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The MCRA toolbox of models and data to support chemical mixture risk assessment



Hilko van der Voet^{a,*}, Johannes W. Kruisselbrink^a, Waldo J. de Boer^a, Marco S. van Lenthe^a, J.J.B. (Hans) van den Heuvel^a, Amélie Crépet^b, Marc C. Kennedy^c, Johanna Zilliachus^d, Anna Beronius^d, Cleo Tebby^e, Céline Brochot^e, Claudia Luckert^f, Alfonso Lampen^f, Emiel Rorije^g, Corinne Sprong^g, Jacob D. van Klaveren^g

^a Wageningen University & Research, Biometris, the Netherlands

^b ANSES, French Agency for Health and Safety, France

^c FERA, Sand Hutton, York, United Kingdom

^d Karolinska Institutet, Stockholm, Sweden

^e INERIS, METO Unit, Verneuil-en-Halatte, France

^f BfR, German Federal Institute for Risk Assessment, Department Food Safety, Germany

^g RIVM, National Institute for Public Health and the Environment, the Netherlands

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ABSTRACT

A model and data toolbox is presented to assess risks from combined exposure to multiple chemicals using probabilistic methods. The Monte Carlo Risk Assessment (MCRA) toolbox, also known as the EuroMix toolbox, has more than 40 modules addressing all areas of risk assessment, and includes a data repository with data collected in the EuroMix project. This paper gives an introduction to the toolbox and illustrates its use with examples from the EuroMix project. The toolbox can be used for hazard identification, hazard characterisation, exposure assessment and risk characterisation. Examples for hazard identification are selection of substances relevant for a specific adverse outcome based on adverse outcome pathways and QSAR models. Examples for hazard characterisation are calculation of benchmark doses and relative potency factors with uncertainty from dose response data, and use of kinetic models to perform *in vitro* to *in vivo* extrapolation. Examples for exposure assessment are assessing cumulative exposure at external or internal level, where the latter option is needed when dietary and non-dietary routes have to be aggregated. Finally, risk characterisation is illustrated by calculation and display of the margin of exposure for single substances and for the cumulation, including uncertainties derived from exposure and hazard characterisation estimates.

1. Introduction

Human activities have drastically increased the number of chemical substances to which we are exposed and which might have a negative impact on our health. Chemical risk assessment has focused traditionally on potential risks of single substances. However, multiple substances can have the same health effect, so their combined effects on the same phenomenological endpoint should be assessed (Drakvik et al., 2020). Consequently, the need was perceived to develop risk assessment methods for combined exposure to multiple substances, i.e. mixtures of substances. The current legislative requirements for risk assessment of mixtures were recently reviewed (Rotter et al., 2018).

The tasks for mixture risk assessment are not trivial. First, decisions

are needed regarding which chemical substances should be evaluated together in an assessment group (AG) when considering a specific adverse outcome (AO). For those substances, data or assumptions on both exposure and hazard are needed. A specific human population group has to be defined as the object of protection. Exposure might need to be aggregated over several sources, such as dietary exposure, dermal or inhalation exposure, sometimes for specific population groups, e.g. those working in a risky profession like pesticide spraying. Hazard data can be obtained from *in vivo*, *in vitro* and *in silico* approaches. The latter two categories require biological modelling, e.g. using adverse outcome pathways (AOPs), to assess the relevance of responses for the defined *in vivo* AO. The most common assumption for cumulating effects is the dose addition (DA) model, but its validity might need to be checked

* Corresponding author.

E-mail address: hilko.vandervoet@wur.nl (H. van der Voet).

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(EFSA, 2013a, 2019; OECD, 2018). Under the DA model the relative potencies of substances are expressed as relative potency factors (RPFs). It should be noted that RPFs are typically different at the external or internal biological level. Kinetic modelling can be used to bridge the gap between external and internal doses by constructing *in vitro* to *in vivo* extrapolation (IVIVE) models. Inevitably, limitations in data availability lead to the necessity to make model assumptions and to uncertainty (EFSA, 2018). Many parts of the data will be uncertain, but this has often been ignored in practical work, notably for AG membership and RPF estimates.

One of the major aims of the EuroMix project was to integrate hazard, exposure, toxicokinetic and toxicodynamic modelling approaches for mixtures of chemicals together with example data sets into a web-based model and data toolbox openly accessible for stakeholders. The system is able to assess quantifiable uncertainties and their influence on the results of cumulative and aggregated risk assessment. For this, it uses a 2D Monte Carlo approach based on quantifiable variability and uncertainty in the inputs, where an inner loop estimates variability distributions for specific outputs, characterising the variability between individuals or individual-days. Then an outer loop estimates uncertainty distributions for specific outputs, e.g. 95% confidence limits for a percentile of a variability distribution.

In the EuroMix project, the toolbox has been developed as version 9 of the Monte Carlo Risk Assessment (MCRA) platform (see van der Voet et al., 2015 for a description of the previous version MCRA 8). The toolbox also integrates the method innovations recently developed for EFSA in their approach to cumulative exposure assessment and implemented as well in MCRA version 8.3 (van Klaveren et al., 2019a). MCRA is a web-based platform (<https://mcra.rivm.nl>) which employs a high-performance computation cluster to run simulations. For a full description of the toolbox we refer to the online reference documentation (MCRA, 2019). The toolbox can be used in conjunction with the EuroMix handbook (Zilliacus et al., 2019a). In this paper the aim is to provide an overview of the available methods and illustrate the use of the toolbox with several examples for hazard identification, hazard characterisation, exposure assessment and risk characterisation. These examples are not full case studies, but are only intended to illustrate existing and new functionalities that are available in the toolbox.

In section 2.1 of this paper we describe the toolbox of models and data that has resulted from the EuroMix project. The data collected in the EuroMix project are summarised in section 2.2. In sections 2.3-2.6 short descriptions are given of the methods implemented in the toolbox for the four areas of risk assessment, i.e. hazard identification, hazard characterisation, exposure assessment and risk characterisation, respectively, and the data for some simple examples are described. Sections 3.1-3.4 then show the results for the examples. The results and the intended use of the toolbox are discussed in section 4.

2. Methods

2.1. Description of the MCRA toolbox

The toolbox for mixture risk assessment has been built according to the modular design shown in Fig. 1. The modules are listed in Appendix A1. Modules are of three basic types: 1. Scoping modules regarding primary entities on which the risk assessment is built; 2. Data modules, specifying groups of data sources needed or optional for the assessment; and 3. Calculator modules, which calculate results of a certain type. Note, that calculator modules can in principle also act as data modules if the results are already available from previous work.

The primary entities in the toolbox relate to the agents of risk (Substances), the sources of dietary exposure (Foods), the objects of protection (Populations), the potential hazards (Effects), and how these are measured (Responses in Test systems). The data and calculation modules on the left side of Fig. 1 are related to exposure assessment,

those on the right side to hazard identification and hazard characterisation. The module for risk assessment is in the middle of the diagram, integrating exposures and hazard characterisations.

The exposures module can aggregate non-dietary exposures (which then need to be provided as data) and dietary exposures. Dietary exposures are calculated from consumptions and concentrations, possibly incorporating many detailed aspects. Food consumptions may need to be redefined by conversion of food codes from the codes used in consumption surveys to the codes used in concentration monitoring data. Occurrence data (concentrations and substance authorisations) may need to be converted due to differences between active substances and measured substances (in the pesticide field known as complex residue definitions), and such data may be condensed in concentration models (e.g. a lognormal distribution with a spike of non-detects). Total diet study (TDS) concentration data require a conversion to appropriate food codes. Exposures may also be adjusted for food processing effects and for the greater concentration variability in units of consumption compared to the composite samples used in the monitoring program. In addition, modelled exposures may be inspected for co-occurrence of substance combinations as exposure mixtures, and they may be compared to human monitoring data.

Hazard identification is concerned with identifying the active substances related to a given health effect. AOP networks may be used to identify relevant effects connected to the adverse outcome of interest in the assessment. Identification of active substances as belonging to the relevant AG may be just specified as data, or it may be derived from available toxicity data or from *in silico* models (QSAR and/or molecular docking). Effect representations data will link observed responses to effects of interest and may specify benchmark responses, which are toxicologically relevant levels for those responses, to be used in the modelling of dose-response data to obtain benchmark doses (BMDs). BMDs as well as other points of departure (POD) specified as data can then be used for hazard characterisation, either directly or after applying assessment factors for POD type, species differences and/or within-species variability. Kinetic models (or simple absorption factors) may be used to extrapolate between external and internal doses. We use the term hazard characterisation (HC) as a generic term which can be any form of POD or health-based guidance value, depending on the purpose of the analysis. For the assessment of mixtures, RPFs are calculated as the ratio of HC for an index substance to HC for a specific substance.

The toolbox distinguishes between two types of runs: the nominal run and the uncertainty analysis loop. The nominal run represents a single calculation in which the aim is to compute the most likely, if possible unbiased estimates for the model at hand. E.g., when computing dietary exposure distributions, the nominal run computes one exposure distribution, using, as much as possible, fixed values for all uncertain inputs, and summarises the exposure distribution by point estimates of statistics such as the mean exposure or specific percentiles of variability. In the uncertainty analysis, on the other hand, the calculation run is repeated a number of times, each time with a different uncertainty scenario obtained using bootstrapping, parametric resampling, and/or re-calculation of uncertain values, yielding uncertainty distributions and confidence intervals for specific outputs. Making the distinction between the nominal run and the uncertainty loops has the practical advantage that it allows the user to setup and evaluate complex simulations first using only the nominal runs to quickly obtain a picture of the results and identify possible errors in the data or in the model settings before running the more time-consuming uncertainty analysis loop.

User work is organized in workspaces. A workspace is a collection of work items that are logically grouped together. A workspace has a name, description and, optionally, a number of tags. Users are the owners of their own workspace folders. An action is started with the module of the corresponding action type (e.g. dietary exposures), but also links to other modules that are needed for its completion (e.g.

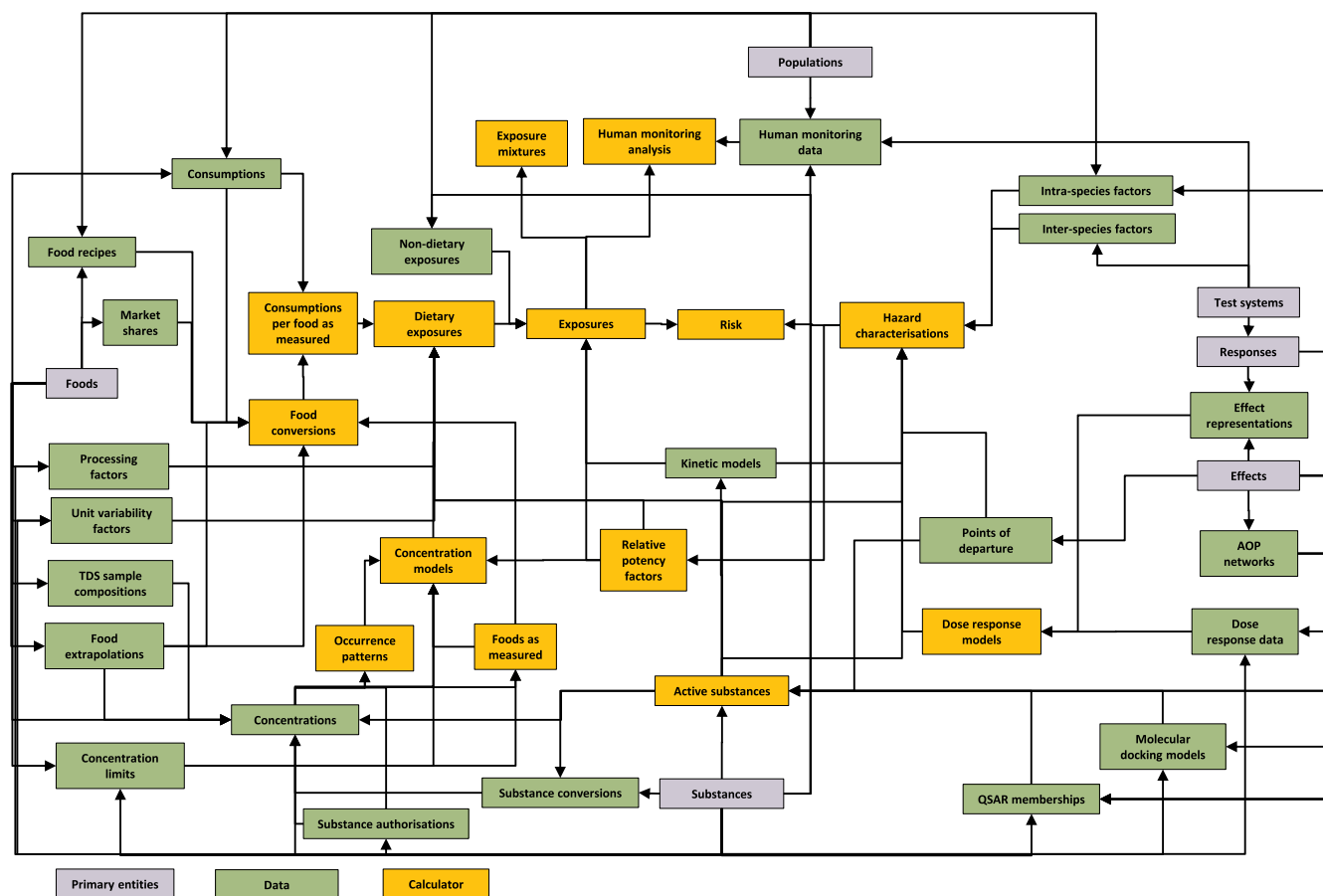


Fig. 1. Modular design of the MCRA toolbox. Not all links are shown in this graph. See [Appendix A1](#) for a complete list.

consumptions per food as measured, consumptions, foods, etc.). An action can be available in two forms: 1) a data selection action and 2) a calculation action. A data selection action comprises the selection of already available data of that action type, optionally the specification of selections on that data, and in some cases some pre-processing of data. A calculation action is an action in which the data of that action is calculated based on relevant input and specific calculator settings. Within a workspace, multiple actions can be created. When running an action, a task is spawned that produces output. Output is available in the form of reports or in the form of data that can be used as input in other actions. Actions have multiple outputs when settings are changed. Output reports are presented as screen reports or print reports and structured according to the modules of the modular design.

2.2. Data – example data organised in the EuroMix project

The toolbox contains a repository for relevant data. Data can be uploaded by individual users or by representatives of a larger user group. Data can be shared with other users or user groups. The data administrator for each data set can decide on use, read, or read/permission for users. The data collected during the EuroMix project (Data/EuroMix repository) are shared with project participants and other stakeholders and are summarised in [Table 1](#). These data collected in the EuroMix project were the basis for the examples shown below.

2.3. Hazard identification: AOP-based assessment groups, probabilistic memberships from *in silico* data or expert elicitation

2.3.1. Implemented methods for hazard identification

In the context of mixture risk assessment, hazard identification includes the task of identifying and grouping substances that may lead to a specified adverse outcome (AO) considered in a risk assessment. The set of such substances together form the AG for the AO. The toolbox offers various methods to establish AGs. In this work we will focus on some of these methods. The *Active substances* module of the toolbox includes several possibilities for defining AGs. First, the substances belonging to an assessment group related to a given AO can be directly specified. Secondly, only the substances for which POD data are available can be selected. Thirdly, substances can be selected based on predictions for the given AO from QSAR or molecular docking models ([Cotterill et al., 2016](#); [Rorije et al., 2019](#)). Under the first and third options, missing PODs can be imputed ([Kennedy et al., *subm.*](#)).

In probabilistic modelling, substances can also be identified with a membership probability for the assessment group. Membership probability can be derived from expert knowledge elicitation, as in recent EFSA reports ([EFSA et al., 2019a,b](#)), and entered as data in the toolbox. Probabilities can also be based on the fraction of positive QSAR or molecular docking models, either as a simple ratio estimate or using a Bayesian calculation that includes the sensitivity and specificity of the QSAR models when available ([Kennedy et al., *subm.*](#)).

Table 1

Data in the toolbox, as collected in the EuroMix project. For specific data regarding modules that were not in the focus of the EuroMix project and therefore not included in this table, references are van Klaveren et al. (2019 ab) for Unit variability factors, Substance authorisations, Substance conversions, Concentration limits and Food extrapolations, Kolbaum et al. (2019) for TDS sample compositions, van der Voet et al. (2009) for Intra-species factors and Inter-species factors.

Module	Description data sets
Foods	2289 foods-as-eaten and foods-as-measured coded in FoodEx1 (EFSA, 2011) 32 processing types
Substances	1629 substances, classified in categories PPPs, Biocides, Alkaloids, EnvironmentalPollutants, FoodAdditives, Mycotoxins (Kyriakopoulou et al., 2017)
Effects	48 effects in 7 AOP networks related to liver steatosis, reproductive toxicity and craniofacial malformations
AOP networks	
Populations	15 population groups from 10 countries (different age groups) (Crépet et al., 2019a)
Test systems	14 test systems, 477 responses, 162 effect representations (Luckert et al., 2019; Schreiber et al., 2019ab)
Responses	
Effect representations	
Consumptions	11 files with food consumption data in 10 countries (Crépet et al., 2019a)
Food recipes	5555 records specifying food ingredients in the FoodEx1 system or conversions (Boon et al., 2015)
Concentrations	Food monitoring data 2010–2014, SSD formatted data (Crépet et al., 2019a)
Processing factors	667 processing factors for pesticides and environmental pollutants (derived from Verarbeitungsfaktoren_3-0.xls, downloaded 01-09-2015 from https://www.bfr.bund.de/de/a-z_index/verarbeitungsfaktoren-8400.html)
Non-dietary exposures	Simulated non-dietary exposures from Browse and Bream2 (Kennedy et al., 2019)
Human monitoring data	Norwegian biomonitoring study (Husøy et al., 2019)
QSAR membership models	26 QSAR models applied to all substances in the EuroMix chemical inventory (Rorije et al., 2019)
Molecular docking models	20 Molecular docking models applied to all substances in the inventory (Rorije et al., 2019)
Kinetic models	EuroMix Generic PBTK model parametrised for 9 substances based on htkk and for all substances in the inventory based on QSAR (Tebby et al., 2019)
Points of departure	144 NOAEL or LOAEL values related to Steatosis-liver (Crépet et al., 2019a)
Dose response data	28 files describing experiments with single substances or mixtures, on 15 responses (or groups) in 9 test systems in 6 laboratories (Luckert et al., 2019; Schreiber et al., 2019ab)

2.3.2. Example

The EuroMix inventory list of 573 pesticides (Kyriakopoulou et al., 2017) was analysed for possible hazard with respect to the adverse outcome steatosis. From data available at EFSA, steatosis-specific PODs were available for a minority of pesticides. Additional indications for steatotic activity were derived from *in silico* models such as QSAR model predictions and molecular docking binding energies (Cotterill et al., 2016; Rorije et al., 2019). An AOP network for steatosis (Vinken, 2015; Mellor et al., 2016) was assessed (Luckert et al., 2018) and has been graphically outlined (Fig. 2). This network was uploaded to the toolbox in the form of relational tables specifying all effects and effect relations. Based on this data, several options for non-probabilistic (crisp) or probabilistic AG membership assessments that are available in the toolbox will be illustrated.

2.4. Hazard characterisation: dose response modelling, calculation of RPFs, use of kinetic models for IVIVE

2.4.1. Implemented methods for hazard characterisation

For hazard characterisation in the context of mixture risk assessment the RPF calculations are based on POD values for the substances. The POD value can be a BMD from benchmark dose modelling or a No Observed Adverse Effect Level (NOAEL) or Lowest Observed Adverse Effect Level (LOAEL). Health-based guidance values, such as Acceptable daily Intake (ADI) or Acute Reference Dose (ARfD) can also be used instead of POD, i.e. the POD divided by the assessment factors, which should be taken into consideration for comparison to exposure. Because of the many variations possible, the value used for the RPF calculation is generically called HC in this paper and in the toolbox.

Well-known softwares for benchmark dose (BMD) modelling of dose response data are BMDS (<http://www.epa.gov/bmds>) and PROAST (<http://www.rivm.nl/en/proast>) (EFSA, 2017). In module *Dose response models* of the toolbox, dose response data organised in the data repository can be fitted using the various dose response models (Slob, 2002; Slob and Setzer, 2014; EFSA, 2017), either using an internalised version of the PROAST software or using the web-based PROAST version (<https://proastweb.rivm.nl>). In the toolbox connections can be specified between effects, e.g. the adverse outcome or related biological

effects, and responses, which are the measured quantities, *in vivo* or *in vitro*, that are available for BMD modelling. For example, it was proposed to consider the AdipoRed response after 72 h in a HepaRG test system as one of the most appropriate and cost-effective responses in relation to the AO steatosis (Lichtenstein et al., 2019). Part of these *Effect representations* is the specification of an appropriate benchmark response (BMR), i.e. the response level considered suitable for the BMD modelling, often as a limit value for adversity (EFSA, 2017).

Whereas dose response models focus on BMD calculation related to specific responses, further or alternative steps may be needed to obtain an appropriate HC. In the *Hazard characterisations* module, many possibilities are provided to define HCs either as deterministic threshold values, such as NOAEL, ADI or ARfD, or as distributions generated from probabilistic models. HCs can be calculated for acute or chronic risk types, and for different target levels of the human body (external via some route of exposure or internal for a specific defined organ or compartment). It may be needed to align the available information to the desired target level by including assessment factors for inter-species differences, intra-species variation, different expression types (e.g. BMD or NOAEL or LOAEL) and the difference between external and internal exposure. The latter conversion is especially relevant when *in vitro* dose response data are to be used for a HC that should be compared with external, e.g. dietary, exposure data. In general this type of modelling is known as *in vitro* to *in vivo* extrapolation (IVIVE). In this approach, we need human physiologically based toxicokinetic (PBTK) models. Within EuroMix, the Cosmos model was integrated in the toolbox as a general PBTK applicable model (Bois et al., 2019; Tebby et al., 2019).

The conceptual model used for the use of *in vitro* and/or *in vivo* animal study data for human hazard characterisation and risk assessment using IVIVE is shown in Fig. 3. A typical situation is that there are many substances in a proposed AG, which could be measured all *in vitro* but not *in vivo*. The *in vitro* dose response relations are assumed to coincide with *in vivo* relations between internal dose and some early biological response. The *in vitro* data can then be used to derive internal RPFs, i.e. at the tissue or cell level. Then kinetic models (or absorption factors which are considered a lower tier of kinetic model) can be used to adjust external PODs, i.e. expressed as external exposure levels, to internal HCs, or alternatively to translate internal RPFs to external

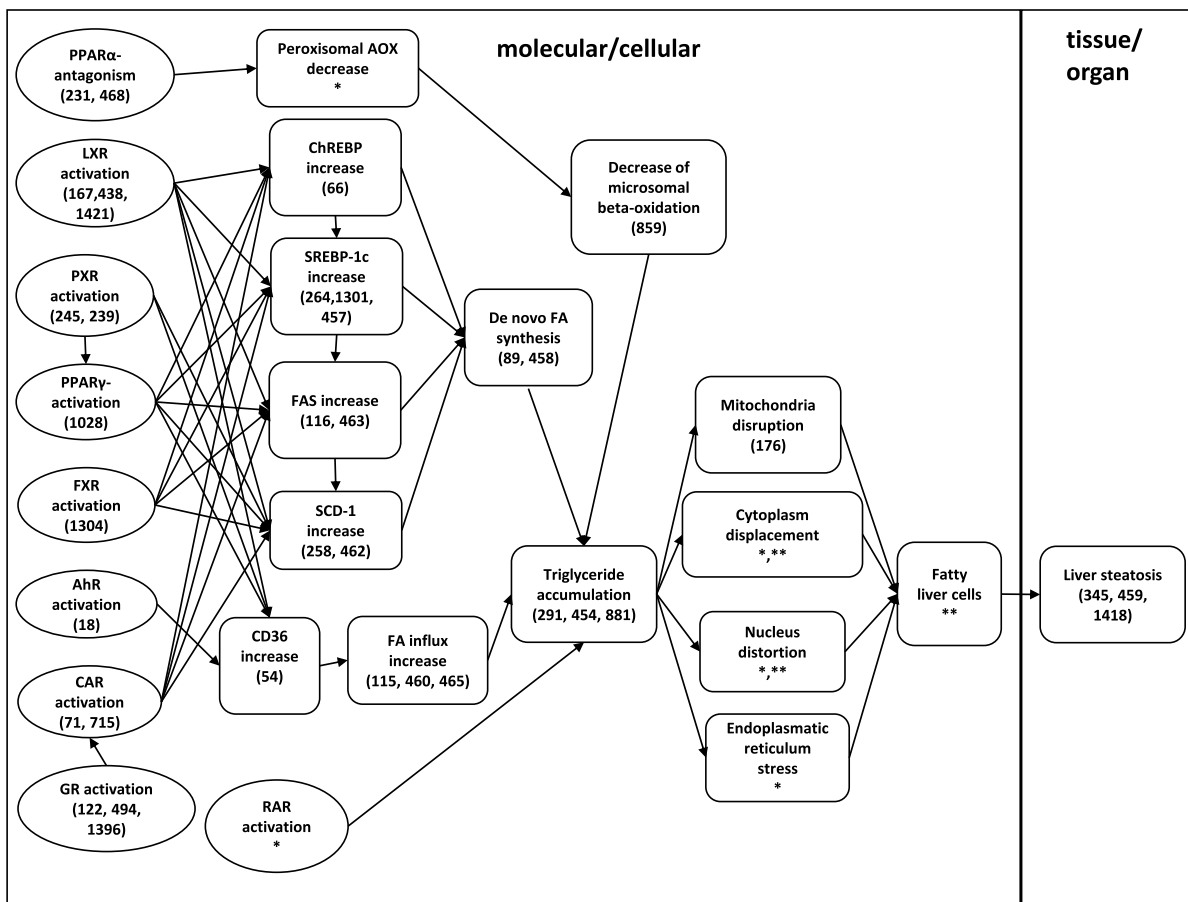
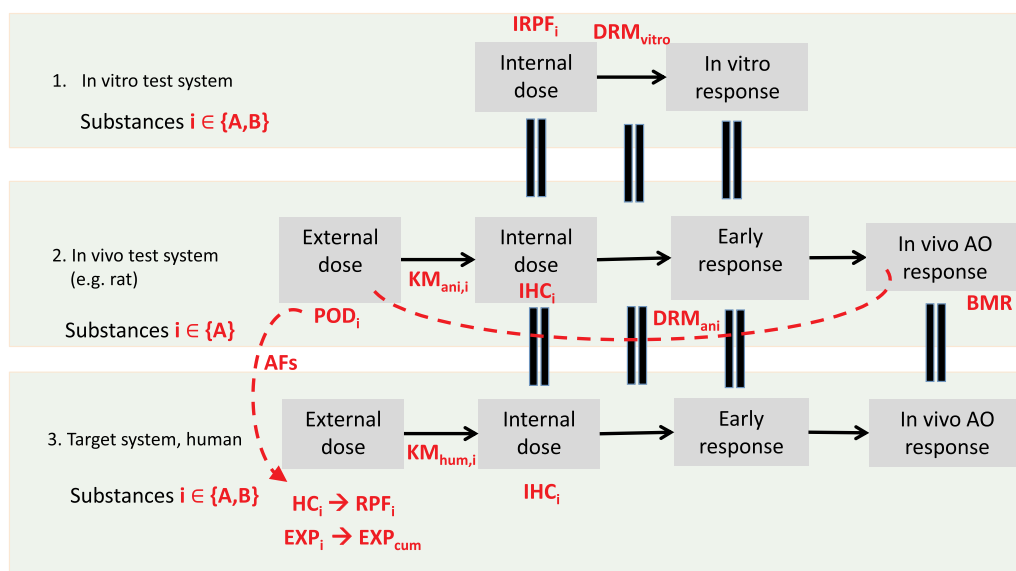


Fig. 2. Adverse Outcome Pathway (AOP) network for the adverse outcome (AO) liver steatosis. The ovals are molecular initiating events (MIEs) and the boxes are other key events (KEs). The arrows depict key event relationships (KERs). The numbers in the ovals/boxes refer to KE numbers in the AOP wiki (<https://aopwiki.org>). * refers to KEs not included in the AOP wiki but described in Mellor et al. (2016). ** refers to KEs not included in the AOP wiki but described in Vinken (2015).



Risk assessment: $MOE = \frac{POD_{index}}{EXP_{cum}}$

AO = Adverse Outcome
 BMR = BenchMark Response
 DRM = Dose Response Model
 POD = Pont Of Departure
 KM = Kinetic Model (animal or human)
 MOE = Margin Of Exposure
 AFs = Assessment Factors
 (I)HC = (Internal) Hazard Characterisation
 (I)RPF = (Internal) Relative Potency Factor
 EXP = Exposure

Fig. 3. Overview of *in vitro* to *in vivo* extrapolation (IVIVE) model components. cum = cumulative; index = index substance; ani = animal, hum - human. Classes of substances: A: substances with *in vitro* and *in vivo* data, B: substances with only *in vitro* data.

RPFs.

In mixture risk assessments, RPFs are calculated by dividing the HC of a chosen index substance by the HC of each specific substance. This implies that RPFs may, and typically will be different depending on whether an external exposure or an internal exposure, e.g. at the target tissue or cell level, was used. In the module *Relative potency factors* both types can be calculated, and modelled uncertainties in the values used to derive the RPFs (e.g. from BMD modelling) are translated to uncertainties about RPFs. It may further be noted that RPFs represent the distance between parallel dose response curves, and can even be calculated when no BMR has been specified in the dose response modelling.

In the toolbox, external doses from animal studies can be adapted to external doses for humans by the use of inter-species and/or intra-species factors. Alternatively, no inter-species and intra-species factors are used, and then the final margin of exposure will have to be considered against an appropriate value representing the combined assessment factors, e.g. 100. We use the latter approach in this paper.

2.4.2. Examples

As a first example, hazard characterisations and RPFs were calculated based on the NOAELs or LOAELs available for 144 substances related to the AO steatosis, using the well-studied risk driver imazalil as an index substance. Standard inter-species and intra-species assessment factors of 10 were used for all hazard characterisations. As in Crépet et al. 2019a,b, for 13 substances where no NOAEL was available, the LOAEL was divided by 3 to obtain an estimated NOAEL. This is a simple approach, and further refinement may be necessary in real applications. The guidance document from WHO/IPCS (2018) states that it would be much better to use dose-response data. In the current project, it was not possible to use the dose-response data for all 144 substances.

In the EuroMix project three chemicals were prioritised in relation to the AO steatosis: imazalil, thiocloprid and clothianidin based on relevance from dietary exposure and other considerations (Crépet et al., 2019a, Lichtenstein et al., *subm.*). We therefore provide more detailed example calculations based on *in vitro* dose response data for these chemicals.

Dose response relations for the intracellular lipid accumulation after 72 h were measured using the AdipoRed assay in the *in vitro* HepaRG test system for three substances in the steatosis AG (Luckert et al., 2019). Using the Proast model (<https://www.rivm.nl/en/proast>; Slob and Setzer, 2014), that is integrated in the MCRA toolbox, a 6-parameter parallel-curve exponential dose response model was fitted to the data. Three parameters represent the lower and upper asymptote and common slope, one parameter is the BMD for the index substance (here imazalil), and the remaining two parameters represent the RPFs for the other two substances relative to the index substance. A 10% increase in AdipoRed response was assumed to be an appropriate benchmark response (BMR) level. The appropriateness of the parallel-curve model was checked by superimposing the shifted dose response curves and the data (Kienhuis et al., 2015). The RPFs are based on *in vitro* doses in molar units. For reverse dosimetry we changed to mass units and mass-based RPFs.

Using the EuroMix Generic PBTK model that is integrated in the toolbox (Bois et al., 2019; Tebby et al., 2019) the internal liver concentration was simulated when a daily dose equal to the BMD is given. In the EuroMix project different parameterisations of the model for each substance have been investigated (Tebby et al., 2019). Here, we rely on kinetic parameters estimated by QSAR but with hepatic clearance values obtained from *in vitro* clearance measurements. The long-term exposure relevant for chronic risks was obtained by averaging over predicted liver concentrations in the period between 15 and 28 days. The ratio of this internal concentration to the external exposure per unit bodyweight was then used as the absorption factor. For all routes, the exposure is taken to be the total amount entering via that route per unit bodyweight and per day.

2.5. Exposure assessment: dietary exposure with large AG, aggregating dietary and non-dietary exposures, comparison with human monitoring

2.5.1. Implemented methods for exposure assessment

The module *Dietary exposures* of the toolbox implements the methods of the EFSA guidance on probabilistic modelling (EFSA, 2012) as well as the most recent EFSA methodology for cumulative dietary exposure assessment for acute and chronic risks of pesticides (van Klaveren et al., 2019a; EFSA et al., 2019a,b). Using the module *Food conversions* consumptions of food-as-eaten as specified in a dietary consumption survey can be recoded in terms of the foods-as-measured. In this paper concentration data according to EFSA's standard sample description (SSD1) (EFSA, 2010) were used with FoodEx1 food classification (EFSA, 2011). The toolbox however has no fixed coding systems, and could equally well use the newer SSD2 system (EFSA, 2013b) provided a table with code conversions for the foods-as-eaten is available.

The module *Dietary exposures* also implements advanced methods for long-term exposure commonly used in nutrition science, such as the logistic-normal normal (LNN) model for episodic intakes (Goedhart et al., 2012; Roodenburg et al., 2013; Boon et al., 2014; van der Voet et al., 2015).

In the module *Exposures* dietary (external) exposures can be translated to internal exposures, using simple absorption factors or integrated kinetic models. Non-dietary exposures from e.g. dermal or inhalatory routes can be aggregated with the dietary exposures (Kennedy et al., 2019; Karrer et al., 2019; Vanacker et al., *in prep.*). There can be multiple instances of a kinetic model (e.g. the human model for imazalil or the rat model for clothianidin). The parameters of each instance can be specified as fixed values or as variable and uncertain quantities. Distributions for variability and uncertainty are characterised by their type (log-normal, logistic-normal) and coefficient of variation.

Modelled exposures in given body compartments, e.g. blood or urine, can be compared to actually measured exposures from human (bio-)monitoring studies in the module *Human monitoring analysis*.

Dose-additivity is a common assumption in mixture risk assessment (EFSA, 2013a, 2019; OECD, 2018). With large number of substances that have the same health effect it is practically impossible to check this assumption for all combinations. It is then important to identify the main groups of substances that contribute to the cumulative exposure. The module *Exposure mixtures* implements a multivariate method, sparse nonnegative matrix underestimation (SNMU), to find such groups (Crépet et al., 2019a).

Uncertainty about assessment group membership for substances can be addressed probabilistically as sketched in section 2.3.1. In the toolbox, such probabilities can be used in an exposure assessment to include or exclude substances in the multiple loops of an uncertainty calculation (see also EFSA, 2019a,b), as described in Kennedy et al. (*subm.*).

2.5.2. Examples

An example is given of dietary exposure from 144 pesticides with a POD (NOAEL or LOAEL) for steatosis (Kyriakopoulou et al., 2017). Consumption data were available for a Dutch population of children (1–19 years) (van Rossum et al., 2011). These were combined with merged European food control and monitoring data 2010–2014 (Crépet et al., 2019a) using a previously established database of food conversions (Boon et al., 2015) and BfR processing factors (https://www.bfr.bund.de/de/a-z_index/verarbeitungsfaktoren-8400.html, downloaded 01-09-2015). As reported in section 3.2, the PODs were used to calculate external RPFs. For the handling of left-censored data (concentrations reported to be below a limit of reporting) the EC-EFSA method based on observed occurrence patterns was applied (van Klaveren et al., 2019ab), thus avoiding the extreme conservatism of the EFSA basic pessimistic model. Exposure percentiles for the basic observed

individual means (OIM) method and the LNN method are compared, and the most important food-substance combinations (risk drivers) are identified.

In a second example, dietary exposures were combined with non-dietary exposures for the three prioritised steatotic substances imazalil, thiacloprid and clothianidin. The dietary exposures were based on consumption data of French adults (Dubuisson et al., 2010) and the same other dietary exposure data as mentioned above. The non-dietary exposures were estimates for adult residents from the Browse model (Kennedy et al., 2019). Internal RPFs for steatosis were based on *in vitro* data from AdipoRed assays (Luckert et al., 2019). For linking external to internal level exposures, the EuroMix Generic PBTk was used, with parameterisations for the human including variability of the parameters (Tebby et al., 2019).

In a third example, the module *Human monitoring analysis* was used to combine questionnaire data on food consumption and personal care product use with monitoring data on bisphenol A (BPA) in food, and compare the resulting predicted exposures with measured urine levels. Human biomonitoring data for BPA were available from a Norwegian survey which measured bisphenols in urine and asked participants for their diet and their use of personal care products (Husøy et al., 2019). Predicted exposures were based on the recorded consumptions and personal care product use in combination with measured or modelled BPA levels (Karrer et al., 2019) using a kinetic model developed for this purpose and integrated in the toolbox (Karrer et al., 2018).

2.6. Risk characterisation: comparing exposure and hazard characterisation distributions

2.6.1. Implemented methods for risk characterisation

Risk characterisation is fundamentally the process of comparing exposure to hazard. Both exposure levels and hazard threshold levels (called HCs in the toolbox) can be variable (between individuals or individual-days) and/or uncertain. The toolbox module *Risks* can be used to display this comparison. More directly, the ratio of HC to exposure, i.e. the margin of exposure (MOE) is calculated and displayed. For mixture exposure, this has also been termed the MOE total (MOET) or combined MOE (e.g. Rotter et al., 2018; EFSA, 2019), but in the toolbox the term MOE is used throughout, for both single-substance and cumulative cases. For example, in a traditional risk assessment human exposures are often compared to a POD derived from an animal study. The product of assessment factors, e.g. 100 resulting from a factor 10 each for inter-species and intra-species differences, may then be used as a threshold for the MOE specified by the user.

In a more advanced calculation such as in the integrated probabilistic risk assessment (IPRA) approach (van der Voet and Slob, 2007; van der Voet et al., 2009), the assessment factors (as well as their variability and uncertainty) are internalised in the probabilistic hazard characterisation and modelled probabilistically. Probabilistic hazard and exposure estimates are then compared and the MOE compares

individual human exposures to individual human HCs. Note that for this case this ratio has previously been termed the individual MOE (IMOE), but in the toolbox the term MOE is used generically, whether the HC includes the assessment factors or not. For practical risk assessment, the distribution of MOE (based on probabilistic exposure or probabilistic hazard or both) can be characterised with a lower percentile of interest, e.g. P1, or with the lower confidence limit on such a lower percentile. For an example of this fully probabilistic approach see Jacobs et al. (2015).

Uncertainty about assessment group membership for substances can be addressed probabilistically as sketched in section 2.3.1. In the toolbox, such probabilities can be used in a risk assessment to include or exclude substances in the multiple loops of an uncertainty calculation (see also EFSA et al., 2019a, b), as described in Kennedy et al. (subm.).

2.6.2. Example

A mixture risk assessment for Dutch children was performed for the group of 144 pesticides with a POD for steatosis (Kyriakopoulou et al., 2017), calculating MOE values based on Dutch consumption data (van Rossum et al., 2011) and European monitoring data (Crépet et al., 2019a,b) and using a user-specified MOE threshold value of 100.

3. Results

3.1. Hazard identification: assessment groups, probabilistic memberships from *in silico* data

Running the *Active substances* module for the 573 pesticides listed in the EuroMix Chemical Inventory, 144 substances (25%) were found to belong to the AG for steatosis based on available PODs for steatosis. The remaining 429 pesticides (75%) may or may not have steatosis as a non-critical effect, but it was not recorded in the dossiers. Therefore QSAR models may be useful to consider possible AG membership. We first illustrate non-probabilistic (crisp) options for membership assignment. From a larger collection of 29 available QSAR models collected in the EuroMix data, the five QSAR models that relate to the AOP network for steatosis were automatically identified (Table 2). Note that three of these models directly relate to the adverse outcome, whereas the remaining two relate to other effects (molecular initiating events or key events) that occur upstream in the AOP network. Using the toolbox it was calculated how many substances would belong to the AG for steatosis. This was based on the optimised FERA model, on all three available steatosis models, or on all five models related to the steatosis AOP network (Table 3). Pesticides for which no QSAR results could be calculated were omitted or added to the AG. Depending on the QSAR models chosen and the treatment of the substances without a QSAR prediction, the number of pesticides with an QSAR indication of a possible effect was between 250 and 525 (see column QSAR-based in Table 3), much higher therefore than the number for which a POD was available ($n = 144$). In a second scenario, the QSAR results were only

Table 2

QSAR models related to the adverse outcome steatosis. Note that some of the 573 substances could be analysed with the QSAR models.

Model	Model description	Effect	Number of substances with QSAR results	Fraction of these substances classified in AG
1	COSMOS Nuclear Receptor model for Steatosis liver nuclear receptors used to predict hepatotoxicity - and to predict steatosis	Steatosis-liver	513	0.60
2	at least one of the 16 Liver NR Docking models from Uni Milano above binding threshold energy	Steatosis-liver	513	0.84
3	FERA developed model using the reference dataset for Steatosis - to predict steatosis	Steatosis-liver	513	0.49
4	OCHEM AhR receptor binding model used to predict hepatotoxicity - and to predict steatosis	AhR-act-liver	512	0.40
5	OCHEM PPARγ receptor binding model used to predict hepatotoxicity - and to predict steatosis	PPARγ-act-liver	508	0.45

Table 3
Classification of pesticides by use of QSAR models related to the adverse outcome steatosis.

QSAR model(s) (see Table 2)	rule for aggregation	include in AG pesticides without QSAR results	Number of pesticides in steatosis AG (% of 573 pesticides)		
			QSAR-based	Restricted to POD present	Expanded with POD present
3 (FERA model)	–	no	250 (44%)	83 (15%)	311 (54%)
		yes	310 (54%)	96 (17%)	358 (62%)
1,2,3 (steatosis only)	any	no	445 (78%)	122 (21%)	467 (82%)
		yes	505 (88%)	135 (24%)	514 (90%)
	majority	no	357 (62%)	111 (19%)	390 (68%)
		yes	417 (73%)	124 (22%)	437 (76%)
1,2,3,4,5 (include AOP-linked effects)	any	no	465 (81%)	128 (22%)	481 (84%)
		yes	525 (92%)	141 (25%)	528 (92%)
	majority	no	295 (52%)	90 (16%)	349 (61%)
		yes	355 (62%)	103 (18%)	396 (69%)

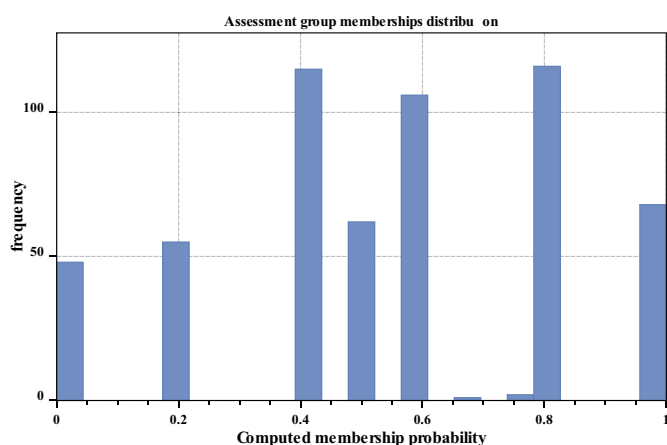


Fig. 4. Probabilistic memberships for 573 pesticides for the Steatosis AG based on predictions from five QSAR models.

used to reduce the set of 144 pesticides having a POD, by omitting all substances without a QSAR prediction of steatosis. Instead of 144 pesticides we then found between 83 and 135 pesticides in the AG. In a third scenario, the QSAR-derived sets were expanded with those pesticides for which a POD was available although no QSAR-signal was obtained. This resulted in between 311 and 528 pesticides in the AG.

Considering the large uncertainty about steatosis AG membership, an alternative approach is to estimate probabilities of membership, and use these in the uncertainty analysis. Here, ratio-based membership probabilities were derived (Fig. 4). In this analysis, a default probability 0.5 was used if QSAR classification was missing. 48 substances were excluded from the AG (all QSAR classifications negative) and 68 substances were included with certainty (all QSAR classifications positive). For the remaining 457 substances a membership probability equal to

the fraction of positive QSAR models was derived. A more advanced Bayesian calculation is also available, and is described elsewhere (Kennedy et al., *subm.*). These membership probabilities can be used in probabilistic assessments by including each substance in iterated uncertainty runs with the calculated probability as proposed by EFSA (EFSA et al., 2019a, b), which method is also available in the toolbox.

3.2. Hazard characterisation: dose response modelling and relative potency factors

The preparation of the NOAEL and LOAEL data of 144 pesticides related to steatosis has been described in Crépet et al. (2019a). Hazard characterisations varied between 0.25 µg/kg bw/day (ethoprophos) and 20 mg/kg bw/day (metosulam). A complete overview is given in the supplementary material, Appendix A2. Based on the hazard characterisation of 40 µg/kg bw/day for the index substance imazalil, the RPFs varied between 160 (ethoprophos) and 0.002 (metosulam). For the three substances also used in the second example, the RPFs from *in vivo* NOAELs were 1, 0.148 and 3.33 for imazalil, clothianidin and thiacloprid, respectively.

For imazalil, clothianidin and thiacloprid, dose response relations for the AdipoRed response after 72 h were measured in the *in vitro* HepaRG test system. Using the integrated Proast model in the toolbox, a 6-parameter parallel-curve exponential dose response model was fitted to the combined data of the three experiments, where three parameters represent the lower and upper asymptote and common slope, one parameter is the BMD for the index substance (here imazalil), and the remaining two parameters represent the RPFs for the other two substances relative to the index substance. Fig. 5 and Table 4 show the results. In Fig. 5b the doses for all three substances are expressed as equivalents of the index substance, $d_{eq} = RPF \cdot d$.

On visual inspection the data show no major deviations from the parallel curve model, but the variation around the fitted curve is large,

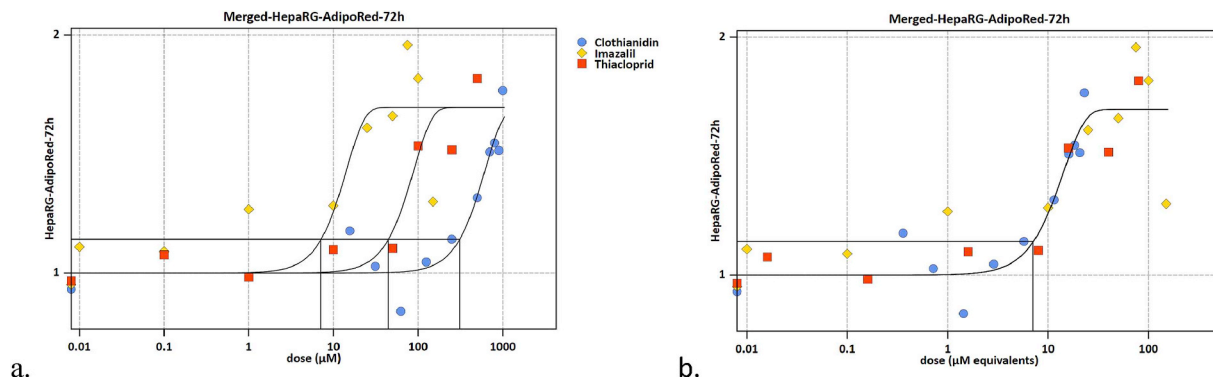


Fig. 5. Dose response model AdipoRed in HepaRG test system (a) Parallel curves fitted for three substances. (b) Doses for all substances expressed in equivalents of the index substance Imazalil. BMDs for a BMR of 10% increase are shown.

Table 4

Benchmark doses (BMD, in μM) with lower (P5) and upper (P95) bounds (BMDL, BMDU), calculated from a parallel-curve exponential model to the AdipoRed dose response data, and internal and external RPFs (with bootstrap-based 90% confidence intervals).

Substance	BMD <i>in vitro</i> (μM)	RPF internal (mol based)	Molecular mass	RPF internal (mass based)	Absorption factor from kinetic model	RPF external (mass based)
Clothianidin	309 (160–595)	0.023 (0.015–0.037)	249.68	0.027 (0.018–0.042)	0.013	0.00038 (0.00025–0.00058)
Imazalil	7.1 (3.18–11.8)	1	297.18	1	0.91	1
Thiacloprid	44.4 (23–85.5)	0.16 (0.09–0.26)	252.73	0.19 (0.12–0.35)	0.32	0.066 (0.041–0.14)

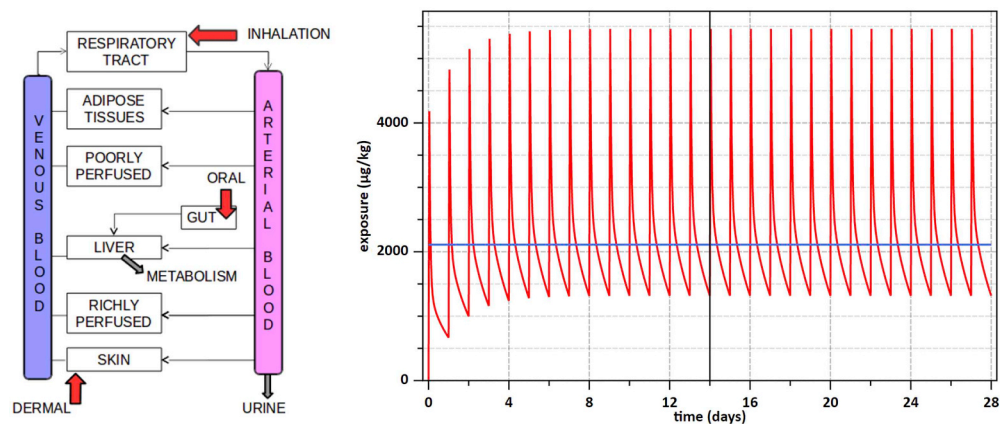


Fig. 6. EuroMix Generic PBTK model and example of use to derive internal to dietary (oral) external ratio (imazalil, human model), based on one dose per day leading to an internal concentration equal to the *in vitro* BMD (zero dermal and inhalation exposures are assumed). The horizontal line indicates the mean internal exposure in the selected interval between 14 and 28 days.

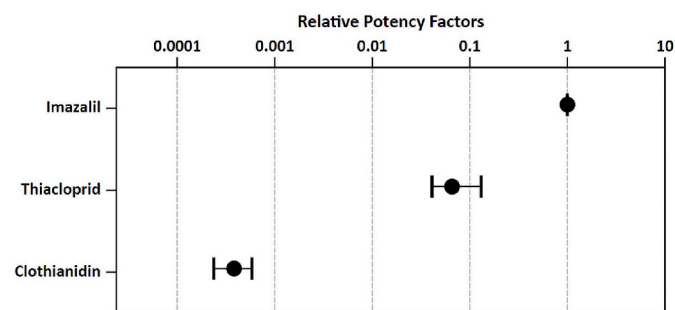


Fig. 7. External, mass-based relative potency factors calculated from *in vitro* BMDs, with 90% confidence intervals based on BMD uncertainties. Index substance imazalil.

which translates to wide confidence intervals for BMDs and RPFs. For example, the RPF for thiacloprid is 0.16, but is uncertain by almost a factor 3 from the 95% confidence interval (0.09, 0.26).

Using the EuroMix Generic PBTK model the internal liver concentration when a daily dose equal to the BMD is given was simulated and averaged over the period between 15 and 28 days to estimate the pseudo-steady-state concentration (Fig. 6). The ratio of this internal concentration to the external exposure was then used as the absorption factor to convert internal to external RPFs (Table 4, Fig. 7). Note that for a given dose imazalil has the highest concentration in the liver

Table 5

Chronic exposure percentiles using two methods: observed individual means (OIM) and model-assisted logistic-normal (LNN) estimates, median and 90% confidence limits.

Percentage	Cumulative exposure ($\mu\text{g}/\text{kg bw}/\text{day}$, as imazalil)		OIM/LNN
	OIM	LNN	
50	1.42 (1.20–1.68)	1.74 (1.46–2.05)	0.82
90	4.51 (4.00–5.06)	4.26 (3.77–4.92)	1.06
95	5.86 (5.26–6.71)	5.37 (4.77–6.27)	1.09
99	9.32 (8.36–10.4)	7.88 (7.02–8.86)	1.18
99.9	15.1 (12.3–17.0)	10.7 (9.64–12.6)	1.42
99.99	19.0 (15.5–22.2)	12.6 (11.3–14.2)	1.51

(higher absorption factor). Consequently, the external RPFs for clothianidin and thiacloprid are much lower than the internal RPFs.

3.3. Exposure assessment: risk drivers, aggregating dietary and non-dietary exposure, comparison with human monitoring

3.3.1. Cumulative dietary exposure and risk drivers for steatosis

Following the traditional approach, the cumulative dietary exposure of Dutch children (1–19 years) to steatosis-related pesticides was calculated at the external level, using external RPFs for dose addition. In Fig. 8 we show an example of cumulative exposure assessment based on

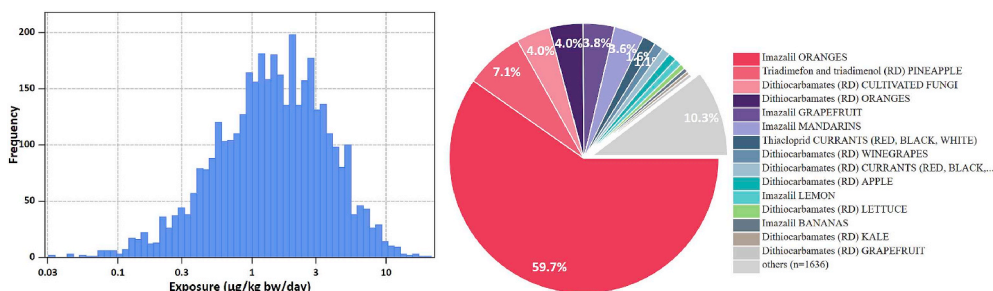


Fig. 8. a) Exposure ($\mu\text{g}/\text{kgBW}/\text{day}$ imazalil equivalents) Dutch population from 144 steatosis-related pesticides using NOAEL- or LOAEL-based dose addition, and OIM method. b) risk drivers in the upper 2.5% of the distribution.

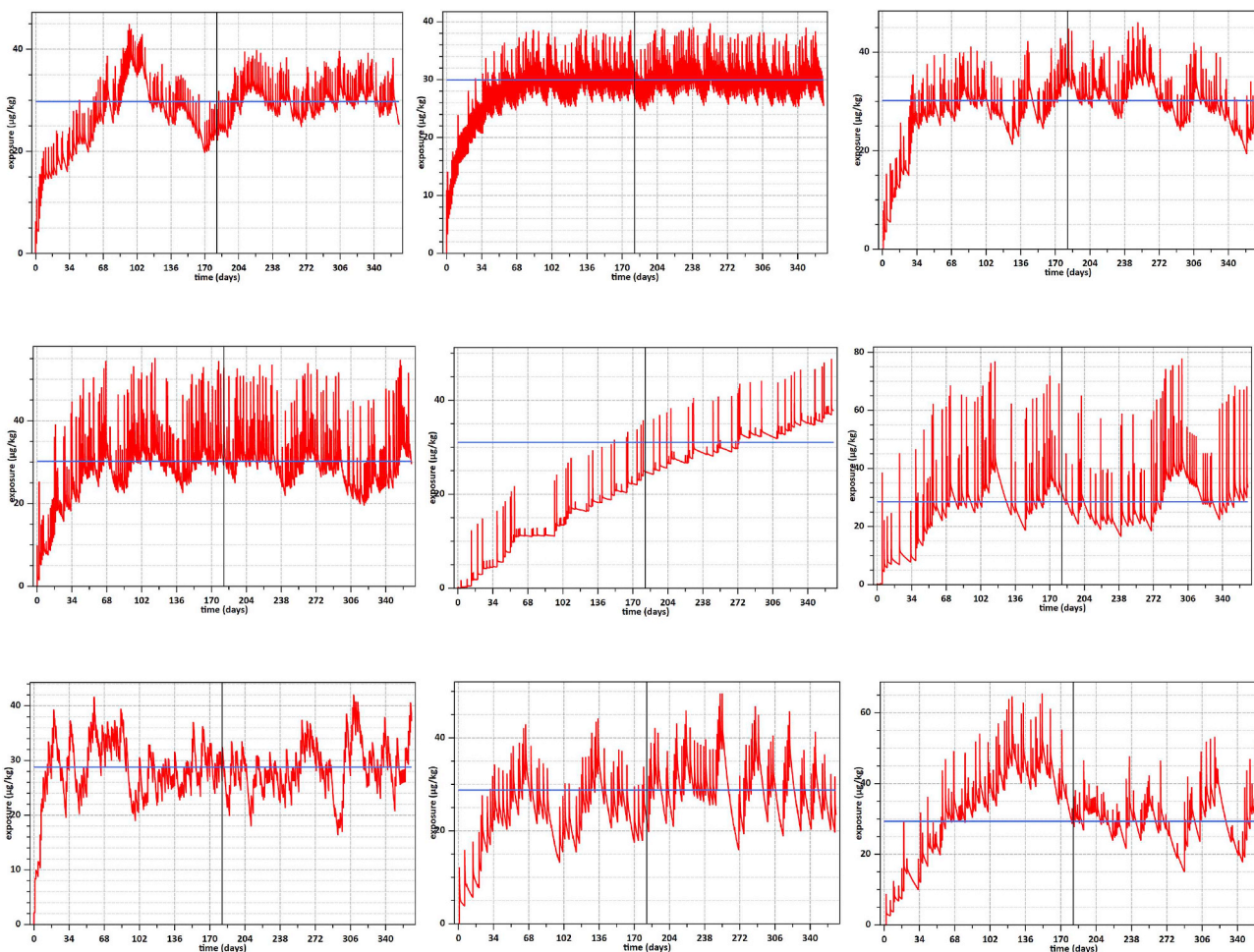


Fig. 9. Simulated kinetics of imazalil for 9 individuals in the French population around the 97.5th percentile of exposure in the cumulative exposure distribution. Note the random draws from the seven survey days for the external doses.

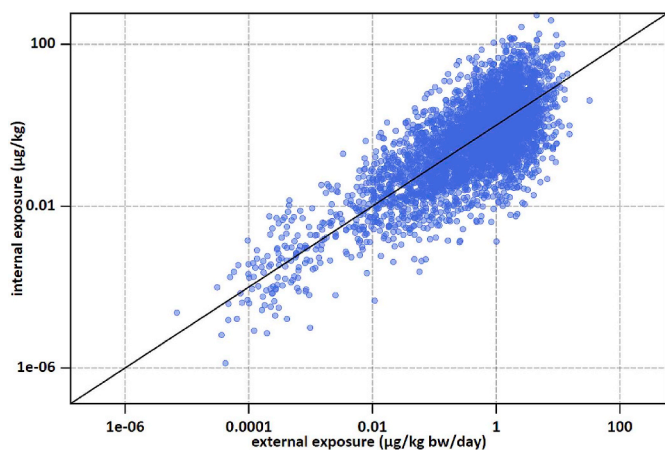


Fig. 10. Internal vs. external exposure.

NOAEL- or LOAEL-based RPFs for the 144 of 573 pesticides that were related to steatosis according to the POD data (see 3.2 and Appendix A2). It can be seen that imazalil in citrus fruits are main risk drivers, where it can be noted that processing factors for the peeling and/or juicing of citrus fruits were missing and therefore suggest a possibly useful refinement of the model. For further details see Crépet et al. (2019b).

Comparing different models for long-term exposure, the model-assisted estimates of the LNN model were found to be lower than the OIM

estimates in the upper tail (Table 5), which confirms that the OIM method underestimates the median, but overestimates the upper tail percentiles (Dodd et al., 2006; Goedhart et al., 2012).

3.3.2. Aggregated cumulative exposure using a kinetic model

With dietary and non-dietary exposures, it is essential to aggregate at the internal level. Consequently, internal RPFs are needed for dose addition. In a simple approach standard absorption factors can be used, e.g. 1 for dietary or inhalation exposure and 0.1 for dermal exposure. See Kennedy et al. (2019) for such an application. Here we illustrate the use of kinetic models in an example with just three substances. In this example the EuroMix Generic PBTK model was used to translate dietary, dermal and inhalation exposures to internal exposure in the liver. Further, for each individual the external exposures on each of the 365 days of the simulation were randomly selected from the seven daily imazalil exposures that were calculated for the seven days of this individual in the French consumption survey. In separate runs for dietary, dermal and inhalation external exposure, the mean absorption factors to the liver were estimated as (0.91, 0.96, 0.92) for imazalil, (0.35, 0.34, 0.32) for thiacloprid and (0.017, 0.019, 0.013) for clothianidin. Fig. 9 shows simulated kinetic curves for the amount of imazalil in the liver for the nine individuals in the French consumption survey that had aggregated cumulative exposures closest to the 97.5th percentile of the distribution. It can be seen that very variable kinetic curves were obtained, and that for some individuals the pseudo steady state is not yet reached after half a year. This is due partly to differences in dietary exposures and partly to the assumed variability of kinetic model

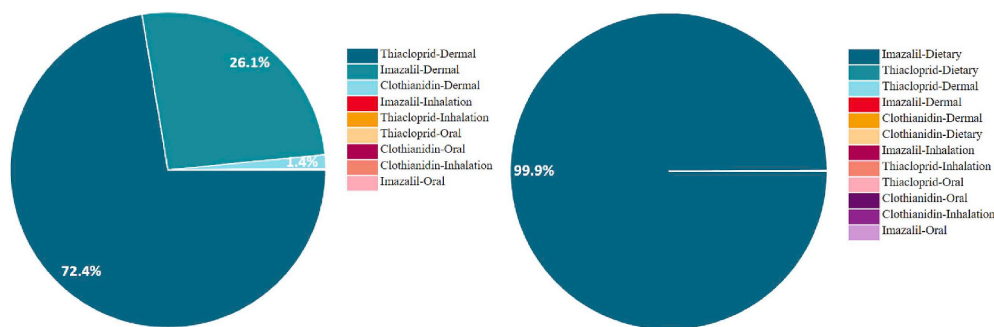


Fig. 11. Contributions by route and substance to (a) the non-dietary cumulative exposure, (b) the total (dietary and non-dietary) cumulative exposure.

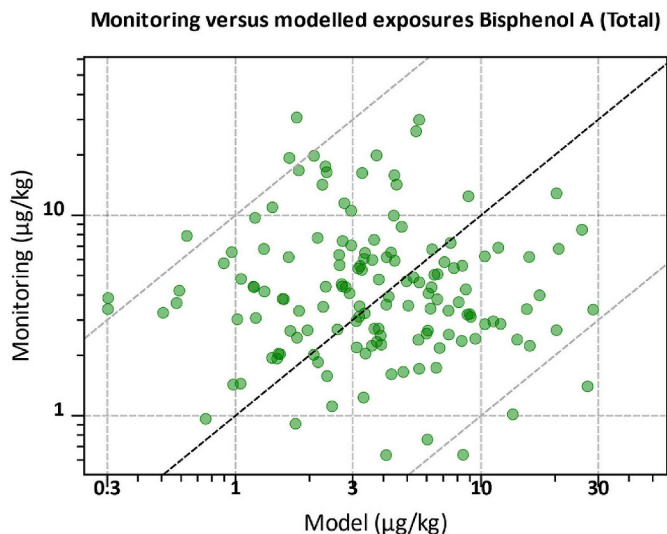


Fig. 12. Bisphenol-A measured in urine vs. predicted from dietary and non-dietary exposures for 144 persons.

parameters. The high variability in absorption is also evident from the plot of internal vs. external exposure (Fig. 10).

Using estimates that respectively 10.3%, 0.9%, 0.8% and 0.1% of the population lives near agricultural fields where wheat, potatoes, sugar beet or dessert apples are sprayed, we observe that for those people thiacloprid and imazalil via the dermal route had the largest contribution in their non-dietary exposure (Fig. 11a). This is in accordance with the results based on fixed absorption factors in Kennedy et al. (2019). However, in the total exposure of the total adult population the non-dietary contributions were minor. Imazalil from dietary exposure had by far the largest contribution in this example (Fig. 11b).

3.3.3. Comparison of modelled exposure with human biomonitoring data

Using the Human monitoring analysis module of the toolbox, human biomonitoring data (BPA measured in urine on a single day) from a Norwegian study (Husøy et al., 2019; Karrer et al., 2019) were compared to chronic exposure predictions based on the dietary consumptions and non-dietary uses of personal care products recorded for the survey participants (Fig. 12). The results showed roughly comparable levels of BPA around 1–10 µg/kg body weight per day, but no strong correlation.

3.4. Risk characterisation: comparing exposure and hazard characterisation distributions

In an assessment of all 144 pesticides with a POD for steatosis, the final risk assessment is shown in two different ways. First, the HCs, which in this case were the NOAELs in or derived from the data repository, were plotted against the exposure distributions for each of the

substances separately, and also cumulated (Fig. 13a). The variability and uncertainty in the exposure also induce variability and uncertainty of the MOE, as represented by the diagonal line sections. Background colours have been applied to indicate possible areas of risk and safety. Note that one line (in the red area) represents equality of exposure and HC (POD), whereas the other line (in the yellow area) represents the user-specified threshold value 100 for the interpretation of MOEs.

A more direct representation of the MOEs is given in Fig. 13b. In both plots it is seen that the cumulative MOE is well above 100, the 5th percentiles of the cumulative distribution is estimated as 649, with a lower 95% confidence limit of 597. Imazalil stands out as the main risk driver.

4. Discussion

This paper has described the modular structure of the MCRA model and data toolbox developed in the EuroMix project. Simple examples have been shown how the toolbox can be used for various aspects of the risk assessment of chemical mixtures. It is stressed that this paper does not intend to present extended case studies. All examples have been given for illustration of the methodology only, and do not represent real hazard, exposure or risk assessments. For example, more study is needed regarding the large differences between the *in vivo* and *in vitro* derived RPFs for clothianidin or thiacloprid relative to imazalil. In fact, it is not the purpose of this paper to propose any specific methodology as an optimal approach for specific case studies, but rather to emphasise that a wide variety of both simple and more complex approaches with varying degrees of conservatism can be explored and compared using an appropriate model and data toolbox.

More possibilities are available in the toolbox than could be illustrated here. For example, the toolbox also contains functionality to use molecular docking models for identifying AG membership (Cotterill et al., 2016; Kennedy et al., *subm.*). Missing HCs can be imputed, e.g. based on thresholds of toxicological concern (Munro et al., 1996; Kennedy et al., *subm.*). More refined exposure models can be applied, including the use of occurrence patterns for the imputation of left-censored data and residue definitions for measured substances which are only indirectly measuring the active substances (van Klaveren et al., 2019a). The most relevant mixtures for which further refinement could be important can be identified (Crépet et al., 2019a). Integrated probabilistic risk assessments where the uncertainty factors are also modelled probabilistically can be run (van der Voet and Slob, 2007; van der Voet et al., 2009; Jacobs et al., 2015).

MCRA 9, developed as the EuroMix toolbox and presented in this paper, is maintained after the EuroMix project at <https://mcra.rivm.nl> and can be used in its current state. Users can upload their own data or can access data which are shared by other platform users or user groups. The possible links between the MCRA toolbox and the IPCHEM platform of the European Commission for supporting the assessment of chemical mixtures have been discussed (Dalla Costa et al., 2018) and the European Commission, EFSA, industry and regulators were trained

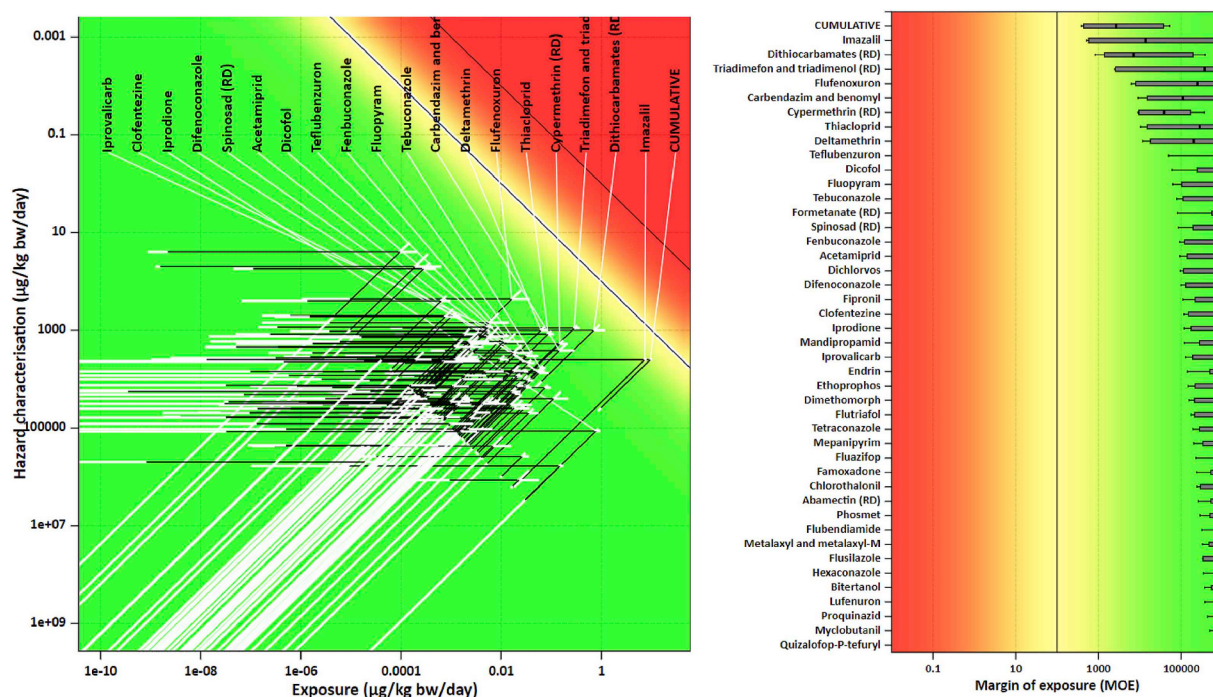


Fig. 13. a. Hazard vs. exposure plot using 100 as a MOE level for risk of steatosis. Exposure ranges and induced MOE ranges are plotted for cumulative (imazalil as index substance) and for the separate substances (only 20 with lowest MOE shown). Black line segments represent the variability range between percentiles p1 and p99, with white extensions representing one-sided 95% uncertainty limits on these percentiles. b. Margin of exposure, cumulative and for the separate substances (restricted to substances with $P1(MOE) < 10^6$), using 100 as a MOE threshold for risk. Bars represent MOE ranges in the population (P1-P99), with whiskers representing one-sided 95% uncertainty limits for P1 and P99.

in the use of MCRA (Bopp et al., 2018; Zilliacus et al., 2019b). The toolbox will also be further developed in cooperation with EFSA and other stakeholders such as national risk assessment institutes. Such development might go in two opposite directions. On the one hand, the use by less-experienced users can be optimised by offering clearly described tiers including pre-sets of options, avoiding the need to specify all settings by hand. On the other hand, the modular design of the toolbox makes it suitable for developing interoperability with other web-based databases and models, and for adding new functionalities. A practical example would be to add the use of expert-elicited uncertainty distributions to adjust the results of risk assessments (EFSA, 2019cd). Another extension would be to include read-across approaches for hazard assessment as have been recently proposed (Escher et al., 2019).

In conclusion, the MCRA model and data toolbox has been found useful to perform case studies of hazard identification, hazard characterisation, exposure assessment and risk characterisation, as shown in this paper and in other deliverables in the EuroMix project (Crépet et al., 2019a,b; Kennedy et al., 2019; Karrer et al., 2019; Kennedy et al., *subm.*; Vanacker et al., *in prep.*). At the same time, the toolbox has been prepared to serve a wider public, and will be tested and further developed in future collaborations.

CRedit authorship contribution statement

Hilko van der Voet: Conceptualization, Methodology, Software, Formal analysis, Writing - original draft, Writing - review & editing. **Johannes W. Krusselbrink:** Methodology, Software, Writing - review & editing. **Waldo J. de Boer:** Methodology, Software, Writing - review & editing. **Marco S. van Lenthe:** Methodology, Software, Writing - review & editing. **J.J.B. (Hans) van den Heuvel:** Software, Writing - review & editing. **Amélie Crépet:** Methodology, Investigation, Writing - review & editing. **Marc C. Kennedy:** Investigation, Writing - review & editing. **Johanna Zilliacus:** Investigation, Writing - review & editing. **Anna Beronius:** Investigation, Writing - review & editing. **Cleo Tebby:**

Investigation, Writing - review & editing. **Céline Brochot:** Investigation, Writing - review & editing. **Claudia Luckert:** Investigation, Writing - review & editing. **Alfonso Lampen:** Investigation, Writing - review & editing. **Emiel Rorije:** Investigation, Writing - review & editing. **Corinne Sprong:** Investigation, Writing - review & editing. **Jacob D. van Klaveren:** Conceptualization, Supervision, Writing - review & editing.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fct.2020.111185>.

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