Neubauer, T. Petry, U. G. Sauer, D. Warheit, K. Wiench, W. Wohlleben, R. Landsiedel, *Regul. Toxicol. Pharmacol.* **2016**, *76*, 234–261; b) J. H. Arts, M. Hadi, A. M. Keene, R. Kreiling, D. Lyon, M. Maier, K. Michel, T. Petry, U. G. Sauer, D. Warheit, K. Wiench, R. Landsiedel, *Regul. Toxicol. Pharmacol.* **2014**, *70*, 492–506.

[89] a) OECD, *Physical-chemical decision framework to inform decisions for risk assessment of manufactured nanomaterials*, can be found under https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2019)12&doclanguage=en **2019**; b) OECD, *Categorisation of Manufactured Nanomaterials—Workshop report*, can be found under https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2016)9&doclanguage=en **2016**.

[90] ECHA, *Guidance on information requirements and chemical safety assessment—Appendix R.6-1 for nanoforms applicable to the Guidance on QSARs and Grouping of Chemicals*, can be found under https://www.echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf/71ad76f0-ab4c-fb04-acba-074cf045eaaa **2019**.

[91] a) G. Janer, R. Landsiedel, W. Wohlleben, *Nanotoxicology* **2021**, *15*, 145–166; b) G. Janer, D. Ag-Seleci, J.-A. Sergent, R. Landsiedel, W. Wohlleben, *Nanotoxicology* **2021**, *15*, 1016–1034.

[92] V. Stone, S. Gottardo, E. A. Bleeker, H. Braakhuis, S. Dekkers, T. Fernandes, A. Haase, N. Hunt, D. Hristozov, P. Jantunen, *Nano Today* **2020**, *35*, 100941.

[93] N. Hunt, *Zenodo* **2021**, https://doi.org/10.5281/zenodo.5534466.

[94] L. Traas, R. Vanhauten, *GRACIOUS framework blueprint*, can be found under https://doi.org/10.5281/zenodo.5497615 **2021**.

[95] a) R. Verdon, S. L. Gillies, D. M. Brown, T. Henry, L. Tran, C. R. Tyler, A. G. Rossi, V. Stone, H. J. Johnston, *Nanotoxicology* **2021**, *15*, 1–20; b) F. Murphy, S. Dekkers, H. Braakhuis, L. Ma-Hock, H. Johnston, G. Janer, L. di Cristo, S. Sabella, N. R. Jacobsen, A. G. Oomen, *NanoImpact* **2021**, *22*, 100314; c) H. M. Braakhuis, F. Murphy, L. Ma-Hock, S. Dekkers, J. Keller, A. G. Oomen, V. Stone, *Appl. In Vitro Toxicol.* **2021**, *7*, 112–128.

[96] F. Loosli, K. Rasmussen, H. Rauscher, R. K. Cross, N. Bossa, W. Peijnenburg, J. Arts, M. Matzke, C. Svendsen, D. Spurgeon, P. A. Clausen, E. Ruggiero, W. Wohlleben, F. von der Kammer, *NanoImpact* **2022**, *25*, 100375.

[97] N. Jeliazkova, L. Ma-Hock, G. Janer, H. Stratmann, W. Wohlleben, *NanoImpact* **2022**, *26*, 100391.

[98] ECHA, *Appendix for nanoforms applicable to the Guidance on Registration and Substance Identification*, Helsinki, **2019**.

[99] GoC, *Chemicals Management Plan implementation table at a glance*, can be found under https://www.canada.ca/en/health-canada/services/chemical-substances/chemicals-management-plan/implementation-table-at-glance.html **2022**.

[100] J. H. Arts, M. Hadi, M.-A. Irfan, A. M. Keene, R. Kreiling, D. Lyon, M. Maier, K. Michel, T. Petry, U. G. Sauer, *Regul. Toxicol. Pharmacol.* **2015**, *71*, S1–S27.

[101] D. M. Mitrano, W. Wohlleben, *Nat. Commun.* **2020**, *11*, 5324.

[102] ECHA, *Intentionally-added microplastics: Annex XV dossier proposing restrictions of the manufacture, placing on the market or use of a substance within the EU*, can be found under https://echa.europa.eu/restrictions-under-consideration/-/substance-rev/22921/term **2020**.

[103] ECETOC, *ECETOC Technical Report* **2019**, 133–1.

[104] WOOD, *European Commission, ENV.B.2—Sustainable Chemicals* **2020**.

[105] EU, *Proposal for an EU-definition of a Polymer of Low Concern (PLC)—version 8*, can be found under https://circabc.europa.eu/ui/group/a0b483a2-4c05-4058-addf-2a4de71b9a98/library/55ff270d-4fd2-45f7-9d48-2627ccb3ff2e/deta **2021**.

[106] ECETOC, *Case Studies Putting the ECETOC Conceptual Framework for Polymer Risk Assessment (CF4Polymers) into Practice*, can be found under https://www.ecetoc.org/wp-content/uploads/2021/10/ECETOC-TR-133-3-CF4Polymers-Case-Studies.pdf **2021**.

[107] ECETOC, *Applicability of Analytical Tools, Test Methods and Models for Polymer Risk Assessment*, can be found under https://www.ecetoc.org/wp-content/uploads/2021/10/ECE-TOC-TR133-2.Polymers-Risk-Asessment.pdf **2020**.

[108] a) D. Bury, J. Head, D. Keller, M. Klaric, J. Rose, *Regul. Toxicol. Pharmacol.* **2021**, *123*, 104964; b) EFSA, *Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment*, can be found under https://doi.org/10.2903/j.efsa.2019.5708 **2019**, *17*.

[109] G. Cramer, R. Ford, R. Hall, *Food Cosmet. Toxicol.* **1976**, *16*, 255–276.

[110] M. Batke, F. M. Afrapoli, R. Kellner, J. F. Rathman, C. Yang, M. T. Cronin, S. E. Escher, *Front. Toxicol.* **2021**, 30.

[111] R. Kroes, A. Renwick, M. Cheeseman, J. Kleiner, I. Mangelsdorf, A. Piersma, B. Schilter, J. Schlatter, F. Van Schothorst, J. Vos, *Food Chem. Toxicol.* **2004**, *42*, 65–83.

[112] I. C. Munro, R. A. Ford, E. Kennepohl, J. G. Sprenger, *Food Chem. Toxicol.* **1996**, *34*, 829–867.

[113] a) T. Yamada, M. Kurimoto, A. Hirose, C. Yang, J. F. Rathman, *Front. Toxicol.* **2021**, 12; b) L. Reilly, R. Serafimova, F. Partosch, U. Gundert-Remy, J. C. Abrahantes, J.-L. M. Dorne, G. E. Kass, *Toxicol. Lett.* **2019**, *314*, 117–123.

[114] I. Lea, L. L. Pham, T. Antonijevic, C. Thompson, S. J. Borghoff, *Regul. Toxicol. Pharmacol.* **2022**, *133*, 105190.

[115] B. Van Ravenzwaay, M. Dammann, R. Buesen, S. Schneider, *Regul. Toxicol. Pharmacol.* **2011**, *59*, 81–90.

[116] a) M. D. Nelms, G. Patlewicz, *Front. Toxicol.* **2020**, *2*, 580347; b) S. F. Chebekoue, K. Krishnan, *J. Occup. Environ. Hyg.* **2019**, *16*, 308–319.

[117] a) R. Kroes, A. Renwick, V. Feron, C. Galli, M. Gibney, H. Greim, R. Guy, J. Lhuguenot, J. Van de Sandt, *Food Chem. Toxicol.* **2007**, *45*, 2533–2562; b) C. Yang, S. M. Barlow, K. L. M. Jacobs, V. Vitcheva, A. R. Boobis, S. P. Felter, K. B. Arvidson, D. Keller, M. T. Cronin, S. Enoch, *Food Chem. Toxicol.* **2017**, *109*, 170–193.

[118] a) R. Safford, *Regul. Toxicol. Pharmacol.* **2008**, *51*, 195–200; b) T. Nishijo, A. M. Api, G. F. Gerberick, M. Miyazawa, M. Na, H. Sakaguchi, *Crit. Rev. Toxicol.* **2022**, *52*, 51–65.

[119] a) EMA, *Concept paper for a guideline on limits for genotoxic impurities*, can be found under https://www.ema.europa.eu/en/documents/scientific-guideline/concept-paper-guideline-limits-genotoxic-impurities_en.pdf **2013**; b) EMA, *ICH guideline M7(R1) on assessment and control of DNA reactive (mutagenic impurities in pharmaceuticals to limit potential carcinogenic risk* **2018**; c) EMA, *Questions and answers on the "Guideline on the limits of genotoxic impurities"*, can be found under https://www.ema.europa.eu/en/documents/scientific-guideline/questions-answers-guideline-limits-genotoxic-impurities_en.pdf **2010**.

[120] EFSA, *Recent developments in the risk assessment of chemicals in food and their potential impact on the safety assessment of substances used in food contact materials*, **2016**.
[121] a) U. Bernauer, G. Heinemeyer, B. Heinrich-Hirsch, B. Ulbrich, U. Gundert-Remy, *Toxicol. Lett.* **2008**, *176*, 68–76; b) N. Ball, R. Bars, P. Botham, A. Cuciureanu, M. Cronin, J. Doe, T. Dudzina, T. Gant, M. Leist, B. van Ravenzwaay, *A Framework for Chemical Safety Assessment Incorporating New Approach Methodologies Within REACH*, **2022**; c) T. Hartung, *ALTEX Altern. Anim. Exp.* **2017**, *34*, 331–351; d) R. Zaleski, M. Embry, R. McKee, L. Teuschler, *Regul. Toxicol. Pharmacol.* **2021**, *127*, 105051.
[122] a) P. J. Borm, K. E. Driscoll, *Part. Fibre Toxicol.* **2019**, *16*, 11; b) H. Stratmann, W. Wohlleben, M. Wiemann, A. Vennemann, N. End, U. Veith, L. Ma-Hock, R. Landsiedel, *Regul. Toxicol. Pharmacol.* **2021**, *124*, 104988; c) A. Jarabek, T. Stedeford, G. Ladics, O. Price, A. Tveit, M. Hayes, R. Tremblay, S. Snyder, K. Salazar, S. Osman-Sypher, *EPA Abstr.* **2021**, *2593*.
[123] EU, *Methodology for derivation of occupational exposure limits of chemical agents : the General Decision-Making Framework of the Scientific Committee on Occupational Exposure Limits (SCOEL) 2017*, doi/10.2767/435199, **2018**.
[124] HSE, *EH40/2005 Workplace exposure limits for use with the control of substances*, can be found under https://www.hse.gov.uk/pubns/books/eh40.htm **2020**.
[125] ECHA, *Occupational exposure limits*, can be found under https://echa.europa.eu/oel#, **2019**.
[126] NIOSH, *Qualitative risk characterization and management of occupational hazards: Control banding (CB); A literature review and critical analysis*, **2009**.
[127] a) ECHA, *Assessing chemicals in groups: faster action on harmful chemicals*, can be found under https://open.spotify.com/episode/5XCT4CdDaKpvp9BgVTfnfk?si=_BJ806V4Ryy0O19BGt7eyw&nd=1, **2021**; b) ECHA, *Prioritisation of substances for further regulatory action*, **2019**.
[128] ECHA, *Faster action on groups of harmful chemicals*, can be found under https://www.echa.europa.eu/documents/10162/5641810/irs_annual_report_2021_en.pdf/b38d8eec-d375-beb2-98b2-1fb0feb3612a?t=1655903126992 **2022**.
[129] ECHA, *Finding the most effective ways to manage chemical risks*, can be found under https://www.echa.europa.eu/web/guest/articles#/article/35848338?utm_source=echa-weekly&utm_medium=email&utm_campaign=weekly&utm_content=20220622 **2022**.
[130] EU, *COMMISSION STAFF WORKING DOCUMENT Restrictions Roadmap under the Chemicals Strategy for Sustainability*, can be found under https://ec.europa.eu/docsroom/documents/49734/attachments/1/translations/en/renditions/native **2022**.
[131] HBM4EU, *HBM4EU Priority Substances*, can be found under https://www.hbm4eu.eu/hbm4eu-substances/hbm4eu-priority-substances/, **2022**.
[132] HBM4EU, *Prioritisation strategy and criteria, Deliverable Report D 4.3*, **2017**.
[133] HBM4EU, *Categorisation of substances*, can be found under https://www.hbm4eu.eu/hbm4eu-substances/categorisation-of-substances/#:~:text=The%20categorisation%20supports%20the%20prioritisation%20process%20and%20indicates,of%20human%20biomonitoring%2C%20related%20to%20possible%20health%20consequences, **2022**.
[134] A. Braeuning, D. Bloch, M. Karaca, C. Kneuer, S. Rotter, T. Tralau, P. Marx-Stoelting, *Arch. Toxicol.* **2022**, *96*, 1661–1671.
[135] EFSA, *Draft Guidance Document on Scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals*, **2021**.
[136] EU, *Regulation (EC) No 1907/2006 on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)* **2006**, *L396*.
[137] EU, *Chemicals Strategy for Sustainability Towards a Toxic-Free Environment*, **2020**.
[138] EU, *Pathway to a Healthy Planet for All EU Action Plan: 'Towards Zero Pollution for Air, Water and Soil'*, can be found under https://ec.europa.eu/environment/pdf/zero-pollution-action-plan/communication_en.pdf, **2021**.
[139] EU, *REACH revision under the chemical agency*, can be found under https://ec.europa.eu/environment/chemicals/reach/reach_revision_chemical_strategy_en.htm **2022**.
[140] EU, *Grouping speeds up regulatory action*, can be found under https://op.europa.eu/en/publication-detail/-/publication/f767c265-0a01-11ec-b5d3-01aa75ed71a1 **2020**.
[141] CEFIC, *Economic Analysis of the Impacts of the Chemicals Strategy for Sustainability Phase I report commissioned by the European Chemical Industry Council (Cefic)*, **2021**.
[142] a) M. Herzler, P. Marx-Stoelting, R. Pirow, C. Riebeling, A. Luch, T. Tralau, T. Schwerdtle, A. Hensel, *Arch. Toxicol.* **2021**, *95*, 2589–2601; b) M. Herzler, P. Marx-Stoelting, R. Pirow, C. Riebeling, A. Luch, T. Tralau, T. Schwerdtle, A. Hensel, *Arch. Toxicol.* **2022**, *96*, 2387–2390; c) S. E. Escher, H. Kamp, S. H. Bennekou, A. Bitsch, C. Fisher, R. Graepel, J. G. Hengstler, M. Herzler, D. Knight, M. Leist, *Arch. Toxicol.* **2019**, *93*, 3643–3667.
[143] J. van Dijk, A. Leopold, H. Flerlage, A. van Wezel, T. B. Seiler, M. H. Enrici, M. C. Bloor, *Integr. Environ. Assess. Manage.* **2021**, *17*, 1105–1113.
[144] T. W. Schultz, M. T. D. Cronin, *Regul. Toxicol. Pharmacol.* **2017**, *88*, 185–191.
[145] a) K. Blackburn, S. B. Stuard, *Regul. Toxicol. Pharmacol.* **2014**, *68*, 353–362; b) T. W. Schultz, A.-N. Richarz, M. T. Cronin, *Comput. Toxicol.* **2019**, *9*, 1–11.
[146] A. Banerjee, M. Chatterjee, P. De, K. Roy, *Theor. Comput. Chem.* **2022**, *29*, 0, 10.1016/j.chemolab.2022.104613.
[147] N. Burden, K. Aschberger, Q. Chaudhry, M. J. Clift, S. H. Doak, P. Fowler, H. Johnston, R. Landsiedel, J. Rowland, V. Stone, *Nano Today* **2017**, *12*, 10–13.
[148] C. Alexander-White, D. Bury, M. Cronin, M. Dent, E. Hack, N. J. Hewitt, G. Kenna, J. Naciff, G. Ouedraogo, A. Schepky, *Regul. Toxicol. Pharmacol.* **2022**, *129*, 105094.
[149] G. Pallocca, M. J. Moné, H. Kamp, M. Luijten, B. Van de Water, M. Leist, *ALTEX Altern. Anim. Exp.* **2022**, *39*, 419–426.
[150] EFSA, *Draft Guidance Document on Scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals*, can be found under https://www.efsa.europa.eu/sites/default/files/2021-10/draft-opinion-scientific-criteria-grouping-chemicals-assessment-groups.pdf **2021**.
[151] N. Spinu, M. T. Cronin, S. J. Enoch, J. C. Madden, A. P. Worth, *Arch. Toxicol.* **2020**, *94*, 1497–1510.
[152] A. Paini, A. Worth, S. Kulkarni, D. Ebbrell, J. Madden, *Comput. Toxicol.* **2021**, *18*, 100159.
[153] EU, *REACH*, can be found under https://ec.europa.eu/environment/chemicals/reach/reach_en.htm#:~:text=REACH%20(EC%201907%2F2006),authorisation%20and%20restriction%20of%20chemicals. **2022**.
[154] C. Pestana, S. J. Enoch, J. W. Firman, J. C. Madden, N. Spînu, M. T. Cronin, *Comput. Toxicol.* **2022**, *22*, 100220.
[155] M. Z. Hauschild, T. E. McKone, K. Arnbjerg-Nielsen, T. Hald, B. F. Nielsen, S. E. Mabit, P. Fantke, *Environ. Sci. Eur.* **2022**, *34*, 11.
[156] ECHA, *In support of the EU chemicals strategy for sustainability: One substance—one assessment*, can be found under https://echa.europa.eu/documents/10162/21877836/efsa-echa-

position-paper-osoa_en.pdf/74b1ae31-290b-a608-85e9-05b340840b34 **2020**.

[157] EMMC, *Materials 2030 Manifesto*, can be found under https://ec.europa.eu/info/sites/default/files/research_and_innovation/research_by_area/documents/advanced-materials-2030-manifesto.pdf **2022**.

[158] a) OECD, *Test No. 442C: In Chemico Skin Sensitisation*, can be found under https://doi.org/10.1787/9789264229709-en **2022**; b) OECD, *Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method*, can be found under https://doi.org/10.1787/9789264242845-en **2021**; c) OECD, *Test No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage*, can be found under https://doi.org/10.1787/9789264203846-en **2020**; d) OECD, *Test Guideline No. 431—In Vitro Skin Corrosion: Reconstructed Human Epidermis (RhE) Test Method*, can be found under https://www.oecd-ilibrary.org/docserver/9789264264618-en.pdf?expires=1664454669&id=id&accname=guest&checksum=0AC155777C5BE982F904-B27ADE8519F5 **2019**.

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