Modified Reference Point Index (mRPI) and a decision tree for deriving uncertainty factors: A practical approach to cumulative risk assessment of food contaminant mixtures

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ABSTRACT

Risk assessment of chemical mixtures remains a challenging task in all areas of food and consumer safety. So far, no general method has been developed that is best suited to several subject areas (e.g. food contaminants, additives and supplements, plant protection products). Especially for mixtures of food contaminants sophisticated methods are typically not applicable due to a general lack of complete toxicological data sets. We developed a new approach, the *modified Reference Point Index* (mRPI), that combines the advantages of the *Hazard Index* and the *Reference Point Index*. Furthermore, we developed a decision tree for the determination of specific uncertainty factors that makes the mRPI an easy to use method for cumulative risk assessment even in a data poor field such as food contaminants. To further characterise the estimated cumulative risks, the *Maximum Cumulative Ratio* (MCR) was adapted to be applied on the mRPI, and the *modified Maximum Cumulative Ratio* (mMCR) was established to identify whether the risks are dominated by a single substance. We present two case studies assessing the nephrotoxic and neurotoxic risks for the Austrian population originating from food contaminant mixtures. Calculations could not rule out potential cumulative risks, yet, they seemed to be dominated by single substances.

1. Introduction

Common health risk assessments of chemical hazards such as food contaminants are typically based on the evaluation of single substances. However, awareness of the relevance of chemical mixtures to potential health risks is growing steadily. Given the comprehensive knowledge of the variety of contaminants found in our food, it is very likely that people are exposed to a cocktail of potentially harmful substances through their

daily diets. Co-exposure to several hazardous substances confronts scientists and experts with challenging questions regarding toxicological effects of chemical mixtures and approaches for appropriate health risk assessments. Great efforts have been made to establish risk assessment methods, and several reviews and recommendations from public authorities and organisations are available (ATSDR, 2004; COT 2002; EFSA, 2008, 2013a, b, 2019; Kienzler et al., 2016; Kortenkamp et al., 2012; Meek et al., 2011; SCHER, SCENIHR and SCCS, 2012; US-EPA, 2000). However, the applicability of the proposed approaches is limited, in particular in the context of food contaminants. Contaminant mixtures are generally very complex compared to deliberately combined substance formulations such as plant protection products or food supplements. Typically, not all contaminants are known or fully characterised. Furthermore, these complex contaminant cocktails are highly unstable and may vary depending on the cultivation area, harvest time or food processing (Adeyeye, 2016; Arvanitoyannis and Dionisopoulou, 2014). For the risk assessment of such mixtures, a so-called component-based approach is recommended, in which the risks are estimated on the basis of the effects and the exposure of individual constituents (ATSDR, 2004; EFSA, 2013a; 2019; US-EPA, 2000). Sophisticated methods such as the *Relative Potency Factor* or the *Hazard Index Interaction* are generally not applicable to food contaminants, as well-founded toxicological data on relative potencies or interactions between compounds are not available in most cases. Other mathematical models for calculating cumulative risks, such as the *Hazard Index* (HI) or the *Reference Point Index* (RPI), have been found to be rather restrictive or general and are therefore not optimally suited for this purpose (detailed descriptions are provided below). In our paper, we present an innovative approach to overcoming the restrictions of the HI and the generalisation of the RPI, the *modified Reference Point Index* (mRPI). In addition, a first cumulative risk assessment is performed for the Austrian population with regard to potential nephrotoxic and neurotoxic health hazards originating from food contaminants.

2. Material and methods

2.1. Hazard identification - selection of food contaminants

For hazard identification, concentration data on food contaminants from the Austrian market, which were analysed by the Austrian Agency for Health and Food Safety (AGES) in the years 2011-2015, were examined. Food categories for which fewer than 20 samples were analysed were excluded from further calculations. Analytical results comprised data on the following contaminants, which were considered in the cumulative risk assessment: 3-monochloropropane diol (3-MCPD) and its fatty acid esters, glycidyl fatty acid esters (GE), acrylamide, chlorate, perchlorate, the sum of the 4 most relevant polycyclic aromatic hydrocarbons benzo*[a]*anthracene, benzo*[a]*pyrene, benzo*[b]* fluoranthene and chrysene (PAH4), mycotoxins - aflatoxins, deoxynivalenol (DON) and its acetylated forms 15-ADON and 3-ADON, nivalenol (NIV), ochratoxin A, patulin, T-2 and HT-2 toxin, zearalenone (ZEN) and metals and metalloids - arsenic (organic and inorganic forms), cadmium, lead, mercury (organic and inorganic forms).

2.2. Hazard characterisation - common assessment group (CAG)

In cumulative risk assessment, the potential hazardous effects of several substances are considered jointly. Consequently, the inclusion of additional substances automatically leads to an increase in the estimated risk. However, thorough toxicological considerations indicate that combined exposure to specific substances does not necessarily increase health risks. For instance, considering two substances, one toxic to the liver and the other toxic to the nervous system, it seems unlikely that the combination of these two substances would lead to increased hepatotoxicity or neurotoxicity. To accommodate this, it is proposed to cluster different substances into assessment groups according to similar health effects, such as common target organs, adverse outcome pathways (AOP) or

modes of action (MoA) (EFSA, 2013a, 2013b, 2019, Bopp et al., 2016; Meek et al., 2011; SCHER, SCENIHR and SCCS, 2012). In 2013, the European Food Safety Authority (EFSA) developed recommendations for so-called Cumulative Assessment Groups (CAG) based on specific target organs (EFSA, 2013b). According to these recommendations, the CAGs for nephrotoxicity and neurotoxicity were selected for an initial cumulative risk assessment of food contaminants for the Austrian population. All food contaminants listed in 2.1 *Hazard Identification* were evaluated for nephrotoxic and neurotoxic properties. For this purpose, literature - mainly by international and national authorities such as EFSA, the World Health Organisation (WHO), the Agency for Toxic Substances and Disease Registry (ATSDR), the National Toxicology Program (NTP) or the National Institute for Public Health and the Environment (RIVM) - was searched for nephrotoxic or neurotoxic reference points including No Observed Adverse Effect Levels (NOAELs), Lowest Observed Adverse Effect Levels (LOAELs) or Benchmark Dose Lower Bound (BMDL).

2.3. Exposure assessment

A deterministic exposure assessment was used to test the applicability of the currently recommended approaches for cumulative risk assessment in the area of food contaminants. The exposure estimates were performed for Austrian adults (18-64 years) and children (6-9 years) based on the occurrence data (see Table 3) and consumption data from the Austrian Study on Nutritional Status 2010-2012 (Elmadfa et al., 2012). These data are also available through the EFSA Comprehensive European Food Consumption Database (https://www.efsa.europa.eu/en/food-consumption/comprehensive-database). Medium and high exposure scenarios were estimated using the lower-bound (LB) - upper-bound (UB) concept for the calculation of mean concentrations of contaminants in food (FAO/WHO, 2009). For the calculation of the LB, all data below the limit of detection (LOD) and limit of quantification (LOQ) were set to zero. For the UB, all data below the LOD and LOQ were set to the respective values of the LOD and LOQ. Point estimates of mean chronic exposure to each contaminant were calculated by multiplying the mean concentration in food and the mean chronic amount of food consumed by the total population for the different population groups (children and adults). The total dietary chronic mean intake of each contaminant was estimated by aggregating the intakes across different food categories.

In order to assess high chronic exposure for each contaminant, it was assumed that individuals were high consumers (95th percentile of consumers only) of the food category contributing most to the total mean chronic intake in the LB and that the remaining food categories were all consumed in average quantities (mean of the total population).

The mixtures of contaminants to which people are exposed through their daily diet can be very diverse and can change permanently. Since it is difficult to identify specific mixtures, the following worst-case assumptions were made: all contaminants that occur in certain food categories always occur together at the level of the LB or the UB, respectively.

2.4. Risk characterisation - calculations on cumulative risks

2.4.1. The modified Reference Point Index (mRPI)

Several methods for calculating cumulative risks have been proposed in the past and are reviewed elsewhere (Kienzler et al., 2016). These approaches have been examined for their applicability to the cumulative risk assessment of food contaminants. Mixtures of contaminants can be very diverse and unsteady and may consist of a multitude of substances. Specific data on interactions between the mixture compounds or on the relative potencies between them are generally not available for all mixtures. Calculation methods that take such interactions or relative potencies into account are therefore not applicable. We previously pointed out that two methods are suitable for calculating cumulative risks from food contaminants, the *Hazard Index* (HI) and the *Reference Point Index* (RPI) (EFSA, 2013a; SCHER, SCENIHR and SCCS, 2012; US-EPA, 2000). Both do not consider

Table 1
Toxicological reference points (RP) on nephrotoxic effects and determined uncertainty factors (UF).

Substance	Effect	Reference point (RP)			UF and annotation number*		References
		Type	Value (μg/ kg bw/d)	Species			
3-MCPD fatty acid esters	Renal tubular hyperplasia	BMDL10	200	Rat	100	(2)	(Cho et al., 2008; EFSA, 2016)
Inorganic mercury	Effects on kidney weight	BMDL10	60	Rat	100	(2)	(EFSA, 2012b; FAO/WHO, 2011; NTP, 1993)
Lead	Chronic kidney disease	BMDL01	0.63	Human	1	(2)	EFSA (2010)
Cadmium	Proximal tubular reabsorption dysfunction (Fanconi Syndrom)	Urinary concentrations, BMDL5 including an uncertainty factor of 3.9 for intra-species differences	0.36	Human	1	(2)	EFSA (2009)
Fumonisins B1, B2 and B3	Nephrotoxicity	NOAEL	250	Rat	100	(2)	(EFSA, 2005; NTP, 1999)
Methylmercury	Chronic nephropathy	NOAEL	30 (Hg)	Mouse	100	(14)	(ATSDR, 1999; Hirano et al., 1986)
Ochratoxin A	Effects on kidney enzymes and kidney function	LOEL	8	Pig	450	(2)	(EFSA, 2006; FAO/WHO, 2001)
Patulin	Altered kidney function	NOEL	800	Rat	100	(3)	(FAO/WHO, 1995; RIVM et al., 1986)

*The annotation numbers refer to specific decisions made in the determination process of the uncertainty factors and are specified in the decision tree in Fig. 1.

Table 2
Toxicological reference points (RP) on neurotoxic effects and determined uncertainty factors (UF).

Substance	Effect	Reference point (RP)			Uncertainty Factor (UF) and annotation number*		References
		Type	Value (μg/kg bw/d)	Species			
Acrylamide	Peripheral nerve (sciatic) axonal degeneration	BMDL10	430	Rat	125	(2)	(EFSA, 2015; NTP, 2012)
Cadmium	Peripheral neuropathy	LOAEL	2200	Rat	1000	(12)	(ATSDR, 2012; Sato et al., 1978)
Methylmercury	Effects on locomotor activity	NOAEL	37 (Hg)	Rat	100	(14)	(Day et al., 2005; EFSA 2012b)
T-2 toxin and HT-2 toxin	Reduced motor activity and performance	NOAEL	400	Rat	100	(14)	(EFSA, 2011b; Sirkka et al., 1992)

*The annotation numbers refer to specific decisions made in the determination process of the uncertainty factors and are specified in the decision tree in Fig. 1.

Table 3

Occurrence data on contaminants/contaminant groups in µg/kg, which were assessed to have nephrotoxic or neurotoxic effects.

Acrylamide

Food category	N	N > LOQ	Mean LB	Mean UB	P95 LB	P95 UB	Min	Max
Coffee (beverage) (FoodEx L2)	49	48	13.0	13.0	24.0	24.0	< LOQ	39.0
Grains and grain-based products	124	77	244.0	244.0	685.0	685.0	66.0	1824.0
Snack food (FoodEx L2) (mainly potato crisps (FoodEx L3))	30	29	474.0	475.0	1162.0	1162.0	< LOQ	1408.0
Starchy roots and tubers (FoodEx L1) (mainly potatoes and potato products (FoodEx L2))	94	71	453.0	465.0	2395.0	2395.0	< LOQ	3827.0

Cadmium

Food category	N	N > LOQ	Mean LB	Mean UB	P95 LB	P95 UB	Min	Max
Alcoholic beverages	167	3	0.0	1.0	0.0	1.8	< LOQ	2.1
Fish and other seafood	1199	608	36.1	37.7	169.5	169.5	< LOQ	1607.9
Fruit and fruit products	58	6	2.5	4.6	12.7	12.7	< LOQ	92.8
Fruit and vegetable juices	476	62	0.6	2.2	4.4	4.5	< LOQ	20.8
Grains and grain-based products	335	327	27.2	27.3	59.8	59.8	< LOQ	127.1
Herbs. spices and condiments	131	31	8.8	38.2	47.9	88.9	< LOQ	190.9
Legumes. nuts and oilseeds	63	61	90.5	90.6	741.6	741.6	< LOQ	1100.4
Meat and meat products	517	86	3.7	6.2	15.6	15.6	< LOQ	280.7
Milk and dairy products	122	0	0.0	1.3	0.0	4.0	< LOQ	< LOQ
Sugar and confectionary	328	198	17.7	19.5	67.9	67.9	< LOQ	426.8
Vegetables and vegetable products	298	263	85.3	85.6	356.0	356.0	< LOQ	3230.3

Fumonisin B1and fumonisin B2

Food category	N	N > LOQ	Mean LB	Mean UB	P95 LB	P95 UB	Min	Max
Beer and beer-like beverage (FoodEx L2)	431	10	0.3	11.4	0.0	40.0	< LOD	91.0
Grains and grain-based products	650	32	46.5	93.7	0.0	40.0	< LOD	7078.0

Lead

Food category	N	N > LOQ	Mean LB	Mean UB	P95 LB	P95 UB	Min	Max
Alcoholic beverages	167	85	4.2	6.2	16.4	18.2	< LOD	35.1
Fish and other seafood	1204	287	12.9	22.0	66.7	66.7	< LOD	591.1
Fruit and fruit products	58	11	3.5	10.3	25.3	30.2	< LOD	38.3
Fruit and vegetable juices	476	180	6.0	10.2	26.1	26.1	< LOD	72.0
Grains and grain-based products	334	52	4.5	17.5	24.5	26.1	< LOD	200.5
Salt (FoodEx L3)	132	99	375.9	404.3	1363.9	1363.9	< LOD	3357.4
Legumes. nuts and oilseeds	81	16	5.0	16.7	22.7	25.00	< LOD	97.9
Meat and meat products	528	186	28.2	36.2	108.6	108.6	< LOD	2700.8
Milk and dairy products	124	15	1.0	5.2	5.7	14.9	< LOD	26.7
Sugar and confectionary	329	92	13.0	25.4	45.0	45.0	< LOD	635.6
Vegetables and vegetable products	240	84	24.6	31.1	98.9	98.9	< LOD	1823.2

Mercury

Food category	N	N > LOQ	Mean LB	Mean UB	P95 LB	P95 UB	Min	Max
Fish and other seafood	1213	1126	61.7	62.3	248.7	248.7	< LOQ	1266.0
Fruit and fruit products	50	0	0.0	5.0	0.0	6.2	< LOQ	< LOQ
Fruit and vegetable juices	263	7	0.2	4.3	0.0	7.5	< LOQ	10.9
Grains and grain-based products	120	0	0.0	9.2	0.0	9.5	< LOQ	< LOQ
Herbs. spices and condiments	124	15	0.2	73.8	0.0	190.0	< LOQ	14.8
Meat and meat products	512	24	0.5	7.2	0.0	12.6	< LOQ	22.4
Milk and dairy products	114	1	0.0	3.2	0.0	8.6	< LOQ	0.9
Sugar and confectionary	214	0	0.0	11.6	0.0	18.8	< LOQ	< LOQ
Vegetables and vegetable products	75	5	1.2	6.3	4.7	8.6	< LOQ	54.7

Ochratoxin A

Food category	N	N > LOQ	Mean LB	Mean UB	P95 LB	P95 UB	Min	Max
Beer and beer-like beverage (FoodEx L2)	430	218	0.0	0.0	0.1	0.1	< LOD	0.4
Chocolate (Cocoa) products (FoodEx L2)	92	35	0.1	0.2	0.5	0.5	< LOD	1.0
Confectionary (non-chocolate) (FoodEx L2)	43	8	0.5	0.6	0.8	0.8	< LOD	19.3
Dried fruits (FoodEx L2)	57	11	6.1	6.1	2.0	2.0	< LOD	333.5
Fruit and vegetable juices	102	14	0.0	0.1	0.2	0.2	< LOD	0.9
Grains and grain-based products	808	118	0.2	0.6	0.5	5.0	< LOD	75.0
(Spices FoodEx L2)	21	15	5.9	6.0	22.0	22.0	< LOD	27.9
Legumes. nuts and oilseeds	50	8	0.1	0.1	0.7	0.7	< LOD	1.1
Meat and meat products	132	4	0.1	0.1	0.0	0.2	< LOD	5.9
Vegetables and vegetable products	528	282	0.4	0.5	2.0	2.0	< LOD	4.7

Table 3 (*continued*)

Patulin

Food category	N	N > LOQ	Mean LB	Mean UB	P95 LB	P95 UB	Min	Max
Fruit and vegetable juices	485	57	2.7	6.0	13.9	13.9	0.0	159.0

Sum of 3-MCPD and 3-MCPD-fatty acid esters

Food category	N	N > LOQ	Mean LB	Mean UB	P95 LB	P95 UB	Min	Max
Animals and vegetable fats and oils	71	71	0.7	0.7	2.4	2.4	0.1	3.3

T2 toxin and HT2 toxin

Food category	N	N > LOQ	Mean LB	Mean UB	P95 LB	P95 UB	Min	Max
Beer and beer-like beverage (FoodEx L2)	429	5	0.1	2.2	0.0	2.0	< LOD	27.6
Grains and grain-based products	952	110	1.7	8.5	11.0	60.0	< LOD	70.0

N: number of samples; LOQ: limit of quantification; LOD: limit of detection; LB: lower bound; UB: upper bound, P95: 95th percentile. Food categories represent FoodEx level 1 (L1), unless specified otherwise.

interactions between substances, but dose addition is adopted as the default assessment approach as recommended by EFSA (EFSA, 2019). Nevertheless, it was found that both HI and RPI have their limitations.

The HI is calculated on the basis of the health-based guidance values (HBGV) of all substances in the mixture and the respective estimated exposures (*Exp*, see formula below). If the calculation yields HI < 1, combined risks are considered acceptable. Typically, such HBGV are established for only one toxicological effect, the most sensitive end-point, i.e. an organ-specific effect occurring at the lowest dose. At higher doses, however, most substances also have an additional effect on other organs. Consequently, using the HBGV to calculate tox-icological effects on an organ other than the most sensitive is a rather conservative approach that tends to overestimate the actual health risks. A single term of the HI, representing a single substance in the mixture, is referred to as *Hazard Quotient* (HQ):

$$HI = \sum_{i=1}^{n} \frac{Exp_i}{HBGV_i}$$

$$HQ_i = \frac{Exp_i}{HBGV_i}$$

The RPI method (see formula below) attempts to overcome this lim-itation. The calculations are based on toxicological reference points (RP) such as NOAELs, LOAELs or BMDLs, which are usually available for most specific effects of a substance. However, the main drawback of the RPI is the application of a generalised uncertainty factor (UF). This stands in contrast to the usual efforts to derive specific UFs for HBGVs of single substances using sophisticated toxicological methods. Consequently, this generalised UF is a potential source of inaccuracies and can lead to over- or underestimation in the risk assessment. If the RPI multiplied by a generalised UF is < 1, combined risks are considered acceptable.

$$RPI = \sum_{i=1}^{n} \frac{Exp_i}{RP_i}$$

We therefore propose a refined method for calculating cumulative risks, the *modified Reference Point Index* (mRPI). This approach also assumes dose addition, and interactions between substances are not considered. A single term of the mRPI, representing a single substance of the mixture, is referred to as *Reference Point Quotient* (RPQ):

$$mRPI = \sum_{i=1}^{n} RPQ_i$$

$$RPQ_i = \frac{Exp_i * UF_i}{RP_i}$$

Similar to HI and RPI, an mRPI < 1 indicates that health risks are considered unlikely. However, the mRPI combines the advantages of both methods: the specificity of HBGVs regarding UFs and the direct consideration of the particular toxicological effect. The key point of the mRPI is the derivation of specific UFs for all available RPs of a sub-stance and not only for the most sensitive endpoint. We have developed a sophisticated, but easy to use method for the derivation of UFs. This procedure is based on some simple questions and is depicted in the decision tree below (Fig. 1).

2.4.2. A decision tree for deriving UFs

The decision tree follows generally accepted conventions (EFSA, 2012a; WHO, 2011), but also addresses considerations for the specific purpose of cumulative risk assessment and takes existing HBGVs into account. In most cases, this approach avoids the need for a sophisticated expert judgement in the derivation of UFs. A collection of questions on the underlying toxicological study guides the derivation of distinctive UFs for a specific RP of a substance. Most questions can easily be an-swered by reviewing the study design. Others, especially the second question, may require further explanation in order to make a decision: Does the reasoning for the UF of the HBGV also apply to the toxicological RP? This question could for example be answered with "yes" if the RP comes from the same study from which the HBGV was derived. In ad-dition, the UFs may only be partly applicable, for example if the study was conducted on the same species as the study used for the HBGV. Then specific UFs for differences between humans and this species concerning toxicodynamics and toxicokinetics should also apply. However, UFs re-garding other parts of the study design (type of RP, extrapolation from sub-chronic to chronic studies, etc.) may not apply.

Some decisions require the consideration of an additional UF before proceeding with the decision tree. To provide a clear overview in such cases, dashed lines from these decisions are drawn to one of the con-cluding steps in deriving the final UF - the multiplication of all con-sidered UFs.

HBGVs are considered protective against all adverse effects of a substance. The specifically derived UF for any adverse effect should therefore not be more conservative than the HBGV. This is checked in the final step of the decision tree by dividing the RP by the derived UF. If this value is lower than the HBGV, the mRPI calculations should simply use the HBGV instead of the RPQ for this substance.

2.4.3. The modified Maximum Cumulative Ratio (mMCR)

To further characterise the estimated cumulative risks, the *maximum cumulative ratio* (MCR), a method by Price and Xianglu Han, 2011, was adapted and applied. This method can be used to analyse whether cu-mulative risks arise due to the combined exposure to several substances or if they are dominated by a single substance. Calculations of the MCR were originally based on the HI and the *maximum Hazard Quotient* (maxHQ) of a specific cumulative risk assessment (Price and Xianglu Han, 2011):
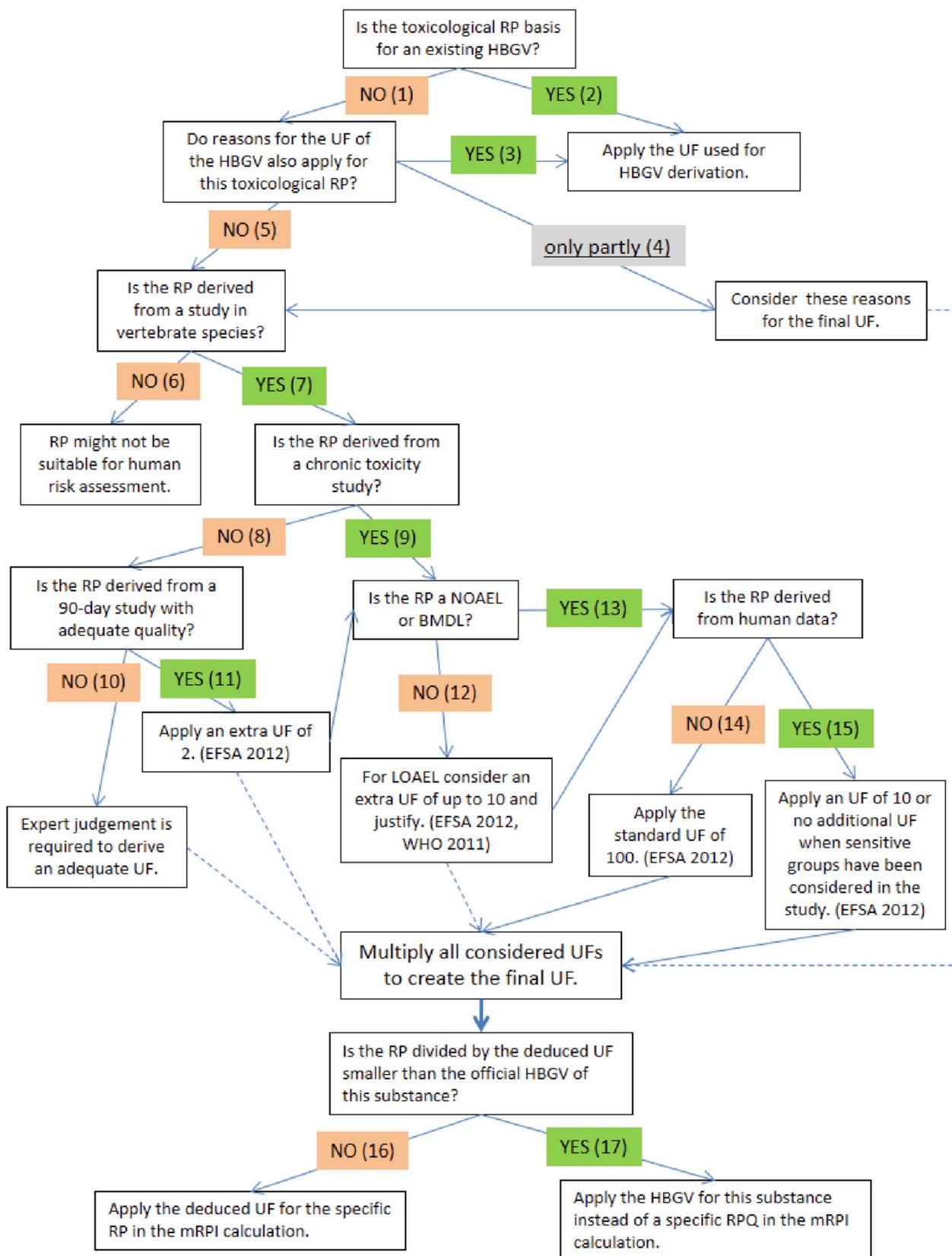
Fig. 1. Decision tree to determine the distinctive uncertainty factors (UF) for a specific reference point (RP) of a substance. Decisions, which require additional UF, should be considered before proceeding with the decision tree and are additionally connected to the crucial finishing step with dashed lines to support a clear overview. HBGV: Health Based Guidance Value; NOAEL: No Observed Adverse Effect Level; LOAEL: Lowest Observed Adverse Effect Level; BMDL: Benchmark Dose Lower Bound; mRPI: modified Reference Point Index; RPQ: Reference Point Quotient.

$$MCR = \frac{HI}{maxHQ_i}$$

Accordingly, our modified MCR (mMCR) is calculated based on the mRPI and the maximum RPQ (maxRPQ) and provides analogous information on the nature of the cumulative risks:

$$mMCR = \frac{mRPI}{maxRPQ_i}$$

Depending on the value of the mMCR and its relation to the mRPI, conclusions can be drawn as to whether the risks are dominated by one substance or not, similar to what is described for the original MCR and the HI by Price and Xianglu Han, 2011. Accordingly, mixture exposures can be classified into the following 4 groups:

Group I - maxRPQ > 1: at least one substance may give cause for concern irrespective of mixture exposures.

Group II - mRPI < 1: low concern regarding the mixture exposure.

Group IIIA - mRPI > 1, mRPI < mCR, mMCR < 2: concerns regarding the cumulative risk are dominated by one substance.

Group IIIB - mRPI > 1, mRPI < mMCR, mMCR > 2: concerns regarding the cumulative risk arise by several substances.

3. Results and discussion

To review the proposed methodology for the cumulative risk assessment of contaminants, the risks of nephrotoxicity and neurotoxicity from contaminant mixtures for the Austrian population were estimated.

The identification of nephrotoxic and neurotoxic compounds was carried out by compiling toxicological reference points (RP) listed in publications of national and international authorities or agencies in the fields of toxicology, health and food safety. Tables 1 and 2 list the two CAGs for nephrotoxicity and neurotoxicity and report the identified contaminants/contaminant groups and their respective RPs. In addition, specifically derived UFs including annotation numbers are provided. These annotation numbers can also be found in Fig. 1, which allows clear traceability of decisions made in the UF derivation process.

Table 3 provides descriptive statistics on occurrence data for each contaminant/contaminant group assessed to have nephrotoxic or neurotoxic effects. Mean and high exposures of children and adults were calculated for each contaminant/contaminant group. The results are presented in Table 4. Compared to EFSA's exposure assessment across all European Union countries, our results show that the Austrian population is exposed to less lead and methylmercury and slightly more inorganic mercury and T2 and HT2 toxins (EFSA, 2010; EFSA, 2011b; EFSA, 2012b). Regarding the exposure to 3-MCPD, Ochratoxin A and cadmium, our calculations are consistent with the EFSA exposure assessment for European countries (EFSA, 2006; EFSA, 2009; EFSA, 2016). The calculated exposure of Austrian children and adults to acrylamide is considerably higher compared to cross-European exposure estimates (EFSA, 2015). However, the exposure assessment approach used can only be considered as a rough estimate. Calculations were based on the food category of level 1 of the EFSA food classification and description system FoodEx1 - "grains and grain-based products" (EFSA, 2011a) (see Table 3) This might have led to an overestimation in the case of acrylamide exposure. A more differentiated assessment could possibly result in lower exposure estimates.

The cumulative risks for nephrotoxicity and neurotoxicity were estimated by calculating RPQs for each compound and then deriving the mRPI. The calculations were based on exposure data in Table 4 and RPs and UFs in Tables 1 and 2 In addition, the percentage of each compound contributing to the estimated mRPI was calculated based on lower bound occurrence data. To analyse whether cumulative risks are dominated by one substance, the mMCR was calculated and each exposure scenario was classified into the respective mMCR group as shown in Tables 5 and 6.

The calculated mRPIs for nephrotoxicity in children and adults are above 1 in all scenarios. For neurotoxicity, all calculated scenarios for children and the scenarios of high exposure of adults likewise result in mRPIs > 1. In line with recommendations from public authorities and organisations, we do not suggest that an mRPI > 1 per se should be interpreted as an indication of possible health risks. The cumulative risk assessment should be refined. Detailed information on suggested tiered approaches to refinement is proposed, for instance, in the WHO/IPCS framework for cumulative risk assessment (Meek et al., 2011). In such a case, as presented here, the primary step towards refining the risk assessment would be a more sophisticated exposure assessment. The presented exposure calculations are mainly based on categories of level 1 of the EFSA food classification and description system FoodEx1 (EFSA, 2011a) (see Table 3). Further calculations based on more detailed categories, for example FoodEx1 level 2 or 3, could reduce the current overestimates of exposure and therefore risk assessment. Alternatively, more precise estimates could be obtained using probabilistic exposure calculations.

Further refinement of the hazard assessment might also be possible, at least theoretically. The calculated risks could be reduced by subdividing the compounds into smaller CAGs. However, sophisticated expert judgement would be necessary to distinguish between the effects of substances that could influence each other and those where interaction is unlikely. This is necessary to maintain an adequate level of conservatism in risk assessment when defining new CAGs. Furthermore, detailed toxicological data on the mode of action of compounds would be required that might not be available for all substances, in particular in the case of food contaminants.

In the scenarios of mean exposure of adults, the estimation of the cumulative risk for neurotoxic effects resulted in an mRPI < 1. Accordingly, already at this stage of a crude estimation and without refinement of the risk assessment, health risks can be assumed unlikely.

Tables 5 and 6 list the calculated RPQs and percentages for each substance contributing to the mRPI and provide information on how the mRPI was calculated. Regarding nephrotoxicity, it becomes clear that

Table 4

Mean and high exposure of children and adults to nephrotoxic and neurotoxic contaminants/contaminant groups in µg/kg body weight/day (LB-UB).

Contaminant/contaminant group	Children		Adults	
	Mean exposure	High exposure	Mean exposure	High exposure
3-MCPD	0.67–0.68	1.45–1.48	0.14–0.14	0.37–0.38
Acrylamide	2.90–2.92	4.46–4.48	1.13–1.13	2.07–2.08
Inorganic mercury	0.01–0.23	0.01–0.29	0.00–0.11	0.01–0.20
Lead	0.26–0.52	0.37–0.64	0.13–0.24	0.20–0.31
Cadmium	0.53–0.58	0.82–0.87	0.25–0.28	0.51–0.54
Fumonisins B1 and B2	0.42–0.84	0.71–1.44	0.17–0.35	0.35–0.72
Methylmercury	0.04–0.04	0.23–0.23	0.01–0.01	0.13–0.13
Ochratoxin A	3.34–7.23[a]	4.96–10.82[a]	1.51–3.07[a]	5.90–6.10[a]
Patulin	0.01–0.03	0.05–0.1	0.004–0.01	0.03–0.06
T2 and HT2 toxin	0.02–0.08	0.03–0.13	0.01–0.03	0.01–0.07

[a] in ng/kg body weight/day.

Table 5
Cumulative risk assessment for nephrotoxicity - calculated Reference Point Quotients (RPQ) and modified Reference Point Indices (mRPI) in lower and upper bound (LB-UB), contribution of single substances to the mRPI in the lower bound (% LB), calculated modified Maximum Cumulative Ratios (mMCR) and classification into mMCR groups. The maximum values of RPQ (maxRPQ) are highlighted in bold.

	Children				Adults			
	Mean exposure		High exposure		Mean exposure		High exposure	
	RPQ (LB-UB)	% LB	RPQ (LB-UB)	% LB	RPQ (LB-UB)	% LB	RPQ (LB-UB)	% LB
3-MCPD	0.334-0.34	12	0.727-0.739	15	0.069-0.07	6	0.185-0.188	6
Inorganic mercury	0.009-0.378	0	0.017-0.476	0	0.004-0.184	0	0.01-0.329	0
Cadmium	**1.478-1.619**	54	**2.283-2.425**	46	**0.687-0.771**	59	**1.426-1.513**	50
Fumonisins B1 and B2	0.167-0.336	6	0.286-0.576	6	0.067-0.142	6	0.139-0.288	5
Lead	0.417-0.832	15	0.584-1.01	12	0.203-0.375	18	0.32-0.498	11
Methylmercury	0.121-0.123	4	0.767-0.774	15	0.046-0.047	4	0.443-0.447	15
Ochratoxin A	0.188-0.406	7	0.279-0.609	6	0.085-0.173	7	0.332-0.343	12
Patulin	0.002-0.004	0	0.006-0.013	0	0.001-0.001	0	0.003-0.008	0
mRPI (sum of RPQ)	2.72-4.04	100	4.95-6.62	100	1.16-1.76	100	2.86-3.61	100
	LB - UB		LB - UB		LB - UB		LB - UB	
mMCR	1.84-2.50		2.17-2.73		1.69-2.28		2.01-2.39	
mMCR Group	I		I		IIIA - IIIB		I	

cadmium accounts for the largest share of the cumulative risk in all calculated scenarios (46-59%). In the mean exposure scenarios, lead is the second largest contributor, whereas in the high exposure scenarios methylmercury accounts for a considerable share. The exposure of children to 3-MCPD also seems to contribute substantially to the cumulative risk of nephrotoxicity. Similarly, for neurotoxicity, one contaminant, acrylamide, represents the major contributor to the cumulative risk in all scenarios. In the scenario of high exposure of children, methylmercury also accounts for a considerable part of the neurotoxic risk.

Finally, the cumulative risks for nephro- and neurotoxicity were additionally calculated using the original method of the RPI. Table 7 compares the results of the RPI and the mRPI calculations. Using the original RPI yields much higher risk estimates for nephrotoxicity and somewhat lower risk estimates for neurotoxicity. The main difference between the methods is that the mRPI uses specific UFs while the original RPI uses a generalised UF (usually 100). In our chosen examples of nephro- and neurotoxicity, the specific UFs for the different toxicological effects of cadmium are mainly responsible for the discrepancies between the two calculation methods. In the case of nephrotoxicity, the original RPI leads to an overestimation of the risk since a UF of 100 is not required for the nephrotoxic effects of cadmium. A UF of 1 is used in corresponding mRPI calculations and in the derivation of the HBGV for cadmium. When estimating neurotoxic effects, the original RPI might underestimate the risk as a UF of 100 is used for cadmium. The available

RP represents a LOAEL for which an additional UF is recommended (EFSA, 2012a). In corresponding mRPI calculations, a UF of 1000 is used for neurotoxic effects of cadmium. The comparison of the two methods clearly shows the advantage of the mRPI over the RPI in terms of possible over- and underestimation of risks.

Furthermore, the mRPI shows clear advantages over the HI approach. Since the HI is based on HBGV, neurotoxic effects of cadmium might not even be considered in a cumulative risk assessment since the HBGV is derived from nephrotoxic effects. Alternatively, this HBGV could be used in HI calculations for a CAG of neurotoxicity. However, this would lead to an overestimation: A more detailed look at the mRPI calculations shows that the RPQ values of cadmium are much higher in the CAG for nephrotoxicity compared to the values for neurotoxicity. This is a result of the specific RPs and UFs applied, as exposure does not differ in the calculations. The markedly lower RPQ in the CAG for neurotoxicity therefore shows the advantage of the mRPI approach over the HI, which would have overestimated the risk for neurotoxicity.

Considering our results as a whole, one substance appears to dominate in several exposure scenarios of neurotoxic and nephrotoxic risks. Similar findings are described in Evans et al., 2015 and Price et al., 2014, where individual substances often account for a sizable share of the cumulative risk. Calculations of the mMCR and the classification of each exposure scenario into mMCR groups confirm that the evaluated cumulative risks tend to be dominated by one substance. For

Table 6
Cumulative risk assessment for neurotoxicity - calculated Reference Point Quotients (RPQ) and modified Reference Point Indices (mRPI) in lower and upper bound (LB-UB), contribution of single substances to the mRPI in the lower bound (% LB), calculated modified Maximum Cumulative Ratios (mMCR) and classification into mMCR groups. The maximum values of RPQ (maxRPQ) are highlighted in bold.

	Children				Adults			
	Mean exposure		High exposure		Mean exposure		High exposure	
	RPQ (LB-UB)	% (LB)	RPQ (LB-UB)	% (LB)	RPQ (LB-UB)	% (LB)	RPQ (LB-UB)	% (LB)
Acrylamide	**0.84-0.84**	71	**1.3-1.3**	57	**0.33-0.33**	69	**0.6-0.6**	50
Cadmium	0.24-0.26	20	0.37-0.4	16	0.11-0.13	23	0.23-0.25	19
Methylmercury	0.1-0.1	8	0.62-0.63	27	0.04-0.04	8	0.36-0.36	30
T-2 Toxin and HT-2 Toxin	0-0.02	0	0.01-0.03	0	0-0.01	0	0-0.02	0
mRPI (sum of RPQ)	1.18-1.21	100	2.29-2.33	100	0.48-0.49	100	1.19-1.21	100
	LB - UB		LB - UB		LB - UB		LB - UB	
mMCR	1.40-1.44		1.76-1.79		1.45-1.48		1.98-2.02	
mMCR Group	IIIA		I		II		IIIA - IIIB	

Table 7
Comparison of two methods for cumulative risk assessment. Both, the Reference Point Index (RPI) and the modified Reference Point Index (mRPI) were calculated for the upper and the lower bound of estimated exposure (UB-LB).

		Children		Adults	
		Mean exposure	High exposure	Mean exposure	High exposure
Nephrotoxicity	mRPI (LB-UB)	2.72-4.04	4.95-6.62	1.16-1.76	2.86-3.61
	RPI*100 (LB-UB)	190.17-246.40	288.58-346.24	89.14-115.02	175.45-202.44
Neurotoxicity	mRPI (LB-UB)	1.18-1.21	2.29-2.33	0.48-0.49	1.19-1.21
	RPI*100 (LB-UB)	0.80-0.82	1.70-1.74	0.31-0.32	0.87-0.98

nephrotoxic effects, maxRPQs were calculated for cadmium in all exposure scenarios. In three out of four exposure scenarios, it exceeded a value of 1, resulting in the classification of the cumulative risk into mMCR group I (Table 5). Cadmium therefore raises possible concerns regardless of mixture effects. Cumulative nephrotoxic risks in the mean exposure scenario of adults are categorised in group IIIA or IIIB, respectively, depending on whether exposure estimates are based on the UB or LB occurrence data. It is therefore not clear whether the cumulative risk is dominated by cadmium.

For neurotoxic effects, maxRPQs were calculated for acrylamide in all exposure scenarios (Table 6). The classification of the cumulative risks for children into mMCR group IIIA at mean exposure and group I at high exposure indicates a clear dominance of acrylamide. However, this dominance is less pronounced in the exposure scenarios for adults. At high exposure, the calculated cumulative risk is classified into group IIIA or IIIB, again depending on whether LB or UB occurrence data is used. At mean exposure, the risk is classified into group II and therefore health risks regarding neurotoxicity are assumed to be of low concern.

4. Conclusion

Our findings show that in most exposure scenarios the cumulative risks are dominated by a single substance. Therefore, it is indicated, that reducing exposure to individual contaminants, which make up larger parts of the risks, could be a practicable way to reduce cumulative risks.

In conclusion, this study shows that the mRPI and the proposed procedure for UF determination provide an easily applicable and transparent approach to cumulative risk assessment of food contaminants, taking into account current scientific expert judgement based on toxicological knowledge while preserving sufficient conservatism.

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Disclosure statement

The authors declare that they have no conflict of interest. This article reflects the views of the authors and should not be construed to represent views or policies of the Austrian Agency for Health and Food Safety (AGES) or the Federal Ministry of Labour, Social Affairs, Health and Consumer Protection.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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