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A case study with three chemical silos

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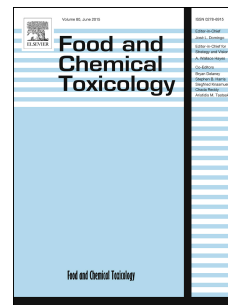
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CRedit authorship contribution statement

Corinne Sprong: Conceptualization, Investigation, Writing – original draft, Writing - review & editing, Visualization

Amélie Crepet: Investigation, Writing - review & editing.

Francesca Metruccio: Investigation, Writing - review & editing

Urška Blaznik: Investigation, Writing - review & editing

Chris Anagnostopoulos: Investigation, Writing - review & editing

Despo Louca Christodoulou: Investigation, Writing - review & editing

Bodil Hamborg Jensen: Investigation, Writing - review & editing

Marc Kennedy: Investigation, Writing - review & editing

Neus González: Investigation, Writing - review & editing

Irena Rehurkova: Investigation, Writing - review & editing

Jiří Ruprich: Investigation, Writing - review & editing

Jan Dirk te Biesebeek: Investigation, Writing - review & editing

Marie Vanacker: Investigation, Writing - review & editing

Angelo Moretto: Investigation, Writing - review & editing

Jacob van Klaveren: Conceptualization, Supervision, Funding acquisition.

Cumulative dietary risk assessment overarching different regulatory silos using a margin of exposure approach: A case study with three chemical silos

Corinne Sprong¹, Amélie Crépet², Francesca Metruccio³, Urska Blaznik⁵, Chris Anagnostopoulos⁶, Despo Louca Christodoulou⁷, Bodil Hamborg Jensen⁸, Marc Kennedy⁹, Neus González¹⁰, Irena Rehurkova¹¹, Jiří Ruprich¹¹, Jan Dirk te Biesebeek¹, Marie Vanacker², Angelo Moretto³⁻⁴, and Jacob van Klaveren¹

¹ RIVM, National Institute for Public Health and the Environment, The Netherlands, PO Box 1, 3720 BA Bilthoven, The Netherlands

² ANSES, French Agency for Food, Environmental and Occupational Health and Safety, Risk assessment department, Methodology and studies unit, 947001, Maisons-Alfort, France

³ ICPS, International Centre for PPRs and Health Risk Prevention, ASST Fatebenefratelli Sacco, Ospedale L. sacco via GB Grassi 74, 20157 Milano, Italy.

⁴ Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan, Italy.

⁵ National Institute of Public Health, Environmental Health Centre, Trubarjeva 2, Ljubljana, Slovenia

⁶ Benaki Phytopathological Institute, Department of Pesticide Control and Phytopharmacy, Laboratory of Pesticide Residues, 8 Stefanou Delta Street, Kifissia, Athens, 14561, Greece

⁷ State General Laboratory, Ministry of, Nicosia, Cyprus

⁸ Technical University of Denmark, National Food Institute, Division of Risk Assessment and Nutrition, Kemitorvet, Building 201, DK 2800 Lyngby, Denmark

⁹ Fera Science Ltd, Sand Hutton, York, YO41 1LZ, United Kingdom

¹⁰ Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira I Virgili, Reus, Catalonia, Spain

¹¹ National Institute of Public Health in Prague, Centre for Health, Nutrition and Food, Brno, Czech Republic

Corresponding Author

Corinne Sprong

Email address: corinne.sprong@rivm.nl

Abbreviations

AMOE	Acceptable margin of exposure
CAG	Cumulative assessment group
DL-PCBs	Dioxin-like polychlorinated biphenyls
Dr,h	External human dose leading to a body burden in humans similar to the body burden in laboratory animals at the external no observed adverse effect level in these animals
EFSA	European Food Safety Authority
FAs	Food additives
HBCDs	Hexabromocyclododecanes
HBGV	Health based guidance value
LOAEL	Lowest observed adverse effect level
LOD	Level of detection
LOQ	Level of quantification
MCRA	Monte Carlo Risk Assessment
MOE	Margin of exposure
MOET	Combined (total) margin of exposure
MPL	Maximum permitted level
mRPI	Modified reference point index
nMOET	Normalised combined (total) margin of exposure
NOAEL	No observed adverse effect level
NDL-PCBs	Non-dioxin-like polychlorinated biphenyls
PBBs	Polybrominated biphenyls
PBDE	Polybrominated diphenyl ether
PFOS	Perfluor octanoic sulphonate
PODI	Point of departure index
POPs	Persistent organic pollutants
PPRs	Plant protection products and residues
RPF(s)	Relative potency factor(s)
RPI	Reference point index
TCDD	2,3,7,8 tetrachlorodibenzo-p-dioxin
TEFs	Toxicity equivalent factors

Abstract

Risk assessment of chemicals occurring in our diet is commonly performed for single chemicals without considering exposure to other chemicals. We performed a case study on risk assessment of combined dietary exposure to chemicals from different regulatory silos, i.e. pesticides (PPRs), persistent organic pollutants (POPs) and food additives (FAs). Chemicals were grouped into the cumulative assessment group (CAG) liver steatosis using a component-based approach. Based on literature, the CAG included 144 PPRs, 49 POPS and 7 FAs for which concentration data were available.

For each silo, chronic combined dietary exposure was assessed for adults and children of nine European countries following the most commonly used exposure methodologies in Europe and by using a relative potency factor approach. For risk characterization, a Margin of Exposure (MOE) was calculated. To overarch the risk across silos, a normalized combined margin of exposure (nMOET) approach was proposed.

This case study demonstrated that risk assessment of combined exposure to chemicals can be performed within regulatory silos. It also highlighted important differences in the conservatism of exposure scenarios, the derivation of point of departures and the subsequent acceptable MOEs between the silos. To overarch the risk despite these differences, a nMOET approach can be used.

Key words: chemical mixtures, cumulative risk assessment, dietary margin of exposure, pesticides, persistent organic pollutants, food additives

1. Introduction

Populations are daily exposed to many chemicals present in their diet and environment. Frequently, risk is assessed for single chemicals and/or single routes of exposure. However, awareness of the need to assess the combined risk of exposure to multiple chemicals through all relevant exposure routes is increasing. Many efforts have been put into development of concepts, methods, guidance and applications for risk assessment of combined exposures to chemicals (e.g. Boobis et al., 2008; EFSA, 2007, 2008, 2019a; Fox et al., 2017; WHO, 2008; Bopp et al., 2018; OECD 2018).

Within the European Union, the European Food Safety Authority (EFSA) proposed to group Plant Protection Product Residues (PPRs) into cumulative assessment groups (CAGs) based on a common adverse outcome (EFSA 2013). Grouping consists of a four-tier system, with each tier being more refined (EFSA 2013). The first one is based on the target organ, the second on the phenotypic effect, the third on the mode of action and the fourth on the mechanism of action. Recently, this system was used by EFSA to perform risk assessment of the combined exposure to pesticides grouped into CAGs related to nervous system and thyroid (EFSA 2019b,c,d,e). Also, this system was used for 155 PPRs belonging to the CAG liver steatosis in order to define principal components in PPR mixtures for further investigation using *in-vitro* tools (Crépet et al., 2019). Within the EuroMix project, a chemical inventory list consisting of 1600 chemicals from several regulatory frameworks, including PPRs, different classes of environmental pollutants and other contaminants, food contact materials and food additives, was established (Kyriakopoulou et al., 2017). This inventory list showed that liver steatosis is an adverse outcome of exposure to chemicals belonging to various chemical/regulatory silos. Therefore, combined exposure should take into account other silos as proposed by Evans et al. (2016).

The present paper describes a case study in which a component-based approach was performed to cumulate the exposure to chemicals regulated in three different frameworks for

which concentration data in food was available: PPRs, the persistent organic pollutants (POPs), and food additives (FAs). For this, chemicals from each silo were grouped into the CAG liver steatosis and relative potency factors (RPFs) were calculated based on the chosen index compound. RPF-normalised chronic exposures of all chemicals within the CAG liver steatosis of a particular silo were summed to cumulate exposure. Subsequently, the margin of exposure (MOE) was calculated per silo by dividing the point of departure of the index compound by the cumulative exposure estimate of the silo. In addition, a normalized combined (total) margin of exposure (nMOET) was proposed for overarching the risk from the MOEs obtained for the three regulatory silos. The results presented for adults and children of nine European countries should not be considered as formal national risk assessments, but as proof of principle in testing the proposed approach. Although data input regarding CAG membership, point of departures, exposure and acceptable MOE could have been refined, this was not done but all these points were indicated as sources of uncertainty.

2. Material and methods

2.1 Data

2.1.1 Grouping into CAG liver steatosis and derivation of point of departure values

The following effects were considered relevant for inclusion of a substance in the CAG liver steatosis (RIVM, ICPS, ANSES, 2016):

- Lipidosis
- Vacuolation
- Steatosis
- Lipid macrovesicular steatosis
- Lipid microvesicular steatosis
- Fatty change
- Fatty deposition

The criteria described by Crépet et al. (2010) were followed for CAG membership. At least one positive study for the specific effect was regarded as sufficient for inclusion in the CAG liver steatosis.

PPRs: the assignment of PPRs as members of the CAG liver steatosis and the determination of the corresponding point of departures, i.e. no observed adverse effect levels (NOAELs) or in case not available the lowest observed adverse effect level divided by three (LOAEL/3),

were derived from three reports supported by EFSA (Nielsen et al. 2012, RIVM et al. 2013, RIVM et al. 2016) following the criteria described by Crépet et al. (2019). Hundred fifty five PPRs were defined as a member of the CAG liver steatosis and listed, together with the characteristics of the study (e.g. animal species, strain, gender) on which the point of departure was based, in the supplementary material of Crépet et al. (2019). It should be noted that, in these reports, grouping was based on the review of summary reports of the studies. Consequently, following detailed evaluation of the original data, a number of compounds might be removed from the CAG. The PPRs were coded using the ParamCodes of the harmonized European Standard Sample Description 1 format (SSD1, EFSA 2010a) following residue definitions for monitoring as described in the EU pesticides database, applicable on the accessed date (6 March 2017). Pesticides with no available ParamCode or point of departure (e.g. copper compounds) were removed from the CAG. Some chemicals sharing the same residue definition for monitoring (benalaxyl-M and benalaxyl, cypermethrin and alpha-cypermethrin, metam and dazomet, metalaxyl-M and metalaxyl, triadimefon and triadimenol) were presented together in the database. This resulted in a total of 144 pesticides. Following criteria described by Crépet et al. (2019), flusilazole was chosen as the index compound.

POPs: Opinions of the European Scientific Committee on Food, EFSA and the European Chemicals Bureau, and publications of NTP studies published before March 2016 were scrutinized for recorded effects on liver steatosis and to select NOAELs or LOAELs using the same criteria as described for PPRs by Crépet et al. (2019). This resulted in 943 congeners and isomers of dioxins (NTP 2006a), dioxin-like polychlorated biphenyls (DL-PCBS; SCF 2001), non-dioxin-like polychlorated biphenyls (NDL-PCBS; EFSA 2005; NTP 2006b), polybrominated biphenyls (PBBs; EFSA 2010b), hexabromocyclododecanes (HBCDs; Kurokawa et al., 1984, as cited in ECB, 2008), polybrominated diphenyl ethers (PBDE; ESFA 2011a; NTP 2016), and perfluor octanoic sulphonates (PFOS; Seacat et al., 2003).

NOAELs from long-term studies were available for dioxins and DL-PCBS, NDL-PCBS. For BDEs and PFOS, only NOAELs from short-term studies (13 weeks) were available. Regarding HBCDs, the LOAEL/3 from a long-term study was used whereas for PBBs, only the LOAEL/3 from short term studies (4.5 to 5 weeks) was available. For simplicity, no additional uncertainty factors were applied for the use of short term studies in the derivation of the point of departure.

Limited toxicity data were available for individual congeners or isomers within a certain chemical subgroup. Toxicity tests were predominantly performed with a purified congener of a particular POP class (2,3,7,8-TCDD, PCB-153 and PFOS) or technical products (NDL-PCBS, HBCDDs, PBBs and PBDE), which were chemical mixtures themselves. Therefore, as a pragmatic approach, the NOAEL obtained from a single congener of a certain POP subclass was assigned to this whole POP subclass assuming equipotency (e.g. the NOAEL of PCB-153 was assigned to all congeners belonging to NDL-PCBs). Similarly, when only toxicity data from technical products was available, the NOAEL of a technical product was assigned to all congeners belonging to the particular POP subclass assuming equipotency (e.g. the NOAEL of the technical product DE-71 was assigned to all PBDEs). An exception was made for the dioxins and DL-PCBs. For these, the NOAEL of TCDD was divided by toxic equivalent factors (TEFs; Supplemental material 1), which were obtained from the World Health Organisation (WHO) (van den Berg et al, 2006), to obtain adjusted NOAELs for the relevant chemicals.

Risk assessment of POPs is usually based on the human body burden. To take this into account, the external human dose leading to a body burden in humans similar to the body burden in laboratory animals at the external NOAEL in these animals ($D_{r, h}$) was assessed. For this, NOAELs or LOAELs/3 derived from animal studies were converted to a test animal NOAEL body burden (*NOAEL BB*) using absorption factors of test animals ($F_{abs, animal}$), half-life in test animals ($t_{1/2, animal}$) and assuming a one-compartment kinetic model (JECFA 2001):

$$\frac{F_{abs, animal} * NOAEL_{animal} * t_{1/2, animal}}{\ln 2} = test\ animal\ NOAEL\ BB \quad (\text{Equation 1})$$

Next, the test animal NOAEL BB was translated to an external human dose leading to the same body burden in humans ($D_{r, h}$) using human absorption factors ($F_{abs, human}$), half-life in humans ($t_{1/2, human}$) and again assuming a one-compartment kinetic model (JECFA 2001):

$$\frac{test\ animal\ NOAEL\ BB * \ln 2}{F_{abs, human} * t_{1/2, human}} = D_{r, h} \quad (\text{Equation 2})$$

Supplemental material 1 summarizes the NOAEL or LOAELs, the absorption factors of test animals and humans, the half-life values in animals and human, the $D_{r, h}$ and RPFs for the 49 POPs congeners and isomers belonging to the steatosis CAG and for which concentration data were available. For the calculation of the RPF, PCB-153 was selected as the index compound.

Food additives: EFSA opinions published before March 2017 were viewed for recorded effects on liver steatosis. The same criteria as described for PPRs by Crépet et al. (2019) were applied to select NOAELs or LOAELs and the subsequent selection of the index compound, except for the criterion that the NOAEL of the index compound should be between 0 and 1 mg/ kg bw/day. While this criterion was originally established to avoid the selection of an index compound eliciting other organ and/or different liver effects at doses lower than those eliciting fatty changes, it was not possible to use it for FAs as all NOAELs and LOAELs were above 1 mg/kg bw/day. In total, 92 EFSA opinions representing 96 food additives were viewed. Five additives were removed from the list because they were no longer authorised (E 431 polyoxyethylene (40) stearate, E 556 Calcium aluminium silicate, E 558 Bentonite, E 559 aluminium silicate, E 912 montan acid esters), yielding 88 food

additives. In total 7 food additives showed liver steatosis: E 161 g 'canthaxanthin' (Buser et al. 1992a,b, 1997 and Rose et al. 1988 as cited in EFSA, 2010c), E 154 'brown FK' (EFSA 2010d), E 242 'dimethyl dicarbonate' (EFSA 2015a), E 310 'propyl gallate' (EFSA 2014), E 914 'oxidised polyethylene wax' (ESFA 2015b), E 474 'sucroglycerides' (as cited in WHO 1990 and EFSA 2004), and E 1208 'polyvinylpyrrolidone-vinyl acetate copolymer' (EFSA 2010e). Supplemental material 2 lists those additives, the inclusion criteria and the selected NOAEL, LOAEL and RPF. Following the criteria described by Crépet et al. (2019), canthaxanthin was chosen as index compound.

2.1.2 Food consumption and studied populations

Dietary exposure was assessed using food consumption data from nine European countries coded according to the FoodEx1 coding system (EFSA 2011b). For each individual, age and body weight was available. Adults aged between 18 to 64 years old and children aged between 11 to 15 years old were selected as study populations, because food consumption data for these age ranges were available for most of the nine countries. A more detailed description of the food consumption data was provided by Crépet et al. (2019).

2.1.3 Concentration data

Concentration data of PPRs and POPs in food and drinking water were obtained by merging annual control and monitoring programs of nine European countries obtained between 2010 and 2014. Data comprised PPRs and POPs measured in raw agricultural commodities and/or processed food (e.g. juices). Samples obtained by objective or selective sampling strategy were included, whereas samples obtained by any other strategies were excluded since they were considered as non-random samples and therefore not representative for the

occurrence of the investigated compounds. Data were coded according to EFSA's Standard Sample Description (SSD1; EFSA 2010a).

PPRs: The merged data set contained 135 PPRs of the steatosis CAG, of which 126 PPRs had at least one sample above the level of detection (LOD) or level of quantification (LOQ), whichever available. It contained 3,161,615 analyses present in 204 raw agricultural commodities, of which 99.28 % of measurements were non-detects (Crépet et al., 2019). The publication of Crépet et al. (2019) provides more detailed information on the data set used. Although residue definition for risk assessment should be used rather than residue definitions for enforcement (EFSA 2012a), only concentration data for the residue definition for enforcement were available. According to the EFSA opinion of 2012a, the residue concentrations for risk assessment can be obtained by applying conversion factors on concentrations obtained from residue definition for enforcement. As the present paper is a case study to test the overarching approach, these conversion factors were assumed to be 1 for simplicity.

POPs: Of the 943 congeners and isomers in the CAG liver steatosis, concentration values were available for 49 POPs, of which 48 had at least one sample above the LOD or LOQ, whichever available. The concentration data set comprised of 64,672 analyses performed in 43 foods (predominantly raw foods of animal origin), of which 40.4% of measurements were non-detects. Concentration data for POPs and their corresponding LOD and LOQ were often expressed on a fat content basis. For intake calculation, these data need to be expressed on a food basis. Since not all received SSD1 data contained the fat percentage of the food in which the chemical was measured, the French mean fat percentage of the corresponding food was used to calculate the chemical concentration in that food as a pragmatic solution.

Food additives: Because limited concentration data were available for food additives in the merged concentration data set, use levels as described in the EFSA opinions of the 7 FAs (EFSA 2010c, 2010d, 2010e, 2012b, , 2014, 2015a, 2015b) were used to estimate the

intakes. Food categories of Annex II of Regulation 1333/2008 were matched with FoodEx1 level 4 codes, for which the food list of EFSA's FAIM template (version V1.0) was used for guidance.

2.1.4 Food translation table and processing factors

To match consumed foods with concentration data in raw agricultural products, a food translation table was used (Boon et al., 2015). This table was based on Dutch recipes and contained conversion factors to convert foods classified according to FoodEx1 to their edible raw agricultural commodity ingredients (e.g. 167 g raw spinach is needed to produce 100 g cooked spinach). The food translation table included information on processing steps, such as cooking, milling and juicing, and enabled use of processing factors to correct for loss or increase in substance concentration upon processing (e.g. loss of water-soluble substances upon cooking in water). Processing factors for PPRs obtained from the Bundesinstitut für Risikobewertung (BfR; accessed 1st September 2015) were used in the exposure assessment. For 46 out of the 144 PPRs, processing factors were available. The available processing factors were listed in the supplemental material of Crépet et al. (2019). A literature search for processing factors was performed and yielded approximately 1000 processing factors for dioxins, PCBs, and PFOS for cooking, grilling and frying of several meat and fish foods, and for cooking or frying of vegetable foods (Schechter et al., 1998; Tsutsumi et al., 2002; Perelló et al., 2010; Vassiliadou et al., 2015). For food additives, concentration levels were directly expressed as food as consumed and therefore can be used for exposure calculations without food translation table and processing factors.

2.2 Cumulative exposure and margin of exposure (MOE) per regulatory silo

Exposure was calculated per silo using currently applicable methodologies in Europe. Main assumptions and model settings of these methodologies are summarised in Table 1. For

PPRs, the optimistic and pessimistic approaches suggested by EFSA (2012a) were used to calculate chronic exposure. These were designed as simple practical implementations to capture the combined effect of different uncertainties affecting different aspects of exposure modelling, resulting in a range of possible exposure values. The two approaches differ mainly in handling missing data and samples with values below the LOD or LOQ (Table 1). Optimistic assumptions may lead to underestimation of exposure, whereas pessimistic assumptions may lead to overestimation of exposure (EFSA 2012a).

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Table 1

Basic assumptions and settings of chronic dietary exposure assessment used for plant protection products and residues (PPRs), persistent organic pollutants (POPs) and food additives (FAs.)

Assessment component	PPRs		POPS		FAs	
	Optimistic	Pessimistic	Lower bound	Upper bound	Authorization scenario	Non-brand loyal scenarios
Modelling food consumption data	OIM ^a	OIM	OIM	OIM	OIM	OIM
Linking food to concentration data	Food conversion model	Food conversion model	Food conversion model	Food conversion model	Direct	Direct
Origin concentration data	Monitoring data residues	Monitoring data residues, MRL ^b and 0.1 ppb for drinking water for 5 most toxic	Monitoring data	Monitoring data	MPL ^c	Use levels and concentration data

Assessment component	PPRs		POPS		FAs	
	Optimistic	Pessimistic	Lower bound	Upper bound	Authorization scenario	Non-brand loyal scenarios
Treatment samples < LOQ or LOD ^d	Assume 0	chemicals Level of LOQ or LOD when authorized use ^e	Assume 0	Level of LOQ or LOD	NA	Half of the level of LOD or LOQ when authorized usage
Modelling concentration data	Empirical	Parametric	Empirical	Empirical	Empirical	Empirical
Missing values	Set at 0 mg/kg	Complemented with data from other countries, studies or MRL when authorized	Supertype approach ^f	Supertype approach	NA	MPL

Assessment component	PPRs		POPS		FAs	
	Optimistic	Pessimistic	Lower bound	Upper bound	Authorization scenario	Non-brand loyal scenarios
Presence in food [/]	100% ^g	usage 100% ^g	Based on monitoring data	Based on monitoring data	100% when authorised food	100% when authorised usage
Variability between units	No unit variability	No unit variability	No unit variability	No unit variability	No unit variability	No unit variability
Processing factors	Value used in deterministic assessment, fixed	Value used in deterministic assessment, fixed, only included when value ≥ 1 ⁱ	Value used in deterministic assessment, fixed	Value used in deterministic assessment, fixed	NA ^h	NA

Assessment component	PPRs		POPS		FAs	
	Optimistic	Pessimistic	Lower bound	Upper bound	Authorization scenario	Non-brand loyal scenarios
Uncertainties						
Sampling uncertainty food consumption data	Bootstrap	Bootstrap	Bootstrap	Bootstrap	Bootstrap	Bootstrap
Sampling uncertainty /concentration data	Bootstrap	Bootstrap	Bootstrap	Bootstrap	NA, fixed value used for concentration	NA, fixed value used for concentration
Proportion < LOD or LOQ	Bootstrap	Parametric	Bootstrap	Bootstrap	NA	NA

^a OIM = Observed Individual Mean

^b MRL = maximum residue limit; Animal commodities

^c MPL= maximum permitted levels

^d Limit of detection (LOD) and limit of quantification (LOQ), whatever available from monitoring data

^e Established by availability of MRLs in the MRL database on the website of the European Commission

^f The supertype approach links missing concentration data of a certain food to the food group the food belongs to (e.g. if concentration data for spinach is missing, the data of leafy vegetables will be used).

^g The percentage crop treated was set at the level of 100% due to lack of information on this input variable.

^h NA= not applicable

ⁱ Deviates from EFSA guidance for pessimistic, chronic model run where it is stated that 'Distribution of estimates for mean processing factor, obtained by bootstrapping measured values' should be used.

The lower bound and upper bound scenarios were assumed for POPs, and these scenarios differed only in the way samples with values below the limit of detection or quantification are treated i.e. assuming these to be 0 in case of the lower bound scenario and the value of the limit of detection or quantification, whichever is available, in the upper bound scenario (Table 1). The lower bound scenario may lead to underestimation of exposure, whereas the upper bound scenario may lead to overestimation of exposure.

For food additives, we calculated EFSA's authorization scenario using legal maximum permitted levels and the non-brand-loyal scenario using mean use levels (EFSA 2017a). The scenario with the legal maximum permitted levels generally leads to an overestimation of exposure and the non-brand-loyal scenario can be regarded as refined and more realistic.

For all chemical silos, cumulative exposure was assessed using the Monte Carlo Risk Assessment tool (MCRA version 8.2, de Boer et al., 2016, <https://mcra.rivm.nl>). Chronic (long-term) exposure was calculated using the simple Observed Individual Means (OIM) model. Briefly, for each individual i in the food consumption data base, the consumed amount of a certain food f averaged over the total number of consumption days q_{if} was multiplied with the average concentration of each chemical present in that food c_{ifs} . This was done for all consumed foods per individual. The subsequent obtained exposures per food were summed for each chemical s per individual over the F numbers of food consumed and divided by the bodyweight of the individual bw_i , which yielded the chronic exposure E_{is} to chemical s of the individual i .

$$E_{is} = \frac{\sum_{f=1}^F q_{if} c_{ifs}}{bw_i} \quad (\text{Equation 3})$$

The chronic exposure of each chemical s in the CAG E_{is} was then multiplied by the RPF of the chemical (RPF_s) and summed per individual to obtain the cumulative exposure per individual $Cum E_i$.

$$Cum E_i = \sum_{s=1}^S E_{iS} * RPF_s \quad (\text{Equation 4})$$

where S is the total number of chemicals considered. For PPRs and food additives the relative potency factors were calculated by dividing the NOAEL of the index compound ($NOAEL_{ind}$) by the $NOAEL_s$ of each chemical s :

$$RPF_s = \frac{NOAEL_{ind}}{NOAEL_s} \quad (\text{Equation 5})$$

For POPs the $D_{r,h}$ values were used to calculate RPFs:

$$RPF_s = \frac{D_{r,href}}{D_{r,hs}} \quad (\text{Equation 6})$$

The calculation resulted in an exposure distribution from which exposure percentiles can be obtained. The MOE for each silo was calculated by dividing the point of departure (NOAEL or the $D_{r,h}$) of the index compound by the cumulative exposure estimate for a particular exposure percentile:

$$MOE = \frac{NOAEL \text{ or } D_{r,h}}{CumE} \quad (\text{Equation 7})$$

Then, the MOE was compared to an acceptable margin of exposure (AMOE) defined for each silo taking into account the uncertainty associated to hazard data. If the MOE was higher than the defined AMOE, a risk can be ruled out. If the MOE is smaller than the AMOE, a risk may exist or refinement of the assessment is required, depending on the conservatism of the input data.

2.3 Normalized combined (total) margin of exposure (nMOET) across the regulatory silos

The MOEs calculated per silo can be combined in a combined (total) margin of exposure (MOET), (EFSA 2019a). The MOET is defined as the reciprocal of the sum of the reciprocal MOEs of different chemicals or in our case regulatory silos:

$$\frac{1}{MOET} = \frac{1}{MOE_{PPRS}} + \frac{1}{MOE_{POPS}} + \frac{1}{MOE_{FAS}} + \dots \quad (\text{Equation 8})$$

In case of different AMOEs, the MOET cannot be directly used without a normalisation for the AMOE. Following our case study, the MOE of each silo was divided by its AMOE and subsequently summing the reciprocals into the reciprocal of the normalised MOET (nMOET):

$$\frac{1}{nMOET} = \frac{1}{\frac{MOE_{PPRS}}{AMOEP_{PPRS}}} + \frac{1}{\frac{MOE_{POPS}}{AMOEP_{POPS}}} + \frac{1}{\frac{MOE_{FAS}}{AMOEF_{FAS}}} \quad (\text{Equation 9})$$

Which also reads as:

$$\frac{1}{nMOET} = \left(\frac{AMOEP_{PPRS}}{MOE_{PPRS}}\right) + \left(\frac{AMOEP_{POPS}}{MOE_{POPS}}\right) + \left(\frac{AMOEF_{FAS}}{MOE_{FAS}}\right) \quad (\text{Equation 10})$$

Based on (Equation 7) this can equivalently be expressed as:

$$\frac{1}{nMOET} = \left(\frac{1}{\frac{NOAEL_{PPRS}}{EXP_{PPRS}}}\right) AMOEP_{PPRS} + \left(\frac{1}{\frac{Dr,h_{POPS}}{EXP_{POPS}}}\right) AMOEP_{POPS} + \left(\frac{1}{\frac{NOAEL_{FAS}}{EXP_{FAS}}}\right) AMOEF_{FAS} \quad (\text{Equation 11})$$

which simplifies to

$$\frac{1}{nMOET} = \left(\frac{EXP_{PPRS}}{NOAEL_{PPRS}}\right) AMOEP_{PPRS} + \left(\frac{EXP_{POPS}}{Dr,h_{POPS}}\right) AMOEP_{POPS} + \left(\frac{EXP_{FAS}}{NOAEL_{FAS}}\right) AMOEF_{FAS} \quad (\text{Equation 12})$$

If the nMOET is larger than 1, a risk can be ruled out. If the nMoet is smaller than 1, then a risk may exist or a refinement of the assessment is needed, depending on the conservatism of the input data.

Note that when the AMOEs equal uncertainty factors (e.g. an AMOE of 100 equals a standard uncertainty factor of 100), the reciprocal of the nMOET approach equals the modified reference point index (mRPI) approach proposed by Vedjosky et al (2019) which is the sum of the reference point index (RPI) multiplied by the uncertainty factor (UF) assigned to the silo. The RPI is also referred as the point of departure index (PODI; EFSA 2019a). Because in EuroMix the wording point of departure rather than reference point is used, we

use PODI instead of (m)RPI for harmonisation with other papers published in this special issue. In that case, the nMOET is the reciprocal of the $\sum PODI * UF$:

$$PODI = \frac{EXPi}{PODi} \quad (\text{equation 13})$$

13)

and

$$\sum PODI * UF = \left(\frac{EXP_{PPRS}}{NOAEL_{PPRS}} \right) UF_{PPRS} + \left(\frac{EXP_{POPS}}{Dr,h_{POPS}} \right) UF_{POPS} + \left(\frac{EXP_{FAS}}{NOAEL_{FAS}} \right) UF_{FAS} \quad (\text{equation 14})$$

14)

The overarching nMOET was calculated for mean exposures and for P95 exposures. As a worst case scenario, the nMOET was calculated from data obtained from the pessimistic approach for PPRs, the upper bound scenario for POPs and the MPL scenario for FAs. In addition, for a less conservative scenario, the nMOET and the $\sum PODI * UF$ were calculated from results obtained from the optimistic scenario for PPRs, the lower bound scenario for POPs and the refined scenario with mean use levels for FAs. For the purpose of this case study, a standard acceptable MOE of 100 was chosen for PPRs and FAs. For POPs, a standard acceptable MOE of 10 was arbitrarily selected (see discussion of POPs and overarching).

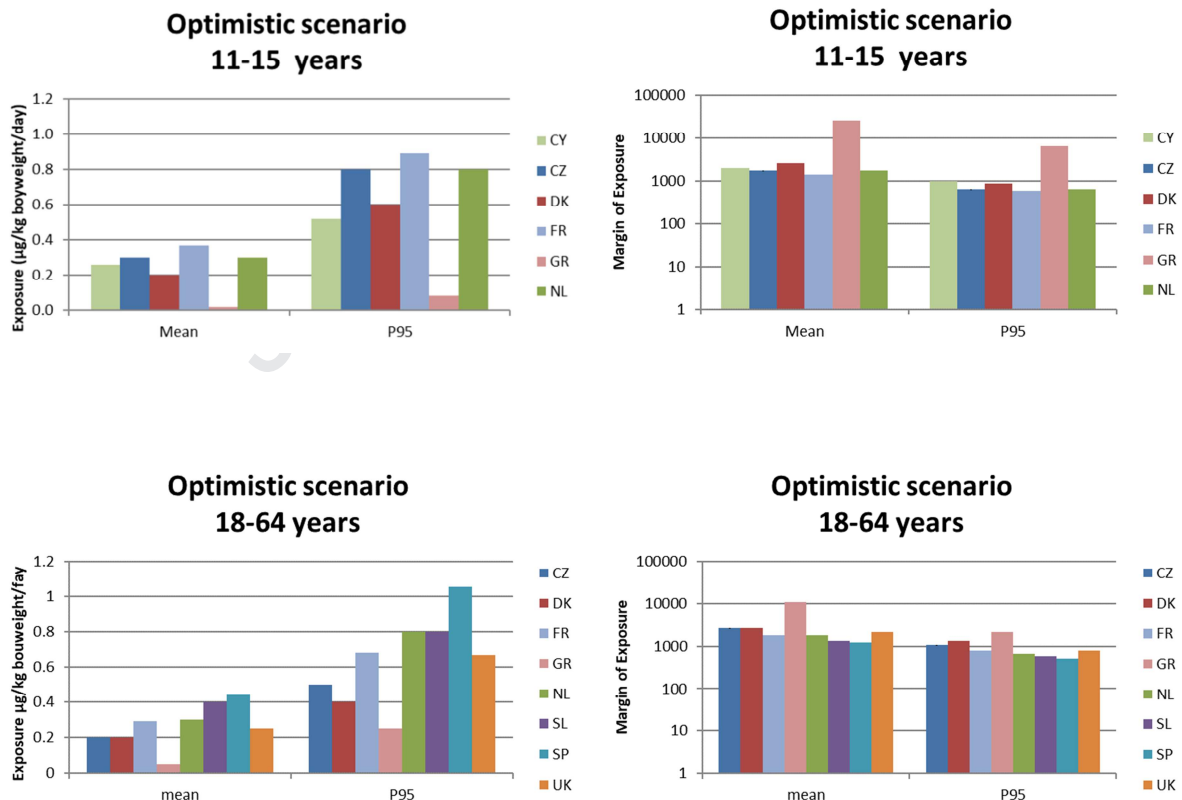
3. Results

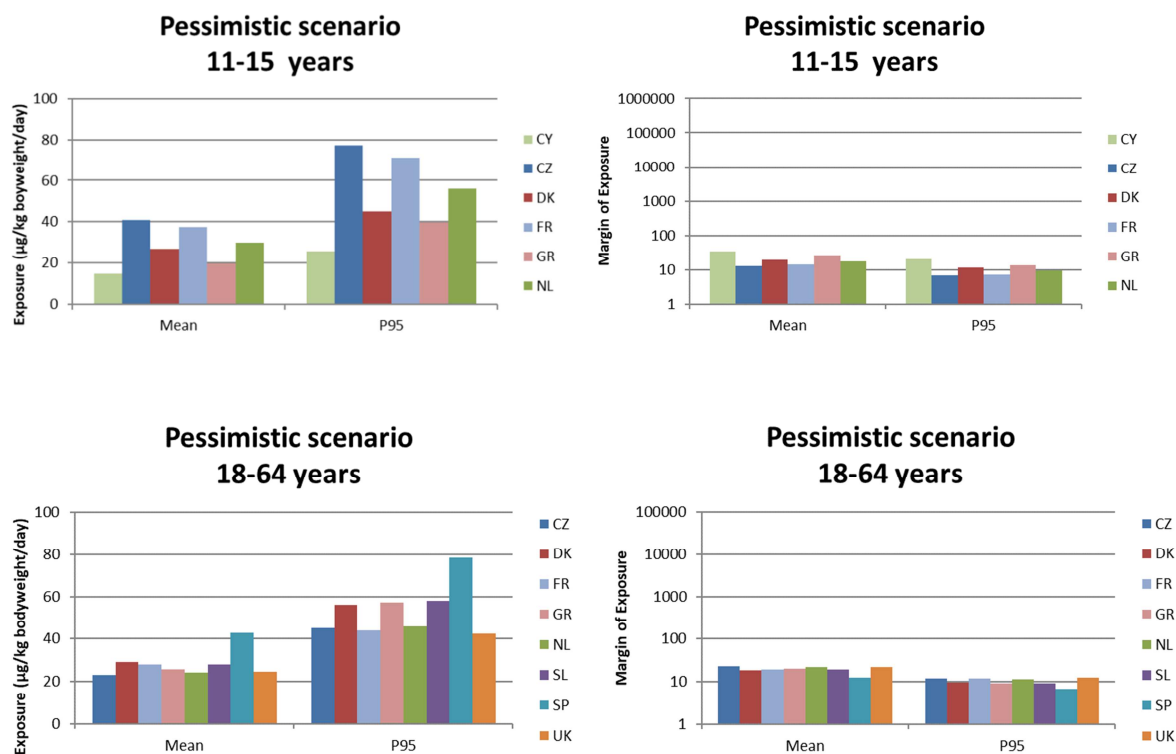
3.1 PPRs

Fig. 1 shows the estimates and MOEs for chronic exposure to PPRs belonging to the CAG liver steatosis for children (11-15 years) and the adult population (18-64 years) following the optimistic and pessimistic approach, respectively. For the optimistic approach, mean cumulative exposure estimates ranged from 0.02 μg to 0.4 μg flusilazole equivalents/kg bw

per day, depending on country and age group, which corresponded to a MOE between 1200 and 26000. With the pessimistic approach, mean exposure estimates varied from 14.7 to 42.7 μg flusilazole equivalents/kg bw per day depending on country and age group. This corresponded to a MOE between 13 and 35. Regarding the 95th percentile of exposure, the exposure estimated ranged from 0.08 to 1.1 μg flusilazole equivalents/kg bw per day and from 25.7 to 78.6 μg flusilazole equivalents/kg bw per day for the optimistic and pessimistic approach, respectively. For the optimistic approach, the MOE of the 95th percentile varied between 503 and 6500 and for the pessimistic approach between 7 and 20.

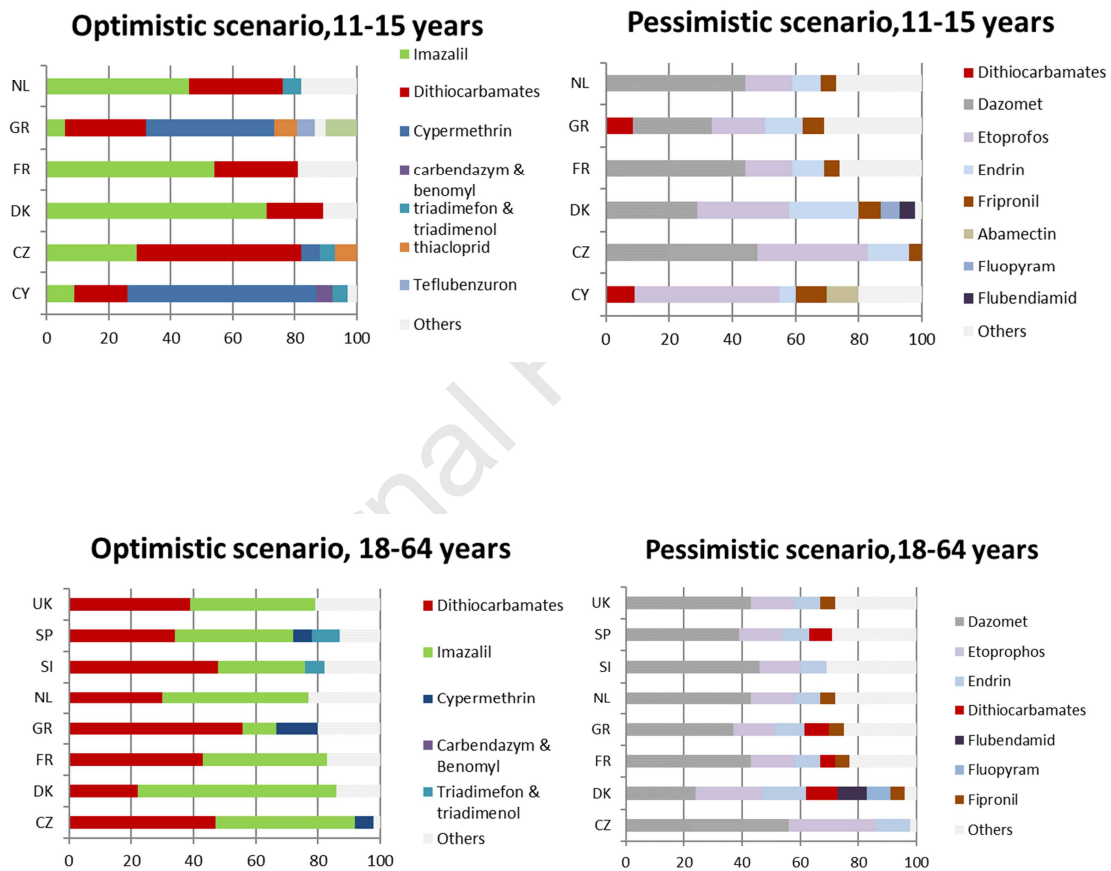
Fig. 1. Mean and the 95th percentile cumulative exposure estimate to 144 plant protection products and their residues belonging to the CAG liver steatosis (left panels) and their corresponding margin of exposures (right panels) calculated for six European populations in the age of 11 to 15 years and eight European populations in the age of 18 to 64 years using an optimistic and a pessimistic model run, respectively.





Main chemical contributors to total exposure to PPRs belonging to the CAG liver steatosis are shown in Fig. 2. For the optimistic approach, imazalil and dithiocarbamates were observed among the major contributors to chronic cumulative exposure to PPRs belonging to the CAG liver steatosis in all populations. The other major contributors to exposure varied between populations and were cypermethrin, carbendazim & benomyl, triadimefon and triadimenol, thiacloprid, teflubenzuron, and/or metalaxyl and metalaxyl M. From these chemicals, only dithiocarbamates appeared to be important contributors to exposure in the pessimistic scenario. Ethoprophos and endrin appeared major contributors to exposure in all populations in the pessimistic scenario. Dazomet was also an important contributor to exposure for all populations in the pessimistic scenario, except for Cypriot children 11-15 years. Fipronil was also an important contributor to exposure, except for Cypriot adults. Other main contributors to exposure varied between populations and were abamectin, fluopyram, and flubendiamid.

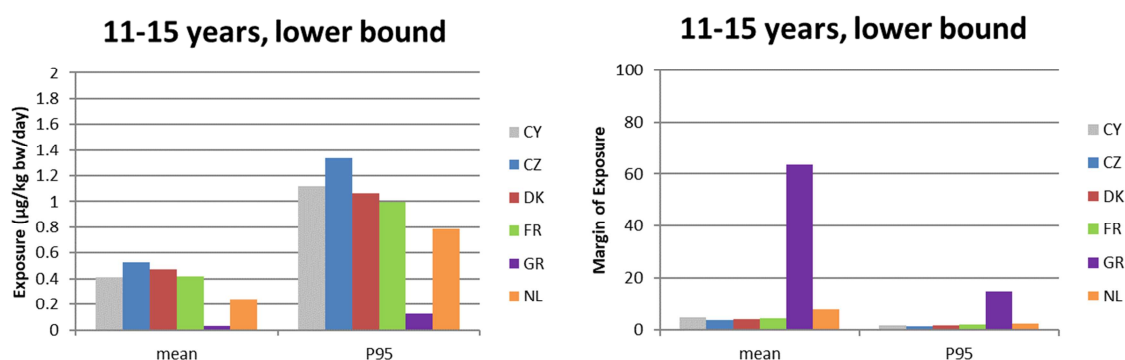
Fig. 2. Main chemicals contributing to the total cumulative exposure estimate of 144 plant protection products and their residues belonging to the CAG liver steatosis calculated for six European populations in the age of 11 to 15 years and eight European populations in the age of 18 to 64 years using optimistic and pessimistic models, respectively

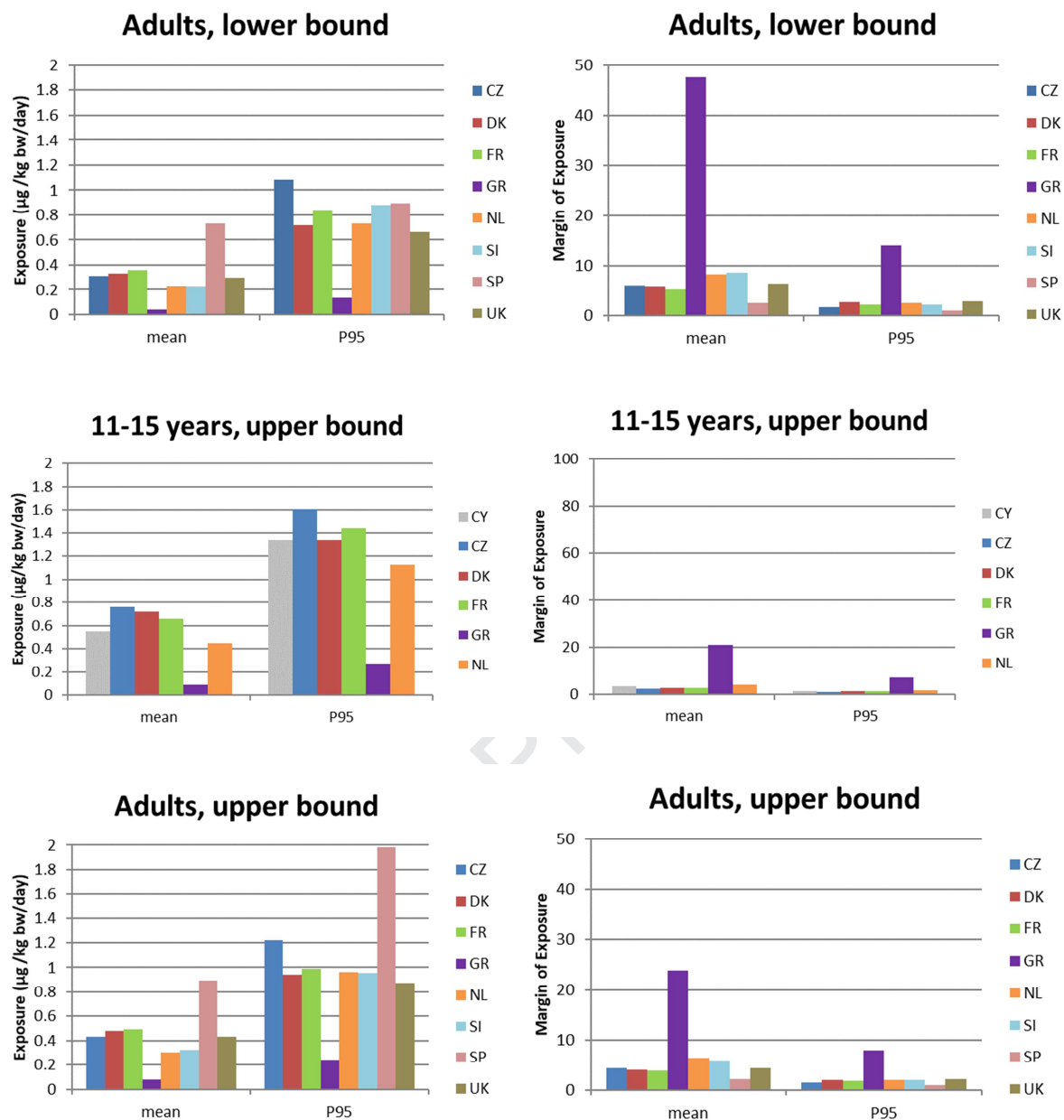


3.2 POPs

Chronic exposure estimates to POPs belonging to the CAG liver steatosis, together with their corresponding MOEs, are shown in Fig. 3. Mean exposures depended on age and country and varied from 0.03 to 0.73 μg PCB-153 equivalents/kg bw per day and from 0.08 to 0.89 μg PCB-153 equivalents/ kg bw per day for the lower and upper bound scenario, respectively. P95 exposures ranged from 0.1 to 1.7 μg PCB-153 equivalents/kg bw per day and from 0.2 to 1.9 PCB-153 equivalents/ kg bw per day for the lower and upper bound scenario, respectively. For the lower bound scenario, the MOE for the mean exposure varied between 3 and 63 and that of P95 exposure between 1 and 15 respectively. MOEs of the upper bound scenario varied between 2 and 24 for the mean exposure and 1 to 8 for the 95th percentile of exposure (Fig. 3).

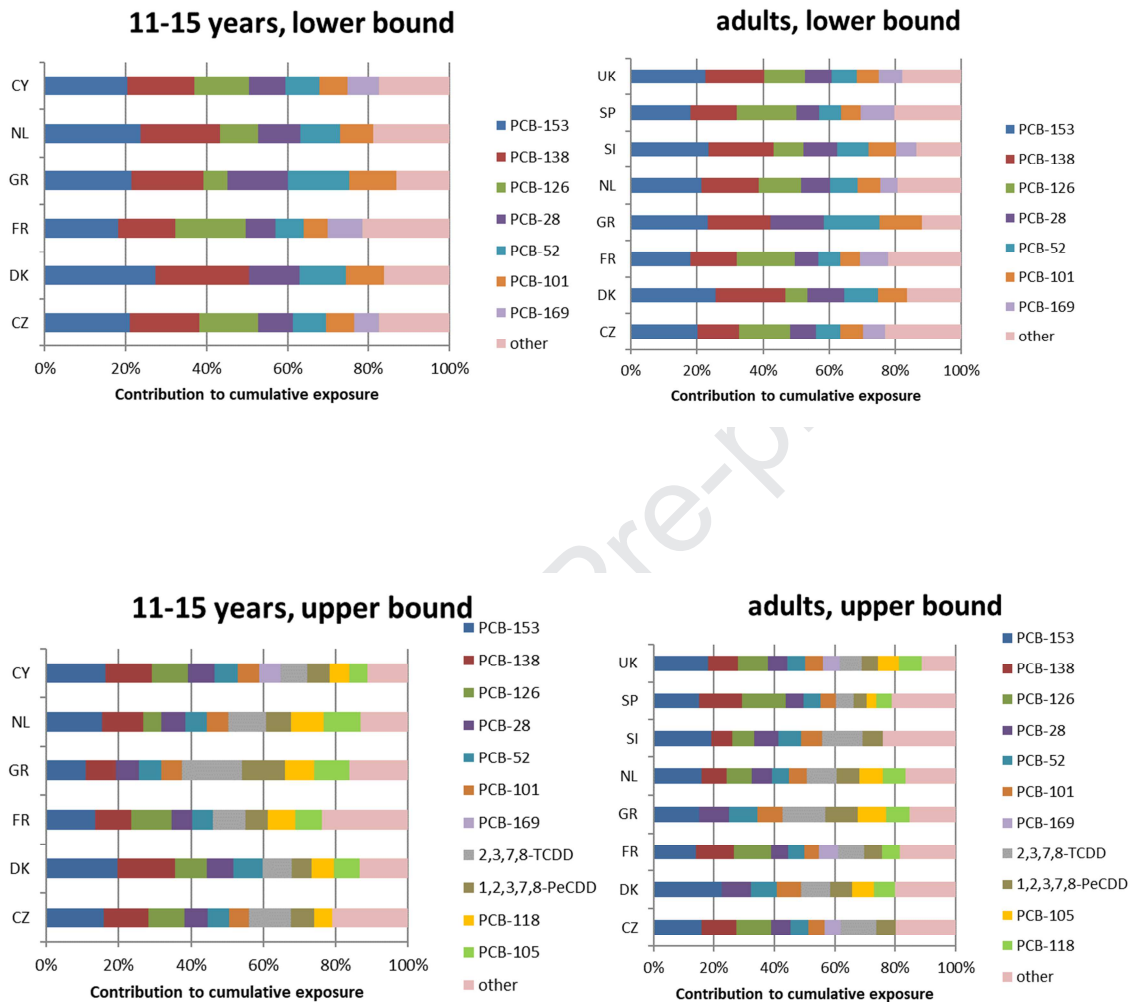
Fig. 3. Mean and the 95th percentile cumulative exposure estimate to 49 persistent organic pollutants belonging to the CAG liver steatosis (left panels) and their corresponding margin of exposures (right panels) calculated for six European populations in the age of 11 to 15 years and 8 European populations in the age of 18 to 64 years using a lower and upper bound scenario, respectively.





Main contributors to total POPs exposure are shown in Fig.4. For the lower bound scenario, 5 indicator congeners of NDL-PCBs (the -153, -138, -28, -52 and -101 congeners) and 2 DL-PCB congeners (-126 and -169) were main contributors to exposure for most populations. These chemicals were also observed in the upper bound scenario, together with 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD and two other DL-PCBs (-105 and -118).

Fig. 4. Main chemicals contributing to the total cumulative exposure estimate of 49 persistent organic pollutants belonging to the CAG liver steatosis calculated for six European populations in the age of 11 to 15 years and eight European populations in the age of 18 to 64 years using an optimistic and a pessimistic model run, respectively.

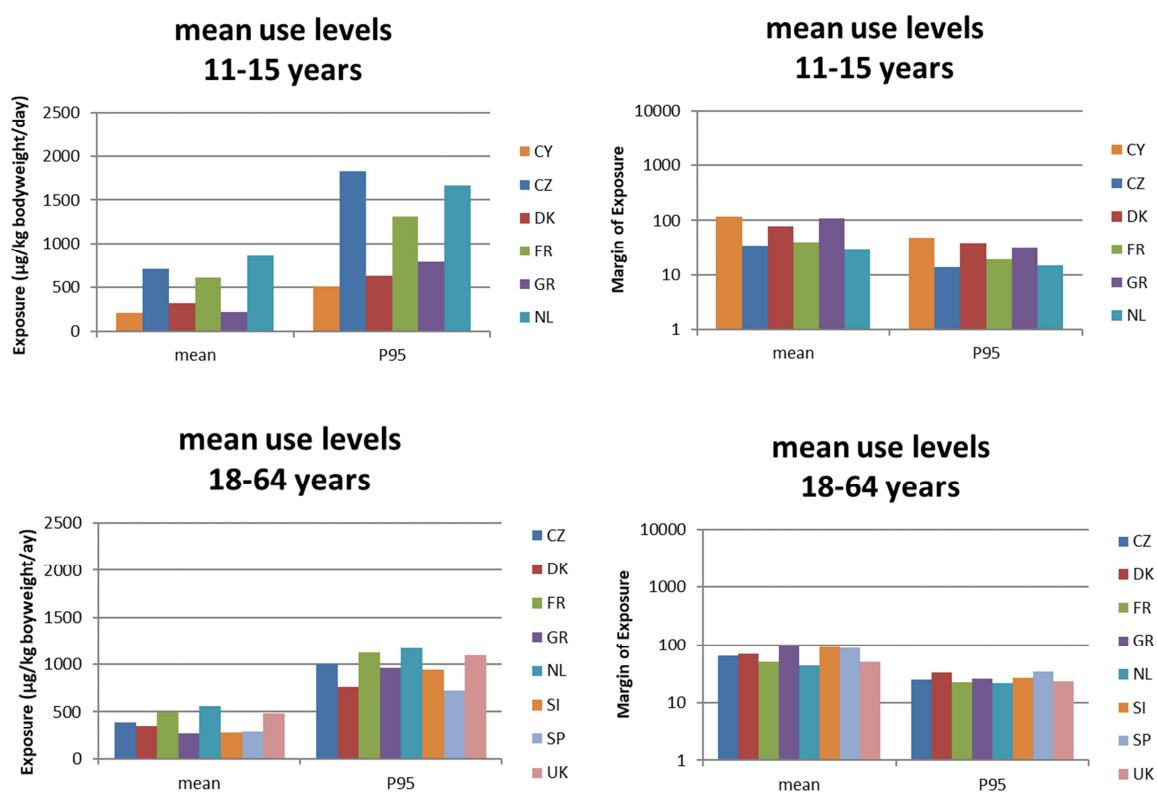


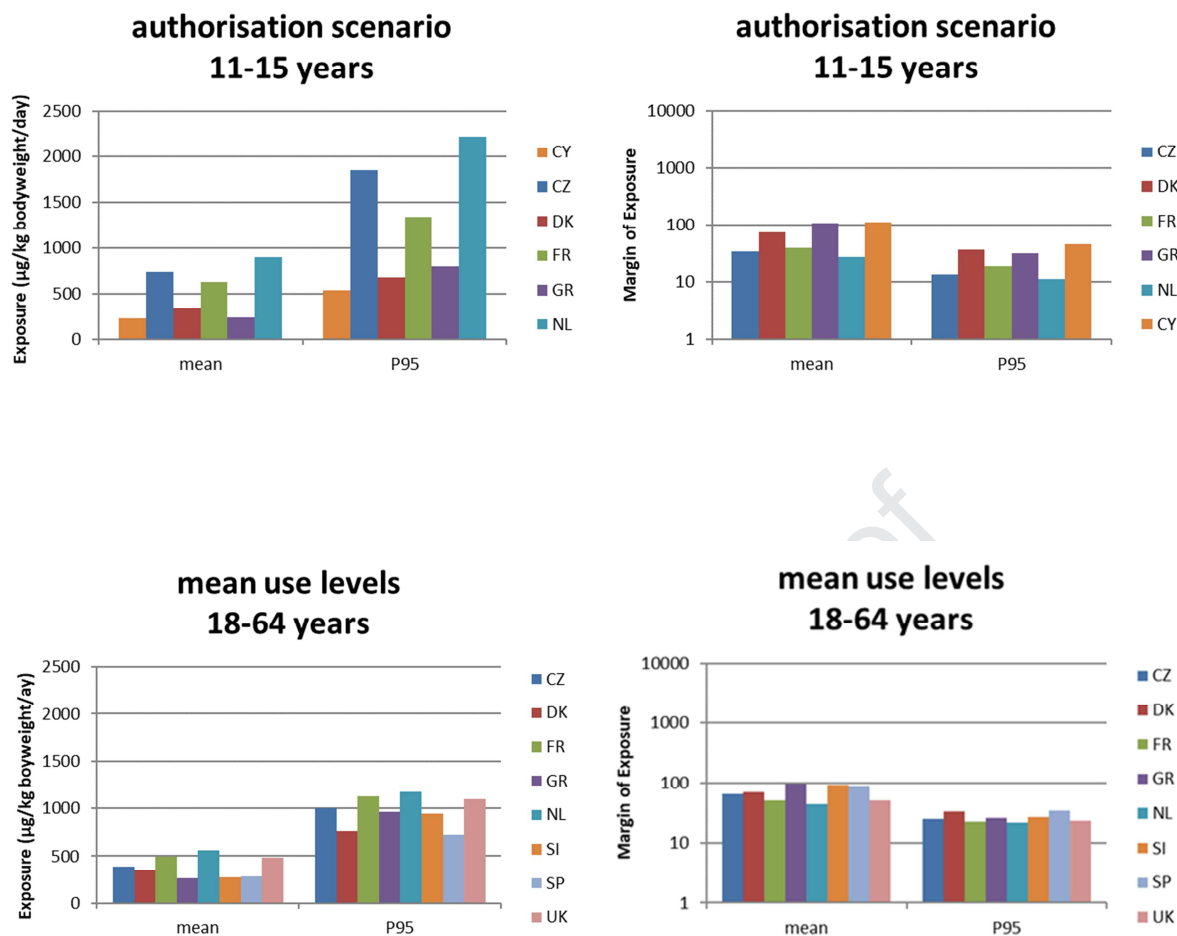
3.3 Food additives

Mean chronic exposure estimates varied from 0.2 mg to 0.8 mg canthaxanthin-equivalents/kg bw per day for both the non-brand-loyal and authorization scenario. High exposure (P95) varied between 0.5 to 1.8 mg canthaxanthin-equivalents/kg bw per day and 0.5 to 2.2 mg canthaxanthin-equivalents/kg bw per day for the non-brand-loyal and authorization scenario respectively (Fig. 5).

The calculated MOEs for the different scenarios are shown in Fig. 5, and varied between 19 and 117 for mean cumulative exposure to food additives and between 11 and 95 for the 95th exposure percentile, depending on population and exposure scenario (Fig. 5).

Fig. 5. Mean and the 95th percentile cumulative exposure estimate to 7 food additives belonging to the CAG liver steatosis (left panels) and their corresponding margin of exposures (right panels) calculated for six European populations in the age of 11 to 15 years and eight European populations in the age of 18 to 64 years using an authorization and a non-brand-loyal refined scenario, respectively





Regardless of the calculation scenario, sucroglycerides dominated the cumulative exposure to food additives in all populations. Its contribution to exposure varied between 81 and 98%. Dimethyldicarbonate also contributed to the cumulative exposure to food additives, but to a lesser extent (2-16%, depending on population and scenario).

3.4 Overarching

Table 2 shows the nMOET for the nine countries. The nMOET was smaller than 1 regardless of exposure scenario (worst case versus less conservative), population and country. Table 2 also shows the contribution of a particular silo to the 1/nMOET metric. For the cumulation of the less conservative scenarios, PPRs contributed up to 3%, POPs between 15-76% and FAs 22-85% depending on population, country and exposure statistics. For cumulation of the worst case scenarios the contribution of PPRs varied between 37-76%, that of POPs between 7 and 37%, and that of FAs between the 8-28%.

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Table 2. Overarching risk characterisation using a normalised total combined margin of exposure (nMOET) assuming a less conservative and a worst case scenario, and the percentage of pesticides (PPRs), persistent organic pollutants (POPs) and food additives (FA) contributing to the 1/nMOET metric

	Less conservative ^b				Worst case ^c			
	nMOET	% contribution to 1/nMOET			nMOET	% contribution to 1/nMOET		
		PPRs	POPs	FAs		PPRs	POPs	FAs
Children aged 11-15 years								
<i>Mean exposure</i>								
CY ^a	0.3	2	71	28	0.2	42	44	14
CZ	0.2	1	49	50	0.07	53	27	20
DK	0.3	1	65	34	0.1	50	37	13
FR	0.2	1	47	52	0.08	54	27	19
GR	0.9	0.4	15	85	0.2	73	9	18
NL	0.2	1	27	72	0.09	49	20	31
<i>P95</i>								
CY	0.1	1	73	25	0.07	53	24	24
CZ	0.07	1	48	50	0.03	50	25	25
DK	0.1	1	68	31	0.05	61	19	19
FR	0.09	2	49	49	0.04	56	22	22
GR	0.3	0.4	17	82	0.08	54	23	23
NL	0.09	1	38	61	0.04	37	31	31
Adults 18-64 years								
<i>Mean exposure</i>								
CZ	0.3	1	50	48	0.1	53	28	19
DK	0.3	1	54	44	0.1	58	27	15
FR	0.3	1	48	50	0.1	53	26	20
GR	0.8	1	16	83	0.2	76	7	17
NL	0.3	2	34	64	0.1	54	19	28
SL	0.4	3	49	47	0.1	65	21	14

	Less conservative ^b				Worst case ^c			
	nMOET	% contribution to 1/nMOET			nMOET	% contribution to 1/nMOET		
		PPRs	POPs	FAs		PPRs	POPs	FAs
SP	0.2	2	76	22	0.07	58	34	8
UK	0.3	1	44	55	0.1	52	25	22
<i>P95</i>								
CZ	0.1	1	58	41	0.05	45	34	21
DK	0.1	1	55	44	0.05	57	27	17
FR	0.1	1	49	50	0.06	46	29	25
GR	0.2	1	15	84	0.06	68	8	24
NL	0.1	2	44	54	0.05	47	27	26
SL	0.1	2	54	44	0.05	55	25	20
SP	0.08	2	75	24	0.04	53	37	11
UK	0.1	2	44	55	0.06	47	27	26

^a Abbreviations used CY Cyprus, CZ Czech Republic, DK Denmark, FR France, GR Greece, NL the Netherlands, SL Slovenia, SP Spain, UK United Kingdom.

^b the less conservative scenario is obtained by summing risk percentiles of plant protection products and their residues obtained with an optimistic scenario, persistent organic pollutants obtained with a lower bound scenario, and food additives obtained with a mean use level scenario.

^c Worst case scenario is obtained by summing plant protection products and their residues obtained with a pessimistic scenario, persistent organic pollutants obtained with an upper bound scenario, and food additives obtained with a maximum permitted level scenario

4. Discussion

This work demonstrated that the component-based approach for performing cumulative exposure assessment and the subsequent risk characterisation can be applied within a given regulatory silo. It also highlighted that each silo has its own specific approach and associated uncertainties summarised in Table 3 and discussed in more detail below. It also showed that a nMOET approach, which is comparable to the mRPI approach proposed by Vejdovszky et al. (2019), can be used for overarching the risk characterisation of the silos. In the sections below, the cumulative exposure and risk assessment will be discussed for each silo, followed by the overarching approach using nMOET.

Table 3. Uncertainties related to risk assessment of combined exposure to plant protection products and residues (PPRs), persistent organic pollutants (POPs) and food additives (FAS).

	PPRs	POPs	FAS
CAG membership	Limited studies for the specific effect	Limited studies for the specific effect	Limited studies for the specific effect
	Grouping based on parent compounds vs presence of residues in food	Grouping based on one congener per POP class or on technological products, which are mixtures themselves	Difference in grouping benzoic acid (CAG member as PPR database but not FAS database)
RPF derivation	Based on NOAELs or LOAEL/3	Based on D_r, h calculated from	Based on NOAELs or LOAEL/3

		NOAELs or LOAEL/3 using absorption factors and half-lives.	
	Limited studies to derive point of departure for several chemicals	Limited studies to derive point of departure for most chemicals	Limited studies to derive point of departure for most chemicals
	Point of departures obtained from studies with different duration	Point of departures obtained from studies with different duration	Point of departures obtained from studies with different duration
		WHO-TEF 2005 factors for dioxins and DL-PCBs need to be updated (EFSA 2018). Body burden TEFS not available (Van den Berg et al., 2009) Limited or no studies for individual congeners or isomers	
Concentration data	Residue definition monitoring vs that of risk assessment	Lack of concentration data for several isomers	Limited concentration data and use levels
Processing factors	Limited available processing factors	Limited available processing factors for some POPs	Processing factors lacking for some FAs

4.1 PPRs

PPRs assessment was based on hazard data obtained from *in-vivo* studies based on similar methodologies, therefore, a MOE of 100 was considered acceptable by EFSA (EFSA 2019a). For the optimistic scenario, the MOE of mean and P95 exposure were all above 100, whereas for the pessimistic scenario the MOE of mean and P95 exposure were below 100 (Fig. 1). A MOE smaller than 100 could indicate a health risk or that refinement of the assessment is needed. The large difference between results of the optimistic and pessimistic scenario indicate a large impact of replacing missing concentration values by maximum residue limits and/or replacing non-detect concentration data by the LOD or LOQ values. This is in agreement with the findings of Boon et al. (2015). The EFSA guidance to probabilistic exposure (EFSA 2012a) recommends refinement of exposure assessment when the optimistic approach resulted in an acceptable MOE whereas the pessimistic approach did not. EFSA's newly developed tier II (EFSA 2019b,c, van Klaveren et al 2019a,b) could be used for such a refinement. This EFSA tier II assumes non-detect values to be equal to half the value of corresponding LOD or LOQ and takes into account assumptions for agricultural use of authorised pesticide-food combinations based on the occurrence percentage of pesticides in food. It also includes modelling of the conversion of the residue definition for monitoring into residue definition for risk assessment. As a real risk assessment was not the purpose of our study, these refinements were not performed.

As for all risk assessment, the present assessment was subject to uncertainties. Recently EFSA recognised 34 sources of uncertainty in their assessments for the nervous system and 32 sources in their assessments for thyroid effects (EFSA 2019b and c). Uncertainties in hazard and exposure data for the PPRs grouped into the CAG liver steatosis was already addressed by Crépet et al. (2019). These can be divided into generic uncertainties and PPR-specific uncertainties. Generic uncertainties include CAG membership because of knowledge

gaps for several chemicals. Recently, EFSA used a method for determination of CAG membership based on expert knowledge elicitation (EFSA 2019d,e). The EuroMix project developed a tool to include a CAG membership probability into uncertainty assessment (Kennedy et al., 2020), which is available in the EuroMix toolbox implemented in MCRA 9.0. Probabilities can be set from expert knowledge or calculated based on QSAR model results. Future assessments can be refined using this tool. Other generic uncertainties include the use short-term studies for the derivation of the point of departure, the use of NOAELs or LOAELs divided by 3 to derive RPFs. A more refined way of deriving points of departure is the bench mark dose approach (EFSA 2017b). This approach takes uncertainty into account by providing a confidence interval around an estimated bench mark dose. Another generic uncertainty is the assumption of dose-addition.

A major PPR-specific uncertainty in cumulative exposure assessment of PPRs is that processing factors are not available for all chemical/food/process combinations and extrapolation of processing factors (e.g. a processing factor available for peeling of mandarins to peeling of lemons) is not common practice. The impact of missing processing factors was assessed for the Dutch population aged 18 to 64 years after adding newly available processing factors for the combination imazalil/oranges, grapefruits, lemon/juicing (Scholz et al. 2018). This resulted in a 1.3 to 1.7 fold reduction of exposure and consequently an increase of the MOE and imazalil changing from being the major contributor to exposure to the second main contributor (not shown). Recently, the EFSA subcontractors RIVM, BPI and BfR started building a database on processing factors for PPRs using the harmonized food coding FoodEx2 and suggested possible extrapolations (Scholz et al., 2018, van Donkersgoed et al., 2018). However, the particular database contains only a limited amount of the available data (Scholz et al 2018). An update of the table containing more PPR residues would facilitate future (mixture) risk assessment. Another option to reduce uncertainty in processing is the use of total diet studies which directly give the concentration in the food “as consumed” (Vin et al, 2014).

Another important PPR-specific uncertainty in cumulative exposure assessment is the establishment of different residue definition for enforcement and risk assessment. Residue definitions for enforcement can be converted to that for risk assessment using conversion factors obtained from pre-registration studies. These are described in EFSA peer review reports and reports of the Joint FAO/WHO Meetings on PPR Residues (JMPR). Conversion factors may vary depending on the compound and/or crop, resulting in many concentration conversions to be manually performed. As this would require significant resources, we assumed conversion factors to be equal to 1, which may lead to an underestimation of exposure, as in the residue definition for risk assessment usually more metabolites are included compared with the residue definition for enforcement. A harmonized database with conversion factors facilitating automated inclusion in cumulative exposure assessment would be helpful for future PPR mixture risk assessment.

To reduce the uncertainties listed and to subsequently refine the combined exposure, focus on risk drivers could reduce the research burden. Priority can be given to the risk drivers of combined exposure to PPRs as shown in Figure 2.

4.2 POPS

According to the EFSA guidance on combined exposure risk assessment, the assessment performed for POPs can be regarded as part of a higher tier risk characterization, since kinetic data are taken into account (EFSA 2019a). The EFSA guidance on combined exposure risk assessment does not provide information on which metric should be compared within a higher tier risk characterisation. Since the $D_{r,h}$ takes into account differences in kinetics between test animals and humans, a MOE smaller than 100 might be acceptable for POPs. Examples of acceptable MOEs or uncertainty factors for derivation of health-based guidance values in literature are 9.6 for dioxins and DL-PCBs (EFSA 2015c), a factor 8 for HBCDDs, (EFSA 2011c), a factor 5 or 6 for PBBs (EFSA 2010b) and a factor 2.5 for BDE-47,

-99 and -153 (EFSA 2011a). Since our study was performed for the purpose of demonstrating overarching dietary chemical mixture risk assessment and not a real risk assessment, we did not determine the value of an acceptable MOE but arbitrarily used a value of 10 for calculation of the nMOET. For a real combined exposure risk assessment, however, this should be addressed. If the outcome is that different acceptable MOEs apply for the different POP classes, as a conservative approach the largest acceptable MOE could be taken, followed by more refined approaches if the observed MOE is deemed too small. Recently, a decision tree for the derivation of uncertainty factors for cumulative exposure assessment was proposed (Vejdovszky et al., 2019). This decision tree can also be used for the derivation of acceptable MOEs.

The same generic uncertainties as mentioned above for PPRs apply for POPs. Regarding POP-specific uncertainties, the use of WHO 2005-TEFs for relative potency of dioxins and DL-PCBs in our study could have contributed to the uncertainties around the RPF. These WHO 2005-TEFs are based on Ah receptor activation and are not considered to be target organ-specific (Van den Berg et al., 2006). Because AhR activation may lead to liver steatosis (Luckert et al., 2018), we considered it justified to apply WHO 2005-TEFs. It should be noted that TEFs from an administered dose may differ from those in situ in relevant tissues or cells and therefore systemic or body burden TEFs would be preferred, but data for establishing systemic or body burden TEFs are not sufficient (Van den Berg et al., 2006). Also, a revision of the WHO 2005-TEFs may be needed because of newly available *in-vivo* and *in-vitro* data as recommended by EFSA (EFSA 2018). In addition, EFSA concluded that more research and understanding is needed on reported congener-specific effects of PCDD/Fs and DL-PCBs, including their relevance at low doses (EFSA 2018).

Uncertainty around the RPF of individual congeners/isomers of the different POP classes due to limited hazard data available for each individual congener/isomer may also account for the other POPs. As described in section 2.1.1, NOAELs were obtained from

technological mixtures of a certain POP subclass (e.g. PBDEs, PBBs) and extrapolated to all relevant congeners of that subclass assuming equipotency. Also, NOAELs were obtained from one congener of a certain POP subclass and extrapolated to the whole POP subclass to which they belong (e.g. PCB 153 to all non-DL PCBs) assuming equipotency. However, in reality potency of congeners within a POP subclass may differ. Gaps in toxicological data for individual congeners could be reduced by the use of *in-silico* and *in-vitro* data, as proposed by (EFSA 2019a), and OECD (OECD 2018). The EuroMix project has developed a toolbox consisting of *in-silico* and *in-vitro* methods aligning an adverse outcome pathway for liver steatosis (Luckert et al., 2018, Rorije et al., 2018). Future studies could be performed to study the feasibility of using these tools in mixture risk assessments of POPs. The major contributors to exposure as shown in Fig. 4 could be priority candidates for further *in-silico* and *in-vitro* testing.

Lack of concentration data for several congeners is another POP-specific uncertainty. For example, out of 197 NDL-PCBs only 6 indicator PCBs are usually analysed, which together are assumed to account for about 50% of the total exposure to NDL-PCBs (JECFA 2016, EFSA 2005). In addition, analytical data were available for only 7 PBDEs and 3 PBBs. Regarding the latter POP class, EFSA considered in 2010 that PBBs are of low priority for further research or monitoring because these chemicals are no longer produced or used in Europe and because of low and declining environmental concentrations (EFSA 2010b). To overcome such missing exposure distributions, an additional assessment factor (e.g. a factor 2 for NDL-PCBs) could be taken into account. Another option might be the use of a feature for imputation of missing exposure distributions implemented in the EuroMix toolbox (Kennedy et al, 2020).

4.3 Food additives

The exposure assessments performed for FAs can be regarded as part of a tier 2 risk characterization, for which a MOE of 100 is generally considered acceptable (EFSA 2019a). As for PPRs, a MOE smaller than 100 could indicate a health risk, but could also indicate that refinement of the assessment is needed. In the case of FAs, it indicates that refinement is needed because of data poverty. Although the non-brand-loyal scenario used in our study is regarded as refined (EFSA 2017a), the exposure estimates obtained with the non-brand-loyal scenario and the concomitant MOEs in our study were marginally different from the conservative authorization scenario. Due to limited real use levels and/or concentration data reported to EFSA (EFSA 2010c, 2010d, 2010e, 2012b, 2014, 2015a, 2015b), MPLs were used in the non-brand-loyal scenario if no other data were available, and, as a consequence, this scenario actually appeared not different from the authorization scenario. The 7 food additives used in our case study were among the first re-evaluated chemicals, when data provision to EFSA was far from optimal. Along the re-evaluation program, data collection of use levels and concentration data has been improved, although in some cases information on real uses and use levels is still limited.

To obtain an example for FAs, some other pragmatic decisions were taken that influenced the outcome of the exposure assessment. Canthaxanthin is only authorised in 'saucisse de Strasbourg' (EFSA 2010c), for which no FoodEx 1 coding was available and therefore not included in the exposure assessment. It is also authorised as colouring matter in feeding stuff and this use could contribute to exposure by carry over into food of animal origin (EFSA 2010c). While this is not food additive use, we used the reported concentrations in meat (EFSA 2010c) to be able to include canthaxanthin in the example of food additives. Dimethyl dicarbonate is authorised for use in beverages, in which it hydrolysis to carbon dioxide and methanol or reacts to dimethyl carbonate, methyl ethyl carbonate and methyl carbamate. The latter one is causing fatty infiltration in rat liver (EFSA 2015a). When using use levels of dimethyl dicarbonate rather than analytical values of methyl carbamate, conversion factors would be required. These conversion factors were not available, so we

used the use levels of dimethyl dicarbonate. This could have resulted in a large overestimation, as methyl carbamate is only a minor reaction product in beverages formed in trace amounts (EFSA 2015a). The FAs E 914 'oxidised polyethylene wax' and E 474 'sucroglycerides' are used for surface treatment of some fruits, which implies that exposure of these chemicals may be lower due to peeling or other kinds of processing. Therefore, in absence of analytical data in processed foods, processing factors should be used for the exposure assessment, but were not available. All these factors contributed to uncertainties of the cumulative exposure assessment and resulted in an overestimation of the cumulative exposure estimate for food additives.

Regarding hazard data, the same generic uncertainties as mentioned above for PPRs and POPs apply for FAs. Particular uncertainty around the RPF due to limited hazard data was applicable to FAs because only one toxicity study was available for each food additive, except for cantaxanthin and propyl gallate. This may contribute to uncertainties in the derived RPFs. Uncertainties around CAG membership also exist for FAs. For example, the FA benzoic acid was in the PPR list of chemicals that may cause liver steatosis (Nielsen et al., 2012), but liver steatosis was not reported in EFSA's re-evaluation of benzoic acid as FA (EFSA 2016). This may be due to different dossiers delivered to distinct EFSA Panels. Chemical inventories and toxicity databases, such as the one from the EuroMix project used in our study, can help to include toxicological data available from other chemical silos. It should be noted that the real number of food additives belonging to the steatosis CAG could be larger, as only available opinions published within the re-evaluation programme of EFSA until March 2017 has been used. Therefore, the exercise could be repeated once the re-evaluation programme of EFSA is finalised and/or including other data sources, such as JECFA opinions. When repeating the exercise, the uncertainties listed in this paper for the FAs included in our studies could be taken into account. For this, priority can be given to the risk drivers of combined exposure to FAs, i.e. sucroglycerides and dimethylcarbamate.

4.4 Overarching

The case study demonstrated that cumulative exposure to mixtures and risk characterisation using MOE can be performed within a particular regulatory silo. It also highlighted that the combined (total) margin of exposure (MOET) approach proposed by EFSA must be normalized into a nMOET approach for overarching the risk across the different regulatory silos. The main difference between the mRPI proposed by Vejdovszky et al. (2019) and the present nMOET is that these authors cumulated single substance exposure calculated with deterministic models based on consumption statistics, whereas we performed cumulative exposure assessment by silo using more refined modelling for cumulative exposure and cumulated the exposure of the silos. Our refined modelling was based on individual food consumption data and the use of relative potency factors for chemicals grouped into the CAG liver steatosis. Another approach for cumulating single substance exposure across chemical silos is the hazard index, which was used by Evans et al. (2016). In the hazard index approach, exposure to a chemical is expressed as a fraction of the health based guidance value of the chemical, the hazard quotient, and subsequently sums all hazard quotients without addressing a specific organ or effect. This is clearly less refined than our approach.

The calculated nMOET was smaller than 1 regardless of exposure percentile, scenario, population or country, which is not surprising because the MOE of FAs only in the less conservative mean use level scenario was already near or below the AMOE. For the more conservative scenarios (pessimistic approach, upper bound and MPL scenario for PPRs, POPs, FAs), the calculated MOE were also below their acceptable AMOEs. This underpins the need for realistic scenarios for the different chemical classes in overarching risk

assessment. The contributions of the chemical classes to the 1/nMOET metric can be used as a tool to identify priority chemical classes for refinement.

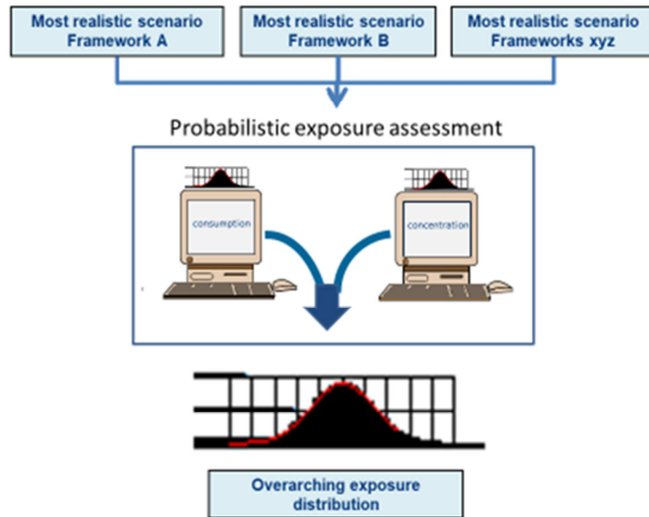
A disadvantage of the nMOET applied on population exposure percentile and not on the individual level, is that cumulation of (high) exposure percentiles may lead to overestimation of exposure and its associated risk. For example, a person with a high exposure to PPRs because of a high vegetable and fruit consumption does not necessarily have a high POP exposure due to high meat consumption. Ideally, cumulative exposure to chemicals belonging to different silos should be performed within a single computational run at the level of individual food consumption, yielding one potency-adjusted exposure distribution for all chemicals. However, the commonly used exposure models for PPRs, POPs and FAs are based on different assumptions (Table 1). This implies that overarching exposure assessment using a single computational run for a single CAG consisting of all relevant PPRs, POPS and FAs and taking into account relevant exposure scenarios for the particular class is currently not possible. To enable this, harmonisation of methodologies between silos is needed. If complete harmonisation of methodologies is not possible (e.g. taking into account percentage of crop treated is relevant for PPRs, but not for POPs or FAs), a new computational tool that automatically assigns the correct exposure methodology to chemicals of different silos may facilitate overarching risk assessment. This is envisioned in Fig. 6. Such a tool recognizes to which chemical silo a certain chemical belongs and calculates the exposure to this chemical for each individual in the food consumption database according to the (refined) methodology and/or scenario that is commonly used for the particular silo. This is done for all chemicals and all chemical silos. Subsequently, exposures are expressed as toxicity equivalents of one selected overarching reference compound taking into account the uncertainty factors applicable for the particular chemical (silo). Finally, potency-adjusted exposures are summed for each individual in the database. This yields an overarching potency-adjusted exposure distribution, from which exposure percentiles can be obtained. The ParamCodes used by EFSA (EFSA 2010) may facilitate such a discriminative tool

automatically assigning the correct exposure methodology for overarching exposure, as these codes use abbreviations that identifies a certain chemical class (e.g. PPR, ORG and ADD for PPRs, POPs and FAs, respectively).

Until such a tool becomes available, the nMOET can be used for risk characterisation of the combined exposure to chemicals belonging to different silos.

It should be noted that combined exposure risk assessment must not be limited to the three silos studied in this work. Indeed, the EuroMix inventory list revealed that chemicals belonging to other regulatory silos such as mycotoxins, food contact materials, flavourings and (veterinary) medicine also have liver steatosis effects and should be included in future overarching risk assessment (Kyriakopoulou et al. 2017).

Figure 6. Proposed computational tool to combine exposure from different chemical silos into one computational run. Such a tool recognizes to which chemical silo a certain chemical belongs and calculate the exposure to this chemical for each individual in the food consumption database according to the methodology and/or scenario that is commonly used for the particular silo. This is done for all chemicals and all chemical silos. Subsequently, exposures are expressed as toxicity equivalents of one selected overarching reference compound, and the potency-adjusted exposures are summed for each individual in the database. This yields a distribution of an overarching potency-adjusted exposure distribution, from which exposure percentiles can be obtained.



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4. Conclusion

Our work showed that cumulative exposure assessment to chemical mixtures and the subsequent risk characterisation can be performed within regulatory silos, but that refinement of the exposure scenarios with respect to conservative assumptions and/or uncertainties is needed for realistic overarching risk assessment. Due to differences in derivation of point of departures and the subsequent acceptable MOEs between silos, we proposed to modify the currently available MOET approach into a nMOET approach for overarching mixture risk assessment based on exposure percentiles obtained from the different regulatory silos. Further developments are needed to perform realistic overarching exposure calculations within a single computational run.

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Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: