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Should the scope of human mixture risk assessment span legislative/regulatory silos for chemicals?

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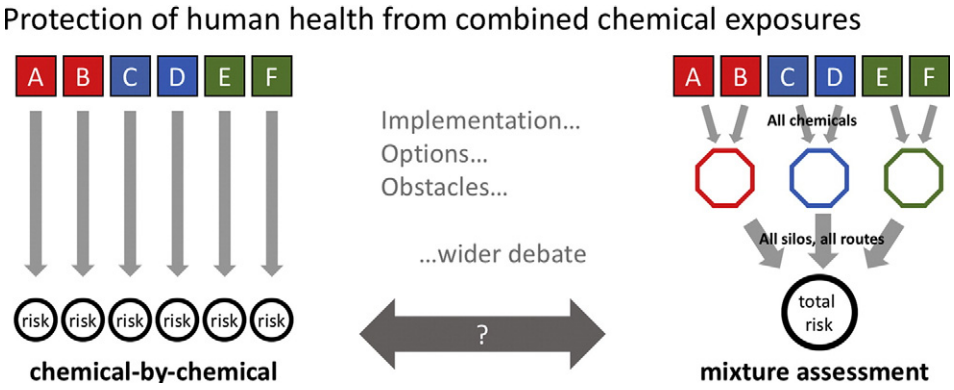
HIGHLIGHTS

- Humans are exposed to multiple chemicals covered by different EU regulations.
- Mixture effects that have been shown experimentally are not currently regulated.
- Combined human health risk from multiple chemicals/routes is not routinely assessed.
- Presented examples show the need for MRA to bridge regulatory 'silos'.
- A wider debate of options and obstacles in MRA implementation is desirable.

GRAPHICAL ABSTRACT



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ABSTRACT

Current chemicals regulation operates almost exclusively on a chemical-by-chemical basis, however there is concern that this approach may not be sufficiently protective if two or more chemicals have the same toxic effect. Humans are indisputably exposed to more than one chemical at a time, for example to the multiple chemicals found in food, air and drinking water, and in household and consumer products, and in cosmetics. Assessment of cumulative risk to human health and/or the environment from multiple chemicals and routes can be done in a mixture risk assessment (MRA). Whilst there is a broad consensus on the basic science of mixture toxicology, the path to regulatory implementation of MRA within chemical risk assessment is less clear.

In this discussion piece we pose an open question: should the scope of human MRA cross legislative remits or 'silos'? We define silos as, for instance, legislation that defines risk assessment practice for a subset of chemicals, usually on the basis of substance/product, media or process orientation. Currently any form of legal mandate for human MRA in the EU is limited to only a few pieces of legislation. We describe two lines of evidence, illustrated with selected examples, that are particularly pertinent to this question: 1) evidence that mixture effects have been shown for chemicals regulated in different silos and 2) evidence that humans are co-exposed to chemicals from different silos. We substantiate the position that, because there is no reason why chemicals allocated to specific regulatory silos would have non-overlapping risk profiles, then there is also no reason to expect that

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MRA limited only to chemicals within one silo can fully capture the risk that may be present to human consumers. Finally, we discuss possible options for implementation of MRA and we hope to prompt wider discussion of this issue.

1. Introduction and background

The current approach to chemical regulation routinely depends on assessment on a chemical-by-chemical basis, however there is concern that this approach may not be sufficiently protective if two or more chemicals have the same toxic effect on humans (Boobis et al., 2008; Kortenkamp et al., 2009). Under EU law, the only notable exception to the chemical-by-chemical paradigm is the Toxic Equivalency Quotient/Factor (TEQ/TEF) approach (van den Berg et al., 1998) in which dioxin-like chemicals, including selected polychlorinated biphenyls, dioxins and furans, are assessed collectively in regulations concerning maximum limits in food items (Regulation EC No 1881/2006 on setting maximum levels for certain contaminants in food). Nonetheless, this approach, as conceived, is limited to the risk assessment of a particular set of compounds (halogenated aromatic hydrocarbons) with a particular property (toxicological similarity to dioxin as manifested by AhR activation).

It is incontrovertible that humans are exposed to more than one chemical at a time, for example to the multiple chemicals found in food, in air and drinking water, and in household and consumer products and cosmetics. The unintentional exposure of humans to multiple chemicals through multiple routes constitutes the 'mixture' situation that is the focus of our interest in this discussion pieces; and our discussion does not apply directly to commercial products that contain multiple, defined ingredients, sometimes called 'intentional' mixtures. Although the existence of a mixture per se does not always indicate a risk to human or environmental health, experimental evidence of mixture effects with chemicals combined at low, ineffective levels (Kortenkamp, 2014) highlights that this should become the topic of assessments that examine whether more accurate estimations of risk will be produced by considering all of the chemicals that are present.

Mixture risk assessment (MRA) is the assessment of the cumulative risk to human health or the environment from multiple chemicals via multiple routes. Whilst there is a broad consensus on the basic science of mixture toxicology (Kortenkamp et al., 2009; DG Health and Consumer Protection, 2011), the path to regulatory implementation of these considerations, as an MRA, in chemical risk assessment is less clear. In the United States, guidelines on the Health Risk Assessment of Chemical Mixtures have existed for some time (EPA, 1986; 2000). In Europe, options were outlined in an opinion of the European Food Safety Authority (EFSA, 2008) and, currently, proposals for MRA approaches include a Framework developed by WHO/IPCS for "Risk assessment of combined exposure to multiple chemicals" (Meek et al., 2011), a decision tree of the European Commission Scientific Committees (DG Health and Consumer Protection, 2011) and an approach examining the contribution of individual mixture components to the joint effect, termed maximum cumulative ratio (Price et al., 2014). The German Federal Institute for Risk Assessment (BfR) have drafted a concept for how to take account of cumulative aspects in the context of the regulation of plant protection products and biocides (Stein et al., 2014).

In this discussion piece we pose an open question: should the scope of human mixture risk assessment (MRA) cross legislative remits? We have defined legislative remits, or regulatory 'silos', as the scope of single pieces of legislation that define the collection of toxicology or monitoring data for a subset of regulated chemicals, for example pesticides, biocides, food contaminants, food contact materials, pollutants and pharmaceuticals (Table 1). In the European Union, silos may be based on substance- or product-oriented regulations (e.g. pesticides, food contaminants, pharmaceuticals), media-oriented (e.g. water, soil etc.) or process-oriented pieces of legislation (e.g. industrial emissions). Currently any form of legal mandate for MRA in human health is limited to only a few pieces of legislation, or silos (e.g. maximum residue limits for pesticides in food; registration, evaluation and authorisation of chemicals (Kortenkamp et al., 2009)), and so it is likely that the scope of an MRA will naturally be set within a silo unless the need for a wider scope is recognised.

If the aspiration is the protection of human health from risks of all chemicals by all routes and uses, then two lines of evidence are

Table 1
Examples of regulatory remits ('silos') in European Union law.

	Remit ('silo')	Legislation	Type
General chemicals control	Authorisation of chemicals (REACH)	Regulation (EC) No 1907/2006	Substance-oriented
	Classification, labelling, packaging (CLP)	Regulation (EC) No 1272/2008	Substance-oriented
Special uses of chemicals	Pesticides authorisation	Regulation (EC) 1107/2009	Substance-oriented
	Biocidal products	Regulation (EU) 528/2012	Substance-oriented
	Human medicines	Directive 2001/83/EC	Substance-oriented
	Herbal medicines	Directive 2004/24/EC	Substance-oriented
	Veterinary medicines	Direction 2001/82/EC	Substance-oriented
Emission control	Pollution prevention and control	Directive 2008/1/EC	Process-oriented
	Industrial emissions	Directive 2010/75/EU	Process-oriented
	Environmental impact assessment	Directive 85/337/EEC	Process-oriented
Quality of environmental media	Water framework	Directive 2000/60/EC	Media-oriented
	Drinking water	Directive 98/83/EC	Media-oriented
	Air quality	Directive 2008/50/EC	Media-oriented
Food law	Food additives authorisation	Directive 89/107/EEC	Substance-oriented
	Food contact materials	Regulation (EC) No 1935/2004	Substance-oriented
	Pesticide residues	Regulation (EC) No 396/2005	Substance-oriented
	Food contaminants	Regulation (EC) No 1881/2006	Substance-oriented
	Feed additives authorisation	Regulation (EC) No 1831/2003	Substance-oriented
	Feed additives assessment	Directive 2001/79/EC	Substance-oriented
Non-food consumer products	General product safety	Directive 2001/95/EC	Substance-oriented
	Cosmetics	Directive 76/768/EEC	Substance-oriented
Occupational health	Workplace health and safety	Directive 89/391/EEC	Process-oriented

particularly pertinent to this question: 1) evidence that mixture effects have been shown for chemicals regulated in different silos and 2) evidence that humans are co-exposed to chemicals from different silos. We now describe selected examples to demonstrate the evidence available, without attempting an exhaustive review. We substantiate the position that, because there is no reason why chemicals allocated to specific regulatory silos would have non-overlapping risk profiles, then there is also no reason to expect that MRA limited only to chemicals within one silo can fully capture the risk that may be present to human consumers.

2. Mixture effects have been shown for chemicals regulated in different silos

When chemicals from two or more silos have been combined in in vitro or in vivo experiments, mixture effects have indeed been observed. In this case 'mixture effects' are defined as either an effect that was greater than the most potent single chemical in the mixture, or an effect that was additive (i.e. conformed to a prediction made using a mathematical concept of additivity such as dose/concentration addition) or synergistic (Kortenkamp et al., 2009). For example, in vitro studies using human cell lines have shown additivity for the end-point of anti-androgenicity for mixtures of up to 30 components including chemicals used as pesticides, antioxidants, UV-filters, preservatives and plasticizers, and chemicals that are synthetic musks, parabens, polycyclic aromatic hydrocarbons, perfluorinated compounds and polybrominated diphenyl ethers (Ermler et al., 2011; Orton et al., 2014). These substances can be assigned to multiple regulatory domains in general chemicals control, special uses of chemicals, food law or non-food consumer products (see Table 1). Similarly, for the in vitro endpoint of estrogenicity, additivity has been shown for a mixture of 14 chemicals including endogenous hormones, pharmaceuticals, flame retardants, polycyclic aromatic hydrocarbons, phytoestrogens, cosmetic ingredients (parabens, UV filters) and plasticizers (Evans et al., 2012). The available evidence is not limited to endpoints related to receptor interactions, and extends, for example, to in vitro genotoxicity. A clear mixture effect was observed for the endpoint of micronuclei induction in Chinese Hamster Ovary (CHO) cells when five substances (the antihelminthic pesticide flubendazole and pharmaceuticals doxorubicin, etoposide, melphalan and mitomycin C) were combined at levels in the region of their individual effect thresholds (Ermler et al., 2014).

In vivo studies have also shown mixture effects for developmental toxicity endpoints such as nipple retention and reduced ventral prostate weight in male rats. For example, the EU project CONTAMED showed mixture effects of 13 chemicals including plasticizers, pesticides, UV-filters, cosmetic ingredients and pharmaceuticals (Christiansen et al., 2012; Isling et al., 2014; Axelstad et al., 2014). Interestingly, one in vivo study of endpoints relating to male sexual development observed a mild synergistic mixture effect (a mixture effect greater than that predicted by the concept of dose addition) for a mixture of four anti-androgens, including a plasticiser, di(2-ethylhexyl) phthalate, a pharmaceutical, finasteride, and two fungicides, vinclozolin and prochloraz (Christiansen et al., 2009). The chemicals included in these in vivo experiments can be assigned to multiple regulatory domains, such as general chemicals control, food law, consumer products and pharmaceuticals (see Table 1).

These examples show that chemicals such as pharmaceuticals, food contaminants, pesticide residues and cosmetic ingredients are able to produce mixture effects. The possibility that chemicals from diverse regulatory silos can act together to produce mixture effects is not considered in current chemicals regulation. Even chemicals within certain silos, such as regulations concerning maximum limits for food contaminants (Regulation EC No 1881/2006), are evaluated without considering mixture effects, with the exception of polychlorinated dioxins, furans and biphenyls.

We have previously supported the use of common toxic effect as the basis for grouping chemicals in an MRA, instead of using common structural features or criteria related to usage or regulation, in proposals for cumulative assessment of phthalate plasticizers (National Research Council (NRC), 2008) and pesticides (Kortenkamp et al., 2012). EFSA have recommended that pesticides that produce common adverse outcomes on the same target organ/system should be grouped together for the purpose of assessing cumulative risk in relation to maximum residue limit (MRL) setting (EFSA, 2013a). These proposals were developed in the context of distinct regulatory silos, but the underlying principles could also be applied across silos.

2.1. Chemicals that evoke the same toxic endpoint are regulated in different regulatory silos

Groups of chemicals that are known to evoke a particular toxic effect have been shown to belong to different silos. For example, chemicals known to be developmental neurotoxicants include industrial chemicals, persistent organic pollutants (POPs), metals and pesticides (Grandjean and Landrigan, 2014). Examples of developmental neurotoxicants from different silos are given in Box 1. Grandjean and Landrigan also identified 218 chemicals as neurotoxic, of which 27 were metals or inorganic compounds (some of which are regulated as food contaminants), 41 were organic solvents, 48 were other organic substances, and 102 were pesticides (Grandjean and Landrigan, 2014); shown visually in Box 2.

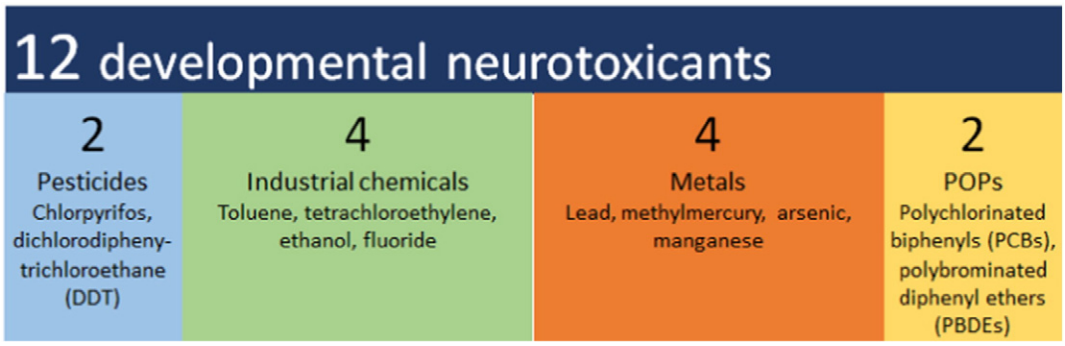
As a further example, Maffini and Neltner have listed over 300 chemicals with the potential to harm the developing brain, including through effects on the thyroid (Maffini and Neltner, 2015). The chemicals concerned belong to multiple regulatory silos relating to food and food quality, such as Pesticides, Food contact materials and Food additives including flavourings, colourings and preservatives; see Box 3.

These examples show that common, similar or related toxic effects can be shown for different chemicals that are regulated in different silos; and that the combined effects of these chemicals across silos are not currently considered by regulation.

3. Humans have been shown to be co-exposed to chemicals from different regulatory silos

Studies that measured multiple chemicals in human tissues have shown the presence of chemicals from several silos. For example, a biannual monitoring programme in the United States, the National Health and Nutrition Examination Study (NHANES), measures around 200 chemicals in blood and urine samples from around 10,000 people. The measured chemicals include persistent organic pollutants (POPs), such as polychlorinated biphenyls, dioxins and furans and polybrominated diphenyl ethers, phthalates, phenols, phytoestrogens, pesticides, volatile organic compounds and heavy metals. By way of a focused example, we identified one individual in whom 92 out of 136 chemicals were present at levels above the limit of detection (NHANES 2003–2004). Fig. 1 lists the detected chemicals, their groupings and shows the individual's exposure level (expressed numerically as a percentile of the exposure levels measured in the whole cohort) with a colour scale used to indicate exposures towards the top of the cohort distribution in red and orange, through exposures in the middle of the distribution in yellow and light green, to exposures towards the bottom of the distribution in dark green. Fig. 1 reveals that this individual's highest exposure percentiles were not restricted to any one class of chemicals because red shading is not limited to one group, and that their exposure level varied within each group of chemicals as shown by most groups having a range of colours from red through yellow to green — indicating that this individual was highly exposed to some chemicals of each group, but not to others.

Human breast milk is both a tissue and an exposure source for breast-fed infants. Breast milk has been found to contain chemicals



Box 1. Developmental neurotoxicants. Graphic shows four groups of chemicals identified as developmental neurotoxicants (Grandjean and Landrigan, 2014). Widths of coloured blocks are in proportion to the number of chemicals. The chemicals shown are subject to different pieces of legislation (Table 1), including pesticide residues (pesticides), REACH (industrial chemicals) and food contaminants (metals, PCBs). POPs are subject to a global treaty, the Stockholm Convention on Persistent Organic Pollutants, to which the EU are signatories.

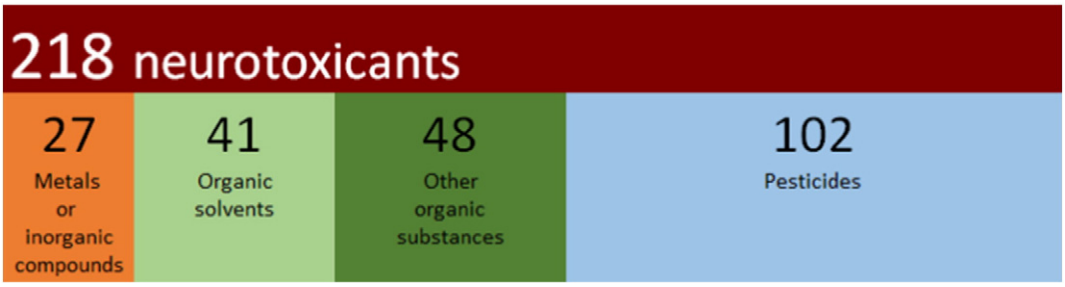
regulated as pesticides; as cosmetics, including UV filters, parabens and phthalates; and as persistent organic pollutants (POPs), including polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) (Schlumpf et al., 2010). Schlumpf et al. carried out a mixture risk calculation for infants consuming breast milk, using the Hazard Index (HI) approach (Teuschler and Hertzberg, 1995). The calculated HI was 66 and indicates that, because this value exceeds 1, the mixture risk is greater than acceptable at this level of assessment - a value of 1 would indicate a risk similar to that of a single chemical being present at its Reference Dose (the metric used as the 'acceptable' level in the HI calculation). Our presentation of this analysis in Fig. 2 makes it clear that the overall risk identified would not be accurately reflected by considering either the individual chemicals (grey bars) or the individual groupings (orange bars) alone. The chemical groupings include chemicals that are not found in any silo, and those found in different and multiple silos, for example: both parabens and phthalates are regulated as cosmetics whilst phthalates are also regulated as food contact materials; the maximum permitted residues of organochlorine pesticides and PCBs are regulated as pesticides and contaminants respectively. PBDE levels are not currently regulated under food law, however, the international Stockholm Convention on Persistent Organic Pollutants, which has been signed by the EU, applies for tetra- and pentabrominated diphenyl ethers, and REACH applies for PBDEs still in commercial use; in addition, quality criteria for PBDE levels in fish have been set under the Water Framework Directive for the purpose of protection of human health.

It is widely accepted that humans are exposed to multiple chemicals through multiple routes, and these selected examples show that multiple, diverse chemicals can indeed be measured in human tissues, including blood and breast milk. However, despite this, the joint risk to the human body from the total chemical load is not managed by regulation, or even routinely monitored.

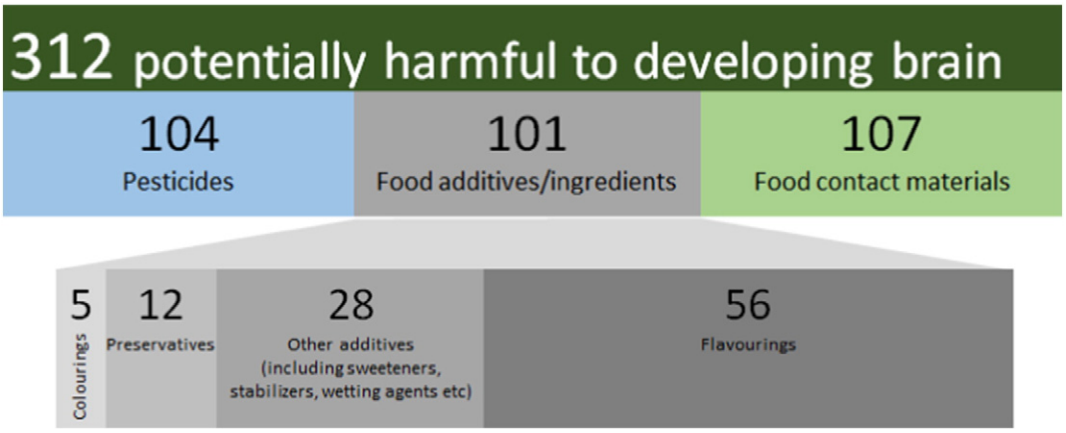
Exposure assessments are a major part of MRA, and there is a need for both better modelling to predict exposure, especially combined exposures and correlated exposures; and for wider data collection and surveillance. The mixture that 'matters' most is the one at the target tissue, and so neither intake (e.g. dietary exposure) nor biomonitoring (usually of blood or urine as proxies for the other human tissues) are sufficient alone. Considering this, it seems that data collection at this scale and level of complexity may well be impractical and so better modelling, that is precautionary but not overly risk averse, is likely to be crucial.

4. Discussion

We have presented two lines of evidence; firstly, that mixture effects have been observed and can be expected for chemicals that are regulated in different 'silos' and secondly, that humans are exposed to chemicals from different silos, and chemicals from different silos can be detected in human tissues. We contend that efforts to regulate chemicals with the aim of protecting human health therefore need to contain an approach that can transcend regulatory silos. The silos that we have referred to here exist to provide a mandate for regulatory action, often in response to a clearly identified need, such as recognition of a toxic effect in an exposed population, or concern that a high risk is possible if certain activities occur. It is therefore not surprising that current silos each delimit their own purview, and do not 'act on' or apply to variables (such as other chemicals and activities) that are outside of their domain. Almost none of the current silos were defined with mixtures in mind, so it is unsurprising that the status quo does not deal with mixtures; however, if the scientific case for MRA, and with a wide scope, can be made, then a broader consideration of possible risks from combined exposures should follow. This is particularly relevant to pieces of European Union regulations with some scope for MRA, such as those on setting maximum residue limits for pesticides (Regulation (EC) No 396/2005) or contaminants (Regulation (EC) No 1881/2006) in food, or all media-oriented regulations (Table 1), to name a few examples.



Box 2. Neurotoxicants. Graphic shows four groups of chemicals identified as neurotoxicants (Grandjean and Landrigan, 2014). Widths of coloured blocks are in proportion to the number of chemicals. The chemicals shown are subject to multiple pieces of legislation (Table 1), including REACH (industrial chemicals), food contaminants (metals) and pesticide residues (pesticides).



Box 3. Chemicals potentially harmful to the developing brain. Graphic shows 312 chemicals identified being potentially harmful to the developing brain, based on in vivo or in vitro evidence for effects on the brain or thyroid system, and grouped according to food-related use (Maffini and Neltner, 2015). Widths of coloured blocks are in proportion to the number of chemicals. The chemicals shown are subject to at least three different pieces of legislation (Table 1), including food contact materials, pesticide residues and food additives.

Two significant advances in the field of mixture toxicology have been 1) the acceptance of dose or concentration addition as suitable default models (DG Health and Consumer Protection, 2011) and 2) a shift towards the use of common effects or toxicological profiles as the inclusion criteria rather than detailed mechanistic considerations. A concrete example of the latter shift is the proposal to form cumulative assessment groups (CAGs) of pesticides on the basis of their toxicological profile (EFSA, 2013b). Since a review of the state of the art in mixture toxicology (Kortenkamp et al., 2009) there have been recent reviews of regulatory requirements and guidance (JRC, 2014) and scientific methodologies (JRC, 2015) for the assessment of mixtures. EFSA have recently organised a Colloquium on the harmonisation of human and ecological risk assessment mixtures (EFSA, 2015). The best foundation for legislation is a robust science and evidence base. These recent reviews and reports have provided a solid understanding of the experimental evidence base in mixture toxicology and of the related regulations and methods, and can serve as a basis for truly evidence-based legislation.

Several major challenges in mixture toxicology include whether, and how, to integrate cost-benefit analyses in MRA, for example to assess mixtures that contain components with beneficial effects as well as undesirable ones, and interactions. Interactions, cases in which a mixture has an effect that is greater (synergistic) or smaller than (antagonistic) the effects predicted by additive models are not yet handled by the existing proposals for MRA. The most concerning situation for risk assessment would be a synergy occurring at low concentrations of the mixture components, and a review of exactly that scenario concluded that "…the magnitude of synergy, at low doses, did not exceed the levels predicted by additive models by more than a factor of 4" (Boobis et al., 2011).

Given the number of possible mixture assessments that could be conceived of, it is clear that criteria are needed for prioritising those assessments that are most needed. Potential criteria include whether potential exposure is significant, frequent or large scale; whether potential effects are severe; whether mixture components persist in the human body; whether interactions are suspected; if chemical similarity is predicted; and if any components are assumed to lack an effect threshold (DG Health and Consumer Protection, 2011; European Commission (EC), 2012). Other factors such as the percentage of the mixture composed of chemicals with specific or high concern could be assessed for their suitability as criteria for setting priorities. The manufacturers of components of mixtures identified as priorities could be asked to provide toxicity and other data to inform the MRA process.

We now discuss the options that are available for addressing the identified need for MRA, and which range from amending existing legislation to the creation of a new piece of over-arching legislation. Considerations include whether an approach is scientifically sound and will achieve the protection of human health, and also whether technical and pragmatic factors will affect the implementation of an approach.

4.1. Could MRA be adequately incorporated into existing chemicals assessment paradigms?

There are at least three options that build on existing approaches and activities. Firstly, certain silos have some scope already for dealing with mixture effects, but currently no methods are implemented in practice. Good examples of this are pieces of regulation in food law, such as Regulation EC No 1881/2006 or Regulation (EC) No 396/2005, and media-oriented regulations such as the Water Framework Directive (2000/60/EC) and others concerned with drinking water quality and air quality (Table 1).

Secondly, two of the existing silos, regulations EC No 396/2005 and 1881/2006, for pesticide and food contaminant residues, respectively, operate by setting maximum residue levels (MRLs). Mixture considerations could therefore bridge these two silos if the process of setting of each MRL was able to include considerations of exposures to all food contaminants and pesticide residues. A proposal as to how information about co-exposure to pesticides can be accounted for when setting MRLs within the plant protection product 'silo' has been worked out by the European Food Safety Authority (EFSA, 2008), and this generic approach could be extended to include food contaminants and other food-relevant chemicals. However, this approach would not address mixture risk arising from other chemical groups covered by non-food regulations, and would naturally focus on exposure via food rather than other routes. Consequently, there is no logical reason to expect this to be entirely protective against the plethora of chemicals in use in the EU and which could contribute to human exposures. However, this option should be achievable and would be a pragmatic advance. Despite not addressing all chemicals and routes, it would include many that are of concern.

Thirdly, there is the option of an additional mixture assessment factor (MAF) to be used in connection with single chemical assessment factors (AF). AF are already commonly used in chemical registration or authorization processes, for example in the context of REACH (Regulation (EC) No 1907/2006) or the Plant Protection Products Regulation (Regulation (EC) No 1107/2009). The existing single chemical AFs are expected to cover issues such as the uncertainty in extrapolating data from animals to human, or the variability within the human populations, however they do not yet include mixture effects (Martin et al., 2013). A specific mixture assessment factor, or MAF, has been discussed in order to safeguard against unwanted mixture effects from multi-

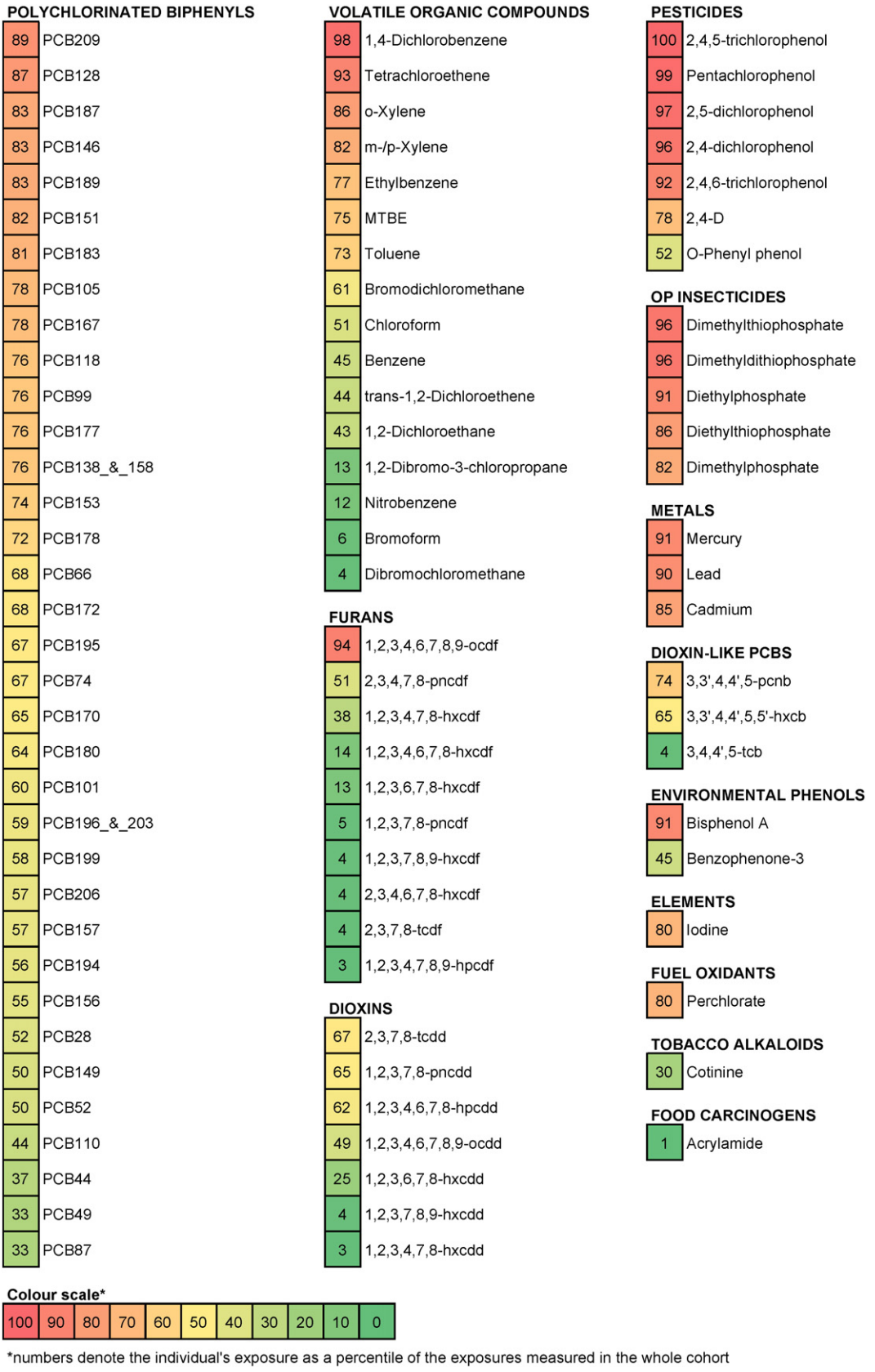


Fig. 1. Exposure percentiles for 92 chemicals measured in one individual. The figure shows the exposure of one individual to 92 chemicals, each exposure expressed as the percentile of all the exposures recorded in the 2003–2004 NHANES cohort. Colour coding indicates percentiles from 100 (red), through orange, yellow, light green to 0 (dark green), see colour scale in figure. The chemical abbreviations and groupings used are those given by NHANES. The chemicals shown are subject to multiple pieces of EU legislation (Table 1), including pesticide residues (pesticides, insecticides), food contaminants (PCBs, furans, dioxins, metals, perchlorate, acrylamide), REACH (volatile organic compounds), food contact materials (Bisphenol A), cosmetics (Benzophenone-3).

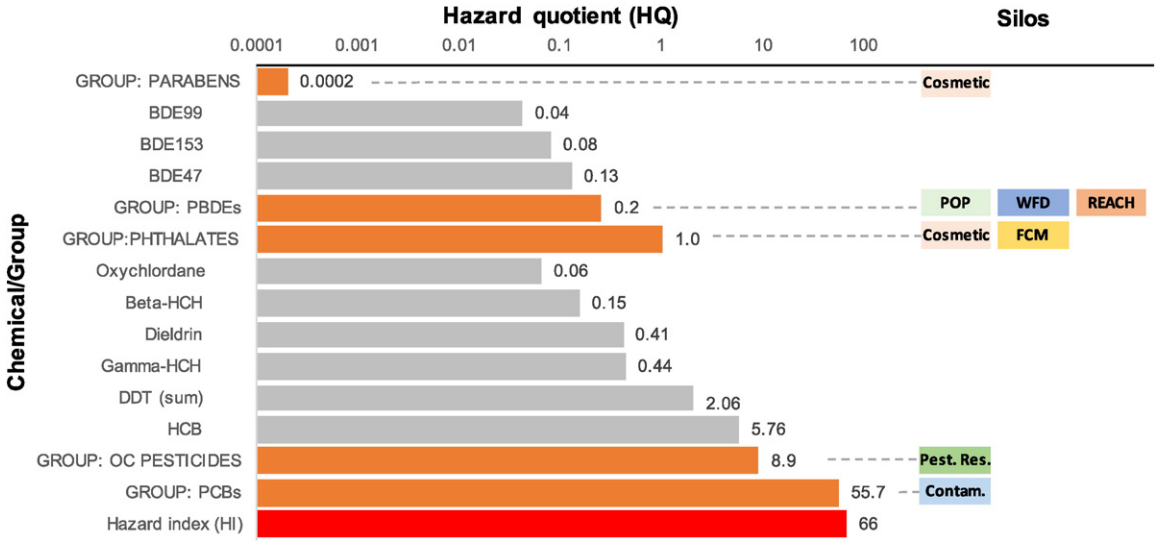


Fig. 2. Hazard Index analysis of human breast milk. Graph shows values for the Hazard Quotient (intake divided by Reference Dose) for single chemicals (grey bars), groups of chemicals (orange bars) or the sum of all quotients, the Hazard Index (HI, red bar). Bars for single chemicals are shown above the bar for the group to which they belong whenever this information was provided in the original publication (Schlumpf et al., 2010). Coloured rectangles at the right of the graph indicate regulatory silos (see Table 1) to which the chemical groupings belong. Abbreviations used: POP, Stockholm Convention on Persistent Organic Pollutants; WFD, Water Framework Directive; REACH, registration, evaluation, authorisation and restriction of chemicals; FCM, food contact material; Pest. Res., pesticide residue; Contam., contaminant.

component mixtures of partly unidentified composition and the lack of knowledge about composition would require the MAF to incorporate a certain amount of conservatism (Backhaus et al., 2010; 2013). As well as the special case of a mixture of unidentified composition, the wider use of an MAF could be envisaged. The advantages of this approach are that the use of AFs is well-established, common practice and easily understood, if not always supported from a strictly scientific perspective (Martin et al., 2013). Although introduction of a MAF may be the most pragmatic way of addressing the need for MRA to be introduced into chemicals risk assessment, there is a major challenge to this approach in that defining scientifically sound criteria for setting the magnitude of this factor may be extremely difficult. Setting a sufficiently protective MAF requires knowledge of the identity and number of chemicals that make up relevant exposure scenarios. This information is at best fragmentary and most often missing entirely, as is toxicity information that can be fed into the process of MRA. Whether or not the MAF could be set sufficiently large to provide conservative protection of human health, without being unacceptably restrictive on chemical use, is a major question.

4.2. Should legislation be enacted that treats the human body as a single receiving point of chemicals?

To complement incremental modifications to existing chemical regulations, is there a case for a new, single piece of legislation that deals with the human body as a single receiving point for chemicals? Such legislation could follow the example of the EU Water Framework Directive (WFD) and could have flexibility in the approaches that it would mandate in pursuit of the goal of protection of human health from combined chemicals exposures. One avenue could be to provide a mandate for human biomonitoring to allow an evidence-based assessment of the extent and frequency of any mixture risk to human populations. New legislation could complement the implementation of mixture considerations to existing product- or media-oriented legislation or guidance, with the aim of scrutinising whether the protection goal has been attained by these regulations. This endeavour would reflect the operation of the EU General Food Law Regulation, (EC) No 178/2002, which provides an overarching and coherent framework with general principles, procedures and requirements for the development of food and feed legislation. The General Food Law Regulation also mandates necessary infrastructure, such as an independent agency for scientific advice and support (EFSA) and procedures for management of crises and emergencies. However, any advantages of the new legislation approach need to be weighed against the complexities and challenges of introducing any piece of legislation, and the possible lack of appetite for such an endeavour.

Further considerations include whether enforcement is possible or practical, the challenges for analytical chemistry and biomonitoring, and whether such legislation would have implications for personal privacy. Biomonitoring using untargeted chemical analyses, which are not limited to chemicals selected by the analyst, may allow the chemicals that should be included in, or prioritised for, an MRA, to be identified. In addition, biomonitoring of effective doses could allow the detection of scenarios in which a mixture effect is present, even when the effect would not be predicted from single chemical information, and could warrant further investigation, for example with effect-directed fractionation to identify components that should be targets for regulatory attention.

4.3. Will MRA be ready for toxicology in the 21st century?

Future developments in toxicology, for example in the drive towards what is called '21st Century Toxicology' (National Research Council (NRC), 2007), may provide both an opportunity and a need to ensure that toxicology in the future is capable of dealing with mixtures and that mixture toxicology keeps pace with the cutting-edge of computational and high-throughput toxicology. Major aspirations for the future direction of toxicology include a scientific and ethical desire to move away from animal testing, and an improved predictive capability. Features such as high throughput screening will provide experimental data for many more mixture components, and moves towards probabilistic exposure and toxicity/risk assessment should provide both data and methods that can be used in the assessment of mixtures resulting in fewer data gaps, less reliance on bridging concepts and default assumptions and a better grasp of whether an assessment is actually over-cautious or under protective.

In conclusion, we feel that a wider discussion on the aspirations for MRA, and on the best path to implementation of MRA, is necessary to

translate the scientific consensus on mixture toxicity into practicable and appropriate regulatory approaches. We hope that this discussion piece will prompt comment and engage the scientific and regulatory communities in a wider, open debate on the implementation of MRA.

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