Existing knowledge on methods for taking mixtures into account in the areas of health risk assessment and the setting of reference values

Reflection on the setting of reference values

Request « 2016-SA-0101 – IAQG for mixture »
Request « 2018-SA-0152 – TRV for BTEX »

Collective expert appraisal REPORT

« Characterisation of substance hazards and toxicological reference values »

« Health reference values»

« Assessment of the risks related to air environments»

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1 Acronyms and abreviations

ACGIH	:	American Conference of Governmental Industrial Hygienists
ADI	:	Acceptable daily intake
AhR	:	Aryl hydrocarbons receptors
AOEL	:	Acceptable Operator Exposure Level
ARfD	:	Acute reference dose
ARR	:	Residual risk analysis (in French : analyse des risques résiduels)
ATSDR	:	Agency for Toxic Substances and Disease Registry
BBDR	:	Biologically based dose-response
BBP	:	n-butyl benzyl phthalate
BDE	:	Bromodiphenylethers
BHI	:	Biological hazard index
BLV	:	Biological limit value
BMD/C	:	Benchmark dose/concentration
BMDL	:	Lower 90 or 95% confidence limit of the benchmark dose
BMR	:	Benchmark Response
B[a]P	:	benzo[a]pyrene
BPA	:	Bisphenol A
BRV	:	Biological reference value
CAG	:	Cumulative assessment group
CAS	:	Chemical Abstract Service
CCl_4	:	Carbon tetrachloride
CEFIC	:	European Chemical Industry Council
CES	:	Expert comittee
CRPF	:	Cumulative relative potency factors
CT	:	Tolerable concentration
CYP P450	:	Cytochrome P 450
DBP	:	Dibutyl-phthalate
DBT	:	Dibutyltin
DCE	:	1,1-dichloroethylene
DDD	:	Dichlorodiphenyldichloroethane
DDE	:	Dichlorodiphenyldichloroethylene
DDT	:	Dichlorodiphenyltrichloroethane
DEHP	:	Diethylhexyl-phthalate
DEP	:	Diethyl-phthalate
DGS	:	Direction générale de la santé
DGPR	:	Direction générale de la prévention des risques

DINP	:	Diisononyl-phthalate
DIBP	:	Di-isobutyl-phthalate
DMEP	:	Bis(2-methoxyethyl) phthalate
DNEL	:	Derived No-Effect Level
DOT	:	Di-n-octyletain
EC/ED50	:	Effective concentration/dose 50%
ECHA	:	European chemicals agency
EDC	:	Endocrine disruptor compound
EDCH	:	water intended for human consumption (in French : eaux destinées à la consommation humaine
EFSA	:	European Food Safety Authority
ERC	:	Cumulative risk assesment (in French : Evaluation de risque cumulé)
E(Q)RS	:	Health risk assessment (in French : Evaluation (quantitative) de risque sanitaire)
ERI	:	Excess Individual risk (in French : Excès de risque individuel)
ERU	:	Unit risk (in French : Excès de risque unitaire)
FDA	:	Food and Drug Administration
GD	:	Gestational day
GIS	:	Geographic Information System
GTMM	:	Generalized physiologically-based toxicokinetic modeling for mixtures
HCBD	:	hexachloro-1,3-butadiene
HCSP	:	Haut Conseil de la Santé Publique
HEI	:	Health Effects Institute
HI	:	Hazard index
HSDB	:	Hazardous Substances Data Bank
IAQG	:	Indoor air quality guideline
ICDE/ICED	:	Index chemical equivalent dose
ICPE	:	Installations classées pour la protection de l'environnement
IEM	:	Interpretation of the State of the Environment (in French : Interprétation de l'état des milieux)
IGHRC	:	Interdepartmental Group on Health Risks from Chemicals
ILSI	:	International Life Sciences Institute
INCA	:	Étude Individuelle Nationale des Consommations Alimentaires
VTi	:	Indicative toxicological value (in French : valeur toxicologique indicative)
INERIS	:	National Institute for Industrial Environment and Risks (in French : Institut National de l'Environnement Industriel et des Risques)
INRAE	:	Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (anciennement Inra et Irstea)
INRS	:	Institut National de Recherche et de Sécurité
IPCS	:	International Programme on Chemical Safety

IRSST	:	Institut de Recherche Robert-Sauvé en Santé et en Sécurité du Travail
ITER	:	international toxicity estimates for risk
JMPR	:	Joint FAO/WHO Meeting on Pesticide Residues
LASSO	:	Least Absolute Shrinkage and Selection Operator
LCI	:	Lowest concentration of interest
LD/LC50	:	Lethal Dose/Concentration 50 %
LNH	:	Non-Hodgkin lymphomas
LOAEL/C	:	Lowest observed adverse effect level/concentration
MCR	:	Maximum cumulative ratio
MCRA	:	Monte Carlo Risk Assessment
METDB	:	Multiple effects toxicity database
MHI	:	Multipathway hazard index
MOE	:	Marge of exposure
MPR	:	Maximum permissible risk level
MRL	:	Maximum residues level
MRL	:	Minimal Risk Level
NHANES	:	National Health and Nutrition Examination Survey
NIST	:	National Institute of Standards and Technology
NOAEL/C	:	No observed adverse effect level/concentration
NRC	:	National Research Council
OEL	:	Occupationnal exposure limit
OQAI	:	Observatory of indoor air quality (in French : Observatoire de la qualité de l'air intérieur)
OMC	:	Octyl-methoxycinnamate
ORP	:	Overall risk probability
OSHA	:	Occupational Safety and Health Administration
PAH	:	Polycyclic aromatic hydrocarbons
PBDE	:	Polybromodiphenylethers
PBPK	:	Physiologically based pharmacokinetics.
PBTK	:	Physiologically Based Toxicokinetics
PCB	:	Polychlorobiphenyles
PCDD	:	Polychlorodibenzo-p-dioxines
PCDF	:	Polychlorodibenzofuranes
PFAS	:	per- et polyfluoroalkyles
PFC	:	perfluorocarbures
PFOS	:	perfluorooctanesulfonic acid

PND	:	Postnatal day
POD	:	Point of departure
PODI	:	Point of departure index
QSAR	:	Quantitative structure-activity relationships
REACH	:	Registration, Evaluation and Autorisation of CHemicals
RfD/RfC	:	Reference Dose/Concentration
RfPI	:	Reference point index
RIVM	:	Rijksinstituut voor Volksgezondheid en Milieu
RPF	:	Relative potency factor.
RNV3P	:	National Network for the Monitoring and Prevention of Occupational Diseases (in French : Réseau national de vigilance et de prévention des pathologies professionnelles)
SCHER	:	Scientific Committee on Health and Environmental Risks
SFSE	:	Société Française de Santé et Environnement
SMRI	:	Similar mixtures risk indicator
SPF	:	French agency for public health (in French : Santé publique France (anciennement Institut de veille sanitaire))
STEL	:	Short term exposure level
TBT	:	Tributyltin
TCDD	:	2,3,7,8-tetrachlorodibenzo-p-dioxine
TCE	:	Trichloroethylene
TCTFP	:	1,1,2-trichloro-3,3,3-trifluoropropene
TDI	:	Tolerable daily intake
TDS	:	Total Diet Study
TEF	:	Toxicity equivalency factor
TEQ	:	Toxicity equivalency quantity
THM	:	Trihalomethanes
TLV	:	Threshold limit values
TPT	:	Triphenyltin
TRV	:	Toxicological reference value
TUS	:	Toxic unit summation
UF	:	Uncertainty factor
US EPA	:	United States Environment Protection Agency
VOC	:	Volatile organic compounds
SVOC	:	Semivolatile organic compounds
TVOC	:	Totale volatile organic compounds
WHO	:	World health organization
WG	:	Working group
WoE	:	Weight of evidence

1

Terms, definitions

Preamble: Existing definitions in the glossaries of the ANSES methodological report relating to the setting of Toxicological reference values (TRVs) [1] (ANSES, 2017a), of the National Institute for Industrial Environment and Risks (INERIS) on facilities classified for environmental protection (ICPE)[2], from the website of the European Food Safety Authority (EFSA) [3] and from the website of the US Agency for Toxic Substances and Disease Registry (ATSDR)[4] have been included and summarised if necessary in this chapter.

The question of human exposure to mixtures and the associated health risk requires a prior definition of the terms used, which refer in particular to the concepts of exposure and hazard.

Aggregate exposure is commonly used to define exposure to a contaminant *via* the different sources (food, water, air, consumer products) and routes of exposure (ingestion, inhalation, dermal) from which it may arise. Thus, aggregate risk corresponds to the risk associated with exposure to a single contaminant from different routes.

Combined exposure or co-exposure refers to simultaneous exposure to several contaminants *via* one or more routes of exposure.

The presence or absence of interaction of the contaminants present in a mixture will define the cumulative risk assessment. The definitions considered in this report are general definitions that do not describe the interaction phenomena that may appear. This report does not address the existing data that allow the nature of the interactions between contaminants to be assessed.

Typology of (inter) actions (US EPA, 2000)

Interaction type	Effects	Actions	
WITHOUT INTERACTION	Additivity of doses	Simple similar actions	
	Additivity of responses	Simple dissimilar actions = independent actions	
WITH INTERACTION	Synergy	Complex similar actions	**Effect > Additivity**
	Potentiation	Complex dissimilar actions	
	Antagonism	Complex similar actions	**Effect < Additivity**
	Inhibition	Complex dissimilar actions	

[1] https://www.anses.fr/fr/system/files/SUBSTANCES2017SA0016Ra.pdf
[2] https://www.ineris.fr/sites/ineris.fr/files/contribution/Documents/drc-guide-ers-2013-v4d-complet-lienscompact-1378197912.pdf: Annexe 1
[3] https://www.efsa.europa.eu/fr/glossary-taxonomy-terms
[4] http://www.atsdr.cdc.gov/glossary.html

Additivity	In a cumulative risk assessment, additivity is the summation of the doses, concentrations, or biological responses of each contaminant in a mixture, in order to assess the overall risk of the mixture. This approach is only valid if the individual components have similar toxicological properties on a target organ or system.
Antagonism [3]	Describes a contaminant having an opposite effect to that of another contaminant and thus canceling or diminishing its action (for example, a hormone which, when released in the body, prevents another hormone from working).
Potentiation	Situation where a contaminant exacerbates the effects of another contaminants, without producing these effects itself (Kortenkamp *et al*., 2009).
Synergy	Interaction of several contaminants or processes whose effect is greater than the sum of the individual effects of each contaminant or process.
Human biomonitoring	French Agency for public health (SPF) defines it as surveillance activities, using biomarkers, that focus on environmental exposures, diseases and / or disorders and genetic susceptibility, and their potential relationships.
	Biomarker[2]:
	Biomarker of exposure: can be defined as a chemical contaminant or its degradation products present in a biological matrix. Its measurement corresponds to the level of concentration of the contaminant or degradation products in the biological matrix (s) under consideration.
	Biomarker of effect: biological response to this contaminant. For example, characterization of early biological effects such as a variation in enzymatic activity, circulating levels of hormones, DNA alteration or biomarkers of immunity.
LCI	(Lowest concentration of interest): LCI is a limit value of emission for a given substance from a consumer product, including building and decorative materials: limit concentration which aims to prevent the occurrence of health effects during long-term exposure to VOC emissions (Anses, 2015). It cannot be used as an air quality guideline value, toxicological reference value or occupational exposure limit value.
Contaminant	An element, such as a solid, liquid or gaseous material, radiation, sound, vibration, heat or odor, which can harm the health of live species or altering the quality of the environment.
Hazard [2,3]	Property of an agent, or situation, that may cause adverse effects to the exposed organism. Example: toxicity of an emitted substance. Situation or possibility for a substance, because of its intrinsic characteristics or properties, to cause damage to people, property and the environment. Adverse health effect such as a change in biological function or value, in the appearance or morphology of an organ, fetal malformation, transient or permanent disease, disability or incapacity, death (1st Health risk assessment stage).

ERS[2]	HRA - Health risk assessment : A four-step process that includes identification of the potentially adverse health effect , dose-response assessment , exposure assessment and risk characterization. Procedure to calculate or estimate the risk for an organism, a system or a (sub) population, including the identification of the related uncertainties, arising from exposure to a particular contaminant, taking into account both the characteristics of the agent in question and the specific target.
	ARR - residual risks analysis: Name of the quantitative health risk assessment approach proposed in the management methodology for polluted sites and soils proposed by the French directorate general for risk prevention (DGPR) in 2017.
	ERI [1] - Excess of individual Risk: Probability of occurrence of an effect on the health of subjects exposed to the contaminant studied over a lifetime compared to the baseline risk.
	HQ - *Hazard Quotient*: *Quotient de danger* (QD in French) ; Ratio between the exposure dose (or concentration) and the reference dose (or concentration), used to characterize the risk of threshold effects related to contaminants.
	MOE [2] - *Margin of Exposure*: Margin of exposure is a tool used in risk assessment to explore the safety issues posed by the presence of a contaminant in food or feed. The ratio of the reference dose to the exposure dose must be compared to a reference margin of exposure (cf. Chapter 3.3.1.3) .
ERC[3]	CRA - Cumulative risk assessment: Method for assessing the health or environmental risks posed by mixtures.
	HI - Hazard Index: Risk index used when assessing the risk of a mixture under the assumption of additivity. It corresponds to the sum of the hazard quotients (*HQ*) of each component of the mixture (see chapter 3.3.1.2).
	This can be modified to take into account the interactions between the compounds as proposed by the "method Weight of evidence" (WoE) and HI_{int} (cf. Chapter 3.3.2.1).
	BHI - Biological Hazard Index: Biological hazard index, based on biomonitoring data; does not take into account possible interactions between the components of a mixture (see. chapter 3.3.2.1).
	MCR - Maximum Cumulative Ratio: Index to highlight the components of a mixture that contribute mainly to the overall risk (see chapter 3.2.1.2).
	PODI - Point of Departure Index: similar to the *Hazard Index (HI)* by replacing the reference dose of contaminants in a mixture by the point of departure (PODs) (for the same effect) (see chapter 3.3.1.3).
	TEQ - Toxic Equivalent Quantity[2]: Toxic equivalent: sum of the concentrations of different substances of the same family, weighted by the Toxic Equivalency Factor (TEF) assigned to each, expressed in

	relation to the reference substance. For example: TCDD equivalent for dioxins or B[a]P equivalent for PAHs (see chapter 3.3.1.4).
	TUS - *Toxic Unit Summation* : similar to the *PODI* originally defined for the ecotoxicological risk, in which the hazard quotients are based on the effective concentration (EC_{50}) of the constituents. Corresponds to the EC_{50} of the mixture (see chapter 3.3.1.1).
Exposure [2,3]	Bringing a contaminant into contact with a target (organism, system or (sub) population). Concentration or quantity of a given substance in contact with a person, population or ecosystem at a specific frequency, within a given time interval.
Exposome	Concept based on a broad vision of the exposure, integrating a temporal component from conception to death, in particular the key exposure periods in life (childhood, puberty, pregnancy, etc.). The concept of exposome also integrates socio-economic, geographic and demographic factors.
IEM [2]	Interpretation of the State of the Environment: evaluation process to be implemented to assess the acceptability of the impacts of a site or an installation on its environment. More generally, this management approach makes it possible to check the compatibility between the state of sites and environments and their uses.
Mixture	the concomitant presence of at least 2 contaminants of all sources at the same place and over the same time frame, leading to cumulative exposure of the population.
Mode of action	Hypothesis about the sequence of key measurable events by which a contaminant exerts its biological effects. It is often confused with or used analogously to the mechanism of action but is considered to be broader. The mechanism of action is a sequence of molecular events that produces a specific biological effect (Kortemkamp, 2009).
PBPK [1]	Physiologically Based Pharmacokinetics are mathematical models that describe the absorption, distribution, metabolism, and excretion of a contaminant in a given organism. The body is described as a set of compartments (conceptual model) which may or may not be grouped together according to their physiological characteristics. The interconnections between these different compartments are represented by the blood exchanges (systemic circulation) between the different organs. The flow of contaminants is modelled by a system of differentiated equations describing the quantity of a contaminant in the different organs as a function of time. Physiological parameters such as blood flow, the volume of organs, the partition coefficients or ventilation rates are used to parameterise the model (Anses, 2017a).
	Similarly, a PBTK (*Physiologically Based Toxicokinetics*) model is defined in the context of toxicological risk analysis.
POD	*Point of Departure*. Indicator (dose, concentration) generally experimental to derive a toxicological reference value (TRV); most often, it is NOAEL, LOAEL, BMD or BMDL.

	BMD - Benchmark Dose [1] Dose producing a measurable effect corresponding to a predefined level of response compared to a control group.
	BMDL Lower limit of the confidence interval of the benchmark dose (generally 90 or 95 %).
	BMR Benchmark Response. Level of response to a stressor (for example 10 % of the maximum effect) from which a BMD can be derived.
	LOAEL [1] *Lowest Observed Adverse Effect Level :* Minimum Dose with Observed Adverse Effect (LOAEL) : Minimum dose / concentration leading to a biological or health effect, considered to be harmful and statistically significant compared to the control.
	NOAEL [1] *No Observed Adverse Effect Level* : (NOAEL): Maximum dose / concentration that does not cause an adverse effect and is statistically significant compared to the control group, resulting from the identification of LOAEL / C . In other words, this is the dose tested which directly precedes the LOAEL.
RPF	*Relative Potency Factor*. are based on the additivity of doses for a mixture of contaminants with similar mechanisms ; corresponds to the relative potency compared to a reference compound.
TEF	*Toxic Equivalent Factor* : Toxic *Equivalent Factor* (), defined for families of substances with similar mechanism ; characterizes the relative toxicity of an agent of the group compared to the reference agent of the same group ; originally established for dioxins and dioxin-like compounds and polycyclic aromatic hydrocarbons (PAHs), corresponding to RPF.
QSAR [3]	*Quantitative Structure-Activity Relationships*.are a set of methods by which the effects of different contaminants are associated with their molecular structure. They make it possible to predict the liekly adverse or beneficial effects of a given contaminant, by comparing it with other contaminants that have similar molecular structures.
	They aim to predict an experimental effect (biological activity, toxicity, affinity for a receptor) on the basis of the analysis of activities of chemical compounds previously tested (handles, 2017 a).
Dose-response relationship [2]	Relation expressing the intensity of a biological effect as a function of the dose or the concentration of a contaminant. This relationship makes it possible to determine BMDs, BMDLs, then TRVs, which are integrated into the risk analysis.
	Dose-effect relationship [2]: Quantitative relationship between the dose or concentration of a contaminant administered or absorbed and the nature[5] or the intensity of the adverse effect of this contaminant (2nd step of the health risk assessment).

[5] the nature of the effect: irritant, sensitising, reprotoxic, carcinogenic, neurotoxic, etc. (Anses, 2017a)

Risk [2,3]	Probability of occurrence of an adverse effect under given exposure conditions. Risk Characterization: qualitative or quantitative determination, including the associated uncertainties, of the probability of occurrence of known or potential adverse effects of a contaminant on a target under defined exposure conditions (4th step of the health risk assessment) .
GIS [2]	Geographic Information System. Computer tool for collecting, managing, manipulating, analyzing, modeling and displaying spatialized data.
Toxicity [3]	Intrinsic property of a contaminant that may cause adverse effects on an exposed organism.
VTR	TRV [1] Toxicological Reference value (ANSES, 2017a). Generic name grouping together all the types of toxicological index making it possible to establish a relationship between a dose and an effect (threshold) or between a dose and a probability of effect (non-threshold) in a population human. TRVs are specific for a substance, duration and route of exposure. By definition, a TRV is constructed for the most sensitive effect deemed to be adverse, thus protecting against all the toxic effects observed in the studies available for a given substance. It is expressed as a daily dose or a tolerable concentration (TDI or CT) to describe the threshold effects; or as the inverse of a dose or concentration (ERU) for non-threshold effects. VTi [1] - Indicative toxicological value: that can be used for risk assessment. This is an indicative value that is less robust than the TRV thus presented for a given substance. TDI or ADI - Daily Intake Tolerable or Acceptable : dose of exposure without appreciable risk to humans. It is constructed by dividing the PODs by uncertainty factors. Other names: reference dose (RfD) for US EPA, Minimal Risk Level (MRL) for ATSDR, reference exposure levels (REL) for OEHHA. ERU [2] - Excess Unit Risk: Additional probability, compared to an unexposed subject, that an individual will contract a pathology if he is exposed during his entire life to a unit dose (or concentration) of a contaminant (generally for carcinogenic genotoxic contaminants). The ERU is expressed in (mg / kg / day) $^{-1}$ for the oral route or in (mg.m^{-3}) $^{-1}$ for the inhalation route.
VGAI	IAQG Indoor air quality guideline value. Concentration in air *, associated with an exposure time, below which noadverse effects or nuisances having repercussions on health (in the case of odorous compounds) are in principle expected for the general population. (* or in the case of non-threshold, concentration associated with a level of risk corresponding to a probability of occurrence of the disease).

VLEP	OEL - Occupational exposure limit value.
	o Occupational Exposure Limit -8 hours (OEL-8h), which aims to protect, in the medium and long term, the health of workers regularly exposed to the chemical agent considered, and this for the duration of working lifetime . This limit is, unless otherwise specified, the limit of the time-weighted average of the concentration of a chemical agent, in the air of a worker's breathing zone during a work shift of 8 hours;
	o Short-term exposure limit (STEL-15 min) which aims to protect workers from adverse effects (immediate or short-term toxic effects such as irritation) due to peaks of exposure. This is the limit of the time-weighted average of the concentration of a chemical agent in a worker's breathing zone over a 15-minutes (unless otherwise specified) during the peak of exposure regardless of its duration;
	o Ceiling value: This is the atmospheric concentration limit of a chemical agent in a worker's breathing zone, which must not be exceeded at any time during the work period. It mainly concerns agents recognized as strong irritants or corrosives or which can cause a serious and potentially irreversible effect in the very short term. Specific analytical measures are implemented to measure this value.

Tables

Figures

1 Background, purpose and procedure for carrying out the expert appraisal

1.1 Background

Human exposure to mixtures, and the potential related health risks, are issues that have been raised for many years. Institutional documents and recommendations have been published since the 2000s with a view to taking mixtures into account primarily when assessing risks to human health (US EPA, 2000, 2002, 2006; ILSI, 1999; ATSDR, 2001, 2004; Health Council of the Netherlands, 2002; IGHRC, 2008; EFSA, 2008, 2009, 2013, 2019; IPCS/WHO, 2009; Kortenkamp *et al.*, 2009; SCHER, 2011).

In the area of chemical substances, there were 34 million compounds registered by Chemical Abstracts Service (CAS) in 2008 (Stewart & Carter, 2009). In 2018, more than 17,000 individual substances had already been registered under the European Union (EU) REACH regulation (Regulation (EC) No 1907/2006). The exact number of chemicals on the market in the European Union is not known but the European Chemicals Agency (ECHA) estimated that there were nearly 140,000 substances in 2017 (https://echa.europa.eu/home).

Chemicals are used in a wide range of economic sectors, including food production, the manufacture of medicinal products, the textile industry, and the automotive industry.

Environmental contamination (air, water, soil, etc.) can result from any of the following:

- production and/or packing and packaging processes;
- everyday use of consumer goods containing chemicals;
- unintentional emissions from combustion;
- environmental persistence of substances that may now be prohibited by the regulations, etc.

The management of the risks associated with chemicals is covered on the one hand by REACH and on the other by media-oriented (water, air, etc.) and sector-oriented (medicinal products, cosmetics, biocides, etc.) regulations, from which separate risk analyses have arisen (Evans *et al.*, 2016). Concerning chemical mixtures, there are risk management guidelines in some of these regulations, especially in the area of food Where a tiered approach is proposed for the assessment of cumulative risks.

The issue of mixtures remains complex, but it can now be addressed through expert appraisal procedures given the existence of knowledge and the development of simplified models on which there is consensus. With regard to health risk assessment, some examples of regulatory provisions stand out, in particular for exposure via food (pesticide residues and drinking water) and the impact of industrial facilities on the environment and the surrounding area. Recommendations from institutional organisations (US EPA, ATSDR, EFSA, SCHER) have formalised methodological approaches considering knowledge on whether or not contaminants interact, and have underlined the importance of their implementation. The most highly recommended hypothesis involves the concept of dose or response additivity. Many studies have tested the model of dose (or concentration) additivity for various mixtures of contaminants having similar toxicological properties for a target organ or system and have shown that overall, this model reasonably predicts the toxicity of mixtures at low doses/concentrations. Models integrating notions of antagonism and synergy are necessary to better understand and take into account the mechanistic bases of interactions, as well as exposure to relatively high doses/concentrations. However, it should be noted that at low doses,

interactions remain unlikely to generate a risk very different from that assessed with the additivity hypothesis due to uncertainties inherent in the risk assessment process itself.

Risk assessment aim to guide public decisions but methods used are based on regulatory provisions that in some cases refer to methodological guides that are not all appropriate for the assessment of mixtures, given the number of possible combinations of substances to which the population can be exposed. It therefore appears impossible to document, in a regulatory framework, hazards and interactions between substances for actual exposure.

The evaluation of mixtures can focus on combinations of different contaminants: chemical contaminants, physical factors (noise, temperature), and/or biological contaminants (bacteria, mould, allergens, toxins, etc.). The scope of this report is limited to **chemicals only**.

1.2 Purpose of the request

As part of ANSES's expert appraisal work on reference values, the issue of mixtures was raised for classes of substances such as aldehydes (acrolein, formaldehyde and acetaldehyde) and aromatic hydrocarbons (benzene, toluene, ethylbenzene and xylenes) to which exposure is often simultaneous.

To further investigate these issues, a review of existing methods for taking mixtures into account in the areas of health risk assessment and the setting of reference values was carried out and is the subject of this report. This review covered the guideline values, and more generally the management values[6], proposed by some institutions in order to consider several contaminants to be measured simultaneously.

The purpose of this report is to summarise knowledge on approaches to assessing potential health risks associated with mixtures and deriving reference values. It focuses on risks to human health, but the additional m concerning effects on ecosystems will also be developed in this review.

1.3 Procedure: means implemented and organisation

From 2016 to 2018, ANSES appointed two expert rapporteurs *intuitu personae* from the two Expert Committees (CESs) involved in expert appraisals on reference values to carry out this expert appraisal work:

- the CES on “Characterisation of substance hazards and toxicological reference values” (CES Substances), in charge of establishing toxicological profiles for chemicals with a view to deriving reference values (TRVs, OELs, IAQGs); on 1 September 2017, it became the CES on “Health reference values”, which is responsible for setting and validating the various reference values for which ANSES's expertise is sought (TRVs, OELs/BLVs/BRVs, IAQGs, DNELs);
- the CES on “Assessment of the risks related to air environments” (CES Air), which is in charge of issues involving the assessment of the hazards and risks to human health (general population and workers) associated with the quality of air environments.

The methodological and scientific aspects of the expert appraisal work were regularly submitted to the CESs. The report takes into account the comments and additional information provided by the members of these CESs. This work was therefore conducted by a group of experts with complementary skills.

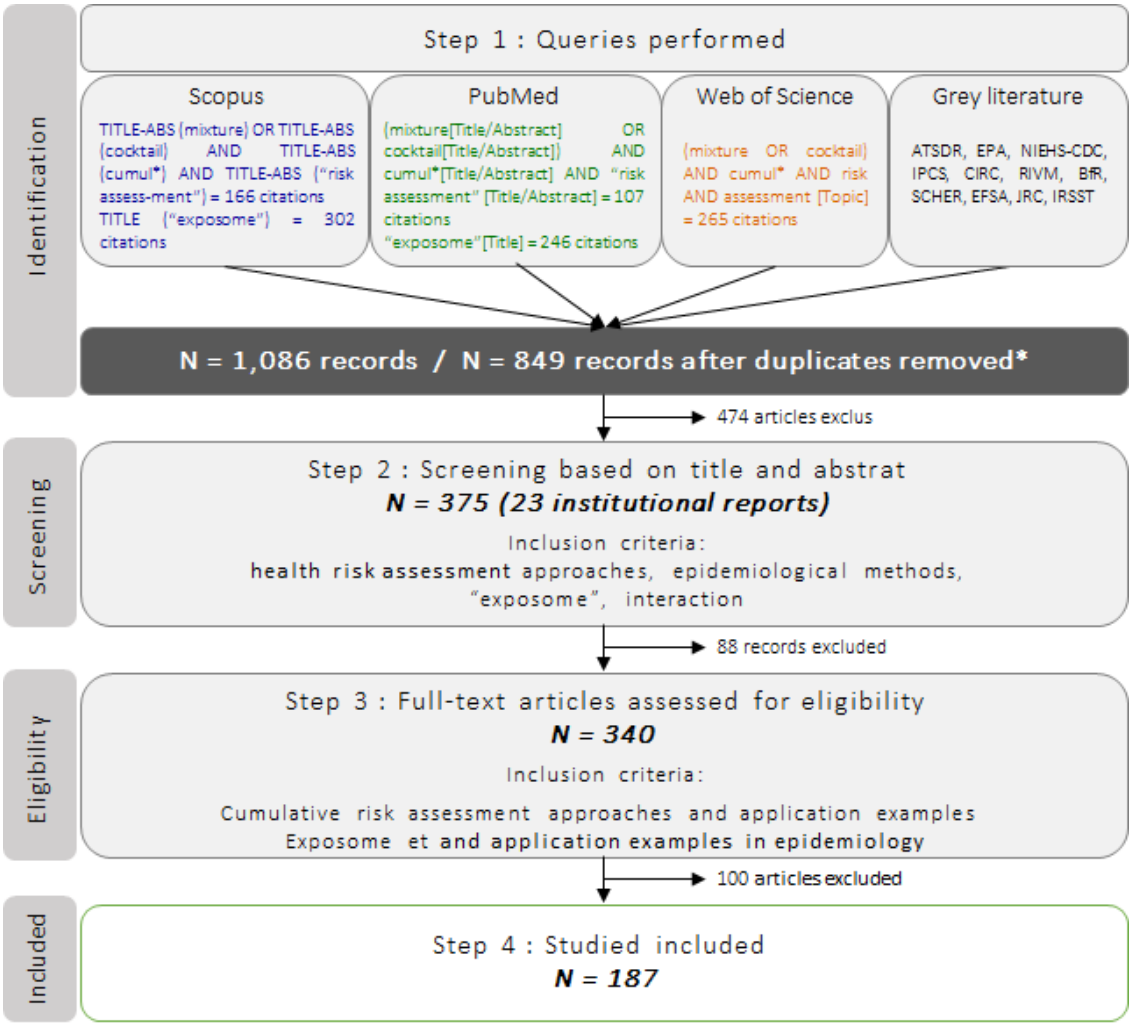
[6] Management values encompass guideline values, whether indicative or regulatory, limit values, and any other values proposed with the aim of implementing an action plan – of any kind – in the event that the exposure limit value is exceeded for a given compound.

The expert appraisal was carried out in accordance with French Standard NF X 50-110 “Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)”.

The information required to conduct this expert appraisal was collected via a literature search (peer-reviewed journals, reference works and grey literature) aiming to identify the methods proposed to take mixtures into account in the assessment of health risks. This search in no way focused on knowledge of interactions for specific mixtures.

The literature review was performed using the PubMed, Scopus and Web of Science bibliographic databases in ***May 2016***; it was then updated by ANSES up to ***September 2020***. It targeted existing approaches in the area of health risk assessment. It was supplemented by the identification of institutional reports dealing with this topic or with the development of reference values, and by a description of methods specifically developed in epidemiology to take mixtures into account by defining queries in the Scopus and PubMed databases with the “exposome” concept.

The steps of the literature review are described in Figure 1, listing the queries performed in the databases, the organisations targeted for grey literature for the identification of references, followed by criteria for the selection of relevant articles in relation to the issue raised in this expert appraisal.



Based on PRISMA Flow Diagram 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Gedda 2015)
* Records from queries carried out at 3 different periods for bibliographical updating with possible overlapping of publication periods

Figure 1: Steps of the literature review for the expert appraisal[7]

Concerning the guideline values and more generally the management values proposed by certain institutions, the documents taken into account in this report are primarily national regulatory texts specific to each country.

The review conducted as part of this report covered the French regulations and those defined within the European Union; some non-exhaustive examples of regulations in other countries that have been described in English-language publications.

1.4 Prevention of risks of conflicts of interest

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

[7] NB: grey literature is information produced on all levels of government, academia, public research, business and industry in electronic and print formats not controlled by commercial publishing (https://www.cairn.info/revue-i2d-information-donnees-et-documents-2015-1-page-30.htm#no1).

2 Existing guideline values for chemical mixtures

For the proposal of guideline values, and more generally of management values, some institutions suggest, or have suggested at a given time, considering several contaminants to be measured simultaneously. This section describes management values, guideline values (whether indicative or regulatory), limit values and any other values proposed with a view to improving the quality of media (water, soil, air, food).

2.1 Drinking water

The management of drinking water (DW), called “water intended for human consumption” in the French regulations, relies on the WHO recommendations, European directives transposed into French law, and the provisions of the French national plans for environmental health and the Grenelle environmental round table laws (Pène & Lévi, 2011). The quality of drinking water is defined based on maximum levels for individual parameters or classes of contaminants, established to protect the health of consumers. A distinction is made between “quality standards”, established based mainly on health criteria, and “quality parameters”, which can be based for example on organoleptic criteria or the proper functioning of water treatment facilities. For the most part, the parametric values correspond to the guideline values established by the WHO (WHO, 2017), which generally represent the “concentration of a compound that does not pose a significant risk to the health of a person consuming the water in question throughout their lifetime” (AFSSA, 2007).

The French Public Health Code (Article R.1321-2), as amended by Decree No 2007-49 of 11 January 2007 on the safety of DW, sets these quality limits, among other things. These are available in Annex I of the Ministerial Order of 11 January 2007 as amended, transposing Directive 98/83/EC on the quality of water intended for human consumption.

The DW regulations address the issue of mixtures for four classes of parameters associated with quality limits taken from the WHO recommendations and based on policy decisions:

- Polycyclic aromatic hydrocarbons (PAHs): 0.1 $\mu g \cdot L^{-1}$ for the sum of the concentrations of benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*g,h,i*]perylene and indeno[1,2,3-*cd*]pyrene. This value comes from the WHO's proposed guideline values published in 1984. It was not based on health criteria but rather on maximum concentrations measured in surface water at a time when treatments were less effective;
- Pesticides: 0.5 $\mu g \cdot L^{-1}$ for the sum of the concentrations of all identified, detected and quantified pesticides. This value is intended to cover all individual active substances and their relevant metabolites, based mainly on their toxicity and/or “pesticide” activities;
- Tetrachloroethylene and trichloroethylene: 10 $\mu g \cdot L^{-1}$ for the sum of the concentrations of these two contaminants. This value is based on a precautionary approach;
- Trihalomethanes (THMs): 100 $\mu g \cdot L^{-1}$ for the sum of the concentrations of chloroform, bromoform, dibromochloromethane and bromodichloromethane. This value is based on a practical approach to reduce chlorination by-products.

As part of the revision of the Drinking Water Directive[8], two new sums of parameters should be introduced concerning:

- Haloacetic acids (HAAs): 60 $\mu g \cdot L^{-1}$ for the sum of the concentrations of monochloro-, dichloro- and trichloro-acetic acid, and monobromo- and dibromo-acetic acid, when disinfection methods that can generate HAAs are used for the disinfection of drinking water. Like for the quality limit for the sum of THMs, the introduction of this parameter and of the

[8] https://data.consilium.europa.eu/doc/document/ST-6230-2020-INIT/fr/pdf

associated quality limit aims to reduce chlorination by-products without compromising the disinfection of water.

- Per- and polyfluoroalkyl substances for which a parametric value will apply once technical guidelines for the monitoring of this parameter have been developed. Member States may then decide to use any of the following parameters:
  - PFAS Total: 0.50 $\mu g \cdot L^{-1}$ for the sum of all per- and polyfluoroalkyl substances
  - Sum of PFAS: 0.10 $\mu g \cdot L^{-1}$ for the sum of per- and polyfluoroalkyl substances considered a concern as regards DW[9].

More broadly, concerning water policy, certain classes of substances, persisting in surface water in particular, are covered by environmental quality standards (EQSs) under the Water Framework Directive (2000/60/EC). These EQSs aim to protect sedimentary organisms and aquatic organisms in the water column from the direct or indirect toxicity of substances by secondary poisoning (environmental component) and also to protect human health from the toxicity of substances in raw drinking water or from secondary poisoning following the consumption of potentially contaminated organisms (health component). The regulations remain based on the assessment of individual substances. EQSs are not suitable for use for the potential toxicity of mixtures because they are established for different targets depending on the substance (Kortenkamp *et al.,* 2019).

2.2 Human food

As part of managing the health risks associated with food contaminants (pesticide residues, food additives, etc.), EFSA publishes acceptable (ADIs) or tolerable (TDIs) daily intakes, some of which are applicable for mixtures of compounds. These values have purely toxicological bases.

This is the case, for example, for:

- Parabens: 0.10 $mg \cdot kg^{-1} \cdot d^{-1}$ for the sum of methyl- and ethylparaben and their sodium salts, noting that these two parabens do not have oestrogenic properties (unlike propylparaben, which is therefore studied separately) (EFSA, 2004b);
- Organotins: 0.25 $\mu g \cdot kg^{-1} \cdot d^{-1}$ for the sum of tributyltin (TBT), dibutyltin (DBT), triphenyltin (TPT) and di-n-octytin (DOT), noting similar immunotoxicity with the same mode of action for these contaminants (EFSA, 2004a);
- Dioxins: 0.2 $pg \cdot kg^{-1} \cdot d^{-1}$ for the sum of dioxins and dioxin-like PCBs (or 0.1 $pg \cdot kg^{-1} \cdot d^{-1}$ when only considering dioxins), after weighting by their toxic equivalency factor (TEF) published by the WHO (WHO-TEF) (EFSA, 2012).

Some of these limit values are included in the regulations.

In the area of food, a residue is a substance found on or in a food product, following the application of pesticides or biocides or the use of veterinary medicinal products. Regulation (EC) No 396/2005 defines maximum residue levels (MRLs)[10] for pesticides in food and feed for each plant protection active substance currently authorised or prohibited. Regulation (EC) No 1881/2006 sets maximum

[9] Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorobutane sulfonic acid (PFBS), perfluoropentane sulfonic acid (PFPS), perfluorohexane sulfonic acid (PFHxS), perfluoroheptane sulfonic acid (PFHpS), perfluorooctane sulfonic acid (PFOS), perfluorononane sulfonic acid (PFNS), perfluorodecane sulfonic acid (PFDS), perfluoroundecane sulfonic acid, perfluorododecane sulfonic acid, perfluorotridecane sulfonic acid

[10] MRL defined as the "upper legal level of a concentration for a pesticide residue in or on food or feed set in accordance with this Regulation, based on good agricultural practice and the lowest consumer exposure necessary to protect vulnerable consumers".

levels for certain contaminants, introducing the concept of toxic equivalency factors (TEFs) to facilitate regulatory controls (Section 5 of the Annex to Regulation (EC) No 1881/2006).

ANSES studied the feasibility of setting an overall maximum level of pesticides in food designed to protect consumers from the cumulative effects of these substances (ANSES, 2017b). It concluded that an "overall" MRL could only be applied appropriately if the aim was the absence of any residue in foodstuffs. It would reduce the assessment of exposure to a substance or substance group to the sole measurement of their concentrations, without completely encompassing the concept of associated risk which alone enables human health to be protected. This work encourages the accelerated development of methodologies for assessing cumulative risks.

2.3 Polluted sites and soils

Under the regulations on polluted sites and soils in the Netherlands (Dutch Soil Protection Act), guideline values for soil quality (intervention values and target values) were proposed based on the risks to human health and ecosystems, to classify sites according to their contamination (Swartjes, 1999). In the area of human health, they are based on the maximum permissible risk (MPR) levels proposed in 1991 and then re-assessed in 2001 and 2009 by the Dutch National Institute for Public Health and the Environment (*Rijksinstituut voor Volksgezondheid en Milieu* (RIVM)) (RIVM, 2001, 2009). In this framework, limit values were proposed for mixtures of compounds (the values are given in $mg \cdot kg^{-1}$ dry matter). The following target values were set out in the regulations (Circular 2000[11]):

- PAHs: the sum of the concentrations of 10 PAHs[12] must not exceed the value of 1 $mg \cdot kg^{-1}$;
- Chlorobenzenes and chlorophenols: 0.03 $mg \cdot kg^{-1}$ for the sum of mono- to hexachlorobenzene, and 0.01 $mg \cdot kg^{-1}$ for the sum of mono- to pentachlorophenols;
- PCBs: 0.02 $mg \cdot kg^{-1}$ for the sum of the congeners (28, 52, 101, 138, 153, 180);
- Organotins: 0.001 $mg \cdot kg^{-1}$ for the sum of TBT, DBT and TPT;
- Certain organochlorine pesticides: 0.005 $mg \cdot kg^{-1}$ for the sum of aldrin + dieldrin + endrin, and 0.01 $mg \cdot kg^{-1}$ for the sum of DDT, DDE and DDD (based on similar hepatic toxicity);
- Phthalates: the value of 0.1 $mg \cdot kg^{-1}$ is provided for the sum of all phthalates.

In France, the regulations do not define limit values similar to those of the Netherlands. The methodology for managing polluted sites and soils was updated in 2017 after 10 years of implementation. Situation analysis values can be defined for various environments, such as soil and soil gases, indoor air and outdoor air. Concerning soil quality, only metals and metalloids individually without considering speciation or mixtures are addressed with the presentation of the ranges of values commonly observed in "ordinary" soils according to a study by the French National Research Institute for Agriculture, Food and the Environment (INRAE, formerly INRA).

11 https://www.esdat.net/Environmental%20Standards/Dutch/annexS_I2000Dutch%20Environmental%20Standards.pdf
Soil remediation intervention values are not presented.

12 Naphthalene, anthracene, phenanthrene, fluoranthene, chrysene, benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*ghi*]perylene, benzo[*k*]fluoranthene, indeno[1,2,3-*cd*]pyrene.

2.4 Ambient air

The aims of public policies on ambient air at EU level are to develop and implement means of improving air quality (control of mobile- and non-mobile-source emissions, fuel quality, environmental protection in the transport and energy sectors).

Regarding emissions in several areas of activity, targets have been set for reducing emissions of various pollutants including volatile organic compounds (VOCs) and dioxins-furans:

- Directive 1999/13/EC of 11 March 1999 on emissions of VOCs due to the use of solvents established limit values by area of activity for channelled and diffuse VOC emissions. For example, the Ministerial Order of 1 June 2010 on emissions of all kinds from classified facilities for environmental protection (ICPEs) mentions a limit value of 20 $mg \cdot m^{-3}$ for a group of more than 40 VOCs due to their contribution to the formation of tropospheric ozone (ozone in the region of the atmosphere closest to Earth).
- Directive 2000/76/EC of 4 December 2000 on the incineration of waste sets an emission limit value of 0.1 $ng \cdot m^{-3}$ for dioxins and furans, after weighting the concentrations by their respective TEFs.
- Directive 2010/75/EU of 2010 on industrial emissions and Directive 2001/80/EC of 2001 on emissions into the air from large combustion plants aim to prevent and reduce pollution. Directive (EU) 2016/2284 on national emission ceilings sets, for each country, annual emission ceilings for five pollutants including non-methane volatile organic compounds. These commitments include those already made internationally by the Member States under the Gothenburg Protocol in particular.

The assessment and management of ambient air quality are based on Directive 2008/50/EC and in particular on compliance with the limit values in ambient air set for the main pollutants, especially PAHs, in Directive 2004/107/EC. Only benzo[*a*]pyrene (B[a]P) has a specific target value, but air quality monitoring should be able to assess the contribution of B[a]P in ambient air compared to that of other PAHs which at least include benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, indeno[1,2,3-*cd*]pyrene and dibenz[*a,h*]anthracene.

2.5 Indoor environments

Since 1 January 2012, the regulations have required that construction and decoration products bear a label that indicates their level of emission of certain chemical compounds (Decree No 2011-321; Articles R.221-22 to R.221-28 of the French Environmental Code). Ten compounds are covered by the labelling requirement, but an emission limit value for total VOCs (TVOCs)[13] of 1 $mg \cdot m^{-3}$ is mentioned in order to be classified in the A+ category (see Annex 1 of the Ministerial Order of 19 April 2011 on the labelling of construction or wall or floor covering products and paints and varnishes with regard to their emissions of volatile pollutants). The concentration of TVOCs is commonly used as an overall indicator of the VOC content of emissions from construction products but this parameter itself has no health value (ANSES, 2009).

2.6 Summary

Table 1 briefly describes the guideline values identified as part of this expert appraisal and indicates whether the establishment methods were based on health, management or metrological criteria.

[13] Sum of VOCs eluting between and including n-hexane and n-hexadecane, detected using the method in the ISO 16000-6 standard, which is an initial level of characterisation of the VOC emissions of a product as part of an overall approach.

Table 1: Summary of the criteria used to establish the identified guideline values

	Type of value	Establishment criteria
Drinking water	Limit values for mixtures	Management criteria: policy-making
Food	Limit values for mixtures	Health criteria
Soil	Target values for mixtures	Risks to ecosystems criterion
Ambient air	Emission limit values	Management criteria: policy-making (emissions)
	Target values in air	Health criteria for benzo(a)pyrene (monitoring)
Indoor environments	Emission limit values	Management criteria: policy-making

To date, although thousands of chemicals are potentially in contact with humans, few guideline values have been proposed for mixtures. The current guideline values and limit values have in some cases been proposed using highly pragmatic approaches, without any clear explanation of the scientific bases. It therefore appeared necessary as part of this expert appraisal to conduct a literature search on cumulative risk assessment (CRA) approaches as a whole. This will be discussed in the next section.

3 Risk assessment approaches for mixtures

3.1 Introduction

As early as the 1970s, the need to assess the overall risk in cases of multiple exposure was highlighted when it became apparent that the population was being gradually exposed to multiple chemicals. However, risk analysis methods evolved slowly due to a lack of scientific knowledge, suitable techniques and funding for this research (Bopp *et al.,* 2019). In 1986, the United States Environmental Protection Agency (US EPA) published guidelines for the risk assessment of mixtures (US EPA, 1986) that then evolved thanks to advances in knowledge in 2000 and later in 2006 (US EPA, 2000, 2006). In the early 2000s, CRA methods received special attention within numerous governmental institutions (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, 2002; Danish Veterinary and Food Administration, 2002, 2003; European Union, 2005, 2007, 2009; Canadian Environmental Assessment Agency, 2007) including the European Union and, on a larger scale, the WHO (WHO, 2008, 2010).

From a toxicological point of view, when the issue of mixtures is being taken into account, two principles related to biological actions may arise:

1) **Additivity**: in the specific case where some of the effects of substances are similar, these may be related to common or independent mechanisms, causing either **dose additivity** or **response additivity** to be suggested;
2) **Interaction:** when the effects of two substances cannot be predicted by the principle of additivity, the word 'interaction(s)' is used. There may be positive (**synergy**) or negative (**antagonism**) interactions.

From the start, and still today, CRA considers "simple" mixtures of contaminants having similar modes of action, using the simplified additivity hypothesis to assess risks.

The **dose additivity** approach assumes that the substances in the mixture act on the same biological target (cell or organ) and via the same mode or mechanism of action, and that only the toxic potential differs. Therefore, the toxicity of each substance is quantitatively estimated in relation to another: it is considered that the dose of the mixture (D_{mix}) equals the sum of the adjusted doses of the various components (aD_i) according to the following simplified equation:

$$D_{mix} = \sum_{i=1}^{n} aD_i \quad (A)$$

where aD_i is the adjusted dose (weighted by the toxic potential of the substance).

Response additivity suggests that the components of a mixture act independently from one another and that it is the response to the mixture (or probability of effect) that can be predicted based on the response to each of the components. It can be expressed by the following equation:

$$E(D_{mix}) = \prod_{i=1}^{n} \left(1 - E(D_i)\right) \quad (B)$$

If the effect decreases as a function of the dose or concentration (e.g. if survival data are considered).

$$E(D_{mix}) = 1 - \prod_{i=1}^{n}\left(1 - E(D_i)\right) \quad (C)$$

If the effect increases as a function of the dose or concentration (e.g. if mortality data are considered).

where $E(D_{mix})$ is the effect at the dose of the mixture and $E(D_i)$ is the effect of the component at dose i.

The model of integrated additivity is an intermediate model that encompasses the approaches of dose and response additivity (Kortenkamp & Faust, 2010; Rider & LeBlanc, 2005; Rider *et al.*, 2010). The method is based on the grouping of substances having the same mechanism of action; a total dose is then calculated for each group using the dose additivity model. Next, the groups are combined using the response additivity model via the following mathematical equation:

$$R = 1 - \prod_{i=1}^{N}\left\{1 - \frac{1}{1 + \frac{1}{\left(\sum_{i=1}^{n}\frac{Di}{ED50i}\right)^{\rho'}}}\right\} (D)$$

where R is the response to the mixture, D_i is the concentration of substance i in the mixture, ED_{50} is the concentration of substance i that causes 50% of the response, and ρ' is the slope of Hill's dose-response curve.

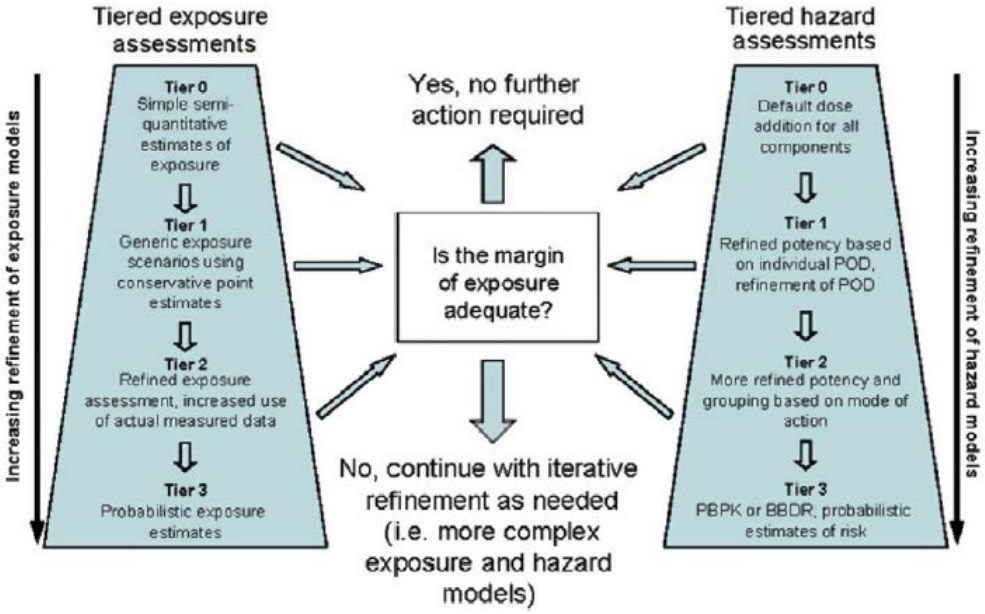
These concepts are all based on the lack of interaction and therefore consider that none of the components of the mixture impact the toxicity of any of the other components. While this hypothesis is simplified with regard to toxicological mechanisms, it is nonetheless considered plausible for environmental exposure to low doses. Studies have tested the additivity model for various mixtures (US EPA, 2000; Rider & LeBlanc, 2005; SCHER, 2011; Boobis *et al.*, 2011; Orton *et al.,* 2014; Scholze *et al.*, 2014); they showed that this model reasonably predicts the toxicity of mixtures having similar toxicological properties for a target organ or system. The dose additivity model appears to be more protective than the response additivity model (Christiansen *et al,* 2009; Orton *et al.*, 2014). Based on 11 studies, Boobis *et al.* estimated that the magnitude of interaction generated results deviating by a factor of 1.5 to 3.5 from the predictions of additive models (Boobis *et al*., 2011). A factor of 3 was also identified in the study by Christiansen *et al.* (2009) for the induction of male genital tract defects during *in utero* exposure to anti-androgenic substances.

It is therefore assumed that at low doses, an interaction (synergy or antagonism), if it occurs, remains unlikely to generate a result other than additivity in light of the uncertainties inherent in the risk assessment process itself. In practice, the interaction is therefore negligible.

Even so, based on current knowledge of mixtures, the absence of interaction cannot be verified in all cases. The default hypothesis of additivity should therefore not be used systematically and should not replace knowledge of specific cases of interactions, as was demonstrated for certain organophosphate pesticides mixed with a cytochrome P450 (CYP 450) inhibitor co-formulant (Rider and LeBlanc, 2005).

The aforementioned hypothesis of additivity is currently recommended by some risk assessment institutions (EFSA, 2013; US EPA, 2006).

Back in 2009, the scientific and regulatory state-of-the-art report on mixture toxicity requested by the European Commission (Kortenkamp *et al.*, 2009) highlighted the need to assess the risks associated with mixtures and develop adequate know-how to take them into account. The authors thus recommended, in addition to conducting research into the issue, exploring options for taking mixtures into account (for example, based on environmental contamination) and using the default dose additivity hypothesis as an initial approach. This type of step-wise procedure or tiered approach was proposed by the WHO in 2011, to advance CRAs based on knowledge of exposure and the related hazards (Figure 2), and by the European Food Safety Authority (EFSA) in 2013 (EFSA, 2019a). The challenges identified by the European Commission aim to improve knowledge on (i) hazards, by understanding mechanisms and developing models to study interactions, and (ii) the characterisation of exposure (Bopp *et al*., 2019).



PBPK: physiologically based pharmacokinetics; BBDR: biologically based dose-response.

Figure 2: Tiered CRA approach based on the refinement of exposure and the related hazards (Meek *et al.,* 2011)

Rotter *et al.* (2018) note that different recommendations appear in the regulations in force in the European Union, the United States and Canada depending on the use of substances and the sector, underlining the lack of harmonised approaches and methods. These differences concern in particular the recommended methodological approach for substances with independent modes of action or for interacting mixtures. The most advanced harmonised approach involves cumulative exposure to pesticides in the United States and Canada. The European SOLUTIONS project (Kortenkamp *et al.,* 2019) encourages the acquisition of data for all pollutants and suggests that the methodological framework should be included in the different regulations on chemicals, in particular REACH, and the regulations on plant protection products and biocides.

In France, ANSES's risks assessment related to plant protection products, DW and food has in some cases taken exposure to mixtures of contaminants into account. This work is described in detail in Annexes 1.2 and 1.3. Moreover, taking mixtures into account is recommended when managing ICPEs and polluted sites and soils according to recently updated methodological guides (see Annexes 1.4 and 1.5).

More recently, the French Society for Environmental Health (SFSE), based on all of this work, also recommended using the existing CRA approaches, with i) an iterative process for taking mixtures into account in health risk assessments, ii) the communication, analysis and institutional recognition of the "mixture" toxicity reference values (TRVs) published in the literature, and iii) the establishment of toxicological profiles for certain frequent cases of co-exposure (SFSE, 2013).

The first step of any CRA involves successively answering the following basic questions (Sexton and Hattis, 2007; Rice *et al.*, 2008).

(i) what contaminant mixtures are the most relevant in terms of public health?
(ii) what is the nature and intensity of the identified populations' exposure?
(iii) what are the mechanisms underlying possible interactions and what are their consequences for human health?

This may involve stating the issue at hand and the objectives of the CRA and identifying all the critical phases of the assessment (Solomon *et al.,* 2018) or assessing possible management measures (especially interventional) that could be taken with stakeholder involvement (MacDonell *et al.,* 2018; EFSA *et al*., 2019a).

The first step, before selecting an appropriate method, is to identify the relevant mixtures that should be taken into account. This step is addressed in the next section.

3.2 Grouping step

Four complementary approaches are generally used when choosing or grouping the contaminants to be taken into account when dealing with mixtures: i) according to the chemical class for substances having similar structures and mechanisms of action; ii) according to a common health effect; iii) according to the exposure of the population; iv) by combining approaches based on environmental contamination/exposure and common effects.

3.2.1 Grouping of contaminants according to their chemical class

This was the first approach that was implemented in the case of mixtures. The best-known historical example of grouping for decision-making in cumulative risk assessment involved 29 compounds, from the chemical class of dioxins (n=17) as well as certain polychlorinated biphenyl (PCB) congeners (n=12, called "dioxin-like" PCBs). It relied on structure-activity relationships based on a common molecular mechanism of binding to the aryl hydrocarbon receptor (AhR) (Safe, 1984; Safe *et al.*, 1985; Eadon *et al.*, 1986). In the infant Total Diet Study (ANSES, 2016), this approach (furans + dioxins + DL-PCBs) was considered inadequate, in particular because there are many substances in food (other than furans, dioxins and DL-PCBs) that also act on the AhR.

More recent risk assessments have also focused on other chemical classes. Some examples have included phthalates (NRC, 2008), perfluorinated compounds (Borg *et al*., 2013), PAHs (Nisbet and Lagoy, 1992; Audebert *et al.,* 2012), pyrethroids (US EPA, 2011) and organophosphates (US EPA, 2006b). However, for these assessments, the approach used to select the contaminants was not specified (except when the chemical classes were made up of substances with similar structures).

3.2.2 Grouping of contaminants according to a common effect

The grouping of contaminants according to a common effect is an approach that has grown in recent years primarily based on the NRC's work on the anti-androgenicity of phthalates (NRC, 2008). For example, as part of a reprotoxic risk assessment, Kortenkamp & Faust (2010) selected multiple contaminants from various chemical classes, all suspected of being anti-androgenic (phthalates; pesticides including fungicides, herbicides and organochlorines; parabens; polybrominated diphenyl ethers (PBDEs); and bisphenol A) (Kortenkamp & Faust, 2010).

More recently, EFSA initiated work to group together all of the active substances in plant protection products based on the available data on the effects on various systems (developmental, reproductive, neurological, thyroid, for example) by defining cumulative assessment groups (CAGs) (EFSA, 2013, 2019b, 2019c; Kennedy *et al*., 2020; Sprong *et al*., 2020; Zoupa *et al*., 2020). The methodology proposed by EFSA and applied to active substances having effects on the nervous system and thyroid is a tiered approach. The contaminants in the mixture to be considered can also be refined according to common specific effects (level 2), modes of action (level 3) or mechanisms of action (level 4) (EFSA, 2013, 2019a, 2019b, 2019c). Along the same lines as EFSA and ECHA, another study proposed grouping contaminants by binary (DEHP + procymidone or BPA + butylparaben) or total (DEHP + procymidone + BPA + butylparaben) mixture for substances having a common effect (reduction of ano-genital distance) (Christiansen *et al*., 2020).

3.2.3 Grouping of contaminants according to the exposure of the population

The grouping of contaminants according to the exposure of the population was developed as part of a French pesticide project (PERICLES research programme). The goals were to identify standard mixtures of pesticide residues to which the French population was the most exposed via food and to test their potential toxic effects. Crépet *et al.* (2013a) thus used a Bayesian nonparametric approach to classify the exposure profiles of 2624 adults and 1455 children for 79 pesticides quantified in at least 10% of samples (from campaigns measuring pesticide residues in food), based on individual food consumption data for the French population (INCA2, ANSES, 2009). The study of correlations between pesticides for the most exposed groups of individuals found seven separate mixtures of two to six pesticides (Crépet *et al.*, 2013a). This work was followed by the implementation of a non-negative matrix factorisation method combined with hierarchical classification that, based on the data of the Total Diet Study (ANSES, 2011), enabled the identification of groups of consumers exposed to pesticide mixtures (Béchaux *et al.,* 2013) and mixtures of various substances (Traoré *et al*., 2016). For example, one of the mixtures contained 10 pesticides, six trace elements and bisphenol A. Exposure to this mixture was related to a diet consisting mainly of fruits and vegetables eaten by a group of individuals who were mainly women (62%) and whose average age was 51 years (Traoré *et al*., 2016). The identification of these standard mixtures has enabled specific toxicological study protocols to be implemented for the evaluation of relevant mixtures (Crépet *et al.*, 2013b). Lastly, this approach was applied to biomonitoring data measuring breast milk contamination (Crépet *et al.,* submitted) with a view to proposing an integrated approach to the risk assessment of mixtures.

More recently, Kapraun *et al.* (2017) applied a frequent itemset mining (FIM) algorithm (like those used for the analysis of shopping baskets) to the biomonitoring data from the 2009-2010 American NHANES survey (over 10,000 subjects, 106 chemicals analysed). They identified 90 standard mixtures in more than 30% of the population, consisting for example of metals, PAHs, and parabens, as well as caffeine, theophylline and derivatives.

As part of the work of the Indoor Air Quality Observatory (OQAI), standard VOC and aldehyde mixtures were identified in French homes in 2003-2005 (Duboudin, 2010). One standard mixture,

observed in 10% of homes, was a mixture of seven compounds on average, all in concentrations two to 20 times higher than those in the complete sample. Two other standard mixtures corresponded to moderate multi-pollution with four to seven VOCs in concentrations around twice as high as those in the complete sample; one of the mixtures mainly contained aromatic hydrocarbons and the other aldehydes. Next, in 24% of the homes studied, there were mixtures characterised by a high concentration of a single VOC (five to 400 times higher than that in the complete sample). Eight sub-mixtures were identified, each of which was associated with a different VOC: 1,4-dichlorobenzene, n-undecane, 1-methoxy-2-propanol, styrene, trichloroethylene, tetrachloroethylene, 2-butoxyethanol or formaldehyde. The final standard mixture (40% of homes) included compounds not detected or found in low concentrations.

"Classification or grouping" approaches can also be used in workplaces to identify occupational uses or worker exposure depending on the area of activity. In France, data on the co-exposure of workers can be extracted from the Colchic database, which is a system used to collect occupational exposure data since 1987. Colchic currently contains 850,000 results for 70 different chemicals. A co-exposure assessment undertaken in 2012 found co-exposure to around 100 standard combinations of chemicals, such as formaldehyde and wood dust, cobalt and tungsten, and carbon and hydrogen sulphide. This co-exposure can be classified according to the number of occurrences, the activity, or the occupation (Vincent & Clerc, 2012).

The main limitation associated with these approaches has involved the choice of substances that were quantified in environments related in particular to analytical capacities at a given time. It therefore appears important to continue developing effective procedures for the measurement of emerging contaminants that are not yet covered by routine measurement campaigns.

3.2.4 Grouping of chemical contaminants by combining exposure data and common effects

Data on population exposure and the similar effects of substances can be relevant when assessing cumulative risks. When no toxicological data are available for mixtures, some approaches take into account both data on the exposure of the population (or on the contamination of an environment) and common effects or mechanisms of action. This was the case of the study by Fournier *et al.* (2014b), which presented a conceptual framework for grouping contaminants and for proposing the most appropriate methodology based on the level of available information on their toxic effects at the systemic level or on target organs or cells, as well as on their mechanism of action (Figure 3).

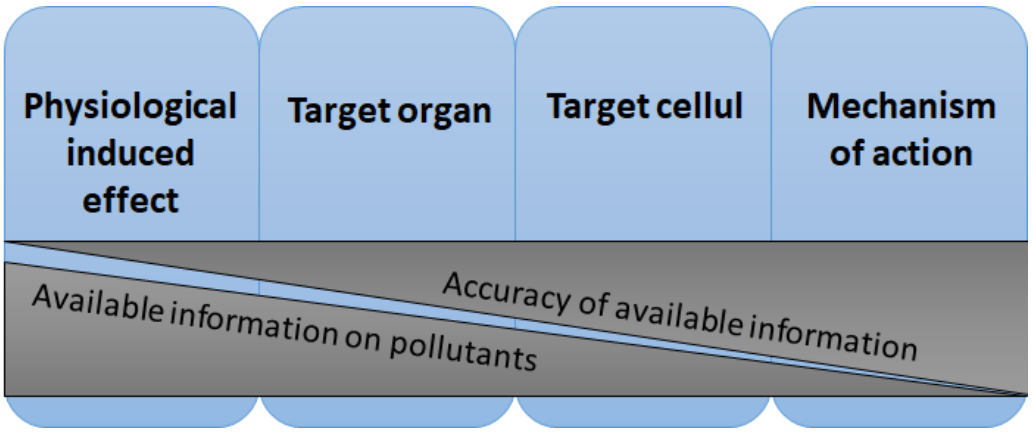


Figure 3: Conceptual framework for grouping contaminants based on their effects (adapted from Fournier *et al.*, 2014b)

This framework was applied to semi-volatile organic compounds (phthalates, PAHs, PBDEs, pesticides, PCBs, etc.) measured in more than 10% of French homes based on their effects at different hierarchical levels of living organisms (clinical to molecular scales). The contaminants were selected based on measurement campaigns (settled dust and airborne particles) in samples of French homes representative of metropolitan France as well as on a review of the literature on effects and action mechanisms. Seven main groups were identified based on their effects on the reproductive or central nervous system; the first five have a common mechanism of action (reducing testosterone synthesis, inhibiting insulin-like factor 3 (INSL3) or connexin 43, reducing dopamine levels) and the last two only have a common cellular or clinical effect (Table 2).

Table 2: Groups of semi-volatile organic compounds identified based on effects on the reproductive or central nervous system (Fournier *et al.*, 2014b)

Group	Description	Clinical or cellular effects	Common mechanism
Group A	DEHP, DiNP, DiBP, BBP, DEP, BDE47, BDE99, BDE100, BPA, lindane, permethrin, cypermethrin	Reprotoxicity	Yes
Group B	DEHP, DBP, DiNP, DiBP, BBP	Reprotoxicity	Yes
Group C	DEHP, DBP, DiNP, DiBP, BBP, BPA, lindane, dieldrin	Reprotoxicity	Yes
Group D	BDE47, BDE99, BDE209, BPA, PCB101, PCB153, lindane, permethrin, cypermethrin	Neurotoxicity	Yes
Group E	BPA, PCB101, PCB153, lindane, permethrin, cypermethrin	Neurotoxicity	Yes
Group F	BDE47, BDE99, BDE100, BDE209, BPA, PCB101, PCB138, PCB153, lindane, permethrin, cypermethrin	Neurotoxicity	Not determined
Group G	DEHP, DBP, DMEP, BPA, lindane	Reprotoxicity	Not determined

Similarly, Su *et al.* (2014) proposed a positive matrix factorisation method for identifying relevant mixtures based on data from the RIOPA study (indoor and outdoor air) on the personal exposure of the population to VOCs in three American cities (Weisel *et al*., 2005). For each of the mixtures, the contribution of each contaminant to the total concentration of the mixture was estimated, in order to verify whether or not the mixture was (spatially or temporally) homogeneous (the more homogeneous a mixture, the more it can be connected to a clearly defined source; in the opposite case, it will be subject to the hazards of human exposure). This multivariate analysis led to the identification of four profiles of mixtures (VOCs related to road traffic or indoor environments, for example) and was supplemented by a study of the literature on the effects and modes of action of substances to group together VOCs with a view to assessing risks of hematopoietic, liver and kidney cancer.

As part of the European “Euromix” project, which aims to develop a cumulative and aggregate (several routes and sources of exposure) risk assessment strategy, an approach based on food exposure and hazard data using CAGs was developed to identify relevant mixtures that will be studied in the project based on their toxicological effects (Crépet *et al*., 2019). Moreover, integrating exposure via food and other non-dietary sources as well as crop treatments and relative toxicity (relative potency factors, RPFs) enabled cumulative risk assessments of pesticide mixtures to be

proposed (Kennedy *et al*., 2019; Vanacker *et al.,* 2020). Kennedy *et al.* (2019) observed that the integration of non-dietary exposure sources modifies the composition of priority mixtures. These tools have been incorporated into a new version of Monte Carlo Risk Assessment (MCRA) software (Van Der Voet *et al*., 2020).

When conducting a review of regulatory methods for the assessment of mixtures, Jonker *et al.* (2004) updated a decision tree for identifying the most relevant contaminants based on the situation (Figure 4). The idea is to narrow the focus to the substances or contaminants posing the greatest risk via a grouping technique based on effects or mechanisms of action.

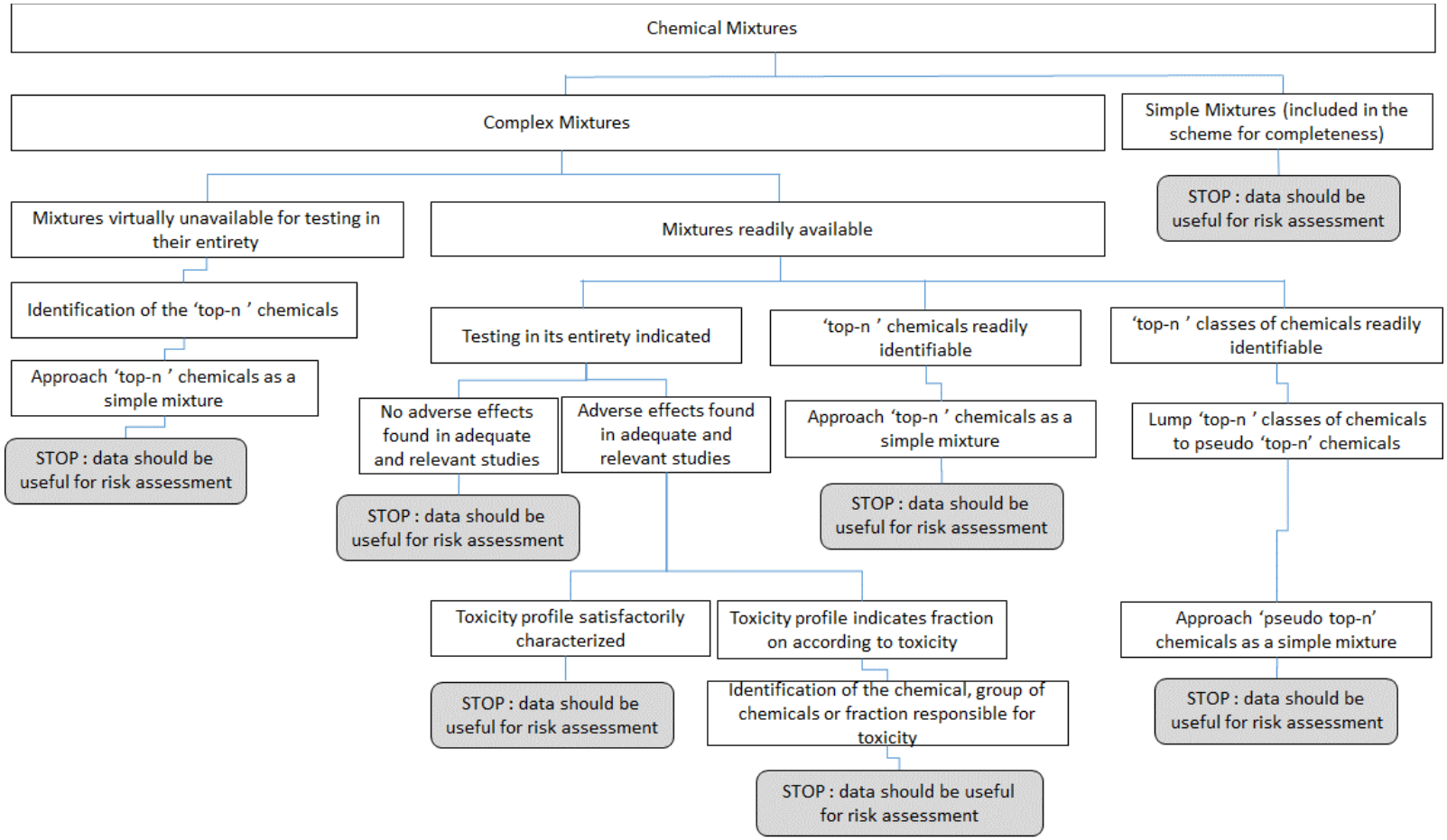


Figure 4: Decision tree for cumulative risk assessment (Jonker *et al.*, 2004). "Top n" contaminants or classes of contaminants: identification of the n contaminants or classes of contaminants most relevant for the risk assessment (not necessarily the most individually toxic)

3.2.5 Outlook

The grouping of substances based on an analysis of effects can be limited by the low availability of mechanistic data for all of the mixtures to which humans can be exposed. Nevertheless, the increasingly widespread use of high-throughput approaches in toxicogenomics is currently enabling large amounts of comparable, low-cost quantitative data to be generated, which may prove useful for improving this cumulative risk assessment step. Martin *et al.* (2007) demonstrated the relevance of using transcriptomics to categorise fungicides and perfluorinated chemicals based on their induction profiles for genes known to regulate nuclear receptors (such as PPAR and CAR/PXR). A similar approach was also developed to classify genotoxic substances based on *in vitro* tests using human cell lymphoblastoid lines (Williams *et al.*, 2015). Similarly, Kongsbak *et al.* (2014) used a proteomic approach to classify pesticides according to their mode of action. The recent studies by Darde *et al.* (2015, 2018) implemented a bioinformatic approach enabling a set of toxicogenomic data to be used for the classification of reprotoxic substances based on their transcriptional signatures; these signatures were then associated with health effects. These tools also have the advantage of being made available via a web interface.

3.3 Cumulation methods for risk assessment

The concept of cumulative risk assessment is currently based mainly on the hypothesis of dose or response additivity (Section 3.3.1). Few methods have yet to be developed to integrate the concepts of synergy and antagonism (Section 3.3.2).

3.3.1 Methods based on additivity

This section gives an overview of the methods developed and used based on the additivity hypothesis; they have already been widely described in several literature reviews (Pelletier *et al*., 2017; Fournier *et al*., 2014a; Sarigiannis *et al*., 2012; Reffstrup *et al*., 2010; Wilkinson *et al*., 2000; Kortenkamp *et al*., 2009; Lipscomb *et al*., 2010; SCHER, 2011; De Zwart & Posthuma, 2013; Pose-Juan *et al.,* 2016; Fox *et al*., 2017; Hass *et al*., 2017). Some are even also used for regulatory purposes (see Annex 1).

3.3.1.1 Toxic unit summation (TUS)

The toxic unit summation method is a direct application of the dose additivity concept. It was proposed in the 1970s in ecotoxicology and is represented by equation (E) where toxicity units are the ratio of exposure to the effect concentration (such as the EC_{50}) of each substance in the mixture for a given effect (Sprague *et al*., 1970).

$$TUS = \sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{\mathrm{DED_i}}{\mathrm{EC_{50_i}}} \quad (E)$$

where TUS: toxic unit summation; TU: toxicity unit; $DED_{i:}$ daily exposure dose for contaminant i; EC_{50i}: effective concentration (for example, 50% mortality in fish) for substance i in the mixture over the course of a day.

Not commonly used today, this method is the foundation of all the dose additivity approaches that have been developed to date and are described below.

Nonetheless, toxic unit summation was recently proposed to extend the applicability, in human health, of the dose additivity model beyond the maximal effect identified for a substance, to allow for the analysis of partial agonists (AhR and oestrogen receptors, for example). The approach was tested with 21 oestrogenic contaminants (epithelial breast cancer cell proliferation test) and the mixture response was correctly predicted based on individual data for each contaminant (Scholze *et al.*, 2014).

3.3.1.2 Hazard index (HI)

The hazard index (HI) method was developed by the US EPA on the same bases as TUS.

The most simplistic approach is defined as the sum of the hazard quotients of each component in the mixture to obtain a hazard index according to the following equation:

$$HI = \sum_{i=1}^{n} \mathrm{HQ_i} = \sum_{i=1}^{n} \frac{\mathrm{DED_i}}{\mathrm{TRV_i}} \quad (F)$$

where HQ_i is the hazard quotient of component i, DED_i is the daily exposure dose for contaminant i and TRV_i is the toxicity reference value of contaminant i.

The advantages of this approach are its simplicity and its ability to be used in all situations, whenever TRVs are available, which is valuable for risk management as part of a decision-making process. This approach can take into account various routes and sources of exposure for a mixture of pollutants, e.g. the inhalation of air in urban areas and the ingestion of contaminated food or water (Ogbeide *et al.,* 2016; Li *et al*., 2016). MacDonell *et al.* (2018) define equations for calculating the multiroute hazard index (MHI) and highlight its relevance when discussing management options.

The main drawback of this approach is that the TRV of each component is based on the critical effect, i.e. the effect that occurs at the lowest doses for the substance of interest. This effect can thus differ from the effect that would be taken into account in a grouping step described in Section 3.2, thus causing the cumulative risk to be overestimated. That is why this type of approach is generally reserved for the screening step (first step of the tiered approaches suggested for use as part of the regulations or scientific expert appraisals) (Gallagher *et al.*, 2015).

This approach can also be improved by deriving *ad hoc* TRVs for a common target organ or specific effect; in this case, an adjusted hazard index (aHI) is calculated (Pose-Juan *et al.,* 2016). The modified reference point index (mRPI) approach proposed by Vejdowszky *et al.* (2019) is similar and combines the advantages of the HI approach and the PODI approach, described in Section 3.3.1.3.

The HI approach is that recommended in methodological guides for the management of ICPEs and polluted sites and soils in France and in the regulations on plant protection products and biocides.

The examples given in Annex 2 show that the use of this approach often considers substances having a common effect. In 33 CRAs identified in the scientific literature that used the HI approach, the most commonly studied pollutants were phthalates, for their anti-androgenic properties (12 of 33), and pesticides (six of 33, for various effects with grouping approaches in some cases). VOCs were investigated in four studies, with two others also including SVOCs; perfluorinated contaminants, PBDEs, drug residues and THMs in water were also studied once. It should be noted that in half of the studies (in 18 of 33), either substances were grouped by target organ and TRV availability, or the TRVs used were specifically derived or taken from the literature, in order to consider a critical effect and thus make the approach more acceptable. However, in five cases, the HIs were estimated based on more disparate data:

- for pesticides: there are no TRVs by group of effect; all the TRVs of the European Commission or the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) are based on the most sensitive effect, which varies from one substance to another for medicinal products: use of the lowest PODs in the literature, for humans and animals (Roden *et al.,* 2014)
- for VOCs: use of lowest concentrations of interest (LCIs) instead of TRVs (Mishra *et al.,* 2015)
- for metals: use of occupational exposure limits (OELs) instead of TRVs (Minigalieva *et al.,* 2017; Omrane *et al*., 2018)
- and lastly, for PCBs, PAHs and PBDEs: use of the regulatory thresholds for quantities of contaminants found in fish (Syberg *et al.,* 2018).

One study investigated a mixture containing both chemical and physical contaminants (VOCs and noise), indicating that the HI can also be used to assess the cumulative risk related to nuisances that can differ in type. For noise, the HQ was estimated by comparing exposure represented by the ambient sound level with the WHO's reference value (70 dB). Another study assessing the impact of noise and pollution in San Francisco, USA on hearing determined the overall HI as the sum of the HQs for VOCs and noise:

$$HI_{\text{Global}} = HI_{\text{VOC}} + \text{HQ}_{\text{Noise}} = \sum_{i=1}^{n} \text{HQ}_{\text{VOC}_i} + \frac{\mu\,[\text{dB}]}{70\ \text{dB}} \quad \text{(G)}$$

where μ is the ambient sound level (in dB) and [70 dB] is the WHO's TRV for noise.

The study concluded that noise was the main contaminant in the (noise + pollution) combination in San Francisco for hearing loss (Evans *et al.*, 2014).

Sometimes, the HI approach is supplemented by a presentation of the largest contributors to risk by using an indicator called the maximum cumulative ratio (MCR), defined as the ratio of the HI to the maximum hazard quotient (maxHQ) of the mixture components (Price & Han, 2011; Han & Price, 2011; De Brouwere *et al.*, 2014; Pose-Juan *et al.,* 2016; Gustavsson *et al.,* 2017; Reyes & Price, 2018; Esposito *et al.,* 2018). It is calculated as follows:

$$MCR = \frac{HI}{\max\,[\text{HQ}_i]} = \frac{HI}{\max\left[\frac{\text{C}_i}{\text{TRV}_i}\right]} \quad \text{(H)}$$

This indicator aims to describe whether the cumulative risk is due to exposure to the mixture as a whole or whether one of the components is a dominant contributor.
If there is a main contaminant, the MCR is close to 1 and the risk assessment by substance would give the same result as the cumulative approach. An MCR above 2 indicates that there is no main component and that 50% of the cumulative risk assessment would not be covered by the single-substance risk assessment.
This "missed toxicity" within an individual approach can also be calculated as follows:

$$Missed\ toxicity = 1 - \frac{1}{MCR} \quad (I)$$

A classification of mixtures into four groups considering the MCR and HI values is proposed below based on a decision tree of the Cefic Mixtures Industry Ad-hoc Team (Price & Han, 2011; Han & Price, 2011; De Brouwere *et al.*, 2014) (Table 3).

Table 3: Classification of mixtures according to HI/MCR values

Group	MCR and HI	Description
I	max[HQ_i] >1 (HI>MCR)	At least one substance in the mixture poses a risk as identified by the single-substance risk assessment
II	HI<1	The cumulative risk associated with the substances is of low concern
IIIA	HI>1; HI<MCR; MCR<2 (MCR between 1 and 2; max[HQ_i]<1)	One substance in the mixture is responsible for the cumulative risk
IIIB	HI>1, HI<MCR, MCR>2 (max[HQ_i]<1)	Several substances in the mixture are responsible for the cumulative risk

This approach was implemented in two studies (Mishra *et al.*, 2015; Diamond *et al.,* 2018).

Lastly, the HI approach has also been used for management purposes. Two examples can be mentioned:

- Health Canada has used this approach when more than one aldehyde is measured in indoor air over a time period of five minutes (considered a short duration) (Health Canada, 1987). The approach consists in adding together the ratios of concentrations of formaldehyde, acrolein and acetaldehyde to their respective guideline values (120, 50 and 9000 $\mu g \cdot m^{-3}$ respectively). The total should be less than or equal to 1 (same principle as for the HI).
- Mixtures have been an issue in workplaces for several years. The American Conference of Governmental Industrial Hygienists (ACGIH) began working on this issue in the early 1960s. The developed approaches assumed that the chemicals to which workers were exposed could act on the same target organ. In 1971, a specific HI equation for mixtures of airborne contaminants was adopted for OSHA's proposal of limit values (1971). More recently, a web tool called MiXie was initially developed in Quebec (first version in 2001, updated in 2005) and was then proposed in France in 2014 thanks to a partnership between University of Montreal, Robert-Sauvé Occupational Health and Safety Research Institute (IRSST) and the French National Research and Safety Institute (INRS) (http://www.inrs-mixie.fr/). Several data analysis phases were followed for the tool's 2005 update (IRSST, 2005). The first phase took a large number of substances (more than 600 regulated substances) into account and led to all effects in similar classes of effects being considered as additive. The second phase, which considered more than 200 selected pairs of substances, aimed to specify the type of interaction for mixtures and was able to identify situations of infra-additivity and supra-additivity. In the end, it was recommended to consider a potential additive effect for situations of infra-additivity. For situations of supra-additivity, reducing exposure to the lowest possible level and establishing a prevention programme were recommended.

  The MiXie tool can rapidly identify whether the chemical agents in mixtures to which professionals are exposed have common effects (based on the target organ). It automatically calculates an exposure index that corresponds to an HI by using the sum of the ratios of concentrations to OELs. In 2020, 12 new substances were added and the classes of effects were updated for around 70 substances taking the European CLP[14] and the international IARC classifications into account.

[14] Regulation (EC) No 1272/2008

3.3.1.3 Point of departure index (PODI)

While the HI is an approach that can readily be used in CRAs whenever TRVs are available, comparing indicators that are not necessarily based on the same effects can be problematic, especially when the threshold of 1 is exceeded, which is often the case in the examples given in Annex 2 (78% of cases). One of the proposed ways to get around this is to compare exposure to substances directly with the animal toxicity indicators identified in the literature for the effect in question, providing a point of departure (POD) (this may be the no observed adverse effect level (NOAEL), lowest observed adverse effect level (LOAEL) or lower limit of the confidence interval of the benchmark dose (BMDL)). This is known as the point-of-departure index (PODI) approach (equation (J)) or the reference point index (RPI) approach (Pose-Juan *et al.*, 2016).

$$\mathrm{PODI} = \sum_{i=1}^{n} \frac{\mathrm{DED_i}}{\mathrm{POD_i}} \quad \text{(J)}$$

where PODI = point-of-departure index, DED_i = exposure, POD = point of departure (critical dose)

According to Wilkinson *et al.* (2000), the toxicity data and the selected POD should ideally reflect the toxicity of the various contaminants in the mixture with regard to a common effect. *A priori*, the same type of critical dose should be used.

This approach also involves applying a single assessment factor for the mixture; the resulting PODI should be less than 1.

As stated in Section 3.3.1.2, the modified reference point index (mRPI) approach proposed by Vejdovszky *et al.* (2019), based on equation (K), combines the advantages of the HI and PODI approaches by identifying the PODs of the different contaminants in the mixture associated with a common effect; uncertainty factors (UF) are also applied for each contaminant based on specific knowledge, resulting in the derivation of TRVs for the common effect. This approach was applied to data on breast milk contamination (Crépet *et al.*, submitted).

$$\mathrm{mRPI} = \sum_{i=1}^{n} \frac{\mathrm{DED_i} \times \mathrm{UF_i}}{\mathrm{POD_i}} \quad (K)$$

Another very similar approach is the margin of exposure for the mixture (MOEmix), as shown in equation (L). This amounts to selecting specific safety factors based on knowledge of the various relevant effects of a substance to assess a mixture's toxicity. It enables the exposure dose to be compared with the minimum dose causing adverse effects. This approach is the responsibility of risk managers and may be different depending on the regulation(s) applicable to the substances in the mixture and the effect in question.

$$\mathrm{MOE_{mix}} = \sum_{i=1}^{n} \mathrm{MOE_i} = \sum_{i=1}^{n} \frac{\mathrm{POD_i}}{\mathrm{DED_i}} \quad \text{(L)}$$

where MOE_{mix} = margin of exposure calculated for the mixture, MOE_i = margin of exposure for contaminant i, and POD = critical dose

This approach has seldom been implemented (Annex 3). Five studies were identified as part of the literature review conducted for this expert appraisal.

These studies assessed the risk for different chemicals that are not in the same class by investigating critical doses (NOAEC, BMCL or LOAEC).

The first one took various data sources into account (ATSDR toxicological profiles, US EPA hazardous air pollutant profiles, ITER, HSDB) and showed a risk for respiratory, neurological, hepatic, renal and immunological effects, which was not the case with the HI approach based on reference concentrations (RfCs), which did not document these last three categories of effects (Fox *et al.*, 2004).

The second considered endocrine-disrupting effects with oestrogenic and anti-androgenic action for which a study in animals was specifically carried out to establish critical doses for sexual differentiation in rats (NOAEL/LOAEL – ano-genital distance or nipple retention). Even at high doses, the mixture in question should not induce any anti-androgenic effects in rats (Christiansen *et al.*, 2012).

The third study showed risks of renal toxicity in adults and children exposed via food, regardless of the exposure scenario, using the mRPI approach (Vejdovszky *et al.*, 2019).

The fourth study used the MOE approach to assess risks associated with dietary exposure to various chemicals (pesticides, persistent organic pollutants and food additives) grouped based on their toxicity into EFSA's CAG for hepatic steatosis. This study showed cumulative risks for the various exposure scenarios and substances considered (Sprong *et al.,* 2020).

The fifth study used the mRPI approach to assess risks associated with neurological and thyroid effects for a mixture of contaminants found in breast milk, in order to use uncertainty factors specific to each substance in the mixture based on data (Crépet *et al*., submitted).

3.3.1.4 Toxic equivalency factors (TEFs)/relative potency factors (RPFs)

The third classic approach is that of toxic equivalency factors (TEFs), which in recent years have become more generally known as relative potency factors (RPFs). This approach implements the dose additivity model where each component can be considered a dilution of the most toxic component of the mixture or that for which the toxicological data involve the least uncertainty. In this framework, the dose of the mixture (D_{mix}) is expressed as the sum of the doses of each component (D_i) weighted by its relative potency factor (RPF_i) or its toxic equivalency factor (TEF_i):

$$\mathrm{D_{mix}} = \sum_{\mathrm{i=1}}^{\mathrm{n}} \mathrm{D_i} \cdot \mathrm{RPF_i} \quad (M)$$

Or

$$TEQ = \sum_{i=1}^{n} TEF_i \cdot C_i \quad (N)$$

The TEF is a method for assessing the toxicity of a specific contaminant that was developed in 1977. It is defined for chemically similar contaminants having the same mechanism of action based on the results of *in vitro* and *in vivo* studies. The first step consists in estimating the toxic potential of a contaminant that will serve as the reference from which the toxic potential of the other contaminants will be established. The quantity, relevance and robustness of the experimental or human data available for each contaminant are taken into account to select the reference compound. A TEF or RPF of 1 is arbitrarily assigned to the reference substance.

The risk assessment (HQ_{mix}) is then conducted based on this equivalency using the following equation:

$$\mathrm{HQ_{mix}} = \frac{\mathrm{D_{mix}}\ or\ TEQ}{\mathrm{TRV_{IC}}} \quad (O)$$

where here, TRV_{IC} is the TRV of the index contaminant selected as the reference (the toxicity of each other component is weighted based on this reference's toxicity). By definition, the reference substance has a TEF or RPF of 1 and the other congeners have factors based on experimental dataor by chemical structural analogy in comparison with the reference substance.

Articles proposing TEFs have mainly dealt with the classes of polychlorinated dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls (respectively PCDDs, PCDFs and PCBs) for which the definition of TEF/RPF has been regularly updated in light of new experimental data. They are listed in the first part of the table in Annex 4.

The WHO International Programme on Chemical Safety (IPCS) has assigned TEFs to various contaminants based on advances in knowledge (van den Berg *et al.*, 1998, 2006). These classes represent a particular situation: a multitude of structurally similar substances activate the same intracellular signalling pathway after binding to the AhR with different potencies. This situation is what led to the development of the TEF concept, where exposure to the mixture is expressed as a toxic equivalency (TEQ) of the most toxic component (2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for dioxins). This concept has been extended to other classes of structurally similar contaminants activating the same biochemical pathway. This is the case, for example, for certain organophosphate and carbamate pesticides that inhibit acetylcholinesterase, for endocrine disruptors that bind to oestrogen or androgen receptors or that inhibit steroidogenesis, and for PAHs with regard to their genotoxicity.

This approach is relevant when common effects resulting from one or more sufficiently known key biological events can be identified. This is why, to date, RPFs or TEFs have only been developed based on a few mechanisms: binding to the AhR (dioxins and PAHs); inhibition of acetylcholinesterase (organophosphates and carbamates); inhibition of voltage-gated sodium channels (pyrethroids); ED activity (inhibition of steroidogenesis, binding to ERs/ARs); and genotoxicity, more specifically histone phosphorylation (PAHs). Although theoretically based on a specific mechanism, most of the TEFs/RPFs arising from this work were developed by comparing a variety of toxicological data whenever a dose-response relationship was available (except for the genotoxic equivalency factors proposed by Audebert *et al.*, 2012) – with different experimental approaches (*in vivo/in vitro*), different exposure durations or routes, and different toxicological indicators (LOAEL, NOAEL, BMD) – which is a major drawback when dealing with the concept of relative toxicity.

Some examples of this approach being used are shown in the second part of the table in Annex 4. They involved the same chemical classes, primarily pesticides (organophosphate and azole contaminants) (Boon *et al.*, 2008; Jensen *et al*., 2013; Payne Sturges *et al*., 2009).

The articles by Fournier *et al.* (2016) and Pelletier *et al.* (2018) focused on mixtures including different types of contaminants in the context of exposure in indoor environments, whereas Chou *et al.* (2017) investigated particulate pollutants in ambient air.

In the study by Teuschler *et al.* (2004), the authors used the US EPA's cumulative relative potency factor (CRPF) approach, which combines the principles of dose and response additivity within a

single method for assessing the risks associated with mixtures for several exposure routes. This method uses information on the specific mode of action of each substance to assign each substance to a subclass of chemicals with a common mechanism of action. These subclasses therefore have different modes of action, but the adverse effect considered is the same for all of the subclasses (Figure 4).

For each of the subclasses, an index chemical equivalent dose (ICED) is calculated using the RPF approach. The ICED concept is used within the CRPF approach at two levels:

- ICED of the substance: refers to the ICED of the substance taken individually in the subclass.
- ICED of the subclass: refers to the ICED for all of the substances in a subclass.

The RPF approach was proposed to characterise the risks associated with a mixture of toxicologically similar substances. The ICED has the same mathematical interpretation as the TEQ for dioxins.

3.3.2 Methods based on antagonism or synergy

Simultaneous or sequential exposure to multiple substances can cause pharmacokinetic and/or pharmacodynamic interactions. Such interactions can modify the dose-response relationship for a substance and therefore its toxicity. There can be antagonistic or synergistic effects.

Several approaches have supplemented some of the methods based on additivity to take these concepts of interaction into account.

3.3.2.1 Weight of evidence approach

The weight of evidence (WoE) method is based on the HI (Mumtaz & Durkin, 1992; Mumtaz *et al.*, 1998; INERIS, 2006). This method proposed by Mumtaz involves weighting the HI by studying interactions between pairs of substances within a mixture. It is based on expressing the relationship between the estimated hazard index of the substances in the mixture and the weight of evidence assessed for the binary interaction of substances in the mixture. The following relationship is obtained:

$$IF_{i,j} + IF_{j,i} = D \cdot W \cdot (HI_i \cdot HI_j)^{0.5} \quad (P)$$

where

$IF_{i,j}$ is the effect of component j on the toxicity of component i, and $IF_{j,i}$ is the effect of i on the toxicity of j;

D is the direction factor for the interaction (where D=0 when there is additivity or lack of interaction; D=1 when there is synergy; and D=-1 when there is antagonism).

W expresses the overall confidence level assigned to the qualitative assessment of the interactions.

HI_i and HI_j are the hazard index values of substance i and substance j respectively.

A weighting factor WoE_M is defined that is expressed based on the sum of the interaction factors of the substances in the mixture ($IF_{i,j}$) that take into account the direction of the interaction and the weight of evidence for this interaction:

$$WoE_M = \sum (IF_{i,j} + IF_{j,i}) \quad (Q)$$

This WoE_M score is then normalised (WoE_N) by dividing it by the WoE_{MAX}, which is the sum of the geometric means of the HIs for the mixture:

$$WoE_{MAX} = \sum (HI_i \bullet HI_j)^{0.5} \quad (R)$$

Thus,

$$WoE_N = WoE_M / WoE_{MAX} \quad (S)$$

WoE_N can have a value within the interval of -1 to 1:

- -1 is the highest possible confidence level for a significant antagonistic interaction
- +1 is the highest possible confidence level for a significant synergistic interaction.

To take into account the level of uncertainty associated with the nature of the interactions between the substances in the mixture, the HI is therefore expressed using the WOE_N method:

$$HI_i = HI \cdot UF^{WOEn} \quad (T)$$

where:

HI_i: Adjusted hazard index

HI: Non-adjusted hazard index, based on the hypothesis of simple additivity

UF: uncertainty factor, having a default value of 10 (Mumtaz *et al.*, 1994)

WoE_N: normalised weight of evidence calculated according to equation (R)

This method was tested by calculating a predictive score for interactions and comparing it with experimental results (study of four nephrotoxic substances with similar modes of action (trichloroethylene, tetrachloroethylene, hexachloro-1,3-butadiene (HCBD), 1,1,2-trichloro-3,3,3-trifluoroproene (TCTFP)) and four other nephrotoxic substances with different modes of action (mercury chloride, lysinoalanine, D-limonene and HCBD). The prediction of interactions for the target organ (kidneys) was relatively satisfactory. However, this method cannot predict the nature of interactions for an organ other than the common target organ.

In some cases, in particular for medicinal products, the US EPA defines factors of interaction between the components of a mixture, taken 2 by 2, by comparing experimental and theoretical LD_{50} values (US EPA, 2003). The United States Food and Drug Administration (US FDA) has established guidelines for assessing drug interactions based on enzymatic induction and clinical pharmacokinetic potential (US FDA, 2012). This leads to the calculation of a cumulative hazard index taking interactions between components into account (HI_{Int}) according to the following formula (U):

$$HI_{Int} = \sum_{i=1}^{n} \left(HQ_i \cdot \sum_{j \neq i}^{n} f_{ij} M_{ij}^{B_{ij}\theta_{ij}} \right) \quad (U)$$

where f is an interaction factor for components i and j, M is a toxic interaction magnitude factor, B is a weight-of-evidence factor dependent on the quality of the toxicological data, and θ depends on the concentration ratio of components i and j in the mixture.

This approach was used by Roden *et al.* in their CRA of drug residues in surface water (Roden *et al.*, 2014).

These interaction factors are based on very high dose indicators where interactions are all the more likely. The LD_{50} data used by Roden *et al.* (2014) are seldom or almost never available for environmental contaminants. Moreover, interactions at the LD_{50} are far from being of the same nature as those that can be observed at low doses. Roden therefore used interaction factors that did not seem very robust.

3.3.2.2 Overall risk probability (ORP) approach

Yu *et al.* (2011) extended the response additivity concept for cumulative risks by quantifying synergistic and antagonistic effects for mixtures of substances. This method for quantifying the effects of mixtures is derived for the cases of independent effects, antagonistic effects, and synergistic effects of the mixture to obtain the overall risk probability (ORP).

When the contaminants in the mixture are independent (do not interact with each other), it is assumed that the ORP of each contaminant remains the same as if the contaminants were in a single-contaminant system.

In this case, the ORP for the mixture is calculated as follows:

$$P_m = 1 - \prod_{i=1}^{n}(1 - P_i) \quad (V)$$

where P_m is the ORP for the mixture, P_i is the ORP for substance i and n is the number of substances in the mixture.

For cases where contaminants in the mixture interact antagonistically and therefore reduce the risk of the other contaminants having an effect, an antagonistic coefficient (a_{ij}) is added to represent the probability of contaminant i reducing the adverse effects of contaminant j.

The ORP is calculated as follows (W):

$$P_i = P_i^0 \prod_{j=1}^{n}\left(1 + a_{ij} P_j^0\right) \quad (W)$$

where P_i^0 is the ORP for contaminant i and P_j^0 is the ORP for contaminant j.

This antagonistic coefficient is calculated through multivariate regression analysis of the experimental data.

For cases where contaminants in the mixture interact synergistically and increase the risk of the other contaminants having an effect, to take this interaction into account, a synergistic coefficient (s_{ij}) is introduced into the calculation, as follows:

$$(1 - P_i) = \left(1 - P_i^0\right) \prod_{j=1}^{n} \left(1 - s_{ij} P_j^0\right) \quad (X)$$

This synergistic coefficient is calculated through multivariate regression analysis of the experimental data.

3.3.2.3 PBPK modelling approach

PBPK modelling is primarily used in mixture toxicology to:

1. estimate an internal or systemic concentration of an individual contaminant relative to external exposure to a complex mixture (this figure is necessary to calculate the biological hazard index (BHI)).
2. investigate possible toxicokinetic interactions between the contaminants in the mixture (e.g. do the contaminants in the mixture behave independently or does an individual contaminant alter the internal or systemic concentrations of the other contaminants?)
3. estimate internal exposure by a given route based on data generated for another exposure route (route transposition).

As early as 2004, the use of PBPK modelling was recommended to quantitatively predict the consequences of interactions between substances in mixtures (Jonker *et al.*, 2004).

There are some studies giving concrete examples of PBPK modelling. In that of Andersen *et al.* (2004), the authors describe known examples of pharmacokinetic and pharmacodynamic interactions for mixtures of substances.

These examples of mixtures can be:

(i) either binary mixtures such as 1,1-dichloroethylene (DCE) and trichloroethylene (TCE), keeping in mind that TCE reduces the toxicity of DCE by competing to bind at the same enzyme site (pharmacokinetic interaction), or carbon tetrachloride (CCl_4) and chlordecone, bearing in mind that in animals, pre-treatment with chlordecone amplifies the toxicity of CCl_4. This is an example of pharmacodynamic interaction probably via the blocking of repair signalling in hepatocytes;

(ii) or other mixtures such as those related to the metabolism of a substance into several metabolites (mixture of the parent substance and metabolites). In rats, for example, n-hexane competes with its own metabolites by pharmacokinetically interacting with its terminal metabolite, 2,5-hexanedione.

In the publication by Sasso *et al.* (2010), the authors describe an overall modelling system based on the use of several PBPK models (referred to as a generalised physiologically-based toxicokinetic modelling system for mixtures (GTMM)) incorporated into the same interface. This system is able to take into account and simulate numerous interactions between heavy metals (cadmium, lead, arsenic) and non-metallic substances (drugs or pesticides) (Table 4). The described interactions are phenomena of induction or inhibition by heavy metals of the CYP enzymes that are involved in the metabolism of substances such as drugs, pesticides and other organic pollutants.

Table 4: Some interactions between metals and CYP enzymes in humans and animals

Metals	Effects on CYP enzymes	Potential substrates
Cadmium	Induced 2A6	Carbamates, drugs
	Induced 2E1	Halogenated aliphates, organophosphates, triazines, VOCs, drugs
	Induced 2C9	Organophosphates, triazines, drugs
Lead	Inhibited 2A6	Drugs
	Inhibited 1A2 (rats)	Arylamines, organophosphates, triazines, VOCs, PCBs, drugs
Arsenic	Induced 1A1 (rats)	Triazines, VOCs, PAHs, PCBs
Metal mixtures	Altered 1A1/2 induction by PAHs/TCDD (rats)	Organophosphates, triazines, VOCs, PAHs, PCBs, drugs

Tan *et al.* (2011) describe the use of PBPK modelling to investigate the PK/PD interactions of substances in mixtures. The examples presented in this article apply PBPK modelling to mixtures of substances as part of a cumulative risk assessment in order to predict the conditions in which PK interactions alter the dose additivity hypothesis. For example, for different ternary mixtures (trichloroethylene/perchloroethylene/methyl chloroform; toluene/xylene/ethylbenzene), PBPK modelling shows that the pharmacokinetic interaction is competitive metabolic inhibition of CYP450. In the case of a binary mixture of CCl_4 and methanol, modelling suggests that there is a pharmacokinetic interaction (enhanced hepatoxic effects of CCl_4 by metabolic induction) and a pharmacodynamic interaction as demonstrated by plasma concentrations of alanine aminotransferase and sorbitol dehydrogenase.

Haddad *et al.* (1999) propose using PBPK modelling to take interactions into account and simulate biomarker concentrations for exposure to a mixture of solvents (toluene, ethylbenzene, xylene), to apply it to the BHI concept. The classic BHI approach uses biomonitoring data without taking into account the toxicokinetic interactions of the components in the mixture, according to the following formula:

$$\mathrm{BHI} = \sum_{i=1}^{n} \frac{\mathrm{MC_i} - \mathrm{BC_i}}{\mathrm{BEI_i} - \mathrm{BC_i}} \quad (Y)$$

where MC_i = simulated concentration or level of excretion of the biomarker

BC_i = background concentration or level of excretion

BEI_i = biomarker concentration in a healthy worker exposed to the reference value (threshold limit value, TLV).

The classic BHI approach without interaction assumes that the toxicokinetics of the components in the mixture are not affected by co-exposure and that the toxic effects are additive.

PBPK modelling can be used to simulate the concentration or excretion levels applied for the classic BHI calculation by taking toxicokinetic interactions between the components of the mixture into account. In this case, the equation for the BHI with interaction is expressed as follows:

$$BHI = \sum_{i=1}^{n} \frac{SC_i}{BEI_i} \quad (Z)$$

where SC_i = simulated concentration or excretion level of the biomarker by PBPK modelling.

This methodology was applied to a mixture of toluene, xylene and ethylbenzene for which interactions by competitive inhibition of hepatic metabolism are known and have been characterised. PBPK modelling with interaction was able to predict the numeric values of the BHI and the simulated concentrations in order to calculate the BHI of the mixture. This was performed for several mixtures of three solvents (toluene, xylene and ethylbenzene) for which the BHI was calculated, and this result was compared with the classic hazard index method. The results seemed to demonstrate that at low concentrations, the BHI results obtained with the classic method were similar to those obtained with the BHI method with interaction, which confirmed, according to the authors, that at low doses, the consequences of an interaction by competitive inhibition of metabolism are negligible. Use of the MiXie tool, which assesses potential interactions between chemicals based on 600 substances, led to a similar conclusion.

3.4 Overall approach

3.4.1 Epidemiological and toxicological data

Epidemiology involves studying several risk factors determining the occurrence, frequency, mode of spread and progression of diseases affecting groups of individuals, requiring that they be integrated in the epidemiological study design and data analysis stages. These risk factors are not limited to chemical factors and can encompass, for example, physical factors (radiation, noise, etc.) and socio-economic characteristics.

Regarding the assessment of exposure, it is desirable to take into account the different individual contaminants in the mixture and study correlations between pollutants.

Levy (2008) and Braun *et al.* (2016) describe the possible contribution of epidemiological studies with regard to the effects of mixtures.

Levy (2008) encourages the use of epidemiological data for cumulative risk assessment, proposing a systematic process that should be applied to determine the relevance of epidemiological data when such data exist (Figure 5).

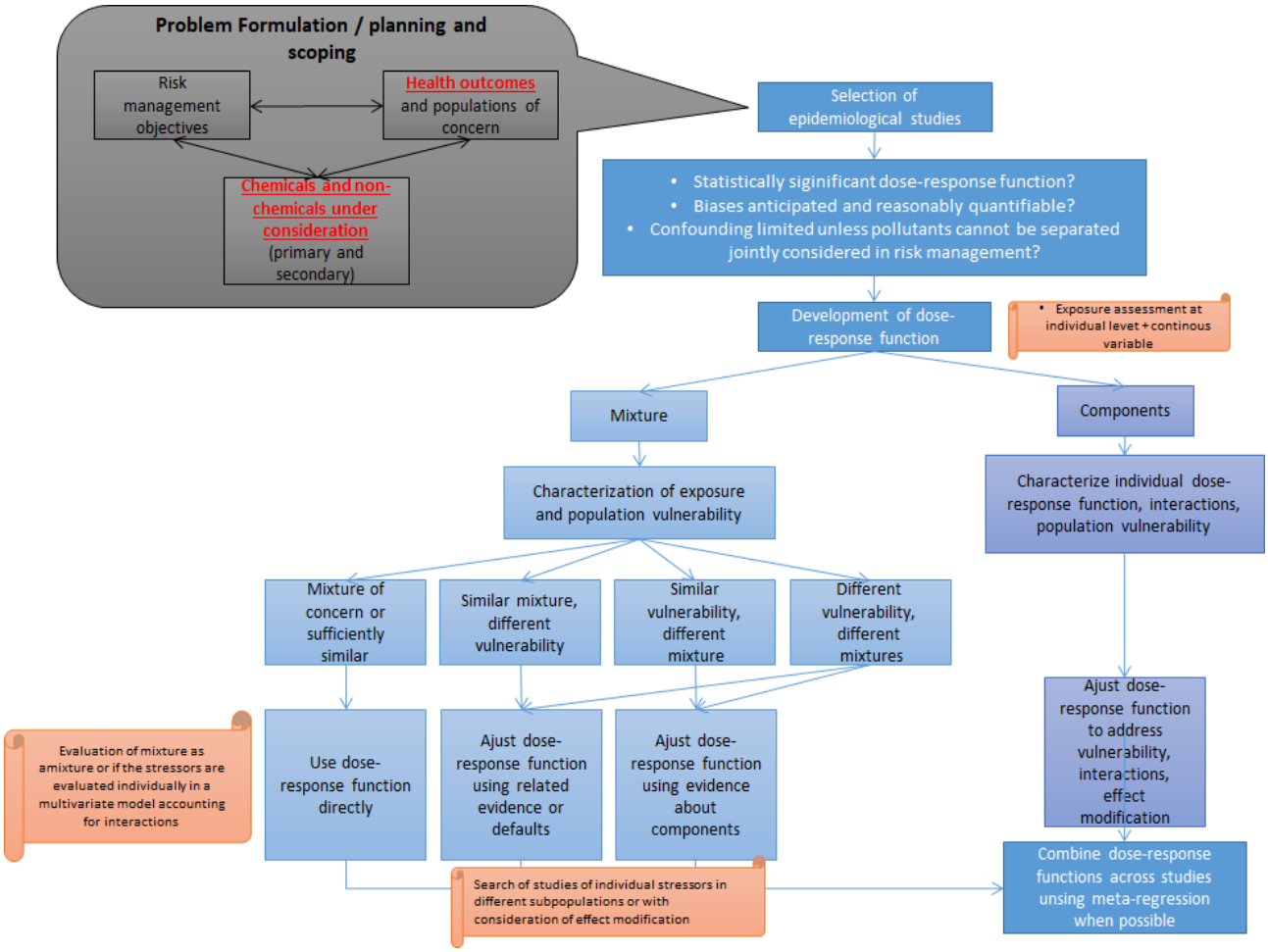


Figure 5: Conceptual approach to the analysis of epidemiological data for cumulative risk assessment (enhanced figure versus the proposal of Levy, 2008)

This diagram shows that to be usable for cumulative risk assessment, epidemiological studies must:

- Study dose-response relationships for broad exposure to multiple pollutants of interest by considering interactions and other effects. The pollutants considered should meet risk management expectations and may contribute to the diseases and symptoms studied.
- Explain and quantify all the dimensions of vulnerability including exposure differences, susceptibility/sensitivity, vulnerability related to the social environment and behaviour, and the ability to study the health effect.
- Study a population similar to those investigated in terms of vulnerability and exposure or at least including relevant sub-populations for these considerations with adequate stratified analyses.

Most epidemiological studies do not meet all these criteria. The conceptual approach therefore proposes a way of analysing data and possibilities for taking them into account. Braun *et al.* (2016) recommend studying each substance with a separate model and then conducting an analysis for several pollutants. This second analysis would require that the most relevant pollutants be chosen or would need to be broadened to the type of correlation between the pollutants.

The review by Chen and McKone (2001) underlines that there is insufficient evidence to conclude as to whether there is any interaction between exposure to ionising radiation and to chemicals.

The most widely documented interaction described in this review is the synergistic effect of exposure to radon and smoking on lung cancer risk: multiplicative interaction followed by supra-additivity or sub-multiplicativity (Hornung, 1998).

Hernandez *et al.* (2017) agree on the relevance of epidemiological data for cumulative risk assessment since these data provide information on human exposure in real conditions, avoiding the need for inter-species extrapolation. They underline the difficulties and limitations of epidemiology and affirm that the quality of studies should be evaluated. They add that systematic reviews and meta-analyses are particularly useful for summarising data on hazard characterisation and for providing more accurate estimates of associations by improving statistical power. The complementary nature of experimental studies, in particular for providing data on the biological plausibility of the associations found in epidemiological studies, is highlighted. This article concludes that it is important to integrate toxicological and epidemiological data to improve the usefulness and robustness of risk assessments for mixtures and affirms that this integration is necessary for decision-making.

The similar mixture risk indicator (SMRI) approach developed as part of the European EDC-MixRisk project considers epidemiological and experimental data when assessing risks using innovative biostatistical methods. This project links the results of human observation studies in the population to data from experimental tests with environmental mixtures to strengthen the weight of evidence related to environmental exposure (Bornehag *et al.,* 2019).

Epidemiological data are used to identify the most harmful mixtures of endocrine disruptors in three areas of health (growth and metabolism, neurological development and sexual development). Experimental data (*in vivo* and *in vitro*) are used to estimate dose-response relationships and determine the lowest doses or concentrations of exposure to mixtures that have disrupted molecular mechanisms in early phases of development. The risk assessment process uses an overall mixture strategy with a statistical measure of similarity to generate a similar mixture risk indicator (SMRI).

Marshall *et al.* (2013) described this approach of similarity of various candidate mixtures to a reference mixture. It involves substituting a mixture for another similar mixture (i.e. with the same components as the study mixture, but in different proportions) whose exposure and toxicity are known. For each candidate mixture and for the reference mixture, dose-response relationship modelling enables benchmark doses (BMDs) to be calculated. The reference mixture is therefore a mixture for which there is an experimental dose-response relationship. The Euclidean distance between the BMD of the reference mixture and the BMD of the candidate mixture is measured.

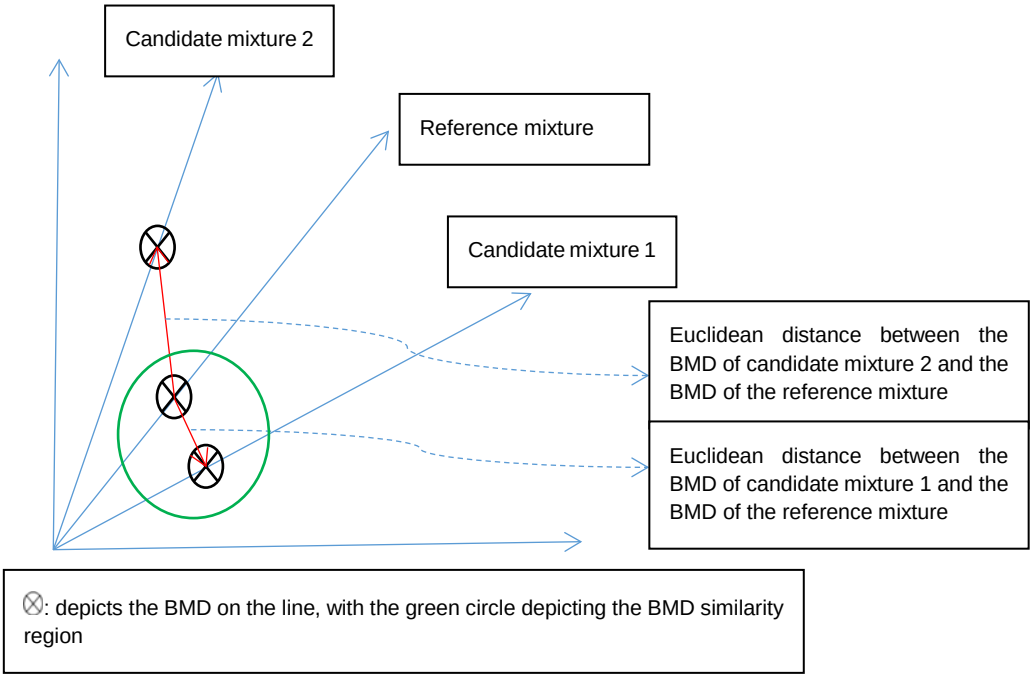


Figure 6: Graphic assessment of the SMRI

The authors estimate the Euclidean distances between the BMDs of the contaminants in the mixtures in an x-dimensional model (x = number of substances in the mixture) and the BMD of the reference mixture (which needs to be known) (Figure 6). Based on the number of data and their values, a similarity distance is defined for a given dose. If the distance between the BMD of the reference mixture and that of the study mixture is shorter than this similarity distance, it is concluded that the mixtures are sufficiently similar, and an SMRI is calculated, either by:

- summation of the ratios of exposure for each contaminant in the mixture compared with the TRV of the *ad hoc* reference mixture [calculated based on the BMDL of the reference mixture divided by UFs] (SMRI/HI approach),
- or determining the ratio of the RPF-weighted sum of exposure to the TRV of the index contaminant in the considered mixture (SMRI/RPF approach).

Marshall *et al.* (2013) illustrate this method with an example using data from the First National Environmental Health Survey of Child Care Centers, where pesticide levels were measured in 168 child care centres in 2001 (national representativeness, US). Only settled dust contamination data for 15 pyrethroids were considered (five pyrethroids accounted for the majority of pyrethroid load: cypermethrin, deltamethrin, esfenvalerate, permethrin and cyfluthrin). The pyrethroid profiles differed for the 168 locations (a specific mixture for each sample) and 126 locations were studied (exclusion of locations where all the data were < LD).

In the first case, the authors used dose-response data for two known mixtures (e.g. for which there were experimental data): one of 11 pyrethroids with proportions determined by BMD_{20} values (Wolansky *et al.*, 2005), and the other of five pyrethroids with proportions determined by the study in child care centres. In the second case, the authors used the proportions of the 126 locations for the 15 analysed pyrethroids. Dose-response function data were available only for the mixture of five pyrethroids used as the reference, not for the 15 pyrethroids. BMDs were therefore estimated using an equation to supplement the dose-response relationships. The authors concluded that the mixtures were similar in 90% of the 126 studied locations, considering at least one of the 15 pyrethroids to be

similar to the reference mixture. The calculated SMRI did not show any risk in this case (SMRI = 0.20<1).

3.4.2 Data from studies on the exposome

Five epidemiological studies dealing with the effects of pollutant mixtures were identified in the literature review conducted as part of this expert appraisal. They included:

- Three studies focusing on occupational exposure assessed via reconstruction using job-exposure matrices (Seeber *et al.*, 1996; Olsson *et al.*, 2010; Moehner *et al.*, 2013). Different indices and scores were used. Seeber *et al.* (1996) referred to the hygienic effect (HE) based on the effect additivity hypothesis. This takes the occupational exposure limits of each contaminant into account.
- Two studies on environmental exposure included a classic analysis considering a single-pollutant model as well as models combining exposure (Winquist *et al.*, 2014; Christensen *et al.*, 2011).

  Winquist *et al.* (2014) studied correlations between different atmospheric pollutants. They found the relationships to be overestimated in classic single-pollutant models and demonstrated the importance of interactions between certain contaminants, considering that these are confounding factors that should be taken into account.

  Christensen *et al.* (2011) studied non-linear and non-monotonic relationships between arterial hypertension and exposure to PCBs. An evaluation of correlation and collinearity between the different blood PCB congeners followed by a clustering analysis determined the most informative congeners regarding the risk of hypertension.

The three parts of Research Report 183 of the American Health Effects Institute (HEI) summarise three commissioned studies investigating epidemiological methods for taking into account the effects of exposure to multiple air pollutants (HEI, 2015, 2016). Bayesian statistical methods were used to integrate earlier and new data such as socio-economic status into the same analysis as covariables.

Numerous environmental factors act as mixtures interacting with other factors (socio-economic, behavioural) to induce changes in the studied phenotypes or increase the risk of developing diseases.

The actual exposure that should be taken into account in epidemiological studies is referred to as the exposome, a concept first described by Wild in 2005. It is based on a broad view of exposure, taking into account the totality of exposure to environmental (non-genetic) factors (Figure 7) over time, from conception to death. Key exposure periods to be documented are proposed in relation to the susceptibility of target organs: pregnancy, childhood, puberty and childbearing years (Wild, 2012; Shaffer, 2017). The exposome concept also incorporates social, behavioural, geographic and demographic factors characterising the living environment (Wild, 2012). A multidisciplinary approach is necessary and should include human and social sciences – especially for issues of health inequalities (Juarez *et al.,* 2014 and 2020) and social justice (Senier, 2017) – and toxicology, to better understand the impact on biological responses (Miller, 2014).

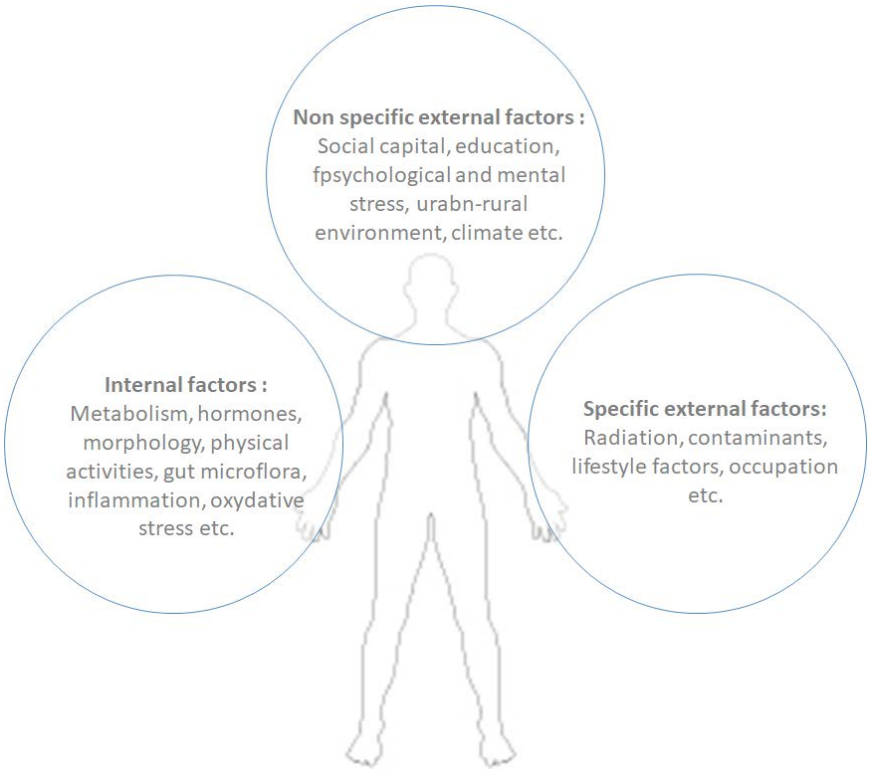


Figure 7: The exposome concept with its three main types of exposure factors (Wild, 2012)

A linguistic analysis of publications on the exposome (261 articles) found that research work in this area has gained momentum since 2011; it also identified the main terms associated with the concept. The top term involved the characterisation of environmental exposure (Kiossoglou *et al*., 2017). Another analysis of publications on the exposome highlighted advances in the area of human health arising in particular from the research projects described below (Haddad *et al*., 2019). The following health indicators were studied: respiratory and allergic symptoms, diabetes, cancer, cerebro-cardiovascular disease, sarcoidosis, Crohn's disease, polycystic ovarian syndrome, sperm quality, oxidative stress and the incidence of occupational diseases. The three types of exposure factors (Figure 7) were included in 48% of the analysed studies, whereas 42% were limited to the dimensions of specific external and internal factors.

The following research projects on exposome have been launched, especially in the European Union:

- the HELIX project (www.projecthelix.eu/fr) combining the environmental risks to which mothers and children are exposed and the study of associations with the growth, development and health of children (Vrijhied *et al*., 2014). Two hundred and thirty-four exposure variables have been evaluated covering different exposure periods (prenatal and postnatal) enabling their correlations, profiles and variability to be studied within and between the six cohorts (Tamayo-Uria *et al.,* 2019). The biological samples collected make up an important biobank for the detection of biomarkers, especially environmental contaminants such as organochlorine, polybrominated and fluorinated compounds, phthalates, phenols, etc. (Haug *et al.,* 2018) and for -omics analyses (Maitre *et al.,* 2018);
- the EXPOsOMICS project (www.exposomicsproject.eu/) developing a new approach for assessing environmental exposure with a focus on air pollution and water contaminants, by studying associations with numerous -omics profiles (Vineis *et al*., 2017);

- the HEALS project (www.heals-eu.eu/) proposing the "functional integration of -omics derived data and biochemical biomonitoring to create the internal exposome at the individual level". The available exposure biomarkers of interest from the HEALS project were specifically studied in relation to the reference levels[15] (Steckling *et al.,* 2018). Nearly 30 risk factors including levels of metals and trace elements in umbilical cord blood were taken into account (Calamandrei *et al.*, 2020).
- The ATHLETE project (https://athleteproject.eu/about/) aims to develop a latest-generation toolbox to study the exposome and set up a prospective exposome cohort in order to systematically quantify the effects of a wide range of environmental risk factors on respiratory, cardiometabolic and mental health and associated biological pathways during the first two decades of life. The project intends to implement feasible and acceptable interventions on the exposome. It may also inform policy recommendations and prevention strategies.

In the United States, the Hercules project (http://emoryhercules.com/) and the NexGen project developed in 2011 by the US EPA aim to provide key structure and expertise to develop and refine new tools and technologies for the discovery, assessment and application of the exposome (DeBord, 2016; Pose-Juan *et al.,* 2016; Jones *et al.*, 2016). The Children's Health Exposure Analysis Resource (CHEAR) programme (https://chearprogram.org) provides researchers with access to laboratories and offers the data analysis capabilities required for the assessment of exposure as part of studies on child health in order to apply the exposome concept (Johnson, 2017).

The exposome concept entails new developments and tools for characterising environmental exposure. The development of biological methods (biomarkers, genetics and -omics) is important for understanding modes of action (inter-species extrapolation and also acute-to-chronic studies) and biological or molecular signs of exposure, with the detection of parent contaminants and metabolites (Fox *et al.,* 2017; Go, 2015; Walker *et al.,* 2019; Thakur *et al.,* 2020; Vineis *et al.,* 2020) or the identification of a key period. For example, measurements in the umbilical cord or in baby teeth are used in biomonitoring to characterise prenatal exposure (Andra, 2015) whereas measurements from hair (Appenzeller *et al.,* 2020) or nails (Bocato *et al.,* 2019) are used to assess chronic exposure; however, the latter two matrices remain debated in the scientific community. There has also been work to combine classic exposure characterisation approaches (external dose of an individual or population) with these new approaches (Peters, 2012).

The analysis of the literature on the exposome highlighted proposals for analytical methods that extend the identification of substances, in particular via mass spectrometry techniques including ion-mobility spectrometry (Metz *et al*., 2017) or using high-resolution techniques (Go, 2015; Jones, 2016; Andra, 2017; Getzinger *et al.,* 2020) with the combination of liquid and gas chromatography or two-dimensional gas chromatography (Weggler *et al.,* 2020) to broaden identification. Targeted analyses are distinguished from non-targeted, unbiased analyses such as -omics analyses. The monitoring of various data analysis steps using computing tools and databanks is necessary for the standardisation of measurements (Xue *et al.,* 2019). The US National Institute of Standards and Technology (NIST) proposes a reference material (SRM 1950) and an online spectral database for

[15] human biomonitoring (HBM) / biomonitoring equivalent (BE): concentration of a chemical or metabolite in a biological matrix (blood, urine, human milk, etc.), consistent with defined exposure guidance values or toxicity criteria.

the analysis of chemicals. The identification of chemicals, especially at the structural level, is a major challenge for the study of the exposome (Johnson, 2017).

Scientific projects and civic initiatives have recently been launched with the use of micro-sensors or portable sensors to monitor the quality of outdoor and indoor air (Jiang *et al.,* 2018) and with smartphone applications on activities performed and meals eaten (Bocato *et al.,* 2019; Martin-Sanchez *et al.*, 2020). They can help improve knowledge of individual exposure (Bean, 2018). For example, two panel studies conducted in pregnant women and children as part of the HELIX project characterised the participants' exposure with the use of a kit featuring this type of technology (Figure 8) over two two-week measurement periods (Donaire-Gonzalez *et al.*, 2019).

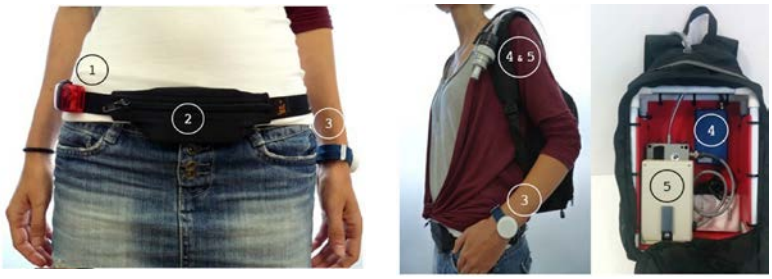


Figure 8: Measurement kit used in the HELIX project to characterise individual exposure (Donaire-Gonzalez *et al.*, 2019)

The generation, compilation and analysis of multidisciplinary data pose major methodological and computing challenges (Juarez, 2014; Sarigiannis, 2017).

Databases have been proposed to provide the information required to study the exposome; some examples include:

- the Toxin and Toxin Target Database (T3DB; www.t3db.ca) containing around 2900 substances to which humans can be exposed; it was created in 2010 and has since been updated on a regular basis (Wishart, 2015)
- Comparative Toxicogenomics Database (CTD; http://ctdbase.org), created in 2004 and initially focused on toxicological data on interactions between substances and genes in connection with diseases. Recent updates, especially those of 2017, have opened a specific module compiling environmental exposure data to connect them with laboratory toxicological data (Davis, 2017)
- the ToxCast database of the US EPA (https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data): regularly updated with data from *in vitro* trials with more than 3000 chemicals (Bessonneau *et al*., 2019)
- the Exposome-Explorer database (http://exposome-explorer.iarc.fr) dedicated to biomarkers of exposure to environmental risk factors, providing detailed information on the nature and concentrations of biomarkers as well as on study populations, measurement methods and correlations with other exposure (Agache *et al.,* 2019). This database, launched in 2012, was released online in 2017. The second version, Exposome-Explorer 2.0, was enhanced for biomarkers of interest for dietary exposure and cancer risk (Neveu *et al.,* 2020)
- the Blood Exposome Database (http://bloodexposome.org) compiling data from the literature (PubMed, PMC) and from databases like the Human Metabolome Database (HMDB) (www.hmdb.ca/) on endogenous and exogenous substances in blood (Barupal *et al.*, 2019)
- the CIL-EXPOSOME database providing an analytical platform for isotopically identifying urinary biomarkers (https://drive.google.com/drive/folders/1i1UNhfwMh_ry97TH6-FKGEm_A-i-oIeU?usp=sharing) (Jia *et al.,* 2019)

The choice of the statistical method is also important when hundreds or even thousands of hypotheses are involved.

Moreover, the hypothesis studied in epidemiology on the effects of exposure to several pollutants is different from that studied in toxicology (additivity, synergy, antagonism), which is based on dose-response curves that can be different (Howard and Webster, 2013).

The number of possible exposure profiles to be studied in exposure-phenotype associations is an analytical challenge (Johnson, 2017). The analysis of exposure correlations is necessary in each study dealing with the exposome, which can vary from case to case and have repercussions on analyses of associations with health indicators and their interpretation (Santos *et al.,* 2020). Correlations can be visualised graphically (maps, networks).

The goal may be to focus on the most frequent co-exposure profiles in the population by estimating the correlation between the different exposure variables and "grouping" highly correlated exposure using "unsupervised learning" techniques (Patel *et al.,* 2015). For example, correlations for 81 environmental exposures in 728 Spanish pregnant women were studied via a principal component analysis. It identified nine strongly correlated exposures (r>0.5) and 26 with a high correlation (r≥0.4). The first principal component included outdoor pollution (air pollution, building density, noise, surface temperature and green spaces). The second involved classes of chemical pollutants (PFASs, PBDEs, phthalates, metals). This study provided a first picture of the structure of the exposome during the *in utero* period (Robinson *et al.,* 2015). Another example involved an unsupervised analysis of a base of around 12,000 environmental, social and health data collected from 1990 to 2010. This study (Juarez, 2014) identified social and environmental predictors of obesity in 3106 US counties with more than 100,000 people (Gittner *et al.,* 2017).

Unsupervised dimensionality reduction methods have also been proposed, such as principal component analysis, factor analysis and non-negative matrix factorisation (Kalia *et al.,* 2020), as have clustering approaches identifying groups of individuals sharing similar characteristics (Santos *et al.*, 2020). An exposome score was constructed using two independent databases to assess the association between exposure to environmental factors and schizophrenia (Pries *et al.,* 2019).

There are three types of statistical analysis methods for studying the association between the exposome and health: (1) single-exposure methods – environment-wide association studies (EWASs) and especially their two-step version ($EWAS_2$), exposome-wide association studies (ExWASs), exposome and metabolome studies (EMWASs), and gene-environment-wide interaction studies (GEWISs), (2) variable selection techniques – Elastic-Net (ENET), Graphical Unit Evolutionary Stochastic Search (GUESS), deletion-substitution-addition (DSA), and penalised regression (LASSO), and (3) dimension reduction techniques – sparse partial least squares (sPLS) regression (Santos *et al.,* 2020). Agier *et al.* (2016) conducted a simulation study showing the limitations of these methods in terms of selecting exposures of interest when exposures are correlated, although GUESS and DSA provide a better balance between sensitivity and specificity than the other methods. The HELIX project across six European birth cohort studies implemented the DSA method to simultaneously study numerous variables of exposure during the prenatal period and in childhood; it also used the ExWAS method to study all of these variables independently (Agier *et al*., 2019; Nieuwenhuijsen *et al.,* 2018; Vrijheid *et al.,* 2020).

When considering scenarios with interactions between exposure factors, and assuming linear effects, it was shown that the Group-Lasso INTERaction-NET (GLINTERNET) and DSA_2 methods are two techniques that can be used (Barrerra-Gomez *et al.,* 2017). Although promising, these

methods still show poor performance when the number of correlated exposures increases and therefore influences the identification of mixtures (Siroux *et al.,* 2016; Patel *et al.,* 2017).

These methods have been implemented in environmental epidemiology in recent years, with EWAS being the most commonly used. The objectives of the studies identified in the literature review are briefly described below:

1. Role of environmental risk factors during the preconception and prenatal periods, within 10 domains (parents' personal characteristics, health, development, education, socio-economic variables, lifestyle, home and social environments, life events and chemical exposure), associated with communication difficulties in nine-year-old children in the ALSPAC study (Steer *et al.,* 2015). In the same study, the influence of transgenerational exposure factors from grandparents' environments and experiences (education, smoking, etc.) was studied in connection with body fat mass in adulthood (Golding *et al.,* 2019). This study underlined the importance of characterising the exposome before conception;
2. Influence of various sources of exposure to metals (air pollution, jewellery, dental crowns, eating habits, smoking) and socio-economic factors on blood levels of metals in 453 Italian adolescents between the ages of 13 and 15 (Pino *et al.,* 2017);
3. Role of environmental contamination due to waste management in urban and suburban environments in the neurological development of 350 children aged three to eight in the HERACLES cohort (Sarigiannis, 2017; Sarigiannis & Karakitsios, 2018);
4. Association between exposure to a mixture of 128 endocrine disruptors measured in urine or serum and seven semen quality endpoints for the male partners of 501 American couples in the LIFE study (Chung *et al.*, 2018);
5. Identification of urinary metabolic signatures associated with exposure to multiple environmental pollutants in 750 pregnant women in the INMA study (Maitre *et al.,* 2018);
6. Study of child exposure (prenatal and childhood periods) by epigenetic analysis based on the methylome (DNA methylation) concept in association with body mass index (BMI) for 1173 children from the HELIX project (Cadiou *et al.,* 2020);
7. Study of the impact of external exposure factors during pregnancy on the risk of hypertension for 819,399 women in Florida; 5784 factors from 10 databases were considered (Hu *et al*., 2020);
8. Link between prenatal exposure to 37 pesticides and 161 metabolites detected in maternal blood and infant birth weight and length of gestation for 102 pregnant women monitored in hospitals in China (Yang *et al.,* 2020).

In France, the National Network for Monitoring and Prevention of Occupational Diseases (RNV3P), created in 2001 with the goal of monitoring occupational exposure-disease associations, analysed observational data with construction of the exposome, combining several exposure factors, and exposure groups. Rieutort *et al.* (2012) illustrated this work with an analysis of data concerning non-Hodgkin's lymphoma (NHL) that addressed multi-exposure and provided new evidence for the hypothesis linking NHL to organic solvents and diluents, agricultural products and ionising radiation, as well as to other exposure groups.

Fox *et al.* (2017) underlined the relevance of epidemiological data for studying non-chemical contaminants. For example, geographic information systems (GISs) can be used to study spatial variations in health indicators and environmental exposure factors, in particular to understand factors associated with health inequalities.

These data are useful for cumulative risk assessment, especially to further explore the independent action approach versus the concentration additivity hypothesis (Fox, 2017; Wishart, 2015).

In conclusion, new statistical methods should still be proposed, and larger datasets based on exposome knowledge should be constructed to help interpret the results and address the complexity and the large number of potential mixtures that may explain phenotypic variability (Siroux *et al.,* 2016; Patel *et al.,* 2017; Kim *et al.,* 2017).

4 Conclusions and recommendations

ANSES develops different reference values that are of use firstly, for assessing health risks and secondly, to enable the public authorities to establish regulatory concentrations of chemical substances that should not be exceeded in order to protect our health. Up to now, it has only proposed values for individual substances and has thus not needed to address the complex exposure of the population. The development of reference values for mixtures will shed light on the possible applications of the various models presented in this report.

In light of this state-of-the-art report, the issue of mixtures remains complex, but it can now be addressed through expert appraisal procedures given the existence of knowledge and simplified models on which there is consensus. With regard to health risk assessment, some examples of regulatory provisions stand out, in particular for exposure via food (pesticide residues and drinking water) and the impact of industrial facilities on the environment and the surrounding area. Recommendations from institutional organisations (US EPA, ATSDR, EFSA, SCHER) underline the importance of implementing these provisions and formalise methodological approaches taking into account knowledge on whether or not various contaminants interact. The most highly recommended hypothesis involves the concept of dose or response additivity. Several studies have tested the dose (or concentration) additivity model for various mixtures and have shown that overall, this model reasonably predicts the toxicity of mixtures of contaminants having similar toxicological properties for a target organ or system, at low doses/concentrations. Exploring the concept of interaction requires models integrating notions of antagonism and synergy to better understand and take into account the mechanistic bases of interactions, as well as exposure to relatively high doses/concentrations. However, at low doses, interactions remain unlikely to generate a very different result due to uncertainties inherent in the risk assessment process itself.

This report is issuing recommendations on setting reference values for a mixture firstly regarding the choice of contaminants and secondly concerning methods to be used to develop reference values for a mixture.

4.1 Choice of substances

The analysis of a mixture presupposes good knowledge of a population's exposure to different chemicals, as well as the identification of classes of substances having similar effects.

The following are recommended:

1. Identifying substances: refer to the most recent national measurement campaigns to find out about exposure in the population;
2. Selecting substances:
    - Use the conceptual diagram of Fournier *et al.* 2014 (see §3.1.4.) to group contaminants based on their effects at various hierarchical levels of living organisms: clinical effects, cellular effects, mechanisms of action;
    - Use the decision tree of Jonker *et al.* 2004 (see §3.1.4.) to choose, if necessary, the most relevant substances to be taken into account;

- Identify the most frequent co-exposure profiles.

4.2 Selection of a construction model

In most cases, environmental exposure to various substances corresponds to low concentration levels and does not involve chemical or metabolic interactions between the mixture's components. In these cases, dose additivity can produce acceptable results with regard to the uncertainty inherent in the risk assessment process, whose model serves as the basis for the proposal of guideline values.

While there are currently models taking interactions into account during risk analyses, these are complex and require knowledge of a number of parameters, which are often not known. It is therefore difficult to implement them on a widespread basis.

Therefore, the following are recommended:

1. Establish toxicological profiles incorporating data on potential interactions for the most frequent co-exposures;
2. Use the additivity hypothesis, if the data collected in the profiles do not call it into question (this should thus be the default approach):
    - using a simplified additivity approach (such as the HI approach) for substances whose mechanism is not sufficiently known, as proposed in the conceptual diagram of Meek *et al.* (2011) (Figure 2). It would be interesting to supplement this approach by identifying the contaminant(s) determining the risk. This helps significantly limit the risk associated with exposure to mixtures by focusing on this or these few determinant substances.
    - using a dose additivity approach as already developed for dioxins (RPF/TEF approach), for all substances having common mechanisms or cellular consequences, for example for certain pesticides/certain classes of congeners (PBDEs, PFCs), and for substances having anti-androgenic action (NRC recommendation even if no common mechanism);

Furthermore, it will be necessary to conduct a complete review of toxic equivalency factors (TEFs) known generically as relative potency factors (RPFs), already available in the literature. A critical analysis by the agency would A allow for institutional recognition, facilitate the development of reference values.

This work led to a second phase of analysis consisting of applying these recommendations to the development of indoor air guideline value for a mixture of aldehydes extended to other irritant substances present in indoor air.

Date of validation of the collective expert appraisal report *by the two Expert Committees*: 8/10/20 and 22/10/2020

[The paper version signed by the Chairs of the WG and CES shall be kept in the archive file for the formal request]

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ANNEXES

Annex 1: Regulatory applications of cumulative risk assessment

- **Annex 1.1 Plant protection products**

The regulations on the marketing of plant protection products (Regulation (EC) No 1107/2009) and on maximum residue levels of pesticides (Regulation (EC) No 396/2005) specify that the cumulative and/or synergistic effects of pesticides shall be taken into account when assessing food risks, when methods allow it.

Since then, the modelling of cumulative risk assessments for consumers has become one of the priorities of the European Food Safety Authority (EFSA), the European Commission and several EU Member States. The development of these methods has progressed and is moving toward the grouping of substances having effects on the same organs and/or sharing mechanisms of action; this involves identifying groups of plant protection active substances showing effects on the same organs and/or having common mechanisms of action.

In 2009, a pilot project was initiated by EFSA for a group of pesticides in the triazole class to evaluate various methodologies for assessing the cumulative effects of these pesticides via food. This exercise helped refine the hazard characterisation, exposure evaluation and risk characterisation steps of the tiered approach to cumulative risk assessment (EFSA, 2009). Since then, several EFSA Opinions have been published specifying the methodology to be applied.

In 2013, EFSA developed a pesticide grouping approach that paved the way for the implementation of cumulative risk assessments (EFSA, 2013a); it was described above in Section 3.2.2. The general methodology used to classify pesticides into cumulative assessment groups (CAGs) relies on the identification of compounds having similar toxicological properties for a specific organ or system. Initially, EFSA's Scientific Panel on Plant protection products and their residues (PPR) applied this methodology to define groups of toxic pesticides for the thyroid and central nervous system.

One of EFSA's Opinions specifically deals with dissimilar modes of action for pesticides that produce a common effect on the same target organ (EFSA, 2013b). In the absence of cumulative risk assessment methods for independent action, EFSA recommends dose additivity as a pragmatic and conservative approach supporting the common effect approach (Fox, 2017).

In parallel, the European Acropolis project led to the development of a software program for assessing cumulative exposure to a group of pesticides. This software addresses most of the constraints identified by EFSA.

- **Annex 1.1a: Biocidal products**

The Biocides Regulation clearly states that all active substances and substances “of concern” should be taken into account when assessing risks for a product.

To that end, a risk assessment should be undertaken to determine the acceptability or unacceptability of all of the identified risks. This assessment should focus on the risks associated with the various relevant components of the biocidal product and should duly take all cumulative and synergistic effects into account.

- **Annex 1.2: Drinking water**

In the DW regulations introduced in Section 2.1, the classes of pollutants considered are PAHs, trichloroethylene and tetrachloroethylene, pesticides and total trihalomethanes.

Health risks associated with the presence of micro-organisms or chemicals in water resources and DW are assessed by ANSES when the maximum levels are exceeded. The work conducted by ANSES involves determining, for certain physico-chemical parameters, a concentration in water that is above the regulatory value and would not pose any risks to the health of a person consuming this water over a limited period of time. The general approach is based on the collection and analysis of toxicological and exposure data for the population, in order to issue recommendations for establishing management thresholds in the event that the limits are exceeded (AFSSA, 2007).

Several mixtures have been considered in these assessments. Generally speaking, as recommended by the WHO in its guidelines, for substances having similar mechanisms or modes of action, it is appropriate to consider the effects as additive. For the example of organic compounds, ANSES's work has used the following approaches:

- For PAHs, the toxic equivalency approach was adopted, considering a mixture of 15 PAHs covered by Standard NF EN 17993 for the measurement of PAHs in water, with the use of toxic equivalency factors (TEFs). The PAHs most frequently detected were fluoranthene, phenanthrene and fluorene, which are not the most toxic (AFSSA, 2007).
- For trichloroethylene and tetrachloroethylene, the risk assessment was conducted for each single compound and for the mixture with, initially, the addition of hazard quotients representing a conservative approach equal to the limit value but not based on experimental data, which are scarce (AFSSA, 2007; ANSES, 2016a).
- For pesticides, the assessment was based on maximum health values (VMAX) determined by ANSES for active substances and metabolites to assess the associated health risks (ANSES, 2019). When different pesticides and metabolites were simultaneously present, the risk assessment considered an additive effect (AFSSA, 2007).
- For trihalomethanes, the assessment focused on the NTP's toxicological data and on epidemiological data on associations between excess risk of bladder cancer in humans and exposure to THM-contaminated water from 50 $\mu g \cdot L^{-1}$. It underlined the need for further studies, in particular on mechanisms of action (AFSSA, 2010).

ANSES also assesses risks associated with drug residues in DW using a proposed general methodology (ANSES, 2013). It takes into account metabolites formed in humans or animals as well as transformation products formed in the environment. When applying this methodology to carbamazepine used in human medicine, it considered that the principal metabolite, 10,11-epoxycarbamazepine, has the same pharmacological activity; therefore, the TRV was determined for the sum of the two substances (parent + metabolite).

- **Annex 1.3: Food**

In the food regulations introduced in Section 2.2, the classes of pollutants considered are mainly chemical classes with examples of reference doses given for parabens, organotins and dioxins.

The Total Diet Studies (TDSs) launched in France since 2000 have quantified the dietary exposure of populations to substances of interest in terms of public health by estimating the composition/contamination of foods “as consumed”. The first two studies focused on the French population over the age of three years and the most recent study specifically targeted children under the age of three years old.

Health risks associated with dietary exposure are assessed by ANSES as part of these studies. Several mixtures have been considered in these assessments (ANSES, 2016b). For the example of organic compounds, ANSES's work has used the following approaches:

- Dioxins and furans (PCDD/Fs): grouping numerous congeners, the toxic equivalency approach was adopted considering the reference dose of 0.7 pg $TEQ_{WHO} \cdot kg^{-1} \cdot d^{-1}$ from the US EPA's reassessment of 2012. Only this class was taken into account.
- Polychlorinated biphenyls (PCBs): of the different congeners, 12 are considered as dioxin-like (DL) from a toxicological viewpoint due to binding to the Ah cellular receptor. Risks for all PCBs were assessed with six "indicator" congeners – PCB-28, 52, 101, 138, 153 and 180 – with a reference dose of 10 $ng \cdot kg^{-1} \cdot d^{-1}$ → the "PCDD/F and DL-PCB" mixture was covered by recommendations underlining the uncertainties associated with this approach. It should not be limited only to PCDD/Fs and DL-PCBs due to the non-negligible existence of other food contaminants having a DL effect.
- PBDEs: seven main congeners (BDE-28, -47, -99, -100, -153, -154 and -183) were considered in mixtures and compared with NDL-PCBs with a threshold of 10 $ng \cdot kg^{-1} \cdot d^{-1}$; BDE-209 was also considered on its own.
- HBCDDs: a mixture of three stereoisomers was assessed by applying the margin of exposure approach compared with the reference dose of 3000 $ng \cdot kg^{-1} \cdot d^{-1}$ for the sum of exposure doses.
- PBBs: there are also numerous congeners, with limited toxicological data. A reference dose of 0.15 $mg \cdot kg^{-1} \cdot d^{-1}$ was applied to the sum of exposure for the three analysed congeners (PBB-52, 101 and 153).
- PAHs: this is a class of several hundred compounds, of which the toxicity of only a small number is known. The risk assessment was undertaken using two approaches overlapping with the previous examples:
    - PAH4: Application of a reference dose of 0.34 $mg \cdot kg^{-1} \cdot d^{-1}$ to the sum of four PAH markers of exposure and effect for PAHs in food: benzo(a)anthracene (BaA), benzo[a]pyrene (B[a]P), benzo[b]fluoranthene (BbF) and chrysene (CHR)
    - PAH11: Use of toxic equivalency factors (TEFs) based on the relative carcinogenic potential of the 11 most toxic PAHs most representative of food contamination: PAH4 + benzo[g,h,i]perylene (BghiP), benzo[k]fluoranthene (BkF), dibenzo[a,h]anthracene (DBahA), indeno[1,2,3-cd]pyrene (IP), anthracene (AN), benzo[j]fluoranthene (BjF) and fluoranthene (FA). This approach is based on the calculation of a reference dose of 5 ng $TEQ \cdot kg^{-1} \cdot d^{-1}$
- Natural steroids: Four steroids were analysed as part of this study. A risk assessment could not be conducted due to the lack of toxicological benchmarks. Nevertheless, the need to implement a mixture/activity approach based on biological measurements (e.g. receptor assays) was underlined.

- **Annex 1.4: Classified facilities for environmental protection (ICPEs)**

The regulations define the content of the impact studies required for industrial facilities subject to authorisation (Articles R.122-5 and R.512-8 of the French Environmental Code). The consequences of the classified facilities plan for the health of populations are assessed in particular.

Since 2000, a health effect analysis has been developed as part of impact studies using the health risk assessment (HRA) methodology, based on guides produced by *Santé publique France* (SPF, formerly InVS) and the National Institute for Industrial Environment and Risks (INERIS).

In 2013, this approach evolved and focused on two tools: HRA and IEM (interpretation of environmental media). The Circular of 9 August 2013 described this new methodology and was accompanied by a new guide, proposed by INERIS.

For cases of simultaneous exposure to several toxic substances, the INERIS guide presents the general rule which consists in adding together the hazard quotients of the substances producing the same effect on the same organ via the same biological mechanism. It describes the addition of HQs for which the effects associated with the TRVs involve the same target organs.

By simplification, it also mentions adding together all of the HQs, for information, if the sum remains less than 1 (justifying that there is no risk of concern).

For no-threshold effects, the rule is to add up all the individual excess risk to calculate an excess risk for all no-threshold effects combined.

- **Annex 1.5: Polluted sites and soils**

There is no specific legal framework for polluted sites and soils. However, their management is mainly based on the ICPE legislation described above, especially on the provisions of the French Environmental Code on preventing pollution, risks and nuisances.

A national methodology for managing polluted sites and soils was developed 10 years ago and then updated in 2017[16]. It considers the use of environments and undertakes to define means of eliminating pollution on a case-by-case basis, in light of the available techniques and their economic costs. The maintenance of residual pollution on a site is related to its compatibility with the selected use (industrial, residential, etc.) and, if necessary, is associated with conditions for controlling the health or environmental impact.

Quantitative health risk assessments are called “residual risk analyses” (RRAs) as they are conducted as part of the validation of management measures aiming to control pollution or eliminate sources or vectors of pollution.

The additivity of risks associated with various pollutants and/or various exposure routes is considered. For threshold effects, this leads to the addition of hazard quotients only for substances having the same toxic mechanism of action on the same target organ; for no-threshold effects, all excess cancer risks are added together. Other environmental contributions are not taken into account.

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1 Annex 2 : Examples of the use of the « *hazard index* » (HI) approach

Reference	Country, context	Contaminants (n)	Target organ or endpoints	TRV used	Results (HI> ou <1)
Pierson *et al.* (1991)	USA, health complaints from employees in a renovated premise	COV (n=9) but then limited to substances with a TRV based on a common target organ : n=3 : cumene, toluene, xylene	Central nervous system	RfC, US EPA	exceeding threshold
Evans *et al.* (2014)	USA (San Francisco), individual measure on 851 persons (NHANES)	COV (n=10) : benzene, toluene, ethylbenzene, *m,p*-xylene, *o*-xylene, 1,4-dichlorobenzene, chloroforme, trichloroethylene, methyl-*tert*-butylether + le bruit	Hearing	RfC, US EPA + limit valueWHO for noise ((HQ for noise = sound intensity divided by the limit value WHO of 70 dB)	Exceeding threshold. Noise Bruit = main contributor
Mishra *et al.* (2015)	Australia, measurement campaign of COV in 23 classeroom at Brisbane	COV (n=49)	All types	CLI, AgBB and Anses	Exceeding the threshold for 2 classeroom on 23. Phenol main contributor (highlighted by MCR)
Nie *et al.* (2018)	China, measurement performed on a compostage facility for solid waste	COV (n=44)	All types	RfC, US EPA IUR	HI < 1 Total risk > 10^{-4}
Pack *et al.* (2018)	South Korea, cigarette smoke of the main 5 sold trademarks	volatiles compounds, non volatiles andt semi-volatiles (n=38)	All types	RfC, US EPA IUR (inhalation unit risk value)	HI between 367 and 1225
Pelletier *et al.* (2019)	France, comtanimation data in indoor environment	COSV (n=32)	Neurotoxicity, reprotoxicity and génotoxicity	RfD, US EPA	HI > 1 for 95 % of children exposed to a mixture of 11 reprotoxic compounds.
Benson *et al.* (2009)	USA, ubiquitous detection of 6 phthalates (data from NHANES)	Phthalates (n=6)	Reproduction	DJT, EFSA ; DNEL, Danish EPA ; ad hoc for DEHP	Exceeding threshold in function of age group or of the exposure value used (median, P95)
Christenseen *et al.* (2014)	USA, general population , urinary measurementHANES (same as Benson 2009)	Phthalates (n=5) : DEHP, DnBP, DiBP, DiNP, BBP	Reproduction	DJT, EFSA ; DNEL, Danish EPA ; ad hoc for DEHP	Exceeding threshold in function of age group or or of the exposure value used (median, P95). DEHP and DBP = main contributors
Koch *et al.* (2011)	Germany, urinary measurements of phthalates schoolchild	Phthalates (n=3) : DEHP, DnBP, DiBP	Reproduction	DJT, EFSA + ad hoc for DiBP	Exceeding threshold for for 28 childson 108
Pan *et al.* (2011)	China, workers exposed to phthalates	Phthalates (n=2) : DEHP, DnBP	Reproduction	RfD, US EPA	Exceeding threshold for 90 % of exposed workers and 2 % of non-exposed
Soeborg *et al.* (2012)	Denmark, urinary measurement on 129 childs et adolescent	Phthalates (n=5) : DiBP, DnBP, DEHP, BBP, DiNP	Reproduction	TDI, EFSA ; RfD-AA (Reference	Exceeding threshold for the percentile 95

Reference	Country, context	Contaminants (n)	Target organ or endpoints	TRV used	Results (HI> ou <1)
				Doses for Anti-Androgenicity), Kortenkamp	
Chang *et al.* (2014)	Taiwan, following food contamination event with DEHP	Phthalates (n=7) : BBP, DEP, DEHP, DiBP, DiDP, DiNP, DnBP	Liver, reproduction	DJT, EFSA ; RfD, US EPA ; TDI, OMS	Exceeding threshold in function of effectsand age group for the high percentiles (95 and 99%)
Dewalque *et al.* (2015)	Belgium, urinary measurements in 138 womenand 123 men in Liege area	Phthalates (n=5) : DEP, DnBP, DiBP, BBP, DEHP	Reproduction	DJT, EFSA ; RfDAA, Kortenkamp	Exceeding threshold at the percentile 95 of the exposition
Kranich *et al.* (2014)	Denmark, urinary measurements in 33 young men	Phthalates (n=5) : DnBP, DiBP, DEHP, BBP, DiNP	Reproduction	DJT, EFSA ; RfDAA, Kortenkamp	HI > 1 forr 2men
Gao *et al.* (2016)	China, mesures urinaires chez urinary measurements in 108 young men	Phthalates (n=3) : DnBP, DiBP, DEHP	Reproduction	DJT, EFSA ; RfDAA, Kortenkamp	Exceeding threshold for high
Reyes & Price (2018)	USA, biomonitoring og general population (NHANES)	Phthalates (n=6 + métabolites)	Not precised	TDI, Efsa.	HI > 1 for 0,8% of the general population.
Ashworth *et al.* (2018)	New-Zealand (Toys contamination)	Phthalates	Developmental toxicity (DIBP, DBP, BBP, DEHP) Hepatotoxicity (DNOP, DINP, DIDP)	TDI, Efsa	For developmental effects : cumulative exposure with phthalates shows an HI>1. For hepatotoxicity : cumulative exposure shows an HI<1.
Appel et al., 2020	Germany, human data from biomonitoring to 1988 until 2015	Phthalates : DBP, DIBP, BBP, DEHP Et DINP	Antiandrogenic effects	RfD from Kortenkamp and Koch (2010, 2020) TDI, Efsa	HI>1 between 1988andt 1996 HI<1 between 1997 and 2015
Borg *et al.* (2013)	Sweden, occurrence of PFAS as environmental contaminant everywhere	c perfluorocarboxylic et perfluorosulfonic acids (n=17)	Liver, reproduction	*Ad hoc*	HI<1
Jensen *et al.* (2015)	Denark, surveillance program for food	Pesticides (n=157)	All types (not grouping)	DJA, CE ; ADI, JMPR, ad hoc. Exclusion of 10 pesticides without ADI	HI < 1. Use of Danish products allow to divide HI by 2. Nine main contributors on the HI (including diazinon, omethoate, methyl-pyrimiphos)
Nascimento *et al.* (2015)	Brasil, composition data in PM2.5	Pesticides (n=12)	Grouping by mode of action Non cancer effects	AOEL, Efsa	HI <1

Reference	Country, context	Contaminants (n)	Target organ or endpoints	TRV used	Results (HI> ou <1)
Li *et al.* (2016)	China, contamination data from fruits and pesticides	Pyrethrinoides	Short and long term toxicity	ADI, ARfD	The cumulative risks to children were greater than the general population. The HIs of seven pyrethroids were all less than 1, even when consuming four fruits at the same time based on the average daily consumption for both the general population and children over time. HIs for cypermethrin, l-cyhalothrin and bifenthrin for the general population exceeds 1 for the short term.
Iturburu *et al.* (2019)	Argentina, contamination of ecosystems in the province of La Pampa	Pesticides (n=44)	Ecotoxicological effects	PNEC	Very high level of risk (HI>10) for 22 sites, high level (HI>1) for 5 sites
Zng *et al.* (2018)	China, contamination of surface water in the Qungshitan reservoir	Pesticides organo-chlorides	Ecotoxicological effects	PNEC	HI>1 in almost all situations of mixtures
Taghizadeh *et al.* (2019)	Iran, contamination of pistachios	pesticides residues (n=18)	6 groups according to toxicity: Neurological effects Developmental and reproductive effects Systemic effects Hematological effects Thyroid effects	ADI (OPenFoodTox), EFSA	Contribution of the consumption of pistachio low / risk link to food. HI> 1 for 5 groups, the highest being for neurological effects
Roden *et al.* (2014)	USA, New Jersey. Measurement campaign of 18 pharmaceutical residues in surface water (30 locations)	Drugs (n=1 à 11 depending to location)	potentially, all types (POD not precised in the article)	*Ad hoc* (classical method POD/UF)	HI < 1
Pérez-Vázquez *et al.* (2015)	Mexico, contamination of soils at San Luis Potosi	Metals (n=4)	Non cancer effects	RfD, US EPA	HI >1 for high exposure (P90 and maximum) in the 4 studied area

Reference	Country, context	Contaminants (n)	Target organ or endpoints	TRV used	Results (HI> ou <1)
Minigalieva *et al.* (2017)	Russia	Binary mixtures and ternary mixtures of metals (n=6)	Organs histology and blood dosages	TLVs, ACGIH	HI<1ou >1 (Classes A eand B,) HI=1 (Class C).
Omrane *et al.* (2018)	Tunisia, 2nd largest city and economic capital with many industrial activities	Heavy metals (n=6)	Alltypes (not grouping)	VTR VLEP (MIXIE tool)	Not calculated HI
Martin *et al.* (2017)	Europe, contamination data in food (EFSA) and dust (various studies in housing) + body burden estimated from biomonitoring (several studies)	PBDE (n=8 à 16 by age groups and sources)	Neurotoxicity and neurological development	*Ad hoc* for 4 PBDE : BDE-47, 99, 153, 209 (classical methodPOD/UF) and « read-accross » approach (use of the TRV of the closest congener)	HI > 1 in breastfeedin g children, young children(→ 3 ans) and adults with high fish consumption
Syberg *et al.* (2017)	Sweden, impacts on coastal waters	PCB, HAP, PBDE	Global effects	authorized limit concentration [17]	Sum of HQ divided by the authorized limit concentration
Genisoglu *et al.* (2019)	Turkey, contamination of drinking water (tap water and bottled in 100 housings	THM (n=4)	Cancer effects	RfD, US EPA Slope factor (SF)	By inhalation and ingestion, risk between 10-8 and 10 4: highest during inhalation showers and drinking water by ingestion
Riva *et al.* (2019)	Italia, contamination of surface water in the Milan basin	Emerging pollutants as markers of anthropogenic activities (n=47)	Ecotoxicological effects	PNEC, European Union	HI>1 in almost all situations of the mixture

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[17] Les quantités de contaminants présents dans les poissons et autres fruits de mer destinés à la consommation humaine ne dépassent pas les seuils fixés par la législation communautaire ou autres normes applicables (Directive 2008/56/CE)

Annex 3 : Examples of point of departure index (PODI) approach

Reference	Country or design	Substances (number)	Health effect studied	POD	Main results
Fox *et al.* (2004)	USA	Air pollutants (n=41)	Several health effect categories : Body weight; Dermal/ocular irritation; Developmental; Endocrine ; Exocrine; Gastro-intestinal/hepatic ; Heart/vascular; Hematological; Immunological; Mortality; Musculo-skeletal; Neurological; Pancreatic; Renal/kidney, Reproductive ; Respiratory ; Splenic	NOAEC, BMC, LOAEC from multiple effect database METDB : 290 critical doses identified. Twelve health effect categories d'effets from METDB database whom 10 from IRIS database (RfC)	Cumulative risk for respiratory and neurological effects and also gastro-intestinal/hepatic, renal, and immunologic effects
Christiansen *et al.* (2012)	CONTAMED (EU funding– 7th FP and Danish EPA)	13 chemicals : phthalates (DBP, DEHP), pesticides (vinclozolin, prochloraz, procymidone, linuron, epoxiconazole, p,p'-DDE), UV-filters (OMC, 4-MBC), bisphenol A, parabens (BP) and the drug paracetamol	Endocrine disrupting effects-action of androgens and oestrogens male sexual differentiation in rats	NOAEL/LOAEL – Anogenital distance and nipple retention Rats Wistar (56 young adults) at GD3 oral gavage adminstration (GD7 to GD 21 PND1-22)	PODI < 1
Vejdovszky *et al.* (2019)	Austria, food contamination	metals and metalloids mycotoxins - aflatoxins, organic and inorganic compounds (n=12)	Nephrotoxicity and neurotoxicity (EFSA's CAG)	BMDL NOAEL LOEL/NOEL	mRPI>1 Cumulative risk from food contaminant mixtures for the Austrian population : Nephrotoxicity in all scenarios. Neurotoxicity in all scenarios for children and the scenarios of high exposure of adults
Sprong et al., 2020	EU	144 pesticides (PPRs), 49 persistent organic pollutants (POPs), 7 food additives (FAs)	Liver steatosis (EFSA's CAG)	NOAEL/LOAEL	MOE + main contributors for different scenarios
Crépet et al. submit	France	32 substances grouping according to exposure data : 3 mixtures identified	Neurological and thyroid effects	TRV, LOAEL, NOAEL	mRPI>1 Cumulative risk for thyroid effect 3 times higher than risk for neurological effects

Reference	Country or design	Substances (number)	Health effect studied	POD	Main results
					4 main chemical contributors for the two effects

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Annex 4 : Examples of toxic equivalency factors (TEF) or relative potency factors (RPF) approaches

4.1. Calculation of TEF

Reference	Substances [parameter]	Biochemical / health effect	POD	POD data	Main results
Eadon *et al.* (1986)	Dioxins and furans, 13 PCDD-PCDF / 2,3,7,8 TCDD [TEF]	Ah-receptor binding / mortality	LD_{50}	Short term bioassay in guinea pig	/
Nisbet *et al.* (1992)	17 PAH [TEF]	Ah-receptor binding / cancer (different types)	Dose-response relationship from mathematical model – two-stage low-dose-linear case	In vivo and in vitro assays TEF's evaluation and comparison with US EPA's TEF[18] based on primary literature	/
Ahlborg *et al.* (1994)	13 PCB [REP/TEF]	Ah-receptor binding / Different effects	Dose-response relationship using linear interpolation of log-doses ED_{50}, LD_{50}, ED_{25}, ED_{12} NOEL, LOEL, Kd	*in vivo*, structure-activity consideration and in vitro studies (activation AhR, induction de CYP1A1) Review of literature with data compilation	/
van den Berg *et al.* (1998)	7 PCDD, 10 PCDF, 12 PCB [WHO_{98} TEF]	Ah-receptor binding / Different effects	Dose-response relationship using linear interpolation of log-doses ED_{50}, LD_{50}, Kd	Reevaluation of TEF based on new scientific data or existing data [REP_{1997}] Subchronic studies with mink	/
Haws *et al.* (2006)	6 PCDD, 10 PCDF, 12 PCB [REP_{2004}]	Ah-receptor binding / Different effects	EC_{50}, LD_{50}, Kd	Re-examine and update the REP from experimental studies compiled in database built upon the database from Van den Berg 1998	/

[18] Chu & Chen (1984) EVALUATION AND ESTIMATION OF POTENTIAL CARCINOGENIC RISKS OF POLYNUCLEAR AROMATIC HYDROCARBONS (PAH). U.S. Environmental Protection Agency, Washington, D.C., EPA/600/D-89/049 (NTIS PB89221329).
Clement (1988) COMPARATIVE POTENCY APPROACH FOR ESTIMATING THE CANCER RISK ASSOCIATED WITH EXPOSURE TO MIXTURES OF POLYCYCLIC AROMATIC HYDROCARBONS. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/R-95/108.

Borgert *et al.* (2003)	Several substances hormonally active agents in the environment [RPF]	estrogenic action : l ER-α and ER-β.	LOEC, IC_{50}, EC_{20}, EC_{50} (ER-α/β)	Review of relative potency - estrogen equivalence	/
Castorina *et al.* (2003)	11 organophosphorus pesticides [RPF]	brain cholinesterase inhibition/ neurotoxicity	Oral BMD_{10}	US EPA data	/
van den Berg *et al.* (2006)	7 PCDD, 10 PCDF, 12 PCB [WHO_{2005}TEF]	Ah-receptor binding / Different effects	Different types of dose-response studies *(in vivo, in vitro,* chronic, acute etc.)	TEF Re-evaluation based on database published by Haws (2006) with weighting /selection criteria	/
Audebert *et al.* (2012)	13 PAH [TEF named Genotoxic equivalent factor (GEF)]	Genotoxicity (assay γH2AX)	Hill model, EC_{50}	*in vitro* (HepG2, LS-174T)	/
Fournier *et al.* (2016)	6 SVOC (BBP, BPA, B[a]P, DEP, DEHP, cypermethrine) [RPF]	Steroidogenesis enzymes. Inhibition / decrease of serum testosterone concentration	Hill models, BMD_i	toxicological studies - oral-route exposure of adult male rodents.	/
Liu *et al.* (2019)	HAP	Genotoxicity (p53), aryl hydrocarbon receptor, oxidative stress (NF-κB)	AC_{50}	ToxCast chemical library *in vitro*	/

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4.2 Application of TEF method

Reference	Country	Substances [parameter]	Biochemical / health effect	POD	POD data	Main results
Boon *et al.* (2008)	The Netherlands	25 organophosphorus insecticides, 8 carbamates [RPF]	AChE Inhibition / neurotoxicity	$NOAEL_{acute}$ /BMD10 acephate as index compound for the OPs and oxamyl for the carbamates	JMPR /EPA dataset data on rat or dog and human studies	Cumulative exposure - P99.9 health risk for OP for young children only
Lemieux *et al.* (2008)	Canada, Sweden, contaminated soil	24 PAH [RPF]	Genotoxicity (Ames test)	oral slope factor for benzo[a]pyrene (BaP) as index compound	*In vitro* (*S. typhimurium*)	Mutagenic hazard and risk with 2 methods assuming additivity hypothesis
Jensen *et al.* (2013)	Denmark	4 pesticides (epoxiconazole, prochloraz, procymidone and tebuconazole) [RPF]	Reprotoxicity (EDC)	BMD Relative toxicity of prochloraz	Rats	Cumulative exposure The results show that there is no reason for concern in relation to cumulative acute risk for Danish consumers to the four endocrine disrupting pesticides.
Kalantari *et al.* (2013)	Sweden, Italy	6 PCB	AhR binding / Decrease in liver retinoïds	BMDL, ED_{50}, NOEL	Rats	Swedish cumulative exposure for women and men. The percentiles 0.1 of estimated cumulative margin of exposure (MOE) for a group of five PCBs is 20 for women compared to 69 for men.
Payne Sturges *et al.* (2009)	USA	Organo-phosphorus pesticides	inhibition of cholinesterases enzymes/ neurotoxic effect	BMD_{10} Relative toxicity of chlorpyrifos	Female Rats	Risk for national population and for different american county for different ages. A higher percentage of children (6-11 years old) for the Monterey County pesticide (62%, ≤ MOEs
Chou *et al.* (2017)	USA	Ambient Particulate matter and PAH	Response mediated by AhR, Nrf2 and p53	EC_{50}	*In vitro* (HTS tox 21 program)	AhR pathways is the most sensitive activated by PAH compared to Nrf2 and p53. Children population is the most sensitive to the risk linked to AhR activation compared to adults.

Reference	Country	Substances [parameter]	Biochemical / health effect	POD	POD data	Main results
Pelletier *et al.* (2019)	France	PAH PCB-DL Phthalates some SVOC	gastro-intestinal tract cancer Binding with AhR anti-androgenic activity Reprotoxic effect with reduction of testosterone and neurons alterations	RPF or TEF		Reprotoxic risk is associated with a decrease of testosterone in children and adults mostly exposed to a mixtures of B[a]P, DEHP, DEP, BBP ; Some risk are expected in highly exposed children to mixtures of PCB-105, PCB-118 due to AhR binding Immunotoxic risk for children exposed to mixture of Chlorpyrifos, P[a]P, DEHP, PCB-52, PCB-153, dieldrin, lindane, BDE 47, BDE 99
Genisoglu *et al.* (2019)	Turkey	Trihalomethanes THM (n=4)	Carcinogenic effects	RPF converted to Index chemical equivalent dose (ICED) Maximum likelihood estimate (MLE) of cancer slope factor of the index chemical (BDCM)		For ingestion and inhalation cumulative risk levels between 10^{-9} and 10^{-5}, while all 95th percentile values were below 10−5, which therefore can be considered as acceptable Lower cumulative risk levels than the method using HI
Mitra *et al.* (2019)	India	PAH	Carcinogenic effects	TEF (Tian *et al*., 2013)		Environmental risk assessments
Dong et al., 2019	Worldwide	355 organic and inorganic chemicals in indoor dust	endocrine-related activity : aryl hydrocarbon receptor (AhR), androgen receptor (AR), estrogen receptor alpha (ERα), nuclear factor of kappa light polypeptide gene enhancer in B cells (NFκB1), and peroxisome proliferator-activated receptor gamma (PPARγ)	AC_{50}	Toxcast database	The result showed that organic pollutants such as phthalates (e.g., DEHP and DINP), plasticizers (e.g.,BADGE and TOCP), flame retardants (e.g., TBOEP), organotins (DBTC), and phenols (e.g., nitro-phenols) significantly contributed to the bioassays with endocrine disruption.

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Notes