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Progress report on the assessment and management of combined exposures to multiple chemicals (chemical mixtures) and associated risks

Accompanying the document

COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS

Chemicals Strategy for Sustainability
Towards a Toxic-Free Environment

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1. INTRODUCTION

Humans as well as other organisms are exposed to a great variety of chemicals occurring in combinations also referred to as 'mixtures'. Scientific evidence of the strengthened toxicity of such mixtures is mounting.[1] Real-life examples of such mixtures are the different chemicals appearing simultaneously or sequentially in e.g. human blood or in a water body. The total risk related to the exposure to a combination of chemicals typically exceeds the risk related to the exposure to each of the individual chemicals in the mixture on their own, at their respective concentration in the mixture[2]. Therefore, exposure to a mixture can give rise to adverse health and environmental effects, even at levels of exposure which are considered 'safe' for the individual chemicals on their own.

Science demonstrates that in the majority of the cases, the toxicity of the mixture can be assessed by adding up the toxicities of the individual chemicals in the mixture. This is called the 'concentration addition model'. In relatively rare cases, so called 'interactions' are observed, where the toxicity of the mixture is stronger or weaker than what would be expected from applying concentration addition[3].

A distinction can be made between intentional and unintentional mixtures. Intentional mixtures are intentionally manufactured and often have a known composition and are contained in one product. Typical examples are products like paint, glue and detergents. Unintentional mixtures arise spontaneously or coincidentally, for example in air, water, soil, as well as in humans and other organisms as a result of co-exposure. They arise whenever chemicals are used on their own, as components in different intentional mixtures or in objects (*i.e.* complex products). Hence, an unintentional mixture typically includes chemicals from a variety of sources. Their compositions are usually more or less unknown and may vary over time and in space. Due to the very large number of possible combinations of chemicals, the risk assessment and management of unintentional mixtures (sometimes also called 'coincidental mixtures' or 'combination of chemicals') represents a particular scientific and regulatory challenge.

Regarding EU policy, already the White Paper for a Future Chemicals Policy[4] from 2001 highlighted exposure to mixtures of chemicals as a research priority to cover knowledge gaps and achieve the goals set out. In 2009, the Environment Council adopted Conclusions[5] on the 'combination effects of known chemicals', which recognised the difficulties and deficiencies surrounding the regulation of mixtures. It also invited the Commission 'to assess how and whether relevant existing Community legislation adequately addresses risks from exposure to multiple chemicals from different sources and pathways, and on this basis to consider appropriate modifications, guidelines and assessment methods, and report back to the Council by early 2012 at the latest'.

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[1] Kortenkamp, A. and Faust M. (2018) 'Regulate to reduce chemical mixture risk - regulatory systems must better provide for risks from exposure to multiple chemicals', Science: 361 (6399):224-225; July 2018; DOI: 10.1126/science.aat9219, with supplemental material. https://science.sciencemag.org/content/361/6399/224.full

[2] Kortenkamp, A., Backhaus, T., Faust, M., 'State of the Art Report on Mixture Toxicity, Final Report, Executive Summary' 22 December 2009, European Commission Study Contract Number 070307/2007/485103/ETU/D.1. http://ec.europa.eu/environment/chemicals/effects/pdf/report_mixture_toxicity.pdf

[3] Interactions, involving either stronger (synergism) or lower (antagonism) effects, appear when the chemicals involved interact on the molecular level. This is relatively rare and its effects relatively small, largely confined to mixture with only a few components.

[4] Commission of the European Communities 'White Paper. Strategy for a future Chemicals Policy' COM(2001) 88 final. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52001DC0088&from=EN

[5] Council conclusions on combination effects of chemicals', 2988th Environment Council meeting Brussels, 22 December 2009. https://www.consilium.europa.eu/uedocs/cms_data/docs/pressdata/en/envir/112043.pdf

The Commission responded to the Council through a Communication in 2012[6], drawing upon the joint opinion of three EU Scientific Committees[7] and taking into account the comprehensive State of the Art Report on Mixture Toxicity[8]. In the Communication, the Commission reviewed the scientific knowledge and the regulatory requirements regarding the assessment of mixtures and committed itself to several actions and to presenting a report reviewing the progress and experience associated with the actions in the Communication.

The EU 7th Environment Action Program[9] stressed the need for appropriate regulatory approaches to address combination effects of chemicals. Recent resolutions of the European Parliament[10, 11, 12, 13], the Conclusions of the Environment Council on the chemicals policy adopted in June 2019[14], and the Commission Communication on endocrine disruptors adopted in November 2018[15], all confirmed the need to better take into account the combined effects of different chemicals for the protection of human health and the environment. Further, the Commission identified the combination effects of chemicals as a target action to be addressed in a Chemicals Strategy for Sustainability as part of the European Green Deal[16].

The purpose of the present report is to describe the progress made since 2012 on the assessment of mixtures (combinations of chemicals) – both intentional and unintentional – to deliver on the follow-up action announced in the Communication and to provide background information and evidence base to actions announced in the Chemicals Strategy for Sustainability. This report presents the relevant legal requirements, guidance documents, methodologies and knowledge base as well as the remaining challenges. It also provides an overview of suggestions made by Member States, scientists and other stakeholders on possible ways forward.

Box 1. Terminology[17, 18, 19, 20, 21]

[6] Communication from the Commission to the Council, 'The combination effects of chemicals, Chemical mixtures' COM(2012) 252 final. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52012DC0252&from=EN

[7] Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENHIR) and Scientific Committee on Consumer Safety (SCCS). 'Joint Opinion on the Toxicity and Assessment of Chemical Mixtures adopted on 14th December 2011'. https://doi.org/10.2772/21444

[8] Kortenkamp, A., Backhaus, T., Faust, M 'State of the Art Report on Mixture Toxicity', Final Report, Executive Summary, 22 December 2009. http://ec.europa.eu/environment/chemicals/effects/pdf/report_mixture_toxicity.pdf

[9] Decision No 1386/2013/EU of the European Parliament and of the Council of 20 November 2013 on a General Union Environment Action Programme to 2020 'Living well, within the limits of our planet'. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013D1386&from=EN

[10] European Parliament, 'Resolution of 17 April 2018 on the implementation of the 7th Environment Action Programme' (2017/2030(INI)). http://www.europarl.europa.eu/doceo/document/TA-8-2018-0100_EN.pdf

[11] European Parliament 'Resolution on Towards a comprehensive European Union framework on endocrine disruptors' (2019/2683(RSP)) of April 15, 2019. http://ec.europa.eu/transparency/regdoc/rep/1/2018/EN/COM-2018-734-F1-EN-MAIN-PART-1.PDF

[12] European Parliament 'Resolution on Chemicals strategy for sustainability' of July 8-10 European Parliament (2020) (2020/2531(RSP)) https://www.europarl.europa.eu/committees/fr/envi/documents/motions-for-resolution

[13] European Parliament resolution of 10 July 2020 on the Chemicals Strategy for Sustainability (2020/2531(RSP)) https://www.europarl.europa.eu/doceo/document/TA-9-2020-0201_EN.pdf

[14] Council conclusions: Towards a Sustainable Chemicals Policy Strategy of the Union, 10713/19, 26 June 2019. http://data.consilium.europa.eu/doc/document/ST-10713-2019-INIT/en/pdf

[15] European Commission, 'Communication to the Parliament and the Council: Towards a comprehensive European Union framework on endocrine disruptors', November 2018 (COM(2018) 734 final). http://ec.europa.eu/transparency/regdoc/rep/1/2018/EN/COM-2018-734-F1-EN-MAIN-PART-1.PDF

[16] Communication from the Commission to the European Parliament, the European Council, the Council, the European Economic and Social Committee and the Committee of the Regions. 'The European Green Deal' , COM(2019) 640 final https://ec.europa.eu/info/sites/info/files/european-green-deal-communication_en.pdf

[17] Bopp, S., Richarz, A., Worth, A., Berggren, E. and Whelan, M. 'Something from nothing? Ensuring the safety of chemical mixtures' , JRC Science for Policy Brief May 2018. https://op.europa.eu/en/publication-detail/-/publication/4bd117ac-6d22-11e8-9483-01aa75ed71a1/language-en

- **Mixture:** (or chemical mixture) in this report means any set of chemicals to which an organism may be jointly exposed, and which may potentially cause an adverse combination effect, regardless of sources and exposure routes.
- **Intentional mixture**: a mixture or solution which is composed of two or more substances (Article 3(2) REACH, Article 2(8) CLP), normally referring to a formulated product (mixture), manufactured and marketed as such, or the result of intentional use of different products (mixtures) together, where the composition is normally known.
- **Unintentional mixture**: mixtures of chemicals co-occurring in environmental media (water, soil, air), biota, feed, food, or human tissues as a result of releases from various sources and through multiple routes of exposure (the synonym term **'coincidental mixture'** is sometimes used). Unintentional mixtures include degradation and transformation products of chemicals released into the environment. The composition of unintentional mixtures is often unknown and varying in time and space.
- **Priority mixture**: a chemical combination/mixture) of high concern to human or environmental health, which is a priority to identify and further assess for risk reduction.
- **Combined exposure**: simultaneous or sequential exposure to multiple substances via single or multiple pathways/routes (the synonym **'cumulative exposure'** is sometimes used)
- **Aggregate exposure**: exposure to a single substance from multiple sources and via multiple pathways/routes.
- **Combination effect** (sometimes referred to as **'cumulative' or 'mixture effect'):** (eco)toxicological effect on an organism arising from exposure to a chemical mixture. Type and strength of the effect will vary depending on the composition of the mixture and the level of exposure.
- **Sources**: places of release of chemicals (e.g. industrial installations, diffuse sources such as products).
- **Routes of exposure**: ways of entering the organism (dermal, oral, inhalation, normally referring to human health).
- **Compartment**: (environmental) media where chemicals are taken up (air, water, sediment, soil, food/feed).
- **Exposure pathway**: includes fate and transport processes by which chemicals move from the original point of release through the environment, and the routes/interaction(s) through which populations or individuals are exposed (oral/ingestion, inhalation, dermal) via various media (water, sediment, soil, air, food/feed, products).

[18] Kienzler, A., Bopp, S., van der Linden, S., Berggren, E., Worth, A., 'Regulatory assessment of chemical mixtures: Requirements, current approaches and future perspectives', Regulatory Toxicology and Pharmacology Volume 80, October 2016, Pages 321-334.

[19] Meek, M.E. (Bette), Boobis, A.R., Crofton, K.M., Heinemeyer, G., van Raaij, M., Vickers, C.,'Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework' Regulatory Toxicology and Pharmacology 60 (2011) S1–S14. https://pubmed.ncbi.nlm.nih.gov/21466831/

[20] OECD (2018). 'Considerations for Assessing the Risks of Combined Exposure to Multiple Chemicals', Series on Testing and Assessment No. 296, Environment, Health and Safety Division, Environment Directorate. http://www.oecd.org/chemicalsafety/risk-assessment/considerations-for-assessing-the-risks-of-combined-exposure-to-multiple-chemicals.pdf

[21] Swedish Government Inquiries (2019). 'Future chemical risk management Accounting for combination effects and assessing chemicals in groups', Swedish Government Official Reports, SOU 2019:45 https://www.government.se/legal-documents/2019/11/sou-201945/

- **Mode of action (MOA)**: the way a chemical exerts its biological effects. A common mode of action is assumed to involve one or several key events between the chemical and its biological target(s), leading to an (eco)toxicological effect.
- **Interaction**: an event where the joint effect of different chemicals in a mixture causes either an enhanced or weaker effect than that is expected based on the application of models such as concentration addition or independent action to predict the toxicity of the mixture.
- **Synergism**: an interaction between chemicals that enhances the toxicological effect beyond that is expected based on the toxicity of the individual components in the mixture.
- **Antagonism**: an interaction between chemicals that weakens the toxicological effect compared to what is expected based on the toxicity of the individual components in the mixture.
- **Whole mixture testing**: an approach where a sample or chosen media containing a mixture of chemicals is tested experimentally for one or several toxicological endpoints.
- **Component-based approaches**: methods for calculating mixture toxicity based on information on the toxicity and exposure to the individual compounds in the mixture. The two common models for mixture assessment are concentration (or dose) addition, and independent action.
- **Concentration (or dose) addition:** method/model for calculating mixture toxicity, based on the toxicity and concentration of the individual substances and under the assumption that they do not interact and have similar mode(s) of action.
- **Independent action**: assumes that mixture components contribute to a common endpoint via dissimilar and fully independent sequences of events, from an initial interaction with different molecular target sites to different diseases or different adverse effects seen at the level of individuals or populations. Consequently, the individual effects can be considered to be independent events in probabilistic sense.
- **Toxicological Equivalence Factor (TEF):** the toxicity weighted summation of the relevant mixture components. The TEF is in some regulations referred to as the 'Summation Method' and may be used to assess if the sum exceeds a pre-defined effect threshold.
- **Default safety factor:** a factor (sometimes referred to as **default uncertainty factor**), normally applied in the hazard and risk assessment of chemicals. Depending on the regulation different uncertainty factors may be applied. It may be used in the extrapolation from experimental animal studies to potential harmful effects on humans. Uncertainty factors may also be applied when choosing the part of the population that represents the exposure used in a study, as input to the risk assessment, as well as for the allocation of exposure to different sources. Various factors are also used in deriving predicted no-effect concentrations (PNECs) in environmental risk assessment.
- **Mixture Assessment Factor (MAF)**: sometimes also called **'Mixture Allocation Factor'**, is an additional safety factor that can be applied in the risk assessment of single chemicals, in order to generically cover for combined exposure without performing a mixture-specific assessment. The possible use of MAFs in risk assessment has long been discussed, but the approach has so far not been applied for regulatory purposes at EU level.

2. WHY ARE MIXTURES A CONCERN?

Chemicals normally appear as mixtures. They are intentionally blended to form products, such as paints, cosmetics, pesticides and biocides, as well as more or less complex materials like plastics and textiles. Chemicals may also be used together (*e.g.* for plant protection purposes or when different biocidal products are combined) and unintentionally emitted in combination and/or sequentially (*e.g.* as discharges during their manufacturing and use as well as from waste management and sewage treatment plants). Hence, humans, biota and environmental compartments are constantly exposed to such mixtures, often of unknown composition, from various sources and via different pathways.

The occurrence of combination effects of chemicals is long known and has accordingly been an area of scientific research, and to some extent subject to regulatory action[22]. A well-known example is the interaction between different pharmaceuticals when a patient is treated with two or more medicines simultaneously.

It is widely documented that the combined exposure to multiple chemicals can trigger stronger (or occasionally weaker) (eco)toxicological effects than exposure to individual chemicals alone[23, 24, 25]. Even exposures at concentrations regarded as safe (*i.e.* where no effects are expected) for the individual chemical can result in adverse (eco)toxicological effects when several chemicals occur together in a mixture[26].

Current regulatory approaches to the risk assessment of single chemicals normally involves establishing a level of exposure considered reasonably safe in relation to the inherent hazardous properties of the particular chemical[27, 28]. When extrapolating from animal test data to a safe level of exposure for humans or organisms in the environment, safety margins are applied. The purpose is to take account of differences in sensitivity between animals and humans, *i.e.* between different species as well as between individuals. In the model for humans applied under REACH, these safety margins add up to a default safety (or uncertainty) factor of 100, commonly used in single chemicals risk assessment. Similar factors of different magnitude are used to establish safe levels of exposure in environmental hazard and risk assessment. However, these default uncertainty factors do not take into account the effect of combined exposures on humans[29] or the environment[30].

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[22] Monosson, E., 'Chemical Mixtures: Considering the Evolution of Toxicology and Chemical Assessment', Environ Health Perspect. 2005 Apr; 113(4): 383–390. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1278475/

[23] Backhaus, T., Karlsson, M., 'Screening level mixture risk assessment of pharmaceuticals in STP effluents', Water Research, Volume 49, 1 February 2014, Pages 157-165. https://www.sciencedirect.com/science/article/pii/S0043135413009044?via%3Dihub

[24] Kortenkamp A., Faust, M., 'Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment', International Journal of Andrology, Volume 33 (2):463-474, April 2010. https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2605.2009.01047.x

[25] De Brouwere K, Cornelis C, Arvanitis A, Brown T, Crump D, Harrison P, Jantunen, M, Price. P, Torfs R., 'Application of the maximum cumulative ratio (MCR) as a screening tool for the evaluation of mixtures in residential indoor air', The Science of the Total Environment 2014, 479-480, 267–76. https://pubmed.ncbi.nlm.nih.gov/24565859/

[26] This was confirmed by the Scientific Committee on Emerging and Newly Identified Health Risks, the Scientific Committee on Health and Environmental Risks, and the and Scientific Committee on Consumer Safety in a joint statement from 2011 (see Box 2).

[27] Risk characterisation is often performed in terms of a risk quotient (RQ) or risk characterisation ratio (RCR). In general, the quotient denotes the ratio between an observed or predicted exposure level and a regulatory acceptable exposure level, which is considered reasonably safe. There are numerous variants and specifications of this approach, depending on the specific protection goals (e.g. human health or the environment) and the specific regulatory context).

[28] ECHA (2016). Guidance on information requirements and chemical safety assessment Part E: Risk characterisation. ECHA-2016-G-04-EN. https://echa.europa.eu/documents/10162/13632/information_requirements_part_e_en.pdf

[29] Martin, O.V., Scholze, M., Kortenkamp, A. (2013), 'Dispelling urban myths about default uncertainty factors in chemical risk assessment – sufficient protection against mixture effects?', Environmental Health 2013, 12:53 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3708776/pdf/1476-069X-12-53.pdf

A large body of research in the EU and internationally over the past decades, including laboratory experiments, human and environmental monitoring and epidemiological studies provides a growing body of evidence clearly showing that exposure to mixtures of chemicals of anthropogenic origin is the norm in the human and natural environment. Further, research shows that combination effects do occur, in real life exposure situations as well as in experimental studies involving realistic exposure levels (see cases in Box 2).

Box 2: Growing evidence of health and environmental concern relating to exposure to unintentional mixtures

Since 2012, the body of evidence indicating that exposure to mixtures is a cause of health and environmental concern has grown considerably. Several studies show that mixtures are a cause of concern at real-life exposure levels and can be linked to effects through epidemiological and experimental means. Below are some selected examples from recent studies.

JRC review of mixture risk case studies

A 2016 report from the Commission's Joint Research Centre[31] reviews 21 case studies on mixture risk assessment conducted since 2014. The case studies include human health and environmental risk assessments and cover several classes of chemical compounds (*e.g.* pesticides, phthalates, parabens, polybrominated diphenyl ethers (PBDEs), pharmaceuticals, food contact materials, dioxin-like compounds and anti-androgenic chemicals). All selected studies involved testing of either real samples or realistic artificial samples. The reviewed case studies included unintentional mixtures like contaminants in breast milk, chemicals in indoor air and in environmental media, such as surface water, ground-water and drinking water. Several of the case studies indicated that exposure to the assessed mixtures posed concerns, especially regarding vulnerable populations. The review thus clearly demonstrates that unintentional chemical mixtures, also across chemical classes and legislative sectors, need to be better addressed. Aspects needing further attention include possible interactions/synergism, bioaccumulation and effects of metabolites appearing in mixtures as well as data gaps hampering a more refined assessment.

Exposure of children, unborn children and pregnant women to endocrine disruptors and neurotoxic substances

In 2017, the Danish Environmental Protection Agency published a report[32] on the exposure of children under the age of three, unborn children and pregnant women to selected endocrine disrupting and neurotoxic chemical substances. The study included 37 endocrine disrupting chemicals (*e.g.* phthalates, perfluoro-substances, bisphenols and UV filters) and 39 neurotoxic chemicals (*e.g.* heavy metals, bisphenol A, PCBs, dioxins and perfluoro-substances), of which seven substances were relevant for both endpoints. Data regarding exposure via food, drinking water, indoor and outdoor environment, cosmetics and various consumer products were combined with human biomonitoring data to estimate exposure levels. The overall risk

[30] Swedish Chemicals Agency KEMI (2015). An additional assessment factor (MAF) – A suitable approach for improving the regulatory risk assessment of chemical mixtures; Report 5/15 https://www.kemi.se/global/rapporter/2015/rapport-5-15.pdf

[31] Bopp, S. K , A. Kienzler, S. van der Linden, L. Lamon, A. Paini, N. Parissis, A.-N. Richarz, J. Triebe, A. Worth (2016). 'Review of case studies on the human and environmental risk assessment of chemical mixtures' EUR 27968 EN; JRC report; doi:10.2788/272583 http://publications.jrc.ec.europa.eu/repository/bitstream/JRC102111/jrc102111_jrc_tech-rep_mix%20case%20studies_2016_vf.pdf

[32] Danish Environmental Protection Agency (2017). 'Exposure of children and unborn children to selected chemical substances'. *Survey of chemical substances in consumer products No. 158*, 2017. http://orbit.dtu.dk/files/136888869/Eksponeringsrapport_ENG_.pdf

associated with the combined exposure was calculated through a concentration addition approach, establishing risk quotients (RQtotal) for the mixtures.[33] The results of the Danish study indicate that the overall exposure of children under 3 years old to mixtures of endocrine disrupting chemicals is of concern (RQtotal >2) with respect to all three types of disruptive effects (anti-androgenic, estrogenic and thyroid hormone disrupting) already at average exposure levels. Similarly, for pregnant women and unborn children there were indications of possible concern (RQtotal≈1) already at average levels of exposure to combinations of endocrine disruptors. Also considering the exposure to combinations of neurotoxic substances, results indicate concern regarding children under 3 years old (RQtotal 61.1) as well as pregnant women and unborn children (RQtotal 7.9).

Environmental risks of pesticide mixtures in inland waters

A study by Gustavsson et al. from 2017[34] (part of the EU-funded SOLUTIONS project), evaluates the environmental risks associated with complex pesticide mixtures occurring in freshwater ecosystems. It is based on 1308 individual samples collected between 2002 and 2013 from rivers and streams in an agricultural area in southern Sweden, monitoring for 141 different pesticides. The study demonstrated that pesticides are present as mixtures, with up to 53 pesticides found in the same sample, with most commonly eight pesticides per sample. These findings correspond with multiple studies demonstrating the presence of complex pesticide mixtures in surface waters in other areas.

Evaluation of the eco-toxicological effects of the pesticides mixtures, applying a statistical method (the Kaplan-Meier method), and based on an assessment using concentration addition and the water quality objectives established for the individual pesticides, shows that the environmental risk exceeded acceptable levels in 73% of the samples. Further, no clear time-trend in the level of risk was detected, but risk appears to remain relatively constant, despite regulatory interventions. A conclusion is that current single-substance risk assessment and mitigation is insufficient to manage risks related to the total and overall use of pesticides.

A limited number of pesticides explains the largest share of toxicity of the mixture (*i.e.* so-called risk drivers). However, the composition of the mixtures of pesticides varies considerably between samples. Hence, 83 of the 141 monitored pesticides has to be included in the assessment to account for 95% of the risk at all sites and years. This highlights the need for continuous monitoring as a component of risk assessment and mitigation. The study also shows that pesticides, in particular insecticides, even if they are only present at concentrations close to detection limits, still contribute to the overall toxicity of the mixture. Further, the component-based approaches applied in the study do not take account of the whole picture, including *e.g.* interactions. Therefore, the authors suggest complementary approaches, including effect-based monitoring and *in-situ* experiments.

Prenatal exposure to chemical mixtures linked to impaired sexual development, neurodevelopment and metabolism in children

The EDC-MixRisk research project (funded through the EU research programme Horizon 2020) studied health and development of early life-exposure to complex mixtures of

[33] In toxicology, the so called Risk Quotient (RQ) is the ratio between the measured exposure in a predicted exposure situation, and the exposure at which no toxic effect on humans is expected ('derived no effect level', DNEL). A RQ for an individual substance, or the summarised RQ's of the components in a mixture (RQtotal), above the value one (1) is normally regarded as indicating risk.

[34] Gustavssons, M., Kreuger, J., Bundschuhb, M., Backhaus, T., 'Pesticide mixtures in Swedish streams: Environmental risks, contributions of individual compounds and consequences of single-substance oriented risk mitigation', Science of The Total Environment, Volume 598, 15 November 2017, Pages 973-983; https://reader.elsevier.com/reader/sd/pii/S0048969717309580?token=57C5E26D539F6B8CD650794BA2105715A136C7D2A770C5EDC93B67A2A1FBED772D43EBA34076330B7633FC1D7BAC57C8

endocrine disrupting chemicals (EDC) through a combination of approaches. Baseline chemicals exposure data were derived from a pregnancy cohort (SELMA). Out of 54 potential endocrine disruptors analysed in blood and urine from the 2,300 pregnant women, 41 (75%) were found above detection levels in a majority of the samples. The researchers used epidemiological and statistical methods to identify which of these chemicals (*i.e.* in mixtures) are associated with adverse health outcomes in children, with regard to sexual development, neurodevelopment and metabolism/growth.

The identified mixtures were tested in experimental models to uncover mechanisms and pathways behind the health outcomes and the analysed dose-response relationships. Hence, the EDC-MixRisk project demonstrated that exposure to the identified mixtures caused effects and dysfunctions in cell and animal models at levels similar to those measured in the SELMA cohort. Observations in the experimental studies included morphological changes to reproductive organs, interference with thyroid hormone signalling, changes in gene expression associated with autism spectrum disorder and intellectual disability in humans, behavioural changes as well as changes in fat cell differentiation and birth weight. Researchers could also link some of the experimentally induced biomarkers to adverse health outcomes in the exposed children of the cohort.

Finally, the human biomonitoring data from the SELMA cohort and the experimental data were compared and analysed using new statistical/analytical approaches developed as part of EDC-MixRisk. Results showed that the approaches developed in the project, comparing and integrating human biomonitoring data and experimental models were more sensitive (*i.e.* indicated risk for adverse effects at lower exposure levels) than traditional additivity or single substance risk assessment approaches, at least for some endpoints. It also showed that the regulatory guideline values for most of the assessed chemicals seem to be insufficiently protective against combined exposures to multiple substances.

Box 3: The joint statement of 2011 by the Scientific Committee on Health and Environmental Risks, the Scientific Committee on Emerging and Newly Identified Health Risks and the Scientific Committee on Consumer Safety[35]

Regarding impacts of mixtures on human health, the Scientific Committees concluded that, under certain conditions, chemicals in a mixture with a similar mode of action may act jointly and produce a combination effect that is larger than that from each component of the mixture used singly, and hence represent a health concern.

No robust evidence was available at the time of the drafting of the opinion for substances with different modes of action to indicate that exposure to such mixtures of chemicals is a health concern if the individual chemicals are present at or below their estimated zero-effect levels. The Committees concluded on the possible health concerns of such mixtures, that 'if the intended level of protection is achieved for each individual substance, the level of concern for mixtures of dissimilarly acting substances should be assumed to be negligible'.

However, for the ecological effects of chemical mixtures, the Scientific Committees concluded that not only exposure to mixtures of chemicals with similar modes of action was a concern. Combinations of dissimilarly acting substances should also be considered as a possible concern, even if the exposure to all the substances involved is at a level where no

[35] Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENHIR) and Scientific Committee on Consumer Safety (SCCS). 'Joint Opinion on the Toxicity and Assessment of Chemical Mixtures adopted on 14th December 2011' https://doi.org/10.2772/21444

[35] Kortenkamp, A., Backhaus, T., Faust, M. (2009): State of the Art Report on Mixture Toxicity, Final Report, Executive Summary, 22 December 2009. http://ec.europa.eu/environment/chemicals/effects/pdf/report_mixture_toxicity.pdf

effects are predicted for the individual substances (i.e. below the individual Predicted No-Effect Concentrations - PNECs).

3. REGULATORY FRAMEWORKS AND REQUIREMENTS REGARDING MIXTURES

The Commission Communication of 2012 concluded that current EU legislation does not provide for a comprehensive and integrated assessment of the combination effects (the Communication uses the synonym term cumulative effects) of different chemicals taking into account different routes of exposure. It also concluded that 'where a mixture of concern is identified and where such a mixture contains chemical substances regulated under different pieces of EU legislation, no mechanism currently exists for promoting an integrated and coordinated assessment across the different pieces of legislation' (the latter aspect is further described in section 6).

In 2014, the European Commission Joint Research Centre (JRC)[36] published a Report reviewing the **existing regulatory requirements for the assessment of mixtures in current EU chemicals-related legislation**. The Report investigated such requirements in selected pieces of the three main categories of chemicals-related EU legislation:

- **legislation focused on chemical substances and/or intentional/commercial mixtures**, normally involving prospective (pre-marketing) assessment, *e.g.* industrial and consumer chemicals, pesticides, biocides, food and feed additives, pharmaceuticals and cosmetics (12 legislative pieces),
- **legislation mainly oriented towards (or specific to) the use of chemicals in products**, normally not involving prospective (pre-marketing assessment), *e.g.* food contact materials and toys (2 legislative pieces), and
- **legislation focused on chemical emissions** from certain activities, *e.g.* industrial emissions and the presence of **pollutants in food/feed, at work place and in certain environmental media**, *e.g.* air, water (13 legislative pieces).

Most pieces of legislation in the first category include some provisions for the assessment of the exposure to and risks from intentional/commercial mixtures, for which the added chemicals are normally well-known and subject to a prospective risk assessment[37] (see below: Intentional mixtures). However, none of the pieces of legislations in this category includes any requirements for the assessment of unintentional mixtures, except for Plant Protection Products and Biocidal Product Regulation in which cumulative and synergistic effects are covered more generally without making a distinction whether they refer to intentional and unintentional mixtures (see below: Intentional and Unintentional mixtures).

Regarding legislation in the second category, a specific provision regarding the assessment of intentional mixtures exists in the Toy Safety Directive[38]. The directive considers any toy to be a mixture as regards the presence of substances which are carcinogenic, mutagenic and, toxic to reproduction (CMR). CMRs are prohibited by default in toys, but by derogation may be present in a toy up to the generic or specific concentration limit (whichever is stricter),

[36] Aude Kienzler, Elisabet Berggren, Jos Bessems, Stephanie Bopp, Sander van der Linden, Andrew Worth (2014), 'Assessment of Mixtures – Review of regulatory Requirements and Guidance', JRC Science and Policy Report, EUR 26675 EN. http://publications.jrc.ec.europa.eu/repository/bitstream/JRC90601/lb1a26675enn.pdf

[37] 'Prospective risk assessment' is performed in the context of pre-marketing assessment/authorisation of a chemical (as distinct to 'retrospective risk assessment', which is generally aimed to identify the causes of adverse effects that have already occurred).

[38] Directive 2009/48/EC on the safety of toys (Toys Safety Directive)

specified in the CLP Regulation. There is however no reference to the assessment of unintentional mixtures in any piece of the analysed products-focused legislation.

The emission and pollution focused legislation mainly looks at the environmental exposure and sometimes risk resulting from the use of chemicals, including to unintentional contaminants present in these chemicals as well as breakdown and transformation products that might form. In line with this, it does not include any requirements for the prospective assessment of single chemicals or intentional mixtures[39]. However, some of the pieces of legislation in this category include legal requirements for the assessment of unintentional mixtures (see below: Unintentional mixtures). The legislation focused on protection of workers from chemicals at work place contains explicit requirement to assess risks of unintentional mixture present at the work place such chemicals in combination (see below: Unintentional mixtures*)*.

Of the 27 pieces of chemicals-related legislation assessed, 10 do not include any explicit reference to the assessment of either intentional or unintentional mixtures[40,41]. However, mixtures are sometimes considered on an *ad hoc* basis also under these pieces of legislation, particularly focussing on groups of substances. There are examples of such cases, for instance in the area of plastics materials regulation for food contact materials[42], but this is the exception rather than the rule.

The regulatory requirements for mixtures have not changed in any significant respect since 2012. Hence, chemicals-related legislation is still largely focused on the risk assessment and management of single chemicals. The background to this predominantly substance-by-substance approach to risk assessment is that many pieces of legislation were developed to provide marketing and use provisions for chemicals used for specific purposes, and to define the responsibilities of individual economic actors involved (*i.e.* producers, importers and users).

The prevailing need to better address chemical mixtures has however resulted in the development of common approaches to assess related hazards, exposures and risks. Several guidance documents for such assessments have also been developed. However, to what extent these approaches are actually applied in the absence of specific legal requirements is not known. (see examples in section 4).

[39] Under the Water Framework Directive (2000/60/EC), Article 16, a single-substance risk assessment is required for the review of so called 'priority substances', which might influence e.g. reauthorisation processes under the respective pieces of legislation under which the uses of these substances are regulated.

[40] Regulation (EC) No 1333/2008 on food additives; Directive (2010/75/EC) on Integrated pollution prevention and control (IPPC) (now replaced by the Directive 2010/75/EU on industrial emissions (integrated pollution prevention and control); Directive 2008/98/EC on waste and repealing certain Directives (including the Waste Stream Directives); Directive 2006/118/EC on the protection of groundwater against pollution and deterioration; Directive 2008/56/EC establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive); Council Directive 98/83/EC on the quality of water intended for human consumption (Drinking Water Directive); Directive 2008/50/EC on ambient air quality and cleaner air for Europe; Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC (Food Contact Material Regulation), Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food (Food Contaminant Regulation); Directive 2002/32/EC on undesirable substances in animal feed (Feed Contaminant Directive).

[41] The Ground Water Directive and the Drinking Water Directive sets out Environmental Quality Standards for individual as well as total presence of pesticides. These are not based on an assessment of the combined risk of the pesticides (i.e. the unintentional mixture), but still contributes to controlling the risk of combination effects.

[42] Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. Examples of cases of regulation of chemical mixtures in plastics for food contact purposes include the groups primary aromatic amines, aromatic isocyanates, organotins and phthalates. (EU 10/2011)

3.1 Intentional mixtures

Various kinds of references to the assessment of intentional mixtures appear in 11 legislative acts out of the 12 pieces of legislation focused on chemical substances and/or intentional/commercial mixtures assessed. Eight of these refer to such assessment for both human health and the environment[43] and three for only human health[44]. These delimitations usually coincide with the overall scope of the legislation regarding health and the environment, *e.g.* environment is out of scope of the Cosmetics Regulation, which refers to REACH regarding environmental risks.

An example of legislation requiring assessment of mixtures with regard to both human health and the environment is the regulation on biocidal products. The regulation on plant protection products considers effects on human health both related to dietary exposure (food, feed, drinking water) and to non-dietary exposure (workers, residents, bystanders). Biocidal and plant protection products are normally intentional mixtures, which apart from the active substance(s) might include solvents, synergists (adjuvants), safeners and surfactants.

The Plant Protection Products (PPP) Regulation[45] covers both the approval of the individual substances used in plant protection products and the formulated products (*i.e.* intentional mixtures). Active substances used in PPPs are approved in a procedure at the EU level. This normally involves testing and risk assessment of the individual substances, and in some cases testing of the whole formulation for the purpose of environmental risk assessment for particular organism groups. The authorisation also includes the requirement to assess maximum residue levels (MRL) of pesticides in food and feed with regard to human health. MRLs are set based on scientific advice of the European Food Safety Authority (EFSA) according to Regulation (EC) No. 396/2005. Formulated plant protection products, in which an approved active substances is used together with other (formulating) substances, are authorised at the Member State level. The whole formulations are generally assessed only for acute toxicity to humans and for organisms in the environment that might come into direct contact with the product, but Member States have the possibility to require submission of any other information they consider necessary. Data to be provided are either from direct testing of the product itself (whole-mixtures approach), data on all components of the plant protection product, or in some cases the application of a non-animal test method.

The Biocidal Products (BPR) Regulation[46] covers the approval of active substances and the authorisation of formulated biocidal products (*i.e.* intentional mixtures). In both cases the regulation requires 'cumulative and synergistic effects' to be taken into account under 'realistic worst case conditions of use' (Article 19(2)). It also requires that 'for biocidal products that are intended to be authorised for use with other biocidal products, the risks to

[43] Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market (Plant protection Products (PPPs) Regulation); Commission Regulation (EU) No 283/2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market; Commission Regulation (EU) No 284/2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market; Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products (Biocides Regulation); Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (CLP regulation); Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH); Regulation (EC) No 1831/2003 on additives for use in animal nutrition (Feed Additive Regulation); Regulation (EC) No 429/2008 as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives.

[44] Directive 2001/83/EC on the Community code relating to medicinal products for human use (Human Medicines Directive); Directive 2001/82/EC on the Community code relating to veterinary medicinal products (Veterinary Medicines Directive); Regulation (EC) No 1223/2009 on cosmetic products (Cosmetics Regulation).

[45] Plant protection products Regulations (Regulations (EC) 1107/2009, (EU) 283/2013, (EC) 284/2013).

[46] Biocidal Products Regulation (EC) 528/2012.

human health, animal health and the environment arising from the use of these product in combinations shall be assessed' (Annex III Section I Point 8.5.4). Further, common principles for the evaluation of biocidal products, including several references to cumulative and synergistic effects, are set out in Annex VI. The risk assessment of the formulated biocidal products is normally based on data for the individual components addressing all relevant endpoints as required by the data requirements for biocides in Annex VI to the Biocidal Products Regulation. Guidance documents for human health and environmental risk assessment relating to biocidal products are available (see Section 4).

Assessment of intentional mixtures is generally conducted in connection with the hazard based classification and labelling under the Classification, Labelling and Packaging (CLP) Regulation[47]. This process relies firstly on test data on the whole mixture when such are available[48]. Alternatively, the classification can be based on *bridging principles*, *i.e.* the use of data on a similar mixture. Finally, in the absence of information on the mixture itself, classification can be based on the toxicological properties of the ingredients in the mixture and application of the summation rule or concentration addition method.

The REACH Regulation[49] covers, in principle, all intentionally manufactured chemical substances, on their own, in mixtures or in products, unless they are specifically exempted under REACH due to being regulated by sector or product-related legislation (*e.g.* pharmaceuticals and food additives) or are radioactive substances or waste. It requires the producer/importer to register a chemical to be marketed or imported in quantities above 1 ton per year and in this context provide test data, hazard assessment and a risk assessment of the identified uses. REACH registration requirements apply to each of the individual chemicals in an intentional mixture, but not to the mixture itself. The registration must include a hazard assessment of the chemical and a risk assessment for all identified uses. The risk assessment must cover the use of a substance in a mixture if this is placed on the market and be documented in a chemical safety report.

REACH is mainly focused on individual substances and contains no specific methodology for the assessment of intentional mixtures, beyond multi-constituent substances (MCSs) and so called UVCBs (see below). In principle, it is however possible to take into account combined effects of intentional mixtures, *e.g.* during the assessment of substances for restriction and authorisation (this is also true for intentional mixtures, see section 5). Further, REACH obliges the supplier of a mixture to provide the downstream user of that mixture with a safety data sheet (SDS), if the mixture is classified as hazardous. The SDS contains (eco)toxicological information and a chemical safety assessment for the mixture, if one has been performed.

Under several pieces of legislation (*e.g.* CLP, REACH, biocides, cosmetics), special consideration is given to impurities in a substance or a constituent in a multi-constituent substance (MCS) as well as in the so-called UVCBs (Substances of Unknown or Variable composition, Complex reaction products or Biological materials). Although such substances can be seen as mixtures from a toxicological point of view, they are considered substances from a legal point of view (e.g. the REACH definition[50]). Hence, they are registered as one

[47] Classification, Labelling and Packaging (CLP) Regulation (EC) 1272/2008.

[48] The CLP regulation 6(3) and (4) include special requirements for CMRs, biodegradability and bioaccumulation. Tests should not be performed for such endpoints, but the information on the individual ingredient of the mixture should be used instead.

[49] Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (EC) 1907/2006.

[50] The REACH substance definition: 'Substance means a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.'

substance under REACH. Procedures for assessing such substances are described in the REACH and CLP guidance. The approach generally involves the testing of the substance itself, but might also involve methods for predicting the overall risk, based on information on the individual components, in particular for CMR properties.

In conclusion, most pieces of legislation focusing on chemicals and intentional/commercial mixtures require a hazard and/or risk assessment of these. The scope and level of detail in these provisions vary, including whether human health and/or environment are considered. Methodologies and guidance documents are generally available, and assessments are usually performed through applying whole mixture approaches or based on information on the individual components in the mixture.

3.2 Unintentional mixtures

The European Commission Joint Research Centre (JRC) report from 2014 analysed whether 12 pieces of legislation focused on chemical substances and intentional mixtures include requirements for the assessment of unintentional mixtures. Such requirements were found to be absent from all these pieces of legislation, except for the Plant Protection Products and the Biocidal Product Regulations which require the consideration of cumulative and synergistic effects (see Box 4). The Plant Protection Product Regulation include such a specific reference only regarding human health (dietary and non-dietary (for workers, residents, bystanders)), while the Biocidal Product Regulation regarding human health and the environment.

The JRC report also analysed 15 further pieces of legislation (products, pollution, food, work-place and environmental-media related legislation) regarding requirements for the assessment of unintentional mixtures. Nine pieces of legislation[51] have no reference to the assessment of unintentional mixtures, while six of them include some reference to such assessment.

- Two pieces of legislation mention the assessment of unintentional mixtures in relation to both human health and the environment in the operative text (Environmental Impact Assessment Directive, and Water Framework Directive)[52, 53].
- One piece of legislation mentions the assessment of unintentional mixtures in relation to only the environment in the operative text, while human health is only mentioned in a recital (Environmental Quality Standards Directive)[54]. However, its consideration is implicit through the link the Water Framework Directive.
- Three pieces of legislation include such a reference only regarding human health (Maximum Pesticide Residue Levels Regulation, Protection of the Health of Workers Directive and Toy Safety Directive)[55, 56].

[51] Integrated pollution prevention and control (IPPC) (Dir 2010/75/EC), now replaced by the Directive 2010/75/EU on industrial emissions), Waste and waste streams (Dir 2008/98/EC), Groundwater Directive (Dir 2006/118/EC), Marine Strategy (Dir 2008/56/EC), Drinking water (Dir 98/83/EC), Ambient Air quality and cleaner air for Europe (Dir 2008/50/EC), Food Contact material (Reg 1935/2004), Food contaminant (Reg 315/93/EEC), Feed contaminant (Dir 2002/32/EC).

[52] Directive 2011/92/EU on the assessment of the effects of certain public and private projects on the environment (Environmental Impact Assessment (EIA) Directive); Directive 2000/60/EC establishing a framework for Community action in the field of water policy (Water Framework Directive (WFD)).

[53] WFD (Dir 98/83/EC) includes only a mentioning of the assessment of unintentional mixtures in the guidance, but not in detail.

[54] Environmental Quality Standards (EQS) (Dir 2013/39/EU).

[55] Plant Protection Products (PPPs) Regulation (1107/2009); Regulation (EC) No 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin (Maximum Residue Levels (MRLs) Regulation); Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work); Directive 2009/48/EC on the safety of toys (Toy Safety Directive).

The references to assessment of unintentional mixtures in products, pollution and environmental media related legislation are however in most cases vague and general. There is usually a lack of detailed and explicit provisions or guidance on when and how such assessment should be performed. As a result, unintentional mixtures are seldom subject to any regulatory assessment, and when this happens, it is on an *ad hoc* basis.

The most explicit requirement to perform assessment of unintentional mixtures is in the Directive on the protection of the health and safety of workers from the risks related to chemical agents at work[57]. It includes an obligation to assess the combined risks to workers of all hazardous chemical agents in the workplace[58] (see Box 4).

The regulation on the maximum residues levels of pesticides in food and feed (MRL Regulation)[59] includes also a relatively clear general requirement for taking into account the possible presence of pesticide residues arising from sources other than current plant protection uses of active substances, and their known cumulative and synergistic effects (see Box 4).

For three of the analysed pieces of legislation, the requirement to assess unintentional mixtures refers exclusively to particular groups of substances. This is true for the Industrial Emissions Directive[60], which requires assessment of mixtures of chemicals belonging to the groups Dioxins and Furans. The Food Contaminants Regulation[61] and its guidance document[62] requires the same for chemicals of the group poly-aromatic hydrocarbons (PAHs). The Ambient Air Quality and Cleaner Air for Europe Directive[63] also assess PAHs as a group, using benzo(a)pyrene as a marker for the carcinogenic risk from the whole group of PAHs in the setting of target values.

Waste and industrial emissions are unintentional mixtures with a more or less complex composition, which may exhibit considerable variability. Hence, applying the principles of mixtures assessment might be challenging, but relevant in these contexts. For emissions and wastes originating from *e.g.* particular industrial processes, the composition can be estimated or measured with some accuracy, while more mixed waste streams like household waste are more complicated.

Under the Waste Framework Directive (2008/98/EC), specific waste streams are classified as hazardous or non-hazardous to ensure that waste management can be carried out without endangering human health or harming the environment by safely handling hazardous properties materials. The classification of waste is largely based on the hazard classes and criteria in the CLP Regulation. Given that waste generally constitutes mixtures of substances, mixture classification rules apply, and are described in Annex III of the Directive. For certain hazardous properties, direct testing of waste is also an option. Calculation and test methods to classify waste are used in conjunction with the so-called 'List of Waste' (Decision 2000/532/EC)[64]. The list covers over 800 specific waste types and specifies the classification

[56] The Plant Protection Products (PPPs) Regulation is in this report considered to include a requirement for the assessment of unintentional mixture with regard to human health (the JRC report 2014 did not make this interpretation).
[57] Protection of the health of workers from chemical agents at work (Directive 98/24/EC).
[58] Article 4.4 in the chemical agents directive: 'In the case of activities involving exposure to several hazardous chemical agents, the risk shall be assessed on the basis of the risk presented by all such chemical agents in combination.'
[59] Regulation on the Maximum residue levels of pesticides (396/2005).
[60] Industrial Emissions Directive (2010/75/EU).
[61] Food Contaminants Regulation (315/93/EEC),.
[62] Website of the European Union, guidance documents for the Food Contaminants Regulation https://ec.europa.eu/food/safety/chemical_safety/contaminants/sampling_analysis_en.
[63] Ambient Air quality and cleaner air for Europe Directive (2008/50/EC).
[64] Commission Decision ((2000/532/EC) of 3 May 2000 replacing Decision 94/3/EC establishing a list of wastes pursuant to Article 1(a) of Council Directive 75/442/EEC on waste and Council Decision 94/904/EC establishing a list of hazardous waste pursuant to Article 1(4) of Council Directive 91/689/EEC on hazardous waste.

as hazardous and non-hazardous. Detailed guidance on how to characterise and classify waste according to the List of Waste has been issued by the Commission in its notice on technical guidance on the classification of waste (2018/C 124/01)[65].

Box 4: Legal requirements for the assessment of unintentional mixtures in the worker protection, pesticides and biocides legislation

Directive on the protection of the health and safety of workers from the risks related to chemical agents at work

Under this Directive, the employer has the responsibility to assess any risk to the safety and health of workers arising from the presence of hazardous chemical agents at the work place, taken into consideration the level, type and duration of exposure, and to set out the necessary preventive and protective measures. Article 4 specifically requires that:

'*In the case of activities involving exposure to several hazardous chemical agents, the risk shall be assessed on the basis of the risk presented by all such chemical agents in combination'.*

A Commission guidance[66] document on the application of a simplified risk assessment methodology (the Homogeneous Exposure Group (HEG) methodology) is available.

Pesticides legislation

The Regulation on the Maximum Residues Levels (MRL) of pesticides in food and feed and the Regulation on the placing on the market of Plant Protection Products (PPP)[67] have general requirements for the assessment of unintentional mixtures of pesticides.

The setting of the MRL have to take into account:

'Human exposure to combinations of active substances and their cumulative and possible aggregate and synergistic effects on human health' (preamble point 6).

'The possible presence of pesticide residues arising from sources other than current plant protection uses of active substances, and their known cumulative and synergistic effects, when the methods to assess such effects are available.' (Article 14, (2) (b)).

A plant protection product shall meet, according to the PPP Regulation, among others, the following requirements:

'It shall have no immediate or delayed harmful effect on human health, including that of vulnerable groups, or animal health, directly or through drinking water (taking into account substances resulting from water treatment), food, feed or air, or consequences in the workplace or through other indirect effects, taking into account known cumulative and synergistic effects where the scientific methods accepted by the Authority to assess such effects are available; or on groundwater;' (Article 4(3) (b).

Until now, cumulative risk assessment according to the requirements in these regulations has not been systematically performed for regulatory purposes, as the methodology is not yet available. The European Food Safety Agency (EFSA) has, however, taken several steps

[65] Notices from European Union institutions, bodies, offices and agencies. European Commission. Commission notice on technical guidance on the classification of waste (2018/C 124/01) https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52018XC0409(01)&from=EN

[66] European Commision (2006). Practical guidelines of a non-biding nature on the protection of the health and safety of workers from the risks related to chemical agents at work. https://op.europa.eu/en/publication-detail/-/publication/b8827eb0-bb69-4193-9d54-8536c02080c1/language-en

[67] Plant Protection Products (PPPs) Regulation (1107/2009)

regarding the development of a methodology and presented a guidance document[68] focussing on human health risks from dietary exposure to chemicals and on environmental risks of chemicals falling under its remit - *i.e.* pesticides, food and feed additives. EFSA has published the results of its two first pilot assessments on the risks posed to humans (effects on nervous system and thyroid) by residues of multiple pesticides in food. [69,70]

Biocides legislation

The Biocidal Products Regulation has provisions for the assessment of unintentional mixtures of biocides.

Article 19 (2) requires that '*the evaluation of whether a biocidal product fulfils the criteria for authorisation shall take into account cumulative and synergistic effects*'.

Article 8 (3) further specifies, that *'where the evaluating competent authority considers that there are concerns for human health, animal health or the environment as a result of the cumulative effects from the use of biocidal products containing the same or different active substances, it shall document its concerns in accordance with the requirements of the relevant parts of Section II.3 of Annex XV to Regulation (EC) No 1907/2006 and include this as part of its conclusions.*

3.3 Grouping approaches

Assessing and managing chemicals in groups, rather than substance by substance, contributes to addressing risks from exposures to some unintentional mixtures[71,72,73,74]. Chemicals are usually grouped for regulatory purposes based on:

- Similarities in their chemical structure as a proxy for their similarity of toxicity;
- Common intrinsic properties such as toxicity/hazard classification, and exposure related properties, *e.g.* persistence, bioaccumulation and environmental mobility

[68] EFSA (European Food Safety Authority) (2019) Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal 17:5634 (77 pp). https://doi.org/10.2903/j.efsa.2019.5634

[69] EFSA (European Food Safety Authority) (2020) Cumulative dietary risk characterisation of pesticides that have acute effects on the nervous system. EFSA Journal 2020;18(4):6087. EFSA Journal 2020;18(4):6087 https://www.efsa.europa.eu/en/efsajournal/pub/6087

[70] EFSA (European Food Safety Authority) (2020) Cumulative dietary risk characterisation of pesticides that have chronic effects on the thyroid, Scientific Report. EFSA Journal 2020;18(4):6088 https://www.efsa.europa.eu/en/efsajournal/pub/6088

[71] Bopp, S.K., R. Barouki, W. Brack, S. Dalla Costa, J.-L.C.M. Dorne, P. E. Drakvik, M. Faust, T.K. Karjalainen, S. Kephalopoulos, J. van Klaveren, M. Kolossa-Gehring, A. Kortenkamp, E. Lebret, T. Lettieri, S. Nørager, J. Rüegg, J.V. Tarazona, X. Trier, B. van de Water, J. van Gils, Å. Bergman (2018): Current EU research activities on combined exposure to multiple chemicals; Environment International 120 (2018) 544–562 https://www.sciencedirect.com/science/article/pii/S0160412018308420

[72] Stephanie K. Bopp, Aude Kienzler, Andrea-Nicole Richarz, Sander C. van der Linden, Alicia Paini, Nikolaos Parissis & Andrew P. Worth (2019) 'Regulatory assessment and risk management of chemical mixtures: challenges and ways forward, Critical Reviews in Toxicology', 49:2, 174-189, DOI: 10.1080/10408444.2019.1579169. https://doi.org/10.1080/10408444.2019.1579169

[73] Bunke, D., Groß, R., Kalberlah, F., Oltmanns, J., Schwarz, M., Reihlen, A., Reineke, N., 2014. 4M, 'Mixtures in the Environment. Development of assessment strategies for the regulation of chemicals under REACH', Environmental Research of the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety. Project No (FKZ) 3711 63 429 https://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/texte_65_2014_aust_hassold_mixtures_in_the_environment.pdf

[74] Evans, R.E., O.V. Martin, M. Faust, A. Kortenkamp (2016): Should the scope of human mixture assessment span legislative/regulatory silos for chemicals? Science of the Total Environment 543 (2016): 757-764. https://www.sciencedirect.com/science/article/pii/S0048969715309785

- Common exposure routes and/or potential for emission/exposure, *e.g.* chemicals which workers are exposed to in occupational settings
- Common areas of use and/or technical functionalities, *e.g.*, chemicals occurring in certain consumer products.

Chemicals belonging to such groups may be more likely to occur together and hence be subject to co-exposure, or have similar toxicities and hence demonstrate cumulative effects. Thus, risk management of such groups of chemicals contributes to management of some unintentional mixtures.

There are some examples of legal provisions applying grouping approaches in the assessment or regulation of certain chemicals together based on physical-chemical (and/or structural) similarities combined with common routes of exposure and/or potential for co-exposure. These provisions address some particular cases of exposure to specific mixtures and/or aggregate exposure, without requiring an actual mixture assessment.

One example is the Drinking Water Directive (98/83/EC), which establishes standards for individual substances and groups of substances, like pesticides. In the context of the revision of the Directive, the possibility of requiring actual mixture assessments was discussed. It was however finally decided to keep the approach with lists of substances rather than move toward assessments of combined exposure for individual water supplies. The revised Directive will however require risk assessments and monitoring of two additional groups of substances: PFAS and haloacetic acids. The Groundwater Directive also includes a standard for total pesticides.

The Industrial Emissions Directive (IED) (2010/75/EU) lacks detailed provisions for risk assessment of chemical pollution. The assessment may be based on knowledge produced in other contexts (e.g. under REACH). This can include grouping or concepts for the assessment of mixtures of structurally similar substances for the setting of limit values, e.g. toxic equivalency factors (TEF), which has been used for the dioxin-group. The same approach has been used for dioxins also under the Water Framework Directive.

Addressing groups of substances is possible and is increasingly becoming normal practice under REACH, which contains specific provisions for grouping.. The grouping of similar substances is mentioned in connection with substance evaluation (Article 47). The testing and assessment requirements may be modified for a group of structurally similar substances, hence limiting the testing to one or a few substances, while predicting the properties of the other substances in the group based on these tests (Article 13; Annex I point 0.4 and; preamble of Annexes VII to X Annex XI). REACH Article 62(3) allows submitting applications for authorisations for groups of substances. There are several examples of authorisations and restrictions of groups of similar substances such as metal compounds, PAHs, phthalates and PFASs (see examples of restriction in Section 5).

3.4 Aggregate Exposure

Aggregate exposure refers to the exposure to the same substance from different independent sources and/or via different pathways. It is related to and hence often considered in connection with unintentional chemical mixtures. Clear legal requirements for performing aggregate exposure assessments across different regulatory sectors currently exist only in some pieces of chemicals legislation (*i.e.* legislation focused on chemical substances and intentional mixtures), and is hence not normally and systematically taken into account.

Under REACH, aggregate exposure may be assessed when authorities evaluate a substance or consider authorisation (REACH, Title VII). There are examples of restrictions under REACH that are based on aggregate exposure of particular chemicals, such as perfluorooctanoic acid and phthalates.

The Cosmetics Regulation requires the consideration of aggregate exposure in the human health risk assessment of certain ingredients in cosmetics. According to the guidelines from the Scientific Committee on Consumer Safety (SCCS)[75], the evaluation of a cosmetic ingredient for the product safety report must take into account aggregate exposure of the ingredient from different types of cosmetic products.

The Regulation on food contact materials also requires a consideration of different sources of exposure to the same substance or group of substance to which a specific migration limit (SML) is applied. Consequently, several substances regulated in particular in plastic food contact materials acknowledge this and include an allocation factor that further restricts the amount of substance permitted to migrate into food.

Legislation oriented towards pollution and certain environmental media (*e.g.* water and air related pieces of legislation) normally consider the aggregate exposure to a substance, regardless of source. However, it does usually not consider exposure via all relevant environmental media or pathways.

3.5 Conclusions on existing legal requirements for intentional and unintentional mixtures, grouping and aggregate exposure

Regarding intentional mixtures, there are requirements in place in most pieces of chemicals legislation (chemical substance and intentional/commercial mixture focused legislation) as well as in one piece of products-specific legislation, namely the Toy Safety Directive.

Requirements on the assessment of unintentional mixtures are broadly absent in chemical substance and mixture focused legislation. The only exceptions, where requirements for the assessment of cumulative exposure to unintentional mixtures exist, is the directive on the protection of the health and safety of workers from the risks related to chemical agents at work, the regulation on the maximum residues levels of pesticides in food, the Plant Protection Products regulation and the Biocidal Product regulation. The requirements under the regulations on maximum residues levels and the Plant Protection Products are however not yet implemented as the methodology is still under development.

Requirements for the assessment of unintentional mixtures exist in a few additional pieces of legislation focused on products, pollution and environmental media. Out of the 15 assessed pieces of such legislation, six include a reference to the assessment of unintentional mixtures, regarding human health or the environment, or both. These references are however usually vague and general, with a lack of provisions and/or guidance for how to perform the assessment, and hence rarely implemented. In four of these pieces of legislation, the requirement only refers to particular groups of substances, *e.g.* dioxins or PAHs.

A few pieces of legislation provide for grouping approaches in the assessment of certain functionally or structurally similar substances, without requiring an actual mixture assessment. Aggregate exposure to the same substance from different sources and/or via different routes of exposure can be considered in substance evaluation and when considering

[75] SCCS notes for guidance point 3.4.3
https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_224.pdf

authorisation and restriction under REACH as well as in the regulations on cosmetics and biocides. It is not considered in any other pieces of chemicals or products related legislation. . However, aggregate exposure is normally considered in pollution and environmental media focused legislation, but usually limited to the particular environmental media or pathway in scope of the legislation.

Overall, the progress on legal requirements for the assessment of unintentional mixtures has been limited since 2012.

4. PROGRESS ON GUIDANCE AND REGULATORY ASSESSMENT

In the 2012 Communication (Section 5.3, paragraph 2), the Commission commits to coordinate the development of technical guidelines in order to promote a consistent approach for the assessment of priority mixtures across different pieces of EU legislation.

Some overarching guidance documents to support consistent methodologies were recently developed. These are based on the framework for the assessment of combined exposures to multiple chemicals presented by WHO/IPCS in 2011[76], and the methodology is summarized in the State of the Art Report in 2012. EFSA[77], likewise, published a comprehensive guidance document on mixture risk assessments in 2019, to support harmonised methodologies for the environment, animal and human health in the fields falling within EFSA's competence. The Commission also contributed to the 2018 Organisation for Economic Co-operation and Development (OECD) report on risk assessment of combined exposure[78], which describes all essential steps to be considered, independent of the legislative framework, the complexity of the mixture (intentional or unintentional), the scope of the assessment, and the assessor.

The approach for the assessment of chemical mixtures is in principle common to all guidance developed so far and applicable to both intentional and unintentional mixtures. It includes:

- Prioritisation methods and/or grouping approaches to select the components to be considered;
- Options to use (eco)toxicity and exposure data on the single components to predict mixture hazard and/or risks, through component-based approaches (Concentration Addition or Independent Action);
- Options to test the whole mixture as such and assess hazard and/or risk based on the test data;
- Tiered approaches to refine the assessment depending on the availability and quality of data and information on the mode of action.

Further **legislation-specific guidance documents for the assessment of mixtures** have been developed for some pieces of EU legislation in recent years. Currently, such guidance is available for plant protection products[79], biocidal products[80], and veterinary

[76] M.E. (Bette) Meek, Alan R. Boobis, Kevin M. Crofton, Gerhard Heinemeyer, Marcel van Raaij, Carolyn Vickers 'Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework' Regulatory Toxicology and Pharmacology 60 (2011) S1–S14 https://pubmed.ncbi.nlm.nih.gov/21466831/

[77] https://doi.org/10.2903/j.efsa.2019.5634 https://doi.org/10.2903/j.efsa.2019.5634

[78] OECD (2018). 'Considerations for Assessing the Risks of Combined Exposure to Multiple Chemicals', Series on Testing and Assessment No. 296, Environment, Health and Safety Division, Environment Directorate. http://www.oecd.org/chemicalsafety/risk-assessment/considerations-for-assessing-the-risks-of-combined-exposure-to-multiple-chemicals.pdf

[79] EFSA Guidance documents on risk assessment of plant protection products for mammals (2009) and bees (2013), EFSA Scientific Opinions (GDs for bees and birds and mammals, Scientific Opinions on risk assessment of plant protection

pharmaceuticals[81], in all cases including references to the risk assessment of intentional mixtures regarding either human health or the environment.

For human health assessment, guidance documents are also available for plant protection products and their residues with respect to Cumulative Assessment Groups (CAGs)[82,83] and probabilistic modelling[84] as well as for biocidal products[85]. Moreover, technical guidance on the classification of waste[86], which includes aspects of mixture assessment, was published in 2018 (see section 3).

In conclusion, there has been some progress on the availability of both general and legislation specific guidance since 2012. All the legislation specific guidance documents presented refer to the assessment of intentional mixtures, except for the guidance on pesticide residues in food and feed and some elements in the guidance on classification of waste.

5. CASES OF REGULATORY ASSESSMENT OF MIXTURES

The 2012 Communication concluded that methodologies for the identification of chemical mixtures of potential concern as well as for the assessment of chemical mixtures are available, although the extent of the assessment is limited by knowledge and data gaps. It also foresaw that information collected in the context of EU legislation, in particular REACH registration will contribute to reducing these gaps.

In 2014, the Commission (JRC) conducted a survey on methodologies for assessing the combined effect of chemicals[87], answered by 58 experts in risk assessment from 21 countries, representing among others, authorities, academia and industry, with experience in mixture risk assessment, in most cases for regulatory purposes. According to responses to the survey, most of the practical experience with mixture assessments exists in the area of plant protection products and, as regards intentional mixtures in the context of CLP and REACH. Concentration addition appeared as the most commonly used component-based approach.

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products for aquatic organisms (2013), sediment organisms (2015), non-target terrestrial plants (2014), non-target arthropods (2015) https://www.efsa.europa.eu

[80] European Chemicals Agency (ECHA) (2017), 'Guidance on the Biocidal Products Regulation - Volume IV Environment - assessment & evaluation', European Chemicals Agency, Helsinki, Finland. (Parts BþC) (Vol. II). https://echa.europa.eu/documents/10162/23036412/bpr_guidance_vol_iii_part_a_en.pdf/05e4944d-106e-9305-21ba-f9a3a9845f93

[81] EMA (2005) Guideline on pharmaceutical fixed combination products (EMEA/CVMP/83804/2005). https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-pharmaceutical-fixed-combination-products_en.pdf

[82] EFSA Panel on Plant Protection Products and their residues (PPR). 2013. Scientific opinion on the relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides. EFSA Journal. 2013;11(12):3472 https://www.efsa.europa.eu/en/efsajournal/pub/3472

[83] EFSA Panel on Plant Protection Products and their Residues (PPR). 2013. Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile (2014 update). EFSA Journal 2013;11(7):3293, 131:3293. https://www.efsa.europa.eu/en/efsajournal/pub/3293

[84] EFSA (2012) Panel on Plant Protection Products and their Residues (PPR); Guidance on the Use of Probabilistic Methodology for Modelling Dietary Exposure to Pesticide Residues. EFSA Journal;10(10):2839. doi:10.2903/j.efsa.2012.2839 https://www.efsa.europa.eu/en/efsajournal/pub/2839

[85] ECHA (European Chemicals Agency) (2017). Guidance on the biocidal products regulation, Volume III Human health - assessment & evaluation, Parts B and C, ECHA-17-G-25-EN https://echa.europa.eu/documents/10162/23036412/biocides_guidance_human_health_ra_iii_part_bc_en.pdf/30d53d7d-9723-7db4-357a-ca68739f5094

[86] Commission notice on technical guidance on the classification of waste (2018/C 124/01). https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52018XC0409(01)&from=EN

[87] Bopp S, Berggren E, Kienzler A, van der Linden S, Worth A (2015): Scientific methodologies for the combined effects of chemicals – a survey and literature review; JRC technical report EUR 27471 EN; doi:10.2788/093511 https://publications.jrc.ec.europa.eu/repository/bitstream/JRC97522/jrc_tech_rep_sci%20meth%20for%20mix_final.pdf

Cases of mixture risk assessment based on combined exposure and combined effects for regulatory purposes are generally important benchmarks for future action. A recent case of risk management for mixtures is the restriction of the four phthalates DEHP, BBP, DBP, DIBP under REACH[88]. The risk assessment of these, considering combined exposure, was based on biomonitoring data. The limit value of this restriction refers to the combined unintentional exposure to the four substances in articles. This is the first clear-cut example of a REACH restriction where the risk assessment considers combined exposure to structurally similar chemicals (*i.e.* in this report referred to as an unintentional mixture). A similar restriction is being introduced in food contact materials on the basis of the assessment of co-exposure to these phthalates by EFSA[89].

There are also some cases where groups of substances were considered and restricted together as a group, without actually assessing the risk from their combined exposure. Instead, the joint restrictions were based on similarities in their chemical structure (*e.g.* lead compounds and poly-aromatic hydrocarbons), hazard profile (*e.g.* CMR in consumer mixtures), uses (*e.g.* CMR in textiles) and exposure pathways. Further examples include the restriction of the phthalates DINP[90], DIDP[91] and DNOP[92] in toys and childcare articles[93], and the ongoing restrictions of chemicals in tattoo inks[94] and PFAS. Such restrictions can manage some risks related to combined exposure, but are also motivated by other aspects, *e.g.* the avoidance of regrettable substitution.

In addition, some experience relating to the risk assessment of unintentional mixtures, probabilistic modelling, and grouping of chemicals in cumulative assessment groups (CAGs) has been gained under the Regulation on Maximum Residue Levels of Pesticides. The first CAGs for pesticides that have acute effects on the nervous system[95] and chronic effects on the thyroid system[96] were recently published (see also Box 4).

Further cases of combined exposure to chemicals considered in a regulatory context relate to the area of food contaminants. These were mineral oil saturated hydrocarbons[97], hexabromocyclododecanes[98], non-ortho polybrominated biphenyls[99], marine biotoxins[100,101],

[88] ECHA (2017). Committee for Risk Assessment (RAC) and Committee for Socio-economic Analysis (SEAC) Opinion on an Annex XV dossier proposing restrictions on four phthalates (DEHP, BBP, DBP, DIBP); ECHA/RAC/RES-O-0000001412-86-140/F, March 2017 https://echa.europa.eu/documents/10162/e39983ad-1bf6-f402-7992-8a032b5b82aa

[89] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2019.5838

[90] diisobutyl phthalate.

[91] di-"isodecyl" phthalate (CAS No 68515-49-1) as well as 1,2benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich (CAS No 26761-40-0)

[92] di-n-octyl phthalate

[93] ECHA (2013): Evaluation of new scientific evidence concerning DINP and DIDP in relation to entry 52 of Annex XVII to REACH Regulation (EC) No 1907/2006 https://www.echa.europa.eu/documents/10162/13579/201308_echa_review_dinp_didp_final_report_en.pdf

[94] ECHA website (2019): Substances in tattoo inks and permanent make up https://www.echa.europa.eu/web/guest/registry-of-restriction-intentions/-/dislist/details/0b0236e180dff62a

[95] EFSA (European Food Safety Authority) (2020) Cumulative dietary risk characterisation of pesticides that have acute effects on the nervous system. EFSA Journal 2020;18(4):6087. EFSA Journal 2020;18(4):6087 https://www.efsa.europa.eu/en/efsajournal/pub/6087

[96] EFSA (European Food Safety Authority) (2020) Cumulative dietary risk characterisation of pesticides that have chronic effects on the thyroid, Scientific Report. EFSA Journal 2020;18(4):6088 https://www.efsa.europa.eu/en/efsajournal/pub/6088

[97] EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012a; Scientific Opinion on Mineral Oil Hydrocarbons in Food. EFSA Journal 2012; 10(6):2704, 185 pp. doi:10.2903/j.efsa.2012.2704 164. https://www.efsa.europa.eu/en/efsajournal/pub/2704

[98] EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on Hexabromocyclododecanes (HBCDDs) in Food. EFSA Journal 2011;9(7):2296, 118 pp. doi:10.2903/j.efsa.2011.2296 https://www.efsa.europa.eu/en/efsajournal/pub/2296

[99] EFSA Panel on Contaminants in the Food Chain (CONTAM), 2005. Scientific opinion on a request from the Commission related to the presence of non-dioxin-like polychlorinated biphenyls (PCB) in feed and food. The EFSA Journal 2005, 284, 137 pp. doi:10.2903/j.efsa.2005.284. https://www.efsa.europa.eu/en/efsajournal/pub/284

ergot alkaloids[102], polyaromatic hydrocarbons[103], pyrrolizidine alkaloids[104] and, more recently, PFAS[105].

Lastly, lists of known synergists (chemicals interacting so that (eco)toxicological effects are enhanced in cases of co-exposure) are being compiled and are supposed to be included in the respective Annexes of the Biocides and Plant Protection Products Regulations, to better address and regulate these compounds.

The findings in the JRC survey and other developments referred to above, demonstrate that knowledge, methods for the assessment of mixtures and experience in applying them are available in several areas. Regarding the MRL Regulation, knowledge and methodology (CAGs) are still being developed. However, as the development has proven to be extremely complex, CAGs are not yet applied for regulatory purposes under that Regulation.

Hence, there is in several areas an established methodology in principle ready to apply in a more systematic manner, although often limited by factors such as data gaps and absence of specific guidance. Grouping approaches can contribute to addressing some unintentional mixtures, and have been applied much more frequently than actual assessments of mixtures, *e.g.* under REACH and in the area of food contaminants.

6. INTEGRATED AND CO-ORDINATED ASSESSMENT OF MIXTURES ACROSS DIFFERENT PIECES OF LEGISLATION

6.1 Interface between different pieces of legislation

As already concluded in 2012 by the Commission, EU legislation does not provide for a comprehensive and integrated assessment of combined effects of different chemicals across the different pieces of legislation.

In 2016[106], the JRC reviewed **21 published case studies on risk assessments of real-life mixtures** for human health and the environment, all conducted between 2014 and 2016. Assessed cases covered a range of different substance classes and media. All studies addressed unintentional mixtures (apart from one looking at aggregate exposure). Most of them were based on monitoring data, a few on modelled exposure. Seven of the cases

[100] EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009a. Scientific Opinion on a request from the European Commission on Marine Biotoxins in Shellfish – Saxitoxin Group. The EFSA Journal 2009, 1019, 1–76. doi:10.2903/j.efsa.2009.1019. https://www.efsa.europa.eu/en/efsajournal/pub/1019

[101] EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009b. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on marine biotoxins in shellfish – pectenotoxin group. The EFSA Journal 2009, 1109, 47 pp. doi:10.2903/j.efsa.2009.1109. https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2009.1109

[102] EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012b. Scientific Opinion on Ergot alkaloids in food and feed. EFSA Journal 2012;10(7):2798, 158 pp. doi:10.2903/j.efsa.2012.2798. https://www.efsa.europa.eu/en/efsajournal/pub/2798

[103] EFSA Panel on Contaminants in the Food Chain (CONTAM), 2008. Scientific Opinion on a request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. The EFSA Journal 2008, 724, pp. doi:10.2903/j.efsa.2008.724. https://www.efsa.europa.eu/en/efsajournal/pub/724

[104] EFSA Panel on Contaminants in the Food Chain (CONTAM), 2011; Scientific Opinion on Pyrrolizidine alkaloids in food and feed. EFSA Journal 2011;9(11):2406, 134 pp. doi:10.2903/j.efsa.2011.2406. https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2011.2406

[105] EFSA (2020), Public consultation on the draft scientific opinion on the risks to human health related to the presence of perfluoroalkyl substances in food https://www.efsa.europa.eu/en/consultations/call/public-consultation-draft-scientific-opinion-risks-human-health

[106] Bopp, S. K.; A. Kienzler, S. van der Linden, L. Lamon, A. Paini, N. Parissis, A.-N. Richarz, J. Triebe, A. Worth (2016), Review of case studies on the human and environmental risk assessment of chemical mixtures. JRC EUR 27968 EN; https://publications.jrc.ec.europa.eu/repository/bitstream/JRC102111/jrc102111_jrc_tech-rep_mix%20case%20studies_2016_vf.pdf

involves chemicals across different classes and legislative sectors. Available risk assessment tools were successfully used in all the reviewed cases. The concept of Concentration Addition was applied as default to predict mixture toxicities, while the Maximum Cumulative Ratio approach was often used for prioritisation purposes and to decide whether only certain substances or a larger number of chemicals were responsible for the mixture risks. Several of the **case studies identified potential risks from mixtures** for human health and/or the environment (see Box 2).

According to the JRC review, the relatively few cases where regulatory assessment of unintentional mixtures is actually performed (see also Section 3), are usually limited to mixtures consisting of chemicals that fall under the same particular piece of legislation. Thus, no systematic identification, of (unintentional) real-life priority mixtures is currently performed. The regulatory risk assessment or risk management of real-life unintentional mixtures is accordingly performed only rarely and on an ad hoc basis, while available scientific case studies are isolated examples.

The 2016 JRC review concluded that 'so far there is no prospective risk assessment concerning chemical substances related to various regulatory sectors and/or uses, and although numerous chemicals fall under several regulatory frameworks (biocides, pesticides, REACH...), the potential for co-exposure is hardly assessed or taken into account in their risk assessment.'

A related issue is how to address aggregate exposure to the same chemical when this is regulated under different pieces of legislation. An example is the restriction of four phthalates under REACH (see also Section 5). The exposure to one of these phthalates (DEHP) from food intake was identified as the major source of exposure according to the assessment. This part of the exposure, falling under the Food Contact Materials Regulation[107], or other sources of exposure such as environmental contamination or food contaminants was not addressed in the REACH restriction.

Article 16(2) of the Water Framework Directive (WFD) specifies how risk assessments under existing legislation should be used as a basis for listing priority substances in surface waters. It also allows the use of a simplified risk-based assessment procedure taking into account evidence regarding the intrinsic hazard of the substance (aquatic toxicity and human toxicity via aquatic exposure routes), evidence from monitoring and other factors which may indicate widespread environmental contamination, such as production volumes or use patterns. Such risk-based assessments inevitably consider aggregate risk from different uses of the same substance, and in some cases also several substances if they are in a group for which the Environmental Quality Standard (EQS) is set for a marker substance or the sum of the concentrations of related substances. The listing of substances in the priority substances list under the WFD on the basis of such risk-based assessments should be taken into account when substances are approved/authorised or reviewed under several of the relevant pieces of sectoral legislation, in particular if the monitoring data subsequently obtained show that the EQS are still not being met. There are references to the WFD objectives in those pieces of legislation, and the obligation to consider the WFD findings was further specified in Article 7a of the EQS Directive in 2013. The risk-based assessment under WFD does not consider exposure via other routes, *e.g.* air, nor combined effects from unrelated substances (although a quality standard for the broad grouping of "pesticides" is set under the Groundwater Directive).

[107] ECHA (2017) Committee for Risk Assessment (RAC) Committee for Socio-economic Analysis (SEAC) Opinion on phthalates restriction, Opinion on an Annex XV dossier proposing restrictions on four phthalates (DEHP, BBP, DBP, DIBP), ECHA/RAC/RES-O-0000001412-86-140/F, Agreed 16 March 2017. https://echa.europa.eu/documents/10162/e39983ad-1bf6-f402-7992-8a032b5b82aa

The above findings and examples highlight the remaining need to develop risk management measures and coordination across all relevant sectors and uses, when chemicals involved are regulated through different pieces of legislation. Such an approach would require mutual acceptance or alignment of procedures and decisions on substances and mixtures as well as improved co-operation, including the sharing of data between involved EU agencies and Member State authorities.

The Commission has recently engaged in a number of evaluations of EU chemicals related legislation, which in several cases look at challenges related to combined exposure and assessment of mixtures, including mixtures consisting of chemicals from across regulatory areas. Evaluations looking at several pieces of legislation together, and the interplay between these, include the fitness check of most chemicals legislation except REACH[108, 109] (covering over 40 pieces of legislation), the fitness check of the EU water legislation[110,111], the evaluation of the EU legislation on plant protection products and pesticides residues[112] as well as the fitness check of the EU legislation on endocrine disruptors[113]. Evaluations of single pieces of legislation that consider how they address mixtures include those of the Occupational Safety and Hygiene (OSH) Directive[114] and the Detergents Regulation[115].

Several of these evaluations point to the need to develop the assessment of mixtures, including across regulatory areas. As such integrated assessment of chemical mixtures has not been achieved yet, further efforts are needed, including closing interface gaps between pieces of legislation, improve data availability, harmonise approaches and conduct further regulatory and scientific case studies on mixture assessment.

6.2 Case studies on priority mixtures

In the 2012 Communication, the Commission committed to develop a consistent approach to the **assessment of priority mixtures** across the different pieces of EU legislation. The Commission referred to a set of criteria and methodologies that could be applied to identify chemical combinations/mixtures that are priorities for further assessment proposed by its

[108] European Commission (2019). Report from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the regions, Findings of the Fitness Check of the most relevant chemicals legislation (excluding REACH) and identified challenges, gaps and weaknesses. COM(2019) 264 final. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52019DC0264&from=EN

[109] European Commission (2019), Fitness Check of the most relevant chemicals legislation (excluding REACH), as well as related aspects of legislation applied to downstream industries. SWD(2019) 199 final/2. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52019SC0199R(01)&from=EN

[110] European Commission (2019). Executive Summary of the Fitness Check of the Water Framework Directive, Groundwater Directive, Environmental Quality Standards Directive and Floods Directive. SWD(2019) 440 final https://ec.europa.eu/environment/water/fitness_check_of_the_eu_water_legislation/documents/SWD_2019_440_F1_SWD_FITNESS_CHECK_EXECUTIVE_SUMMARY_EN_V4_P1_1058675.pdf

[111] European Commission (2019). Fitness Check Evaluation of the Water Framework Directive and the Floods Directive. Executive Summary of the Support Study. https://ec.europa.eu/environment/water/fitness_check_of_the_eu_water_legislation/documents/Study%20report%20exec%20summary%20EN%20%20-%20TRI%20TEC6327EU.pdf

[112] European Commission (2020), Commission staff working document accompanying the document report from the commission to the European Parliament and the Council. Evaluation of Regulation (EC) No 1107/2009 on the placing of plant protection products on the market and of Regulation (EC) No 396/2005 on maximum residue levels of pesticides. SWD/2020/87 final https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52020SC0087&from=EN

[113] European Commission (2020), Commission staff working document on endocrine disruptors SWD (2020) 249 final.

[114] European Commission (2017) Ex-post evaluation of the European Union occupational safety and health Directives (REFIT evaluation). SWD(2017) 10 final. file:///C:/Users/Boijeur/AppData/Local/Packages/Microsoft.MicrosoftEdge_8wekyb3d8bbwe/TempState/Downloads/2_EN_autre_document_travail_service_part1_v3%20(2).pdf

[115] European Commission (2019), Executive Summary of the Evaluation of Regulation (EC) No 648/2004 of the European Parliament and of the Council of 31 March 2004 on detergents. SWD(2019) 299 final. https://ec.europa.eu/transparency/regdoc/rep/10102/2019/EN/SWD-2019-299-F1-EN-MAIN-PART-1.PDF

Scientific Committees SCHER, SCENIHR and SCCS in 2012[116]. The criteria proposed were intended to facilitate a focus on mixtures of potential concern. They included aspects such as: commercial mixtures with several components of concern; potential serious adverse effects of one or more chemicals at the likely exposure levels; likely large-scale and frequent exposure of humans or the environment; persistence in the body or the environment; information on potential interactions; prediction of similar modes of action; and mixtures containing one or more components without an effect threshold.

An experimental laboratory study from 2014[117] evaluated the effects of realistic mixtures of 14-19 different chemicals falling under different pieces of legislation (pesticides, industrial chemicals, biocides and pharmaceuticals) which may jointly occur in surface waters. These were analysed in concentrations based on their respective individual environmental quality standard (EQS) in 35 different standardised *in vitro* and *in vivo* test systems. Although the individual chemicals were all present at concentrations considered safe under current legislative practices, clear adverse effects of the mixtures were demonstrated in a number of organisms, including fish, frogs, crustaceans, algae and micro-organisms. Further, some EU-funded research projects have identified risks of mixtures to the environment, on the basis of available monitoring/exposure modelling methodology and hazard data (SOLUTIONS)[118], or to human health, based on measurements of human blood samples and *in vitro* and *in vivo* test systems (EDC-MixRisk)[119] (see two cases described in Box 2).

The selection of cases for the assessment of mixtures is usually driven by the availability of data on toxicity and exposure. Therefore, a limited selection of well-studied chemicals tend to be overrepresented in current case studies, which are hence unlikely to be representative of human and environmental exposures. To circumvent this, a more **systematic analysis of cases of mixture risk assessments** is warranted.

In conclusion, the assessment of unintentional chemical mixtures across regulatory areas as well as assessments to identify priority mixtures are rare and only performed on an *ad hoc* basis. Reasons for this include lack of regulatory requirements and coordination between sectors and regulatory bodies as well as absent or incomplete toxicity and exposure data.

7. CLOSING THE KNOWLEDGE GAPS – RECENT ACHIEVEMENTS

To address remaining knowledge gaps, the 2012 Communication[120] set out that the Commission would work further for a better understanding of the chemicals mixtures to which human populations and the environment are actually exposed. This includes reviewing data on toxicity and exposure collected under EU legislation or generated in research projects.

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[116] SCHER/SCENHIR/SCCS (2012): Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENHIR) and Scientific Committee on Consumer Safety (SCCS). Joint Opinion on the Toxicity and Assessment of Chemical Mixtures. Adopted on 14th December 2011. DOI: https://doi.org/10.2772/21444

[117] Carvalho, R.N., A. Arukwe, S. Ait-Aissac, A. Bado-Nilles, S. Balzamo, A. Baun, S. Belkin, L. Blaha, F. Brion, D. Conti, N. Creusot, Y. Essig, V. E. V. Ferrero, V. Flander-Putrle, M. Fuerhacker, R. Grillari-Voglauer, C. Hogstrand, A. Jonas, J. B. Kharlyngdoh, R. Loos, A.-K. Lundebye, C. Modig, P.-E. Olsson, S. Pillai, N. Polak, M. Potalivo, W. Sanchez, A. Schifferli, K. Schirmer, S. Sforzini, S.R. Stuerzenbaum, L. Søfteland, V. Turk, A. Viarengo, I. Werner, S. Yagur-Kroll, R. Zounkov, T. Lettieri (2014), 'Mixtures of Chemical Pollutants at European Legislation Safety Concentrations: How Safe Are They?', Toxicological Sciences, 141(1), 2014, 218–233; doi: 10.1093/toxsci/kfu118. https://pubmed.ncbi.nlm.nih.gov/24958932/

[118] Research project funded under EUs 7th Framework Program for Research and Innovation.

[119] Research project funded under EUs Research and Innovation Program Horizon 2020.

[120] Operational follow-up (Section 5.3, paragraph 3)

The Communication also committed to the creation of a platform for chemical monitoring data, *e.g.* to help link exposure and epidemiological data on humans and the environment.

Further, the Communication also set out how to address some of the other knowledge gaps, in particular: (i) The mode of action of chemicals; (ii) Criteria for grouping chemicals into categories or assessment groups; (iii) Predicting interactions; and (iv) Identifying chemical substances that are the main drivers of mixture toxicity. Below is a brief summary of progress in these areas.

The Communication also concluded that the assessment of chemical mixtures must be carried out with respect to the principle of reducing, refining and replacing testing on vertebrate animals.

To address the identified gaps, the Commission has among other activities funded several research projects, most of them under its Research and Innovation Program Horizon 2020. The outcome of these projects was recently summarised in two scientific publications[121, 122] and is further described below. Some of the findings from two of these research projects are also described in Box 2.

7.1 Effect, exposure and hazard data

The Commission developed the **Information Platform for Chemical Monitoring (IPCHEM)**[123] in response to the major **lack of monitoring and epidemiological data** to understand the co-exposures of humans, animals and the environment to chemicals, as identified in the 2012 Communication. IPCHEM was launched in a demonstration version in 2014 and in a full, publicly available version in 2015 (see Box5).

Recent EU-funded research projects also provide some comprehensive data sets on epidemiological data (*e.g.* EDCMixRisk, EuroMix, HBM4EU, all funded via Horizon 2020) and chemical monitoring and co-exposure modelling data for the environment (*e.g.* SOLUTIONS funded under the 7th Framework Programme for research and innovation).

An important step forward regarding the availability of chemicals toxicity data is the finalisation of the three major rounds of registration of chemicals on the EU market under REACH. However, significantgaps remain in this area (*cf.* below, *Remaining knowledge gaps*).

Currently, databases of the respective European agencies working in the chemicals field provide data on exposure, uses, compositions, substance properties and hazards for regulatory purposes under different frameworks. These include the ECHA databases for REACH and biocides, the EFSA data warehouse, the EEA databases on water as well as data from Member States. Moreover, the EU is also continuously contributing to international activities to bring hazard databases together, such as the OECD eChemPortal and OECD activities towards a 'Global Chemicals Knowledge Base'[124] .

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[121] Bopp, S.K., R. Barouki, W. Brack, S. Dalla Costa, J.-L.C.M. Dorne, P. E. Drakvik, M. Faust, T.K. Karjalainen, S. Kephalopoulos, J. van Klaveren, M. Kolossa-Gehring, A. Kortenkamp, E. Lebret, T. Lettieri, S. Nørager, J. Rüegg, J.V. Tarazona, X. Trier, B. van de Water, J. van Gils, Å. Bergman, 'Current EU research activities on combined exposure to multiple chemicals'; Environment International 120 (2018) 544–562.
https://www.sciencedirect.com/science/article/pii/S0160412018308420

[122] Stephanie K. Bopp, Aude Kienzler, Andrea-Nicole Richarz, Sander C. van der Linden, Alicia Paini, Nikolaos Parissis & Andrew P. Worth (2019) 'Regulatory assessment and risk management of chemical mixtures: challenges and ways forward, Critical Reviews in Toxicology', 49:2, 174-189, DOI: 10.1080/10408444.2019.1579169.
https://doi.org/10.1080/10408444.2019.1579169

[123] https://ipchem.jrc.ec.europa.eu/

[124] OECD (2019) 'A Proposed Vision for a Global Chemicals Knowledge Base, including the evolution of existing related OECD tools'. ENV/JM(2020)2.

The improved **exchange of available data** across regulatory areas and bodies involved is also crucial for the risk assessment of mixtures. To explore the possibility of enhancing the transparency and exchange of data, the Commission has launched a feasibility study on a common open platform for chemical safety data based on a request from the European Parliament[125].

Box 5. The European Commission Information Platform for Chemical Monitoring (IPCHEM)

The 2012 Communication highlighted the need to improve the understanding of human and environmental exposure to chemical mixtures. One of the operational follow-ups set out in response to this need was to promote a more coherent approach to the generation, collection, storage and use of chemicals monitoring data, through the creation of a platform for such data. The Commission developed the Information Platform for Chemical Monitoring (IPCHEM) in direct response to this.

The overall purpose of IPCHEM is to help identify links between chemical exposure, including co-exposure, and the impact on human and ecosystem health. The objectives of the platform include improving access to monitoring data for policy makers and scientists, to host such data and to make it available at a defined quality (regarding spatial, temporal, methodological and metrological traceability). IPCHEM also aims at improving data quality and comparability, thereby facilitating risk assessment practices under EU policies. Other areas where the platform can be useful includes evaluation of policy effectiveness, the design of future monitoring programmes and for the analysing of collected data.

IPCHEM provides a single access point for a wide range of chemical monitoring data, originating from European Commission bodies and Member States, as well as international and national organisations and the research community. An example is the EU-funded human biomonitoring project HBM4EU[126], which shares collected data through the platform. IPCHEM is cross-linked with other reference information systems (*e.g.* the OECD eChem Portal, ECHA-REACH info card, PubChem), thus also facilitating the search for chemicals hazard data relevant to the environment and human health.

IPCHEM is structured into four thematic modules: environmental monitoring, human biomonitoring, food and feed, products and indoor air. From the platform, it is possible to search by chemical substance, environmental media and country. IPCHEM also includes a search tool for joint-occurrences of multiple chemicals at a particular location, which is particularly useful in relation to mixtures.

At present, IPCHEM provides access to over 450 million data records, and continues to grow. The main challenges include harmonising the data and ensure their quality. To address these, specific rules and procedures are implemented, and guidance is provided to data providers as well as end users.

[125] European Commmission (2019) 'Feasibility Study on a Common Open Platform on Chemical Safety Data'. https://etendering.ted.europa.eu/cft/cft-display.html?cftId=5516

[126] www.hbm4eu.eu

The Commission in 2017 arranged a workshop specifically aimed at enhancing the use of IPCHEM for the assessment of mixtures. The resulting report[127] identifies the need for a tighter collaboration and data sharing between research projects, scientific committees, Commission services and EU agencies. To enable this, there is a need to find solutions for limiting the access to restricted data to specific user groups. This requires legal, privacy or contractual obligations, as well as streamlining the efforts to harmonise the management and sharing of data between the different EU institutions involved.

7.2 Knowledge on mode of action, grouping, interactions and drivers of mixture toxicity

The areas mentioned in this sub-section relate to some of the knowledge gaps highlighted in the 2012 Communication (see above) and progress related to these. Improved knowledge in these areas is important to understand and assess the risks related to mixture toxicity, including what methodologies to apply. It is also essential to manage data gaps and to prioritise which chemicals to consider in mixtures assessments.

Information on the **mode of action (MOA) of chemicals** is used to predict possible adverse outcomes, to group chemicals based on their properties, or to close toxicological knowledge gaps via read-across approaches. Some improvements for an easier identification of the mode of action of chemicals have been made by using computer-based methods (sometimes called *in silico* methods, *e.g.* QSAR), as well as *in vitro* approaches and the Adverse Outcome Pathway concept[128].

Grouping of chemicals into categories is important to decide which components to consider in a mixture assessment. The grouping may be based on (eco)toxicological properties, mode of actions, chemical structure or physicochemical properties, as well as the type of product, use or exposure scenarios,*e.g.* joint exposures[129] (see also section 3.3).

In 2018, the Commission (JRC) published a dedicated report[130] on the MOA of existing priority substances under the Water Framework Directive and the possible use of effect-based methods in assessing chemical status. The report discusses how such methods could cover a wider range of chemical substances than currently included in the list by focusing more on their effects than on individual concentrations. The methods might be used in future for exploratory monitoring (*e.g.* for watch-list substances) or to capture better the true chemical status of surface waters based on appropriate trigger values for an adequate range of representative chemical-effect types or MOAs. Knowledge of MOA is important for linking exposure and eco-toxicological effects in the aquatic environment.

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[127] Silvia Dalla Costa, Stylianos Kephalopoulos, Stephanie Bopp, Aude Kienzler, Andrea Richarz, Sander Van der Linden, Peter Korytar, Thomas Backhaus, Erik Lebret, Jacob Van Klaveren, Hilko Van der Voet (2018), 'JRC Workshop on IPCHEM supporting the assessment of Chemical Mixtures - Final report', European Commission, Joint Research Centre, Ispra, PUBSY No.112434.
https://ipchem.jrc.ec.europa.eu/RDSIdiscovery/ipchem/documents/JRC112434_ipchem_mixtures_workshop_final_report_v12_final.pdf

[128] Adverse Outcome Pathway Knowledge Base (AOP-KB). https://aopkb.oecd.org/index.html

[129] Stephanie K. Bopp, Aude Kienzler, Andrea-Nicole Richarz, Sander C. van der Linden, Alicia Paini, Nikolaos Parissis & Andrew P. Worth (2019) 'Regulatory assessment and risk management of chemical mixtures: challenges and ways forward, Critical Reviews in Toxicology', 49:2, 174-189, DOI: 10.1080/10408444.2019.1579169.
https://doi.org/10.1080/10408444.2019.1579169

[130] Dorota Napierska, Isabella Sanseverino, Robert Loos, Livia Gómez Cortés, Magdalena Niegowska and Teresa Lettieri, (2018) 'Modes of action of the current Priority Substances list under the Water Framework Directive and other substances of interest, Review of the Relevant Modes of Action', JRC Technical Report. EUR 29008 EN
https://publications.jrc.ec.europa.eu/repository/bitstream/JRC110117/jrc_tech_report_moa_final__31may2018(8).pdf

Recent research in the area of pesticide residues has advanced the **Cumulative Assessment Groups method**, which is based on the chemicals involved having common target organs and effects (see also section 4). Developing such approaches can also help to assess and manage combined exposure, for instance to a group of structurally similar substances that are considered to have similar toxicological properties. This kind of approach can often be applied without performing an actual mixture assessment which involves the calculation of mixture toxicity (*e.g.* some REACH restrictions take this approach, see section 5). In addition to the specific usefulness of grouping approaches in connection with mixture assessment, they also contribute to the filling of data gaps (*i.e.* through the use of read-across and QSAR methods), to avoid regrettable substitution and to limit the need for animal testing[131]. Several **studies on interactions have been performed** during the last decade. Higher or lower mixture toxicities than predicted by the usual additivity approach may occasionally occur due to interactions between the individual chemicals, for example if chemicals influence each other's metabolism and thus (de)toxification processes in an organism. These phenomena are commonly referred to as synergisms or antagonisms. Several authors have reviewed the evidence of such interactions in real life mixtures. They concluded that interactions are rare at lower concentrations such as exposure via the environment. In observed cases, the interactions have also led to relatively small deviations, usually over or underestimations within a factor of four[132,133] compared to additivity-based predictions.

A systematic literature review on the topic of prediction of interactions was finalised in 2019, through a contract study for the Commission[134]. The review analysed 1220 eco-toxicological as well as mammalian/human health studies related to mixtures which were published since 2007. For a subset of 388 studies involving claims of interaction or providing indications of this, a quantitative reappraisal of the authors' evaluation was conducted. The results indicate that relatively few of the studies claiming synergistic or antagonistic effects show a deviation of observed mixture effects of more than two-fold from concentration addition based predictions if re-evaluated. However, a small proportion of the reviewed experiments that indicated synergism was considered significant and of regulatory relevance. No clear trend in factors, including chemical composition, that tend to be involved in cases of synergism was detected. However, the review confirmed earlier indications that synergism is particularly frequent among pesticide combinations. A conclusion of the study is that the concentration addition concept can be applied as default for predictive assessment of chemical mixtures. This can be complemented on a case-by-case basis by specific considerations for mixtures where interactions are anticipated based on the components.

The above finding confirms the recommendation by EFSA's Scientific Committee in its guidance for risk assessment of mixtures, to use Concentration Addition for the assessment of chemicals with similar as well as dissimilar modes of action in the context of both human health and environmental risk assessment[135].

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[131] Swedish Government Inquiries (2019) 'Future chemical risk management Accounting for combination effects and assessing chemicals in groups', Swedish Government Official Reports, SOU 2019:45 https://www.government.se/legal-documents/2019/11/sou-201945/

[132] Boobis A, Budinsky R, Collie S, Crofton K, Embry M, Felter S, Hertzberg R, Kopp D, Mihlan G, Mumtaz M, et al. (2011) 'Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment'. Crit Rev. Toxicol. 41(5):369–383. https://pubmed.ncbi.nlm.nih.gov/21309635/

[133] Cedergreen N. (2014), 'Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology', PLoS ONE. 9(5): e96580. doi:10.1371/journal.pone.0096580. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0096580

[134] European Commission 2019, 'Systematic Review of Ten Years of Research on Interactions in Chemical Mixtures of Environmental Pollutants - Final Report Service Contract CCR.F.933992.X0'. Olwenn Martin, Martin Scholze, Sibylle Ermler, Joanne McPhie, Andreas Kortenkamp

[135] EFSA Scientific Committee (2019): More SJ, Bampidis V, Benford D, Bennekou SH, Bragard C, Halldorsson TI, Hernandez-Jerez AF, Koutsoumanis K, Naegeli H, Schlatter JR, Silano V, Nielsen SS, Schrenk D, Turck D, Younes M, Benfenati E, Castle L,

Tools and criteria for **prioritising chemicals and identifying the main drivers** of mixture toxicity are also available. In recent EU-funded research projects, a selection of prioritising approaches were applied[136]. The 'Maximum Cumulative Ratio' (MCR)[137,138,139] approach is often used during regulatory and scientific assessments of intentional, as well as unintentional mixtures. In the context of human health assessment, information on Adverse Outcome Pathways and statistical and pharmacokinetic modelling is used. In the context of environmental assessment, the SOLUTIONS[140] project has proposed the use of different lines of evidence based on ecological, effect-based and chemical monitoring to identify priority pollutants in surface waters[141].

To **reduce costs, improve efficiency and replace animal testing**, alternative experimental and computational approaches have increasingly been introduced into (eco)toxicological assessments. In a 2014 Commission (JRC) survey on scientific methodologies for the combined effect of chemicals[142], experts confirmed that there is a high potential in further use of available novel tools and scientific methodologies for the assessment of chemical mixtures. Such methods include methods using *e.g.* cell cultures and biological molecules (*in vitro)*, computer-based simulation methods (*i.e. in silico*) and modelling approaches[143], which can be used in integrated approaches. They allow meaningful information on individual mixture components or whole mixtures to be derived, enabling a better understanding of the underlying mechanisms of mixture effects and prediction of combined effects and risks. On the same theme, the EU-funded project EuroMix[144] has proposed a tiered framework for the assessment of mixtures with a combination of *in silico* and *in vitro* tools.

In conclusion, there has been considerable progress in the development of the calculation and monitoring methods for mixture assessment, as well as approaches to manage data gaps and methods for prioritising substances for assessment. These methodologies so far mainly appear to be used in the research context, less for regulatory purposes. This situation is a

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Cedergreen N, Hardy A, Laskowski R, Leblanc JC, Kortenkamp A, Ragas A, Posthuma L, Svendsen C, Solecki R, Testai E, Dujardin B, Kass GEN, Manini P, Jeddi MZ , Dorne J-LCM and Hogstrand C, 'Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals', EFSA Journal 2019;17(3):5634,77 pp. https://www.efsa.europa.eu/en/efsajournal/pub/5634

[136] Bopp, S.K., R. Barouki, W. Brack, S. Dalla Costa, J.-L.C.M. Dorne, P. E. Drakvik, M. Faust, T.K. Karjalainen, S. Kephalopoulos, J. van Klaveren, M. Kolossa-Gehring, A. Kortenkamp, E. Lebret, T. Lettieri, S. Nørager, J. Rüegg, J.V. Tarazona, X. Trier, B. van de Water, J. van Gils, Å. Bergman, 'Current EU research activities on combined exposure to multiple chemicals'; Environment International 120 (2018) 544–562. https://www.sciencedirect.com/science/article/pii/S0160412018308420

[137] Price, P., Han, X., 2011, 'Maximum cumulative ratio (MCR) as a tool for assessing the value of performing a cumulative risk assessment', Int. J. Environ. Res. Public Health 2011 (8), 2212–2225. https://doi.org/10.3390/ijerph8062212

[138] Price, P., Dhein, E., Hamer, M., Han, X., Heneweer, M., Junghans, M., Rodriguez, C., 2012, 'A decision tree for assessing effects from exposures to multiple substances', Environ. Sci. Eur. 24, 26 2012. https://enveurope.springeropen.com/articles/10.1186/2190-4715-24-26

[139] MCR is the ratio of the cumulative toxicity received by an individual from exposure to multiple chemical stressors to the largest toxicity from a single chemical stressor. The MCR is a quantitative measure of the difference in an individual's toxicity estimated using a chemical-by-chemical approach and using an additive model of toxicity.

[140] https://www.solutions-project.eu/

[141] Brack et al. (2015), 'The SOLUTIONS project: challenges and responses for present and future emerging pollutants in land and water resources management', Sci Total Environ. 2015 Jan 15;503-504:22-31. doi: 10.1016/j.scitotenv.2014.05.143. https://pubmed.ncbi.nlm.nih.gov/24951181/

[142] Bopp S, Berggren E, Kienzler A, van der Linden S, Worth A (2015), 'Scientific methodologies for the combined effects of chemicals – a survey and literature review', JRC technical report EUR 27471 EN; doi:10.2788/093511. https://publications.jrc.ec.europa.eu/repository/bitstream/JRC97522/jrc_tech_rep_sci%20meth%20for%20mix_final.pdf

[143] The JRC report mentions the adverse outcome pathway (AOP) concept, in vitro methods, omics techniques, in silico approaches such as quantitative structure activity relationships (QSARs) and read-across, toxicokinetic and dynamic energy budget (DEB) modelling, and integrated approaches to testing and assessment (IATA).

[144] S. Rotter, A. Beronius, A. R. Boobis, A. Hanberg, J. van Klaveren, M. Luijten, K. Machera, D. Nikolopoulou, H. van der Voet, J. Zilliacus & R. Solecki (2019), 'Overview on legislation and scientific approaches for risk assessment of combined exposure to multiple chemicals: the potential EuroMix contribution', Critical Reviews in Toxicology, DOI: 10.1080/10408444.2018.1541964. https://www.tandfonline.com/doi/pdf/10.1080/10408444.2018.1541964?needAccess=true

consequence of a limitation of the methodologies, which require detailed, sensitive and high quality data on both toxicity and exposure, which is rarely available. This will continue to hamper the certainty of mixture assessment (see 7.3 Remaining knowledge gaps), as detailed data needed will be lacking also in foreseebale future.

One of the most significant scientific advancement in recent years, with direct regulatory implications, is that Concentration Addition can be used as the default method for the assessment of chemical mixtures with similar as well as dissimilar modes of action, in relation to risks to both human health and environment.

7.3 Remaining knowledge gaps

Progress has been made on the development of assessment methodologies, test methods and modelling approaches, as well as on improving the quality of and access to knowledge on toxicity and exposure to chemicals, as described above. Despite this progress, **significant knowledge and data gaps still remain in a number of areas, which will continue to hamper the assessment and risk management of mixtures.** These knowledge and data gaps, and the challenges in closing them, are linked to the wide variety of chemicals from different sources occurring in mixtures to which humans and the environment are exposed.

An important finding is that normally, a limited number of chemicals are responsible for the majority of the toxicity of a mixture. These chemicals are referred to as 'drivers of toxicity'. However, the identities of these chemicals and how much they contribute to the overall toxicity (their potencies) vary greatly between various mixtures and exposure situations. Hence, it is not sufficient to gather data only on high volume or 'priority chemicals'. Additional toxicity data will still be needed for a large number of substances to successfully assess mixtures, *e.g.* through calculation methods and *in-silico* modelling.[145]

The Communication of 2012[146], drawing upon the joint opinion of three EU Scientific Committees[147] and the State of the Art Report on Mixture Toxicity[148], highlighted that extensive knowledge gaps on toxicities and exposures to chemicals were limiting how accurately chemical mixtures could be risk assessed. The Communication mentioned that information to be collected in the context of EU legislation, in particular REACH, would contribute to reducing these uncertainties.

Indeed, the overall quality of and access to toxicity data has improved considerably during the last decade. The registration under REACH of chemicals present on the market (*i.e. phase-in chemicals*), was finalised with the registration deadline in May 2018[149]. Hence, a total of 23,023 unique chemicals, placed on the EU market in quantities over 1 tonne per producer/importer and year, had been registered by June 2020. This number will continue to grow as new chemicals produced or imported to the EU are continuously registered. The REACH database is the most comprehensive available worldwide.

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[145] Van Broekhuizen FA., Posthuma L., Traas TP. (2016). Addressing combined effects of chemicals in environmental safety assessment under REACH – A thought starter. National Institute for Public Health and the Environment, Bilthoven, The Netherlands, RIVM Report 2016-0162. www.rivm.nl/bibliotheek/rapporten/2016-0162.pdf

[146] Communication from the Commission to the Council, 'The combination effects of chemicals, Chemical mixtures' COM(2012) 252 final https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52012DC0252&from=EN

[147] Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENHIR) and Scientific Committee on Consumer Safety (SCCS). 'Joint Opinion on the Toxicity and Assessment of Chemical Mixtures adopted on 14th December 2011' https://doi.org/10.2772/21444

[148] Kortenkamp, A., Backhaus, T., Faust, M 'State of the Art Report on Mixture Toxicity', Final Report, Executive Summary, 22 December 2009 http://ec.europa.eu/environment/chemicals/effects/pdf/report_mixture_toxicity.pdf

[149] https://echa.europa.eu/-/21-551-chemicals-on-eu-market-now-registered

Despite this progress, hazardous properties and occurrence is sufficiently well characterised to allow appropriate mixture risk assessment only for a fraction of all chemicals produced and used. A general challenge is that chemicals are often transformed to other forms along their life-cycles (*e.g.* during use, through degradation in the environment and upon uptake in living organisms). Further, in addition to the exposure to chemicals in current use, there is also exposure to legacy chemicals, still occurring in and released from products and waste, and accumulating in humans and the environment.

It is not known exactly how many chemicals are produced and used worldwide, but estimates span from between 85,000 to over 140,000[150, 151, 152]. These chemicals can in principle occur in the human and natural environment. Another recent study shows that a total of approximately 350,000 individual chemicals and commercial/intentional mixtures are registered under different jurisdictions and traded globally.[153] It should be noted that chemicals of relevance for assessment of unintentional mixtures and combination effects are not only the ones subject to e.g. registration under REACH, but all kinds of chemicals that can occur in the environment, including pesticides, biocides, pharmaceuticals and chemicals used and imported in different kinds of products.

In line with the above findings, several authors conclude that risk assessment of unintentional mixtures continue to be hampered by gaps in, or lack of access to, hazard and exposure data on the individual chemicals that are the components of mixtures[154,155,156,157]. For many chemicals known or suspected to appear in environmental media, information on uses, releases and toxicity is either absent, unavailable or of poor quality. This problem relates to chemicals across uses and regulatory areas. In particular hazard characterizations are frequently incomplete, especially for specific endpoints. This problem is associated with the type and level of information required under different pieces of legislation as well as the lack of alignment of such requirements across legislation[158, 159].

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[150] UNEP (United Nations Environment Programme) (2019b) 'Global chemicals outlook II – from legacies to innovative solutions: Implementing the 2030 agenda for sustainable development'. https://www.unenvironment.org/resources/report/global-chemicals-outlook-ii-legacies-innovative-solutions

[151] The European Environment Agency's (EEA) (2019). The European environment – state and outlook 2020. Knowledge for transition to a sustainable Europe https://www.eea.europa.eu/publications/soer-2020/chapter-10_soer2020-chemical-pollution/view

[152] European Chemicals Agency (ECHA), 'Classification and Labelling Inventory'. Available from: https://echa.europa.eu/information-on-chemicals/cl-inventory-database

[153] Zhang, X., Sun, X., Jiang, R., Zeng, E.Y., Sunderland, E.M., and Muir, D., Screening New Persistent and Bioaccumulative Organics in China's Inventory of Industrial Chemicals. Environmental Science & Technology 2020 54 (12), 7398-7408 DOI: 10.1021/acs.est.0c01898 https://pubs.acs.org/doi/10.1021/acs.est.0c01898?ref=pdf

[154] Bopp, S.K., R. Barouki, W. Brack, S. Dalla Costa, J.-L.C.M. Dorne, P. E. Drakvik, M. Faust, T.K. Karjalainen, S. Kephalopoulos, J. van Klaveren, M. Kolossa-Gehring, A. Kortenkamp, E. Lebret, T. Lettieri, S. Nørager, J. Rüegg, J.V. Tarazona, X. Trier, B. van de Water, J. van Gils, Å. Bergman (2018): Current EU research activities on combined exposure to multiple chemicals; Environment International 120 (2018) 544–562 https://www.sciencedirect.com/science/article/pii/S0160412018308420

[155] Bopp, S. K. , A. Kienzler, S. van der Linden, L. Lamon, A. Paini, N. Parissis, A.-N. Richarz, J. Triebe, A. Worth (2016); Review of case studies on the human and environmental risk assessment of chemical mixtures; EUR 27968 EN; JRC report; doi:10.2788/272583 http://publications.jrc.ec.europa.eu/repository/bitstream/JRC102111/jrc102111_jrc_tech-rep_mix%20case%20studies_2016_vf.pdf

[156] Kortenkamp, A. and M. Faust (2018) 'Regulate to reduce chemical mixture risk - regulatory systems must better provide for risks from exposure to multiple chemicals', Science: 361 (6399):224-225; July 2018; DOI: 10.1126/science.aat9219, with supplemental material. https://science.sciencemag.org/content/361/6399/224.full

[157] Swedish Government Inquiries (2019). 'Future chemical risk management Accounting for combination effects and assessing chemicals in groups', Swedish Government Official Reports, SOU 2019:45. https://www.government.se/legal-documents/2019/11/sou-201945/

[158] European Commission (2019), 'Report from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the regions, Findings of the Fitness Check of the most relevant chemicals legislation (excluding REACH) and identified challenges, gaps and weaknesses', COM(2019) 264 final. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52019DC0264&from=EN

An issue related to the REACH registration requirements is the usually low level of knowledge and/or availability of data on substances produced and used in smaller volumes (1-10 tonnes per producer/importer and year)[160]. Such substances can represent a problem for mixture risk assessment as they despite being produced and used in low volumes can still appear as components of a mixture and thereby contribute to its toxicity. Further, some categories of chemicals including *e.g.* polymers and pharmaceuticals are exempted from registration under REACH. There are also issues related to a high degree of non-compliance and the low quality of registration dossiers under REACH[161,162]. Further, the publically available knowledge on occurrence and concentration of chemicals in articles[163] and related exposure is insufficient. Overall, there is a lack of knowledge of the actual amounts of individual chemicals produced, how much is used for different purposes and how much is released into different environmental media.

Case studies conducted as part of the SOLUTIONS research project[164] illustrate the toxicity knowledge gaps. In one example, a total of 227 chemicals were monitored at different locations in the river Danube. For 27% of these chemicals, experimental toxicity data for acute toxicity was available, assessed through the most common bioassay (Daphnia magna)[165]. For any other bioassay or test species and for chronic effects, data was considerably scarcer.

Regarding exposure data, **knowledge of the composition of the mixtures to which humans and wildlife are exposed and their effects, is often missing**. Overall, the extent of human and environmental monitoring of chemicals is still insufficient to provide adequate knowledge on exposure and occurrence of chemicals in humans, biota and environmental media. Chemical monitoring also has its limitations. For instance, generally only chemicals suspected to be present are detected by targeted or suspect screening analyses. Hence, the chemicals not analysed, including breakdown products or metabolites, which can contribute to a mixture effects might be overlooked. In addition, monitoring data allow retrospective assessments only, while detection of rising levels and effects will be delayed by years or decades after the emissions took place. In order to prospectively model co-exposure to unintentional mixtures, more information on the types and quantities of chemical uses is needed.

Examples of ongoing initiatives at the EU level that will contribute to improved chemicals exposure knowledge and data access includes HBM4EU and IPCHEM (see Section 7.1). Approaches that can contribute to filling toxicity data gaps include grouping and modelling methodologies.

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[159] European Commission (2019), Fitness Check of the most relevant chemicals legislation (excluding REACH), as well as related aspects of legislation applied to downstream industries. SWD(2019) 199 final/2. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52019SC0199R(01)&from=EN

[160] European Commission (2018) 'Commission General Report on the operation of REACH and review of certain elements, Comprehensive Evaluation Report, Conclusions and Actions', SWD(2018) 58 final, 1-7. https://ec.europa.eu/growth/sectors/chemicals/reach/review_en

[161] European Commission (2018) 'Commission General Report on the operation of REACH and review of certain elements. Conclusions and Actions', COM(2018) 116 final. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=COM:2018:0116:FIN

[162] European Chemicals Agency (ECHA), 'Evaluation under REACH: Progress Report 2017 10 years of experience', Reference: ECHA-18-R-05-EN https://echa.europa.eu/documents/10162/13628/evaluation_under_reach_progress_en.pdf/24c24728-2543-640c-204e-c61c36401048

[163] An 'article' is an object which during production is given a special shape, surface or design which determines its function to a greater degree than does its chemical composition' (REACH, Article 3(3). Examples of articles are electronics, vehicles and pieces of clothing.

[164] https://www.solutions-project.eu/

[165] Rico, A., Van den Brink, P.J., Leitner, P., Graf, W. and Focks, A., 2016, 'Relative influence of chemical and non-chemical stressors on invertebrate communities: a case study in the Danube River', Science of the Total Environment, 571, pp.1370-1382. file:///C:/Users/Boijeur/AppData/Local/Microsoft/Windows/INetCache/Content.Outlook/KOMTM6MB/1-s2.0-S0048969716315339-main.pdf

The 2018 EEA report 'Chemicals in European Waters – Knowledge developments'[166] provides an overview of recent findings, challenges and remaining knowledge gaps related to the assessment of chemicals mixtures in the aquatic environment. These include knowledge of the biological actions of pollutants and, information needed to establish the use of effect-based monitoring methods under *e.g.* the Water Framework Directive.

Activities to investigate the feasibility and potential implementations of effect-based monitoring method and tools under the Common Implementation Strategy for the Water Framework Directive started in 2012. The work to provide robust methodologies and to identify threshold values (trigger values) for methods developed is still ongoing, and has resulted in one scientific paper[167] and one report[168], while another report will soon be published.

7.4 Future research and development priorities

Several authors and projects have presented priorities and recommendations on future research and development needs. The project coordinators of the recent EU research projects (SOLUTIONS, HBM4EU, EDC-MixRisk, EuroMix) highlighted mixtures as a high priority for further research in a position paper in 2018[169]. The paper pointed out 12 key issues to be urgently tackled by scientists, regulators and industry. Their conclusions confirm the lack of data and difficulty to access available mixture exposure and hazard data for the components in a mixture (see Section 7.3), while also highlighting the need for validated and standardised test methods, for knowledge on the effects on vulnerable populations and on combined effects with other stressors as key knowledge gaps.

The EFSA Scientific Committee in 2019, in its general guidance on assessment of combined exposure, included a comprehensive set of recommendations on future research and development activities regarding mixture assessment[170]. These range from the development and validation of methods and tools for assessment to the improved access to and sharing of data and cooperation between scientists, EU agencies, member states and international bodies.

Box 6 compiles priorities and recommendations on research and development activities from a number of publications, including the ones mentioned above.

Box 6. Priorities and recommendations on research and development

[166] European Environment Agency (2018): Chemicals in European Waters – Knowledge developments; EEA report 18/2018; doi: 10.2800/265080 https://www.eea.europa.eu/publications/chemicals-in-european-waters

[167] Wernersson, A., Carere, M., Maggi, C. et al., 'The European technical report on aquatic effect-based monitoring tools under the water framework directive', Environ Sci Eur 27, 7 (2015). https://doi.org/10.1186/s12302-015-0039-4

[168] European Commission (2014), 'Technical report on aquatic effect-based monitoring tools', Technical Report - 2014 – 077. https://circabc.europa.eu/sd/a/0d78bbf7-76f0-43c1-8af2-6230436d759d/Effect-based%20tools%20CMEP%20report%20main%2028%20April%202014.pdf

[169] Altenburger, R., R. Barouki, Å. Bergman, W. Brack, E. Dravik, J. van Klaveren, M. Kolossa-Gehring, E. Lebret, J. Rűegg, B. van de Water (2018), 'Position Paper: Preventing risks for people and environment from hazardous mixtures'. https://edcmixrisk.ki.se/wp-content/uploads/sites/34/2018/05/Position-paper-180417-for-the-EC.pdf

[170] EFSA Scientific Committee (2019): More SJ, Bampidis V, Benford D, Bennekou SH, Bragard C, Halldorsson TI, Hernandez-Jerez AF, Koutsoumanis K, Naegeli H, Schlatter JR, Silano V, Nielsen SS, Schrenk D, Turck D, Younes M, Benfenati E, Castle L, Cedergreen N, Hardy A, Laskowski R, Leblanc JC, Kortenkamp A, Ragas A, Posthuma L, Svendsen C, Solecki R, Testai E, Dujardin B, Kass GEN, Manini P, Jeddi MZ , Dorne J-LCM and Hogstrand C, 'Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals', EFSA Journal 2019;17(3):5634,77 pp. https://www.efsa.europa.eu/en/efsajournal/pub/5634

A number of scientific papers, reports and position papers[171,172,173,174,175,176,177] discuss priorities for future research and development to support and facilitate the assessment and management of chemical mixtures. Some priorities and recommendations put forward in these include:

- Common efforts to generate and make toxicity, exposure and chemicals-monitoring data accessible. This includes data on the overall exposure of the European population.
- Structured approaches for collecting data on simultaneous and sequential exposure to mixtures.
- Research and development regarding modelling approaches to support mixture assessment.
- Increasing the availability of validated and standardised test methods and guidance, including such on how to apply new and innovative methodologies (e.g. *in vitro* and computer based methods) for mixture assessment.
- Develop methodologies and harmonised guidance on the grouping of chemicals.
- Guidance for analysing uncertainties in risk assessment of combined exposure to multiple chemicals, e.g. associated with the grouping into assessment groups and assumptions concerning similar MoAs/AOPs and application of dose addition
- Knowledge and methods on how to anticipate synergisms and antagonisms between chemicals, predict when such arise and need to be taken into account, and the development of criteria/guidance on this.
- Methodologies to integrate multiple lines of evidence, such as mixture toxicology and epidemiology, into risk assessment.
- Improve the knowledge about effects of mixture exposure on the heath and resilience of ecosystems, vulnerable populations and the importance of sensitive time-windows of exposure.
- Knowledge building about the combination of chemical stressors with other environmental and social stressors.

[171] Altenburger, R., R. Barouki, Å. Bergman, W. Brack, E. Dravik, J. van Klaveren, M. Kolossa-Gehring, E. Lebret, J. Rüegg, B. van de Water (2018), 'Position Paper: Preventing risks for people and environment from hazardous mixtures'. https://edcmixrisk.ki.se/wp-content/uploads/sites/34/2018/05/Position-paper-180417-for-the-EC.pdf

[172] Bopp, S.K., R. Barouki, W. Brack, S. Dalla Costa, J.-L.C.M. Dorne, P. E. Drakvik, M. Faust, T.K. Karjalainen, S. Kephalopoulos, J. van Klaveren, M. Kolossa-Gehring, A. Kortenkamp, E. Lebret, T. Lettieri, S. Nørager, J. Rüegg, J.V. Tarazona, X. Trier, B. van de Water, J. van Gils, Å. Bergman (2018): Current EU research activities on combined exposure to multiple chemicals; Environment International 120 (2018) 544–562 https://www.sciencedirect.com/science/article/pii/S0160412018308420

[173] EFSA Scientific Committee (2019): More SJ, Bampidis V, Benford D, Bennekou SH, Bragard C, Halldorsson TI, Hernandez-Jerez AF, Koutsoumanis K, Naegeli H, Schlatter JR, Silano V, Nielsen SS, Schrenk D, Turck D, Younes M, Benfenati E, Castle L, Cedergreen N, Hardy A, Laskowski R, Leblanc JC, Kortenkamp A, Ragas A, Posthuma L, Svendsen C, Solecki R, Testai E, Dujardin B, Kass GEN, Manini P, Jeddi MZ , Dorne J-LCM and Hogstrand C, 'Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals', EFSA Journal 2019;17(3):5634,77 pp. https://www.efsa.europa.eu/en/efsajournal/pub/5634

[174] Kortenkamp, A. and M. Faust (2018) 'Regulate to reduce chemical mixture risk - regulatory systems must better provide for risks from exposure to multiple chemicals', Science: 361 (6399):224-225; July 2018; DOI: 10.1126/science.aat9219, with supplemental material. https://science.sciencemag.org/content/361/6399/224.full

[175] UNEP (United Nations Environment Programme) (2019b) 'Global chemicals outlook II – from legacies to innovative solutions: Implementing the 2030 agenda for sustainable development'.

[176] S. Rotter, A. Beronius, A. R. Boobis, A. Hanberg, J. van Klaveren, M. Luijten, K. Machera, D. Nikolopoulou, H. van der Voet, J. Zilliacus & R. Solecki (2019), 'Overview on legislation and scientific approaches for risk assessment of combined exposure to multiple chemicals: the potential EuroMix contribution', Critical Reviews in Toxicology, DOI: 10.1080/10408444.2018.1541964 https://www.tandfonline.com/doi/pdf/10.1080/10408444.2018.1541964?needAccess=true

[177] Swedish Government Inquiries (2019). 'Future chemical risk management Accounting for combination effects and assessing chemicals in groups', Swedish Government Official Reports, SOU 2019:45 https://www.government.se/legal-documents/2019/11/sou-201945/

- Increased collaboration between scientists and regulators on translating science into policy.
- Establish long-term research programs on mixture toxicity on the EU level.

8. INTERNATIONAL ACTIVITIES

An overview of international activities relating to mixture assessment, focusing on available guidance documents and ongoing processes under international organisations, as well as on existing regulation and guidance in some countries outside the EU was prepared by JRC in 2014[178]. The Commission and EU Agencies have participated in several relevant international activities to promote consistent and science-based approaches to the risk assessment of mixtures. Besides the general benefit of the sharing of knowledge, experience and expertise, the joint international activities are important as they can contribute to internationally harmonised approaches to mixtures assessment and management in the long perspective.

The Commission contributed to the 2018 OECD consideration document on the risk assessment of combined exposures to chemicals[179], which describes the essential steps to be considered, independent of legal framework and kind of mixture, scope and assessor (see Section 4). Further, EFSA developed its 2019 guidance[180] on mixture risk assessments based on the WHO/IPCS framework[181] for the assessment of combined exposures to multiple chemicals from 2011, and organised an international consultation (see Section 4).

The involvement of the Commission and EU Agencies in the international activities includes the JRC's co-leading of a working group on Combined Exposures to Multiple Chemicals under the OECD Working Parties on Hazard Assessment and on Exposure Assessment. Further, the JRC together with several EU Agencies, was a member of a WHO informal working group on combined exposure under the WHO Chemical Risk Assessment Network, which was active on the mapping activities, facilitating collaboration, and identifying remaining gaps. The JRC is also coordinating an informal international research group on the testing of mixtures.

Further, the Commission and the EU agencies participate in international cooperation aimed at improving the knowledge base on chemical mixtures, toxicity and exposure information. This includes the OECD-led projects on combining different chemicals-related databases (see Section 7), *e.g.* the OECD eChemPortal[182] and the OECD activities towards a 'Global Chemicals Knowledge Base'.

[178] Kienzler, A., Berggren, E., Bessems, J, Bopp, S., van der Linden, S., Worth, A. (2014), 'Assessment of Mixtures - Review of regulatory Requirements and Guidance', JRC Science and Policy Report, EUR 26675 EN. http://publications.jrc.ec.europa.eu/repository/bitstream/JRC90601/lb1a26675enn.pdf

[179] OECD (2018). 'Considerations for Assessing the Risks of Combined Exposure to Multiple Chemicals', Series on Testing and Assessment No. 296, Environment, Health and Safety Division, Environment Directorate. http://www.oecd.org/chemicalsafety/risk-assessment/considerations-for-assessing-the-risks-of-combined-exposure-to-multiple-chemicals.pdf

[180] EFSA Scientific Committee (2019): More SJ, Bampidis V, Benford D, Bennekou SH, Bragard C, Halldorsson TI, Hernandez-Jerez AF, Koutsoumanis K, Naegeli H, Schlatter JR, Silano V, Nielsen SS, Schrenk D, Turck D, Younes M, Benfenati E, Castle L, Cedergreen N, Hardy A, Laskowski R, Leblanc JC, Kortenkamp A, Ragas A, Posthuma L, Svendsen C, Solecki R, Testai E, Dujardin B, Kass GEN, Manini P, Jeddi MZ , Dorne J-LCM and Hogstrand C, 'Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals', EFSA Journal 2019;17(3):5634,77 pp. https://doi.org/10.2903/j.efsa.2019.5634

[181] Meek, M.E. (Bette), Boobis, A.R., Crofton, K.M., Heinemeyer, G., van Raaij, M., Vickers, C. 'Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework' Regulatory Toxicology and Pharmacology 60 (2011) S1–S14 https://pubmed.ncbi.nlm.nih.gov/21466831/

[182] https://www.echemportal.org/echemportal/index.action

Box 6. Legal requirements, guidance and regulatory assessments in the USA and Canada

Requirements and approaches for a risk assessment and regulation of chemical mixtures also exist outside the EU, including legal obligations and guidance documents[183, 184]. In the USA several legal acts include requirements for the risk assessment of mixtures (*e.g.* the Comprehensive Environmental Response Compensation and Liability Act, the Food Quality Protection Act, and the Safe Drinking Water Act), and several guidance documents are available (*e.g.* health risks assessments for mixtures, simultaneous exposures from food, drinking water and non-occupational use of pesticides).

In Canada, requirements to consider aggregated exposures to pesticides in human health risk assessments (*e.g.* Pest Controls Product Act) as well as guidance documents (*e.g.* for the combined exposures of contaminated sites) are in place and several risk assessments for unintentional mixtures of certain groups have been performed.

International experience of regulatory requirements and implementation of these can be useful in the EU context.

9. POSSIBLE WAYS FORWARD TO ADDRESS CHALLENGES IN THE REGULATORY CONTEXT

Scientists, Member States and industry associations have repeatedly acknowledged the issue of mixtures and the need to address them. They have also provided several recommendations and proposals for approaches to address mixtures in the regulatory context.

On the industrial side, in 2016, the European Chemical Industry Council (Cefic), published a guidance document on a 'lead component identification methodology' (LCID)[185] to improve the communication of classified chemicals and mixtures in REACH safety data sheets. The methodology is also intended for the development of provisions for workers' safety and to establish which quantities are environmentally safe to use during production. Further, Cefic together with the German chemicals industry association (Verband der Chemichen Industrie, VCI) has published a practical guidance and examples of how the results from applying the Lead Component Identification (LCID) methodology can be incorporated into a mixture Safety Data Sheet ("SDS") under REACH[186]. Apart from this 'top-down' LCID method, another, 'bottom-up' approach has also been developed by industry, to identify information to communicate along the supply chain, called 'safe use of mixtures information' (SUMI). Sector organisations can apply this to identify the risk management measures for typical products and uses within the sector[187]. In 2018, Cefic also published a position paper[188],

[183] Kienzler, A., Berggren, E., Bessems, J., Bopp, S., van der Linden, A., Worth, A. (2014), 'Assessment of Mixtures – Review of regulatory Requirements and Guidance', JRC Science and Policy Report, EUR 26675 EN. http://publications.jrc.ec.europa.eu/repository/bitstream/JRC90601/lb1a26675enn.pdf

[184] Rotter, S., Beronius, A., Boobis, A. R., Hanberg, A., van Klaveren, J., Luijten, M., Machera, K., Nikolopoulou, D., van der Voet, H., Zilliacus, J. & Solecki, R. (2019), 'Overview on legislation and scientific approaches for risk assessment of combined exposure to multiple chemicals: the potential EuroMix contribution', Critical Reviews in Toxicology, DOI: 10.1080/10408444.2018.1541964. https://www.tandfonline.com/doi/pdf/10.1080/10408444.2018.1541964?needAccess=true

[185] CEFIC (2016): REACH Practical Guide on Safe Use Information for Mixtures under REACH - The Lead Component Identification (LCID) Methodology. https://cefic.org/app/uploads/2016/03/Practical-Guide-Safe-Use-Information-for-Mixtures-under-REACH_v6-1-1.pdf

[186] CEFIC and VCI, 'Mixtures under REACH – exemplification of the LCID output in the Safety Data Sheet', Final version 1.0 – 30. September 2019 (2016): https://cefic.org/app/uploads/2019/11/Mixtures-under-REACH-–-Exemplification-of-the-LCID-output-in-the-SDS-REPORT.pdf

[187] The Downstream Users of Chemicals Coordination Group (DUCC), 'DUCC/CEFIC pilot project on Exposure Scenarios and communication in the supply chain, Formulators phase: testing the SUMI selection approach

recognizing the need to address combination effects and to engage in the use of available tools for identifying drivers of toxicity, as well as prioritising and assessing mixtures via a tiered approach.

Regarding Member State initiatives, the German Environment Agency in 2017 published a report[189] focused on the risk assessment of chemical mixtures, in particular in the aquatic environment, in the context of REACH and its interfaces with other pieces of legislation. Different regulatory approaches for addressing mixtures are discussed in the report, including tiered approaches for intentional mixtures, the use of Mixture Assessment Factors (MAF) and the whole mixture testing approach.

In 2017, the government of the Netherlands proposed a regulatory approach involving a MAF to account for combination effects in the environmental risk assessment of substances under REACH regulation. This proposal was made based on a report from the Dutch institute for public health and the environment (RIVM)[190] providing detailed methodological considerations for application of this approach.

In 2019, the Swedish Government published a Special Inquiry Report[191] on risk management approaches to account for combination effects and enable assessment of chemicals in groups. The report provides 11 recommendations, including the introduction of specific and crosscutting legal requirements for mixture assessment, the application of a MAF in risk assessment, the increased use of grouping approaches in chemicals legislation and the improved sharing of data.

In 2020, a number of leading scientists in the area of mixtures toxicology published a statement on advancing the assessment of chemical mixtures and their risks. The statement highlights the need to strengthen the legal basis for mixture risk management, to strengthen coordination across regulatory bodies and sectors, to integrate component-based and whole mixture testing in risk assessment frameworks where possible and to apply pragmatic approaches, such as MAF, where conclusive mixture risk assessments cannot be routinely conducted due to significant knowledge and data gaps.[192] The statement also formulates key enablers for mixture risk assessments, such as availability of data and tools, and future research needs.

In 2020, the Netherlands together with the Swedish Chemicals Agency (KEMI) hosted a workshop with the aim of discussing possible pragmatic approaches to address the risks from combined exposure to unintentional mixtures under REACH. The participants agreed that a

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ENES action 4.1. – Report', DUCC, 13 December 2019. https://cefic.org/app/uploads/2020/01/DUCC-CEFIC-pilot-project-on-exposure-scenarios-and-communication-in-the-supply-chain-Pilot-Project-Report-on-Formulators'-phase-testing-the-SUMI-selection-approach.pdf

[188] Cefic Position Paper on Combination Effects of Chemicals, September 2018 https://cefic.org/app/uploads/2019/01/Combination-effects-of-chemicals-position-paper-POSITION-PAPER.pdf

[189] Bunke, D., Groß, R., Kalberlah, F., Oltmanns, J., Schwarz, M., Reihlen, A., Reineke, N., 2014. 4M,'Mixtures in the Environment. Development of assessment strategies for the regulation of chemicals under REACH', Environmental Research of the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety. Project No (FKZ) 3711 63 429 https://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/texte_65_2014_aust_hassold_mixtures_in_the_environment.pdf

[190] van Broekhuizen, F.A., L. Posthuma, T.P. Traas (2016): Addressing combined effects of chemicals in environmental safety assessment under REACH - A thought starter; RIVM Letter report 2016-0162 https://www.rivm.nl/bibliotheek/rapporten/2016-0162.pdf

[191] Swedish Government Inquiries (2019) Future chemical risk management Accounting for combination effects and assessing chemicals in groups. Swedish Government Official Reports, SOU 2019:45 https://www.government.se/legal-documents/2019/11/sou-201945/

[192] Dravik et al. (2020) 'Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment.' Environment International, Volume 134, January 2020, 105267. https://www.sciencedirect.com/science/article/pii/S0160412019331538#!

pragmatic approach is needed and there was a general support for the principle of applying a MAF. No other approaches to deal with unintentional mixtures were identified[193]. A follow up workshop is planned for the autumn 2020 to elaborate on practicalities of such approach.

Recommendations on ways forward including implementation, development of policy and methods as well as knowledge building activities, proposed by Member States, scientists and other stakeholders are summarised in Box 7.

Box 7. Compilation of recommendations by Member States, scientists and other stakeholders on way forward with risk management of chemical mixtures [194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205]

- Continue/step up the implementation of current regulatory schemes, *e.g.* the identification and risk management of substances of very high concern under REACH, thereby controlling individual chemicals and hence reducing some overall risks of combined exposure.
- Step up the use of currently existing regulatory options and methodologies for addressing (assessing and managing) groups of chemicals, and further develop such approaches in the context of relevant legislation.
- Introduce clear and explicit legal requirements for the risk assessment and management of mixtures in all relevant pieces of EU legislation.
- Develop a legal framework and a mechanism to improve the co-ordination across regulatory areas to address mixtures of chemicals subject to different pieces of legislation.

[193] Competent authorities of the Netherlands and Sweden (2020), Discussion paper for CARACAL provided by KEMI and the Netherlands. Towards a pragmatic procedure to regulate the risks of exposure to unintended combinations of chemicals in the EU. 34th Meeting of Competent Authorities for REACH and CLP (CARACAL) Open session, 31 March - 1 April 2020. Doc. CA/MS/34/2020, European Commission 25 Mars 2020. http://files.chemicalwatch.com/24%20-%20CA_MS_34_2020_a.p.10.2_Combination%20of%20chemicals.pdf

[194] Bunke, D., Groß, R., Kalberlah, F., Oltmanns, J., Schwarz, M., Reihlen, A., Reineke, N., 2014. 4M. Mixtures in the Environment. Development of assessment strategies for the regulation of chemicals under REACH. Environmental Research of the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety. Project No (FKZ) 3711 63 429 https://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/texte_65_2014_aust_hassold_mixtures_in_the_environment.pdf

[195] Drakvik et al. (2019): Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. Environment International 134 (2020) 105267 https://www.sciencedirect.com/science/article/pii/S0160412019331538?via%3Dihub

[196] Altenburger, R., R. Barouki, Å. Bergman, W. Brack, E. Dravik, J. van Klaveren, M. Kolossa-Gehring, E. Lebret, J. Rüegg, B. van de Water (2018): Position Paper Preventing risks for people and environment from hazardous mixtures https://edcmixrisk.ki.se/wp-content/uploads/sites/34/2018/05/Position-paper-180417-for-the-EC.pdf

[197] Kortenkamp, A. and Faust, M. (2018): Regulate to reduce chemical mixture risk - regulatory systems must better provide for risks from exposure to multiple chemicals; Science: 361 (6399):224-225; July 2018; DOI: 10.1126/science.aat9219, with supplemental material https://science.sciencemag.org/content/361/6399/224.full

[198] Evans, R.E., O.V. Martin, M. Faust, A. Kortenkamp (2016): Should the scope of human mixture assessment span legislative/regulatory silos for chemicals? Science of the Total Environment 543 (2016): 757-764. https://www.sciencedirect.com/science/article/pii/S0048969715309785

[199] van Broekhuizen, F.A., L. Posthuma, T.P. Traas (2016): Addressing combined effects of chemicals in environmental safety assessment under REACH - A thought starter; RIVM Letter report 2016-0162 https://www.rivm.nl/bibliotheek/rapporten/2016-0162.pdf ;

[200] Swedish Chemicals Agency KEMI (2015): An additional assessment factor (MAF) – A suitable approach for improving the regulatory risk assessment of chemical mixtures; Report 5/15 https://www.kemi.se/global/rapporter/2015/rapport-5-15.pdf

[201] Coors, A. et al. (2018): Prospective environmental risk assessment of mixtures in wastewater treatment plant effluents - Theoretical considerations and experimental verification; Water Research 140:56-66. doi: 10.1016/j.watres.2018.04.031

[202] Bopp et al. (2019): …challenges and ways forward; Crit. Rev. Toxicol.

[203] Kortenkamp, A. and Faust, M. (2018): Science 361

[204] European Environment Agency (2018): EEA report 18/2018

[205] Cefic (2018): Cefic Position Paper on Combination Effects of Chemicals. September 2018 https://cefic.org/app/uploads/2019/01/Combination-effects-of-chemicals-position-paper-POSITION-PAPER.pdf

- Continue the development of guidance on the assessment of intentional and unintentional mixtures for relevant pieces of legislation, with a focus on basic methodologies, including component based approaches, and the selection of an appropriate methodology.
- Combine screening for identifying combined exposures of concern and application of the Maximum Cumulative Ratio (MCR) approach in a tiered approach for the refinement of hazard and exposure assessment of combined exposures, which can be applied where data is available.
- Further elaborate, introduce and apply a MAF in human health and environmental assessment, as a science based, generic mean to take into account combined exposure.
- Perform monitoring of use, emissions and exposure (including co-exposure) to chemicals, through e.g. human, environmental and effect-based monitoring as well as modelling approaches, while improving public availability of the data collected and promoting the use of monitoring data in regulatory assessment.
- Further develop and apply new approaches/methodologies for chemical testing, including read-across and alternative methods to animal testing (e.g. *in vitro* and computational/*in silico* methods).
- Continue the mixture-related research, with a focus on applied research to harmonise and validate methods for concrete and practical approaches (on e.g. modelling and real-life exposure to mixtures, methodology for risk assessment, toxicological modes of action of chemicals, monitoring methods and epidemiological approaches to mixtures).

A common conclusion across many of the recommendations and inputs is that the current system for risk assessment fails to take into account exposure to and effects of unintentional chemical mixtures. Given the large number of chemicals produced and used and the even bigger number of combinations of such, to which people and the environment are exposed, it is not feasible with an approach involving the testing, assessment and/or monitoring of vast number of individual unintentional mixtures. Further, although some progress has been made in improving the quality and access to such information over the last decade, considerable knowledge and information gaps remain regarding the toxicity and exposure to chemicals occurring in unintentional mixtures. These gaps applies to a wide range of different chemicals across classes and areas of use and will for a foreseeable future continue to hamper the detailed assessment of unintentional mixtures. Therefore, to make it possible to broadly and systematically address unintentional mixtures in a relatively near future, a practical and workable approach is needed, which can be implemented despite prevailing knowledge gaps. Several authors propose, as an element in their recommendations, the introduction of a MAF in relevant pieces of chemicals related legislation as such a solution (see Box 8).

Box 8: Mixture Assessment Factor (MAF)[206 ,207, 208, 209, 210]

[206] Kortenkamp, A. and M. Faust (2018) 'Regulate to reduce chemical mixture risk - regulatory systems must better provide for risks from exposure to multiple chemicals', Science: 361 (6399):224-225; July 2018; DOI: 10.1126/science.aat9219, with supplemental material. https://science.sciencemag.org/content/361/6399/224.full

[207] Martin, O.V., M. Scholze, A. Kortenkamp (2013): Dispelling urban myths about default uncertainty factors in chemical risk assessment – sufficient protection against mixture effects?, Environmental Health 12:53. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3708776/pdf/1476-069X-12-53.pdf

[208] Swedish Chemicals Agency KEMI (2015): An additional assessment factor (MAF) – A suitable approach for improving the regulatory risk assessment of chemical mixtures; Report 5/15. https://www.kemi.se/global/rapporter/2015/rapport-5-15.pdf

[209] Swedish Government Inquiries (2019). 'Future chemical risk management Accounting for combination effects and assessing chemicals in groups', Swedish Government Official Reports, SOU 2019:45. https://www.government.se/legal-documents/2019/11/sou-201945/

The application of a 'mixture assessment factor' (MAF), and the related concepts of 'mixture allocation factor' and the 'risk cup' approach, has been proposed by several authors to generically take into account mixture effects in chemicals risk assessments, where the available data are insufficient to allow an assessment of actual co-exposure situations. Such proposals discuss the application in particular under REACH, but also in other regulatory contexts. The MAF is by its proponents considered a pragmatic approach to mixture assessment, in order to manage the current situation of lack of the necessary toxicity and exposure data and make a systematic regulatory management of unintentional mixtures possible in a relatively near future.

When applying a MAF, exposure levels that are considered sufficiently safe for single chemicals are reduced by a certain factor (*i.e.* by MAF) to safeguard against risk from combined exposure to multiple chemicals. Hence, the MAF approach takes into account that the single chemical in real-life will be a component of a mixture as soon as it is released into the environment and that humans and wild organisms will be exposed to such mixtures of different chemicals, all contributing to the risk. The application of the MAF in chemicals risk assessment would target those substances and uses that contributes the most to the toxicity of the mixture (*i.e.* uses of substances with a high toxicity and/or a high exposure, and accordingly a high Risk Quotient). The MAF is proposed to be a way of addressing unintentional mixtures where sufficient knowledge or data are not available to allow an assessment of actual co-exposure situations. If such knowledge or data are available, the MAF can be replaced by scenario-specific factors or by more specific and targeted methodologies, when these are available.

The appropriate numerical value of the MAF is subject to a scientific discussion and different magnitudes have been proposed for its use in different contexts. An important task for science and authorities will be to gain further knowledge in order to set appropriate value(s) of the factor. Further ongoing discussion relates to at what stage of risk assessment should MAF be applied (to the DNEL[211] and PNEC[212] or to the Risk Quotient). Another question is how to use the MAF for substances lacking a dose threshold below which toxic activities does not occur (so called 'non-threshold substances').

The MAF approach has been already successfully applied for regulatory purposes. In the USA, a 'risk cup' approach is applied for pesticides under the Food Quality Protection Act[213]. This approach is also the basis for the allocation factors used for the relative source allocation during the setting of drinking water standards by the World Health Organisation.[214] An example from the EU is the use of a MAF for the derivation of environmental quality criteria for single substances in the Netherlands.[215]

[210] Van Broekhuizen FA., Posthuma L., Traas TP. (2016). Addressing combined effects of chemicals in environmental safety assessment under REACH – A thought starter. National Institute for Public Health and the Environment, Bilthoven, The Netherlands, RIVM Report 2016-0162. www.rivm.nl/bibliotheek/rapporten/2016-0162.pdf

[211] Derived No-Effect Level

[212] Predicted No-Effect Concentration

[213] US Congress (1996),'Food Quality Protection Act of 1996', Public Law No. 104–170, 110 Stat. 1489 (104th Congress, 3 Aug 1996).

[214]World Health Organization (WHO) 2017, 'Guidelines for drinking-water quality, 4th edition', www.who.int/water_sanitation_health/publications/drinkingwater-quality-guidelines-4-including-1st-addendum/en/

[215] Van Vlaardingen, Verbruggen, (2007), 'Guidance for the derivation of environmental risk limits within the framework of ''International and national environmental quality standards for substances in the Netherlands'' ', (INS), Revision 2007. RIVM report 601782001/2007, Bilthoven, The Netherlands. www.rivm.nl/bibliotheek/rapporten/601782001.pdf

10.CONCLUSIONS

The vast majority of **chemicals are used and occur as part of chemical mixtures**. Exposure of humans and the environment to mixtures of chemicals (intentional and unintentional) of anthropogenic origin is consequently the norm rather than the exception. A growing body of scientific evidence shows that combination effects do occur, in real-life exposure situations as well as in experimental studies involving realistic exposure levels, and that some of these co-exposures represent risks to humans and the environment.

Regulatory requirements and management of mixtures have not changed significantly since 2012. Most pieces of legislation focused on chemicals cover also intentional/commercial mixtures and require a risk assessment of such mixtures, although with varying scope (*e.g.* whether considering human health and/or the environment) and level of detail. Methodologies and guidance documents for the intentional/commercial mixtures are generally available.

Requirements to consider unintentional mixtures are broadly absent in legislation oriented towards chemicals and intentional/commercial mixtures. Explicit requirements for the assessment of unintentional mixtures (*i.e.* cumulative effects) exist in the directive on the protection of the health and safety of workers from the risks related to chemical agents at work and the regulation on Maximum Residue Levels of pesticides in food and feed, the Plant Protection Products and the Biocidal Products Regulations require to consider cumulative and synergistic effects. Implementation of the requirements of the regulation on Maximum Residue Levels of pesticides in food and feed and the Plant Protection Products has however not yet started, as methodology is still under development (see below).

Some reference to the assessment of unintentional mixtures as regards human health and/or environmental risks exists in a few additional pieces of legislation focused on products, pollution and environmental media. Except in relation to aggregate exposure to specific substances from several sources, and to certain groups of substances (*e.g.* dioxins or PAHs), many of these references are however rather vague and general; there are few specific requirements or guidance on how to perform the assessment.

Chemicals legislation does not systematically consider aggregate exposure across regulatory sectors (*i.e.* the exposure to one substance from different independent sources and/or via different pathways). Legislation oriented towards environmental media (*e.g.* water and air legislation) normally considers aggregate exposure, although limited to the environmental media/route of exposure on which the particular piece of legislation is focused.

Furthermore, **EU legislation still does not provide for a comprehensive and integrated assessment of the combined effects of different chemicals across different pieces of legislation**. An *ad hoc* working group of relevant Commission services and EU Agencies and Authorities (EFSA, ECHA, EMA and EEA) was established to strengthen co-ordination across the different pieces of legislation and to promote the integrated assessment of priority mixtures, taking into account the risks of human and environmental exposure. This group was used as a platform for information exchange on activities across the different pieces of legislation, while its core objective, to coordinate action on addressing of priority mixtures across legislation, remains to be implemented. Tools and criteria for identifying priority mixtures and main drivers of mixture toxicity are available and can be useful if further integrated into legislative and regulatory practices.

Progress has been made since 2012 on the development of methodologies for mixture assessment. The concept of Concentration Addition is widely accepted as the default

approach to predict mixture toxicities for human health as well as the environment. This includes cases where there are indications of possible interactions (synergism or antagonism).

In the context of setting maximum residue levels for plant protection products, EFSA opted for development of a specific methodology based on cumulative assessment groups with the intention of grouping pesticides based on their effects on all major organs/systems and of considering cumulative effects within those groups. So far, pesticides affecting two organs/systems – the thyroid and the nervous system – have been identified and grouped. A gradual implementation of the methodology is envisaged, starting by using existing groups to guide the monitoring of pesticide residues (as part of a retrospective assessment). In parallel, the methodology will be further developed in the coming years to cover more organs and systems and to cover prospective risk assessment in view of regulatory decisions on MRL setting. With the experience gained in those processes, at a later stage expansion to the approval/authorisation processes for plant protection products could be considered, if feasible.

There has also been **progress on the development of guidance documents**. The basic tools for assessing and managing risks from intentional as well as unintentional mixtures are therefore available to EU agencies and Member State authorities. However, despite the progress on methodologies and guidance for assessing mixtures, a remaining key challenge is, that **as long as the chemical compositions of unintentional mixtures are largely unknown,** including the identity of the chemicals and their concentrations, **mixture assessment methodologies such as concentration addition cannot be generally applied across legislation**. The reason can for example be that information on the composition of mixtures occurring in environmental media (*e.g.* wastewater or waterbodies) may not be available..

Some progress has been made in addressing data gaps on occurrence and exposure to chemicals. The Information Platform for Chemical Monitoring (IPCHEM) was established to promote a more coherent approach to the generation, collection, storage and use of chemical monitoring data in relation to humans and the environment. It provides centralised access to monitoring data held by the Commission Services, EU Agencies and Member States and offers a tool to assess co-occurrence of substances. The platform already provides significant amounts of data but more effort is needed to finalise making data from EU Agencies and Member States available and improve the applicability of the tools available.

Data on hazard and intrinsic properties of chemicals have also been improved. The main reason for this progress is that manufacturers and importers has fulfilled the obligation under REACH to register substances used on their own or in mixtures. The obligation was fulfilled in June 2018 and this has improved the situation as regards the availability of data on the toxicity and exposure to chemicals. However, despite these improvements, **considerable data gaps on the toxicity of components of unintentional mixtures, as well as on exposure to such mixtures remain.** The gaps relates to chemicals across uses and regulatory areas (*i.e.* in addition to chemicals subject to REACH registration, also *e.g.* pesticides, biocides, pharmaceuticals, substances in imported products) that can occur in unintentional mixtures, and these gaps will likely remain for a long time. **This will continue to limit the extent to which unintentional mixtures can be properly assessed**. Approaches, such as grouping of chemicals, read-across, *in-silico* and modelling will be important to fill these data gaps, although a prerequisite for benefiting more from them is to improve our knowledge on where chemicals are used and in what quantities. **Effect-based *in-vitro* methods have also been improved,** and show some promise as tools to assess mixture effects across legislations, but would need to be further improved in terms of sensitivity and their implementation as risk management tools.

Significant progress has been made in the past years to close some knowledge gaps on the impact of mixtures. Although basic knowledge and tools are available and ready to use, more research is still important to further improve the knowledge base, as well as develop assessment methodologies and legal requirements. Continued research is crucial regarding the toxicity of mixture components, toxicological properties of and exposure to mixtures, standardised test methods, and knowledge on the effects on the environment and vulnerable populations. Further areas include human and environmental monitoring methodologies and improved monitoring data, knowledge on epidemiological aspects, modelling methodologies as well as on the combined effects with other stressors.

The Commission and EU Agencies have actively participated in international activities to promote consistent and science-based approaches to the risk assessment of chemical mixtures at a global level and to promote a level playing field around the world. The Commission and the EU Agencies remain involved in several on-going international activities, mainly under the auspices of OECD and WHO and often assume a leading role in these activities.

Additional efforts are needed to adequately address the challenges posed by unintentional mixtures. In particular, there is a need to introduce or strengthen provisions to take account of unintentional mixtures in relevant pieces of legislation, such as REACH, water, food additives, toys, food contact materials, detergents and cosmetics. In the current situation, where knowledge and availability of toxicity and exposure information on unintentional mixtures and mixture components is, and will remain for a long time ahead, fragmented and insufficient, there is a need to apply practical and workable approaches. The application of a mixture assessment factor (MAF) seems to be the most pertinent for industrial chemicals under REACH, but it is applicable also to other regulatory areas, where the available data are insufficient to allow an assessment of actual co-exposure situations. At the same time, there is a need to continue developing more specific and targeted methodologies, such as the one for pesticides, and applying them as soon as feasible. In addition, there is a need to continue filling the gaps on knowledge on exposures and toxicities and to improve coordination to tackle mixtures across different regulatory areas.