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

WP 6 – **Model integration into a web-based model and data toolbox**

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

Abstract

A toolbox has been built to assess risks from combined exposure to multiple chemicals using probabilistic methods. The toolbox has more than 40 modules to address 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 assessment, with special emphasis on the use of in vitro results. Examples shown for hazard identification are the selection of substances relevant for a specific adverse outcome based on adverse outcome pathways and QSAR models. Examples for hazard characterisation are the calculation of benchmark doses and relative potency factors with uncertainty estimates from dose response data, and the use of kinetic models to perform in vitro in vivo extrapolation. Examples for exposure assessment are assessing cumulative exposure at either the external or the internal level, where the latter option is needed in case dietary and non-dietary routes have to be aggregated. Finally, risk assessment is illustrated by graphical displays of the margin of exposure for single substances and cumulated, including uncertainties for the exposure and hazard characterisation estimates.

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1 Introduction

Human activities in the last century 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, but multiple substances can have the same health effect, so their effects on the same phenomenological endpoint should be cumulated. Consequently, the need was perceived to develop risk assessment methods for mixtures of substances. The current legislative requirements for risk assessment of combined exposure to multiple chemicals was recently reviewed by Rotter et al. (2018).

The tasks for mixture risk assessment are not trivial. Decisions are needed which chemical substances should be evaluated together in an assessment group (AG) when considering a specific adverse outcome (AO). Data on both exposure and hazard of those substances are needed. Exposure might need to be aggregated over several sources, such as dietary exposure and dermal or inhalation exposure, sometimes for specific population groups, e.g. 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 standard model for cumulating effects is dose addition (DA), but its validity might need to be checked. Under the DA model the relative potencies of substances are expressed as relative potency factors (RPFs), which can be different at the external or internal biological level. Kinetic modelling might be used to bridge the gap between external and internal doses leading to in vitro in vivo extrapolation (IVIVE) models.

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 uncertainties and their influence on the results of cumulative and aggregated risk assessment.

In this paper we describe the toolbox of models and data that has resulted from the EuroMix project. The toolbox has been developed as a new version 9 of the Monte Carlo Risk Assessment (MCRA) platform that was already available (van der Voet et al. 2015). For a full description of the toolbox we refer to the online reference manual (MCRA 2019). In addition, methods regarding mixture selection from exposure and hazard data are also included in the EuroMix toolbox have been described in the report on Task 6.1 and in Crépet et al. (2018) and the kinetic models used for in vitro in vivo extrapolation are fully described in Deliverable 6.3. The toolbox can be used in conjunction with the EuroMix handbook (Zilliacus et al. 2019). Here, we aim to provide an overview and illustrate the use of the toolbox with some simple examples for hazard identification, hazard characterisation, exposure assessment and risk assessment.

2 Methods

2.1 Description of the EuroMix toolbox

The toolbox for mixture risk assessment has been built according to the modular design shown in Figure 1. The modules are summarised in Appendix A. Summary of modules in the EuroMix toolbox 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.

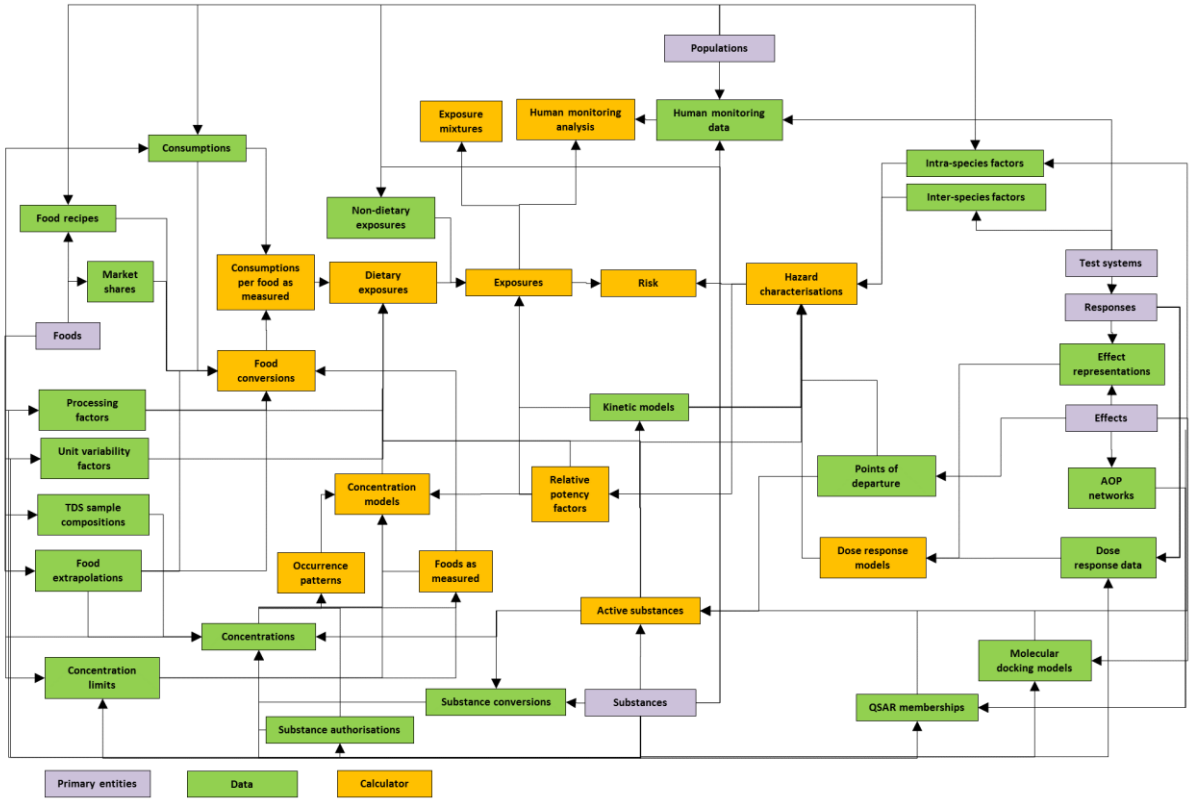


Figure 1. Modular design of the MCRA EuroMix toolbox.

2.2 Data – Example data organised in the EuroMix project

The EuroMix toolbox contains a repository for relevant data. Data can be uploaded by individual users or by representatives of a larger group. Data can be shared with other users or user groups. The data administrator for each data set can decide on read, or read/write permission. The data collected during the EuroMix project and shared with project participants (Data/EuroMix repository) are summarised in Table 1. It will be decided later which of these data can be made available to EuroMix toolbox users outside of the project. These data were the basis for the examples shown below.

Table 1. Data in the EuroMix toolbox, as collected at the end of the EuroMix project

Module	Description data sets
Foods	2289 foods-as-eaten and foods-as-measured coded in FoodEx1 32 processing types
Substances	1629 substances, classified in categories PPPs, Biocides, Alkaloids, EnvironmentlaPollutants, FoodAdditives, Mycotoxins
Effects AOP networks	46 effects in 5 AOP networks
Populations	15 populations in 10 countries (different age groups)
Test systems Responses Effect representations	14 test systems 477 responses 162 effect representations
Consumptions	11 files with food consumption data in 10 countries
Food recipes	5555 records specifying food ingredients in the FoodEx1 system or conversions
Concentrations	Food monitoring data, SSD formatted data
Processing factors	667 processing factors
Non-dietary exposures	Simulated non-dietary exposures from Browse and Bream2
Human monitoring data	[not publically available]
QSAR membership models	26 QSAR models applied to all substances in the inventory
Molecular docking models	20 Molecular docking models applied to all substances in the inventory
Kinetic models	Cosmos model parametrised for 9 substances based on httk and for all substances in the inventory based on QSAR
Points of departure	144 NOAEL or LOAEL values related to Steatosis-liver
Dose response data	28 files describing experiments with single substances or mixtures , on 15 responses (or groups) in 9 test systems in 6 laboratories

2.3 Examples of use

Examples how the toolbox can be used for innovation are given for the various areas of risk assessment, i.e. hazard identification, hazard characterisation, exposure assessment and risk characterisation.

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

In this example we illustrate the use of the *Active substances* module, with additional use of *Substances*, *Effects*, *AOP networks*, *Points of departure* and *QSAR memberships*.

Hazard identification includes the task of identifying substances that may lead to a specified adverse outcome (AO) considered in a risk assessment. The EuroMix toolbox includes several possibilities:

1. Directly specify the substances belonging to an assessment group related to a given AO
2. Select only those substances for which points of departure data are specified
3. Select substances based on predictions for the given AO from QSAR or molecular docking models

Option 1 differs from option 2 because it is possible to force inclusion of a compound and impute any missing points of departure. In probabilistic modelling substances can also be identified with a membership probability that they should be included in the assessment group. Membership probability can be derived from expert knowledge elicitation, as in recent EFSA reports. 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.

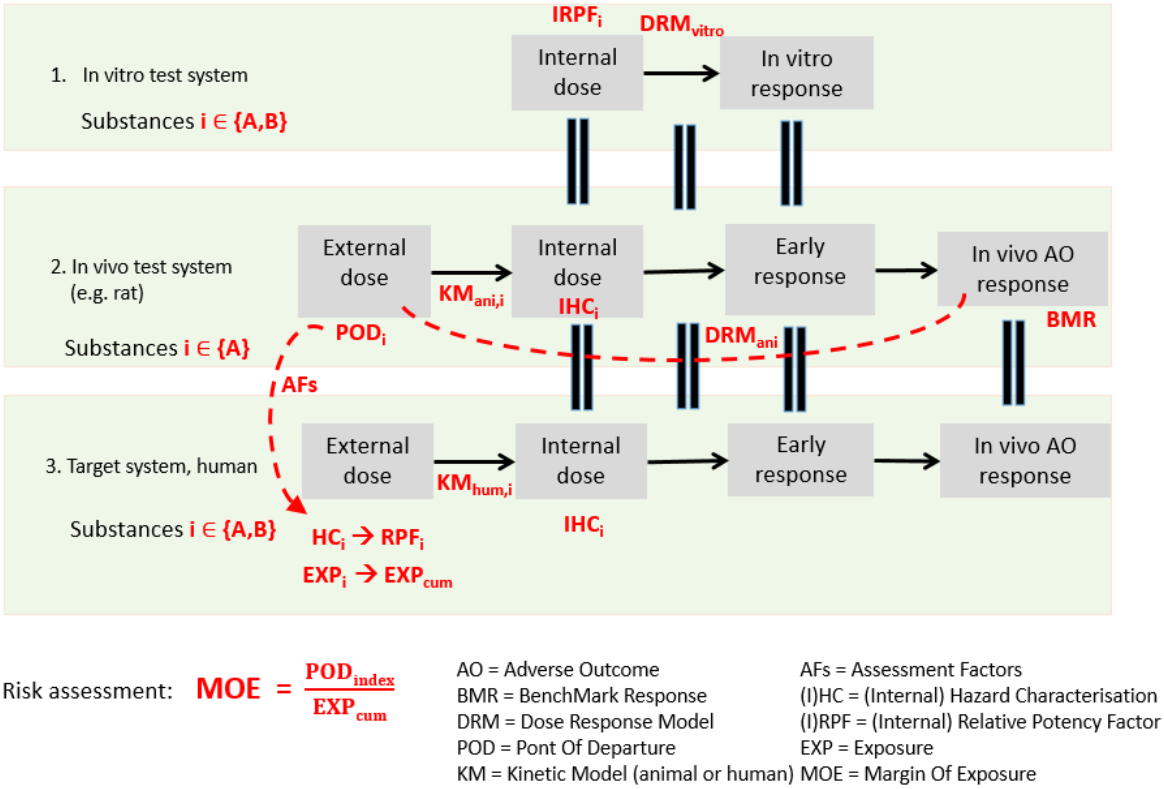
2.3.2 Hazard characterisation: using in vivo or in vitro data, calculation of RPFs, use of kinetic models for IVIVE

In this example we illustrate the use of the *Relative potency factors* module, based on *Hazard characterisations*, *Dose-response models*, *Dose response data*, *Effect representations* and *Kinetic models*.

We consider three chemicals identified to cause steatosis: imazalil, thiacloprid and clothianidin. In the toolbox this can be achieved by Substance selection in the module Substances.

Dose response relations for the AdipoRed response after 72 hours was measured in the in vitro HepaRG test system for three substances in the steatosis assessment group. Using the integrated Proast model in the EuroMix toolbox a 6-parameter parallel-curve exponential dose-response model was fitted to the data, where three parameters represent the lower and upper asymptote and common slope, one parameter is the BMD for the index substance (here Clothianidin), and the remaining two parameters represent the relative potency factors (RPFs) for the other two substances relative to the index substance.

The RPFs are based on mol-based in vitro doses. For reverse dosimetry we want to change to mass-based in vivo doses. The conceptual model used for in vitro in vivo extrapolation (IVIVE) is shown in Figure 2.



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)
AFs = Assessment Factors
(I)HC = (Internal) Hazard Characterisation
(I)RPF = (Internal) Relative Potency Factor
EXP = Exposure
MOE = Margin Of Exposure

Figure 2. Conceptual IVIVE model.

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.

In this approach, we need human kinetic models. Within EuroMix, the Cosmos model was integrated in the toolbox as a general applicable model. We simulated the internal liver concentration when a daily dose equal to the BMD is given, and averaged over the period between 15 and 28 days to estimate the pseudo-steady-state concentration. The ratio of this internal concentration to the external exposure is then used as the absorption factor.

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

In this example we illustrate the use of the *Dietary exposures* module, based on *Concentration models*, *Consumptions per food as measured*, *Relative potency factors*, *Active substances* based on the steatosis FERA QSAR model. Aggregate exposures can be estimated using the *Exposures* module,

integrating *Dietary exposures* and *Nondietary exposures* using *Kinetic models*. We also illustrate the use of the *Human monitoring analysis* module, comparing *Human monitoring data* and modelled *Exposures*.

An example is given with 83 substances, which are the steatosis-related pesticides according to the Fera steatosis QSAR model, and using NOAEL based external RPFs and dietary exposures.
In a second example, we show the use of internal RPFs based on in vitro testing in combination with dietary and non-dietary exposure data.

Human biomonitoring data were available from a Norwegian survey which measured bisphenols in urine and asked participants for their diet and their use of personal care products (Karrer et al. subm, Husoy et al. in prep.). The EuroMix toolbox was used to combine the survey questionnaire data with monitoring data on BPA in food, and compare the predicted exposures with the measured urine levels.

2.3.4 Risk characterisation: comparing exposure and hazard characterisation distributions

In this example we illustrate the use of the *Risks* module, based on comparing *Exposures* and *Hazard characterisations*.

In the example we consider a group of triazole pesticides related to steatosis. We show how traditional margin of exposure (MOE) values can be calculated by not using intraspecies and interspecies factors in Hazard characterisations. Instead, a hazard vs. exposure plot and safety bar are prepared with a user-specified MOE value, e.g. 100.

3 Results

3.1 Hazard identification: Assessment groups, probabilistic memberships from in silico data

Hazard identification includes the task of identifying substances that may lead to the AO considered in a risk assessment. This example considers the AO steatosis, for which an AOP network has been established (Figure 3). This network has been uploaded to the EuroMix toolbox in the form of relational tables specifying all effects and effect relations.

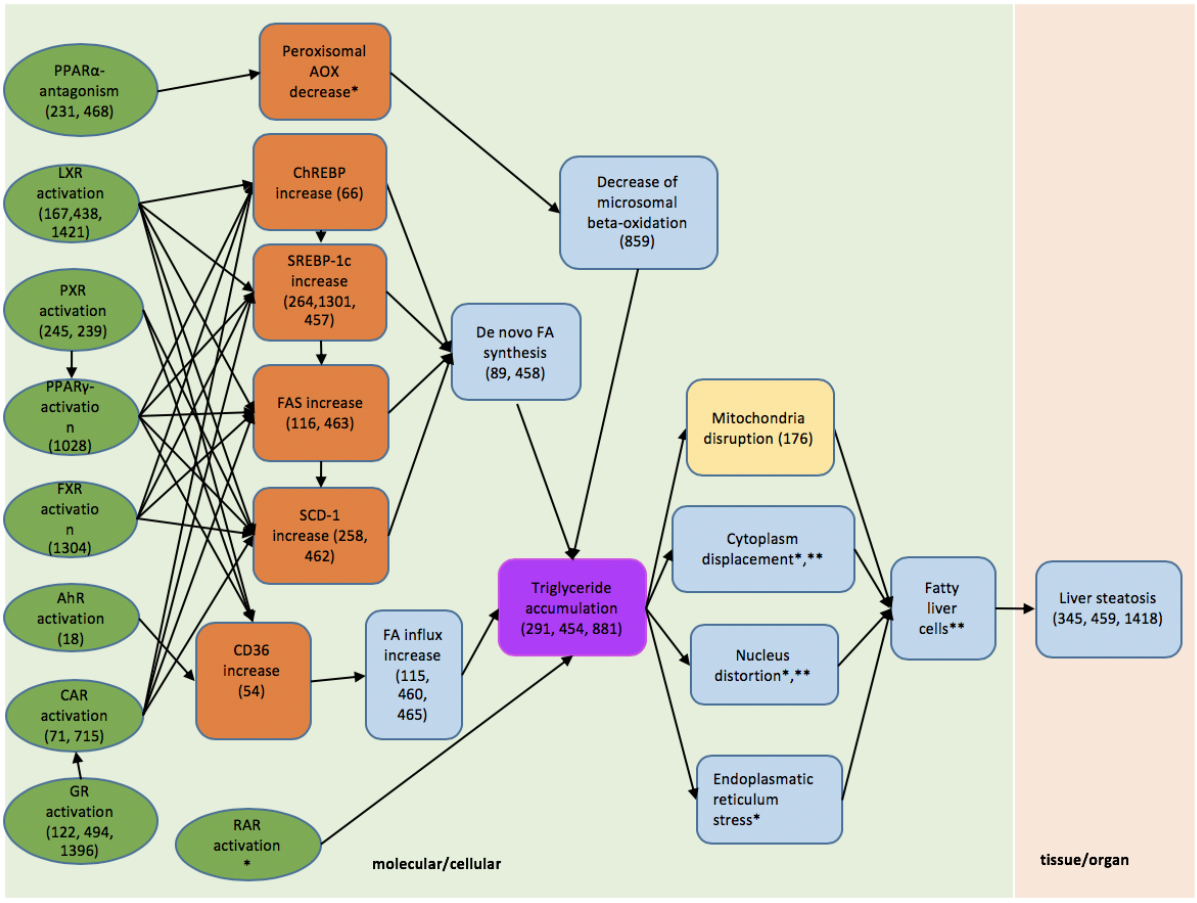


Figure 3. AOP network for the adverse outcome Liver steatosis.

Starting from the 573 pesticides listed in the EuroMix Chemical Inventory 144 substances were in the assessment group (AG) for steatosis based on available PODs for endpoints related to steatosis. An alternative option is to base AG memberships on QSAR models. 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) in the AOP network.

Table 2. QSAR models related to the adverse outcome steatosis.

Model code	Model description	Effect code	Number of substances included in the QSAR model	Fraction positive (included in the AG)
QSAR-COSMOS-NR-Hepatotoxicity	COSMOS Nuclear Receptor model for Steatosis liver nuclear receptors used to predict hepatotoxicity - and to predict steatosis	Steatosis-liver	513	0.60

QSAR-DOCKING-Steatosis-receptors	at least one of the 16 Liver NR Docking models from UniMilano above binding threshold energy	Steatosis-liver	513	0.84
QSAR-FERA-Steatosis	FERA developed model using the reference dataset for Steatosis - to predict steatosis	Steatosis-liver	513	0.49
QSAR-OCHEM-AhR-Hepatotoxicity	OCHEM AhR receptor binding model used to predict hepatotoxicity - and to predict steatosis	AhR-act-liver	512	0.40
QSAR-OCHEM-PPARgamma-Hepatotoxicity	OCHEM PPARg receptor binding model used to predict hepatoxicity - and to predict steatosis	PPARgamma-act-liver	508	0.45

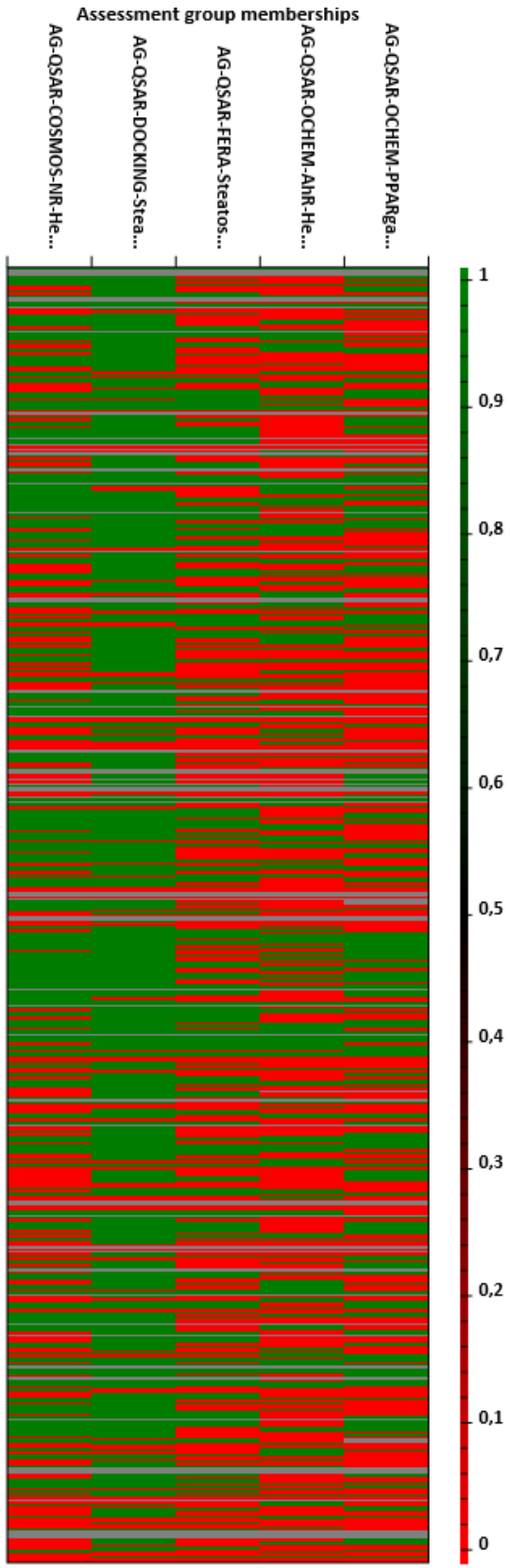


Figure 4. AG memberships for steatosis of 573 substances according to five QSAR models

Several options to combine the results of the various QSAR models are available. Based on the five models 295 pesticides were included in the AG using a majority voting rule. Alternatively, ratio-based

membership probabilities can be derived (Figure 5). In this analysis, a default probability 0.5 was used if QSAR classification was missing. The results were that 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. These membership probabilities can be used in probabilistic assessments by including the substance in iterated uncertainty runs with the calculated probability as proposed by EFSA[1]. This method is also available in the toolbox.

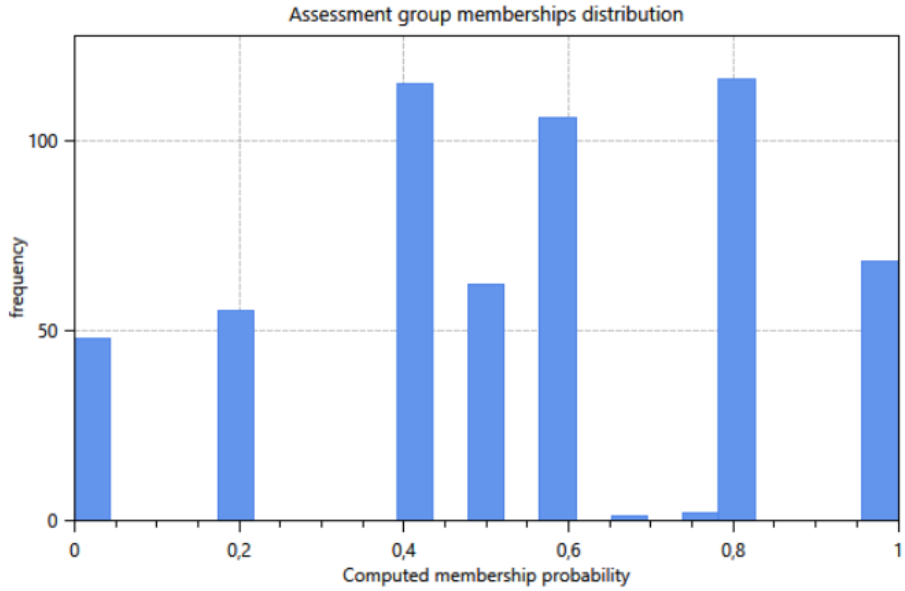


Figure 5. Probabilistic memberships for the Steatosis AG based on five QSAR models.

3.2 Hazard characterisation: Dose response modelling and relative potency factors

Dose response relations for the AdipoRed response after 72 hours was measured in the in vitro HepaRG test system for three substances in the steatosis assessment group. Using the integrated Proast model in the EuroMix toolbox a 6-parameter parallel-curve exponential dose-response model was fitted to the data (Figure 6, Table 3), where three parameters represent the lower and upper asymptote and common slope, one parameter is the BMD for the index substance (here Clothianidin), and the remaining two parameters represent the relative potency factors (RPFs) for the other two substances relative to the index substance.

On visual inspection the data show no major deviations from the parallel curve model, but the variation around the fitted curve is large, which translates to wide confidence intervals for BMDs and RPFs. For example, the RPF for imazalil is 43, but is uncertain with a 95% confidence interval (30, 63).

[1] https://www.efsa.europa.eu/en/consultations/call/180508-0, https://www.efsa.europa.eu/en/consultations/call/190213

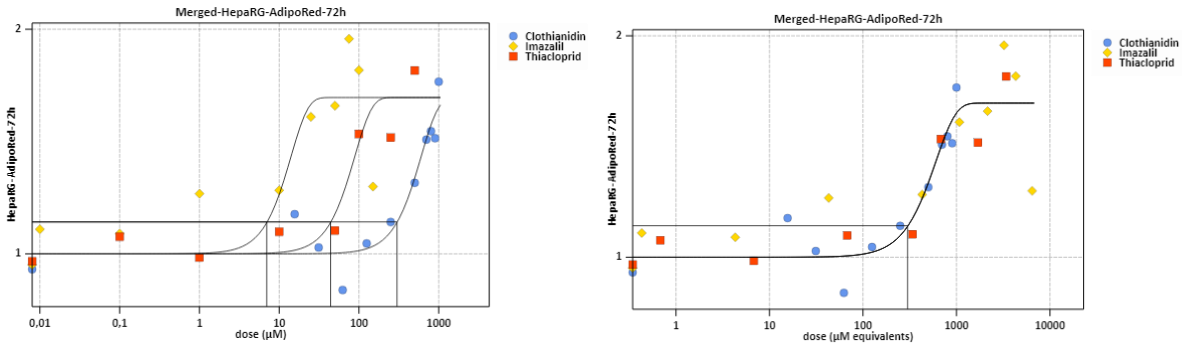


Figure 6 Dose response model AdipoRed in HepaRG test system **(a)** Parallel curves fitted for three substances. **(b)** Doses for all substances expressed in equivalents of the index substance Difenzoquat metilsulfat

Table 3. Benchmark doses (BMD) with lower and upper bounds (BMDL, BMDU) and Relative potency factors (RPF), with lower and upper bounds (RPFL, RPFU) calculated from a parallel-curve exponential model to the AdipoRed dose response data.

Substance name	BMD	BMDL	BMDU	RPF	RPFL	RPFU
Clothianidin	300	177	445	1	1	1
Imazalil	6.98	4.40	11.05	43	29.8	63.4
Thiacloprid	44.1	27.8	69.9	6.8	4.54	9.75

We simulated the internal liver concentration when a daily dose equal to the BMD is given, and averaged over the period between 15 and 28 days to estimate the pseudo-steady-state concentration (Figure 7). The ratio of this internal concentration to the external exposure is then used as the absorption factor. It is used to convert internal to external RPFs (Table 4). Note that in this example, clothianidin is excreted much faster than imazalil, therefore has a much lower absorption factor. Consequently, the external RPF for imazalil is much higher than the internal RPF.

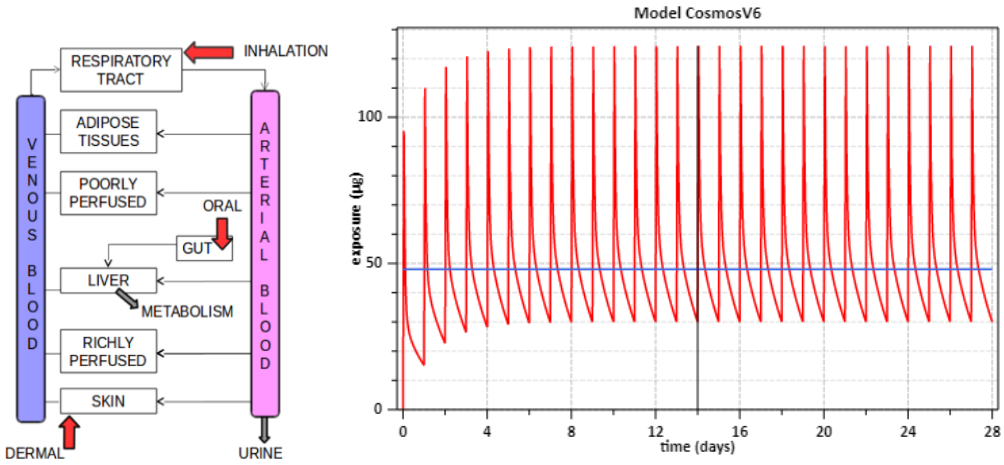


Figure 7. Cosmos model and example of use to derive internal/external ratio (imazalil, human model).

Table 4. Recalculation internal to external RPFs.

Substance name	RPF internal	Molecular mass	Absorption factor	RPF external
Clothianidin	1.00	249.68	0.01	1.00
Imazalil	36.13	297.18	0.91	2582.27
Thiacloprid	6.72	252.73	0.32	168.00

3.3 Exposure assessment: aggregating dietary and non-dietary, comparison with human monitoring

With only dietary exposures the cumulative exposure can be calculated at the external level. Here external RPFs can be used for dose addition. In Figure 8 we show an example of cumulative exposure assessment based on NOAEL-based RPFs for 83 pesticides related to steatosis. It can be seen that imazalil in citrus fruits is the risk driver, where it can be noted that processing factors for the peeling and/or juicing of citrus fruits were missing and therefore indicate a possible refinement of the model. For further details see Crépet et al. (in prep.).

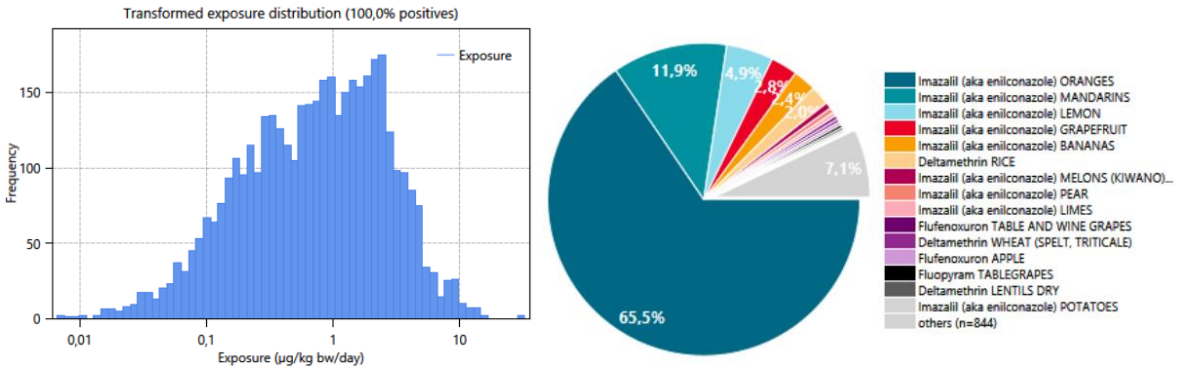


Figure 8. a) Exposure (µg/kgBW/day imazalil equivalents) French population from 83 steatosis-related pesticides using NOAEL-based dose addition. b) risk drivers

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. Figure 9 shows simulated kinetic curves for the amount of imazalil in the liver for the nine individuals in the French consumption survey that had cumulative exposures closest to the 97.5th percentile of the cumulative exposure distribution. In this example the parameters of the Cosmos model used were assumed to be variable according to sampling from a lognormal distribution as specified in Deliverable 6.3. Further the external exposures on each of the 28 days of the simulation were randomly selected from the seven daily imazalil exposures that were calculated for the seven days of the French consumption survey. It can be seen that this leads to very variable kinetic curves, and that for some individuals the pseudo steady state is not yet reached after 14 days.

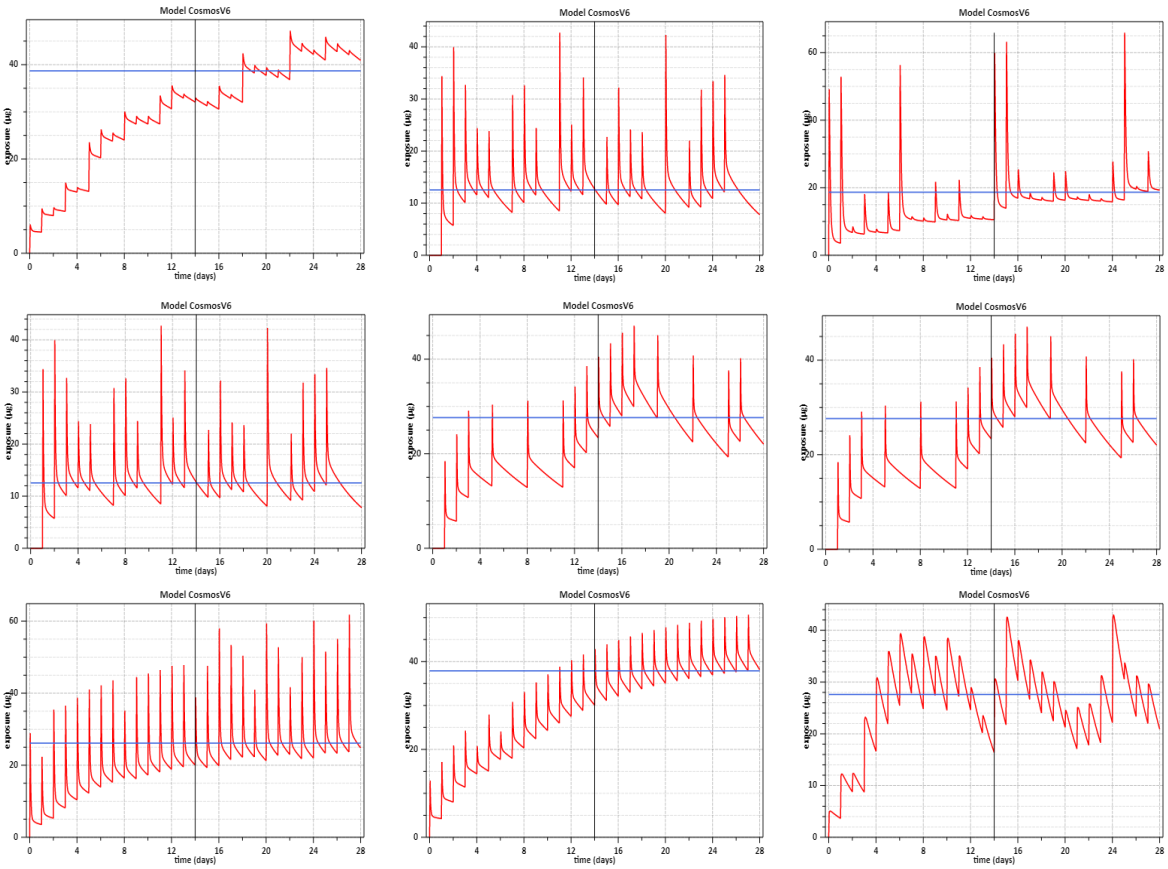


Figure 9. Simulated kinetics of imazalil for 9 individuals in the French population around the 97.5th percentile of exposure in the cumulative exposure distribution.Note the random draws from the seven survey days for the external doses.

Using estimates that 8.9% of the population is a bystander for agricultural fields where crops are sprayed, we observe that for those people inhaled imazalil may have the largest contribution in their non-dietary exposure. This result differs from the results based on fixed absorption factors in Kennedy et al. (2019), where dermal exposure was found to dominate non-dietary exposure. However, in the total exposure the non-dietary contributions are minor. Imazalil from dietary exposure has the largest contribution in this example (Figure 10).

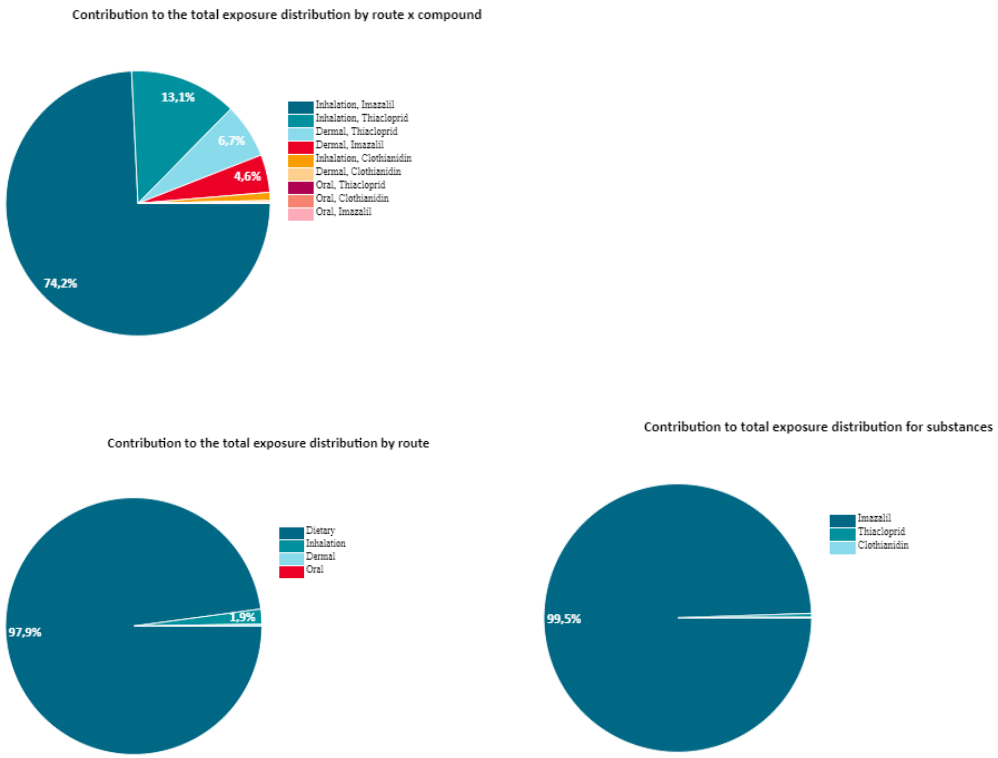


Figure 10. (a) Contributions by route and substance to the nondietary cumulative exposure ; (b) Contributions by route to the total cumulative exposure; (c) Contributions by substance to the total cumulative exposure.

Using the Human monitoring analysis module of the toolbox, human biomonitoring data (bisphenol A measured in urine) from a Norwegian study (Karrer et al. subm., Husoy et al. in prep.) were compared to exposure predictions based on the dietary consumptions and non-dietary uses of personal care products recorded for the survey participants (Figure 11). The results showed roughly comparable levels of BPA, but no strong correlation.

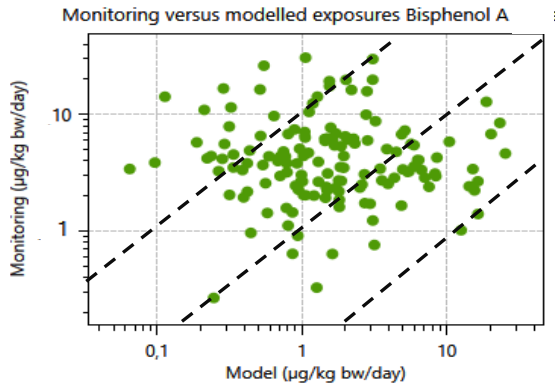


Figure 11. Bisphenol-A measured in urine vs, predicted from dietary and non-dietary exposures.

3.4 Risk characterisation: comparing exposure and hazard characterisation distributions

In an assessment of triazole pesticides related to steatosis, the final risk assessment is shown in two different ways. First, the hazard characterisations, which in this case were thePODs (NOAELs) in the

data repository, were plotted against the exposure distributions for each of the substances separately, and also cumulated (Figure 12). The variability and uncertainty in the exposure also induce variability and uncertainty of the margins of exposure, as represented by the diagonal line sections. Assuming a value of 100 for the interpretation of margins of exposure, background colours have been applied to indicate possible areas of risk and safety.

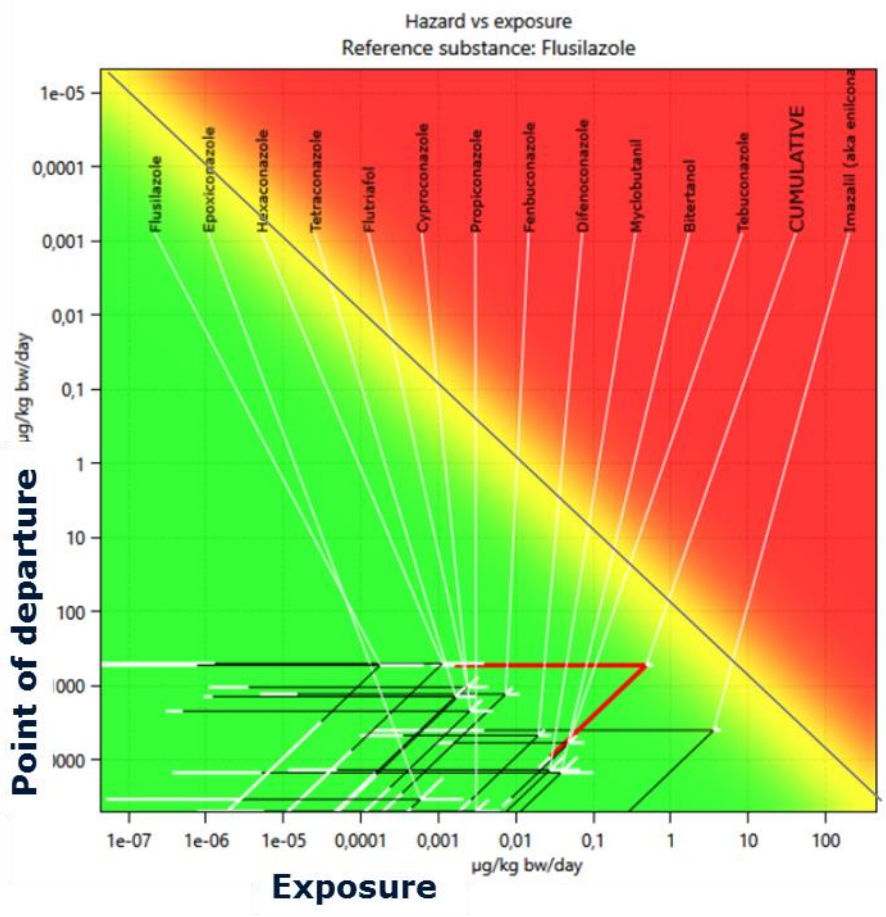


Figure 12. Hazard vs. exposure plot using 100 as a MOE level for risk. Exposure ranges and induced MOE ranges are plotted for cumulative (red lines) and for the separate substances. The ranges represent the variability percentiles p5-p95 in colour, with white extensions representing 95% uncertainty limits on these percentiles.

A more direct representation of the margins of exposure (which are the ultimate quantities for risk assessment) is given in Figure 13. In both plots it is seen that the cumulative margin of exposure is well above 100. Imazalil stands out as the main risk driver.

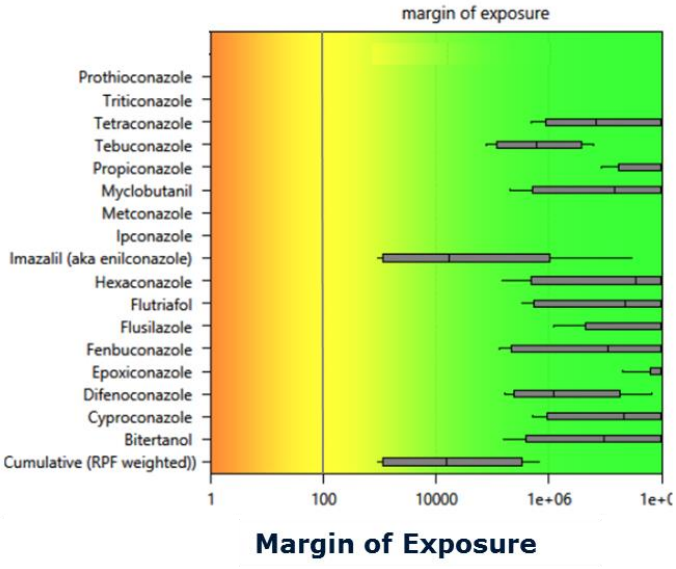


Figure 13. Margin of exposure, cumulative and for the separate substances, using 100 as a MOE level for risk. Bars represent MOE ranges in the population (P5-P95), with whiskers representing 95% uncertainty limits for P5 and P95.

4 Discussion

This paper has shown simple examples of how the EuroMix toolbox can be used for various aspects of the risk assessment of chemical mixtures. It is stressed that all examples have been given for illustration of the methodology only, and do not represent real hazard, exposure or risk assessments.

Many more possibilities are available in the toolbox than we could illustrate here. For example, the toolbox also contains functionality to use molecular docking models, to impute missing hazard characterisations, e.g. by thresholds of toxicological concern (Munro), to apply more refined exposure models 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 (EFSA reports, in prep.), to identify the most relevant mixtures for which further refinement could be important (Crépet et al. 2018), and to include hazard characterisation variability and uncertainty in the risk assessment step using the integrated probabilistic risk assessment (IPRA) model (van der Voet & Slob 2007, van der Voet et al. 2009).

The EuroMix toolbox presented in this paper will be maintained after the EuroMix project and can be used in its current state. However, it is also intended to be further developed. On the one hand, the use by less-experienced users can be optimised by offering clearly described tiers including presets 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.

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Appendix A. Summary of modules in the EuroMix toolbox

Category	Module	Description
Primary entities	Foods	Foods are uniquely defined sources of dietary exposure to chemical substances. Foods may refer to 1) foods-as-eaten: foods as coded in food consumption data (e.g. pizza); 2) foods-as-measured: foods as coded in concentration data (e.g. wheat); 3) any other type of food (e.g. ingredients, e.g. flour).
	Substances	Substances are chemical entities. Substances can refer to: 1) active substances such as investigated in toxicology; 2) measured substances such as defined in specific analytical methods.
	Effects	Effects are biological or toxicological consequences for human health, that may result from chemical exposure and are the focus of hazard or risk assessment.
	Populations	Populations are groups of human individuals that are the scope of exposure or risk assessments.
	Test systems	Test systems are biological or artificial systems used for assessing hazard in relation to chemical exposure from substances in varying doses. Test systems may refer to 1) in vivo test systems (e.g. a rat 90-day study, a human biomonitoring study); 2) in vitro test systems (e.g. HepaRG cells).
	Responses	Responses are measurable entities in test systems. Responses are used to represent effects (see effect representations) and their measured values are collected in dose response data.
Consumption	Consumptions	Consumptions data are the amounts of Foods consumed on specific days by Individuals in a food consumption Survey. For an acute exposure assessment, the interest is in a population of person-days, so one day per individual may be sufficient. For chronic exposure assessments, the interest is in a population of person, so preferably two or more days per individual are needed.
	Market shares	Market shares data specify for a given food percentages of more specific foods (subfoods, e.g. brands) representing their share in a market. Market shares are used when consumption data are available at a more generalised level than concentration data.
	Food recipes	Food recipes data specify the composition of specific foods (typically: foods-as-eaten) in terms of other foods (intermediate foods or foods-as-measured) by specifying proportions in the form of a percentage.
Occurrence	Concentrations	Concentrations data are analytical measurements of chemical substances occurring in food samples. Optionally, concentrations data can be recalculated for active substances, extrapolated to other foods, and/or default values can be added for water.
	Processing factors	Processing factors are multiplication factors to derive the concentration in a processed food from the concentration in an unprocessed food. Processing factors can be given for identified processing types (e.g. cooking, washing, drying).
	Unit variability factors	Unit variability factors specify the variation in concentrations between single units of the same food, which have been put together in a mixture sample on which the concentration measurements have been made.
	Occurrence patterns	Occurrence patterns (OPs) are the combinations (or mixtures) of substances that occur together on foods and the frequencies of these mixtures occurring per food, expressed in percentages. In the context of pesticides, occurrence patterns can be associated with agricultural use percentages. Occurrence patterns are relevant to account for co-occurrence of active substances in exposed individuals. Occurrence patterns may be specified as data or modelled based on observed patterns of positive concentrations.

Category	Module	Description
	Substance authorisations	Substance authorisations specify which food/substance combinations are authorised.
	Substance conversions	Substance conversions specify how measured substances are converted to active substances, which are the substances assumed to cause health effects. In the pesticide legislation such measured substances and the substance conversion rules are known as residue definitions.
	Concentration limits	Concentration limits specify (legal) limit values for substance concentrations on foods and are sometimes used as conservative values for concentration data. In the framework of pesticides the legal Maximum Residue Limit (MRL) is the best known example.
	Concentration models	Concentration models are distributional models of substance concentrations on foods. They describe both the substance presence (yes/no, with no representing an absolute zero concentration) and the substance concentrations. Concentration models are specified per food/substance combination.
	Foods as measured	Foods as measured are foods within the foods scope for which concentration data of substances are available (or expected).
	Focal food concentrations	In some cases the attention in an assessment is on a specific food (focal food), against the background of other foods. Focal food concentrations are separate concentration data for one or more focal food commodities, that will take the place of any other concentration data for the focal food in the ordinary concentrations data.
	Total diet study sample compositions	Total diet study sample compositions specify the composition of mixed food samples, such as used in a total diet study (TDS), in terms of their constituting foods.
	Food extrapolations	Food extrapolations data specify foods (from-foods) that can be used to impute concentration data for other foods with insufficient data (to-foods).
Exposure	Food conversions	Food conversions relate foods-as-eaten, as found in the consumption data, to foods-as-measured, which are the foods for which concentration data are available.
	Consumptions per food as measured	Consumptions per food as measured are consumptions of individuals expressed on the level of the foods for which concentration data are available (i.e., the foods-as-measured). These are calculated from consumptions of foods-as-eaten and food conversions that link the foods-as-eaten amounts to foods-as-measured amounts.
	Dietary exposures with screening	Dietary exposures with screening are just Dietary exposures, but the calculation includes a prior screening step to identify the main risk drivers (food-substance combinations). This allows computations with more substances by suppressing some details for less important food-substance combinations.
	Dietary exposures	Dietary exposures are the amounts of substances, expressed per kg bodyweight or per individual, to which individuals in a population are exposed from their diet per day. Depending on the exposure type, dietary exposures can be short-term/acute exposures and then contain exposures for individual-days, or they can be long-term/chronic exposures, in which case they represent the average exposure per day over an unspecified longer time period.
	Non-dietary exposures	Non-dietary exposures are the amounts of substances to which individuals in a population are exposed via any of three non-dietary routes: dermal, inhalation or oral, per day.
	Exposures	Exposures, possibly from both dietary and non-dietary routes of exposure, to which individuals in a population are exposed per day at a chosen target level. This target level may be external exposure (dietary exposure) or internal exposure.

Category	Module	Description
	Exposure mixtures	Exposure mixtures are mixtures of substances that contribute relatively much to the overall cumulative exposure (potential risk drivers). The occurrence and concentrations of compounds in the same samples may be correlated, which is of importance for acute exposure assessments (Note that chronic assessments only use mean concentration values). Theoretically, this could be modelled and fitted to datasets. However, in practical applications (regarding pesticide residues) the number of positive values is commonly too low to allow such detailed modelling. Co-exposure of compounds is defined as the pattern of compounds occurring together on a single individual day. Co-exposure can enter the risk assessment through the use of mixtures of substances on a single food or by combining different food sources on a single day (through consumption).
	Human monitoring data	Human monitoring data quantify concentrations found in human surveys. Data are provided on the survey, the individuals in the survey, the samples taken, the analyses performed, the analytical methods used, the properties for substances analysed, and the concentrations found.
	Human monitoring analysis	Human monitoring analysis compares observed human monitoring data with predictions made for the same population of individuals from dietary survey data, concentration data and (optionally) non-dietary exposure data.
In silico	QSAR membership models	QSAR membership models specify assessment group memberships for active substances related to a specific health effect (adverse outcome). Memberships should be derived externally from Quantitative Structure-Activity Relationship (QSAR) models.
	Molecular docking models	Molecular docking models specify binding energies for substances in specific molecular docking models related to a specific health effect (adverse outcome).
Kinetic	Kinetic models	Kinetic models convert exposures or hazard characterisations from one or more external routes or compartments to an internal (target) compartment. The reverse conversion from internal to external can also be made (reverse dosimetry).
Hazard	Active substances	Active substances are the substances that may lead to a specific health effect (adverse outcome). Active substances can be either specified directly as data or calculated from QSAR membership models or from Molecular docking models. Optionally, active substances can have assessment group memberships between 0 and 1.
	Relative potency factors	Relative potency factors (RPFs) describe the potency of substances with respect to a defined effect, relative to the potency of a chosen index substance. RPFs can be given as data or computed from hazard characterisations.
	Hazard characterisations	Hazard characterisations are benchmark doses for active substances and for the chosen effect at the chosen target level (external or internal) of the hazard assessment. Hazard characterisations are based on points of departure, such as BMDs from dose-response models or externally specified points of departure (MDSs, NOAELs or LOAELs). The computation may involve inter-species conversion, intra-species factors and the use of kinetic models or absorption factors to convert external doses to internal doses.
	Points of departure	Externally specified points of departure can be used as an alternative to calculated BMDs from dose response models. Points of departure can be of various types, such as NOAEL, LOAEL or BMD.
	Dose response models	Dose response models specify the results of models fitted to dose response data. Dose response models can be provided as data or calculated using a local or remote version of PROAST. The main results for hazard and risk assessment are benchmark doses (BMDs), related to a specified substance, response, optionally covariate value, and the benchmark response (BMR).

Category	Module	Description
	Dose response data	Dose response data are data on response values of test systems at specified doses of substances (or mixtures of substances) from dose response experiments.
	Effect representations	Effect representations are the responses which can be used to measure specified effects and the benchmark response (BMR) that defines a hazard limit for the effect.
	Inter-species conversions	Inter-species conversions specify how to convert a hazard characterisation for a given species to a hazard characterisation for humans. In the simplest approach, this specifies a fixed inter-species factor. In a higher tier, this specifies a geometric mean (GM) and geometic standard deviation (GSD) for a lognormal uncertainty distribution of the interspecies factor.
	Intra species factors	Intra-species factors specify how to convert a hazard characterisation from the average to a sensitive human individual. In the simplest approach, this is a fixed inter-species factor. In a higher tier, lower and upper values for the intra-species factor are used to derive a variability distribution (lognormal around 1) and an uncertainty distribution for the geometric standard deviation related to human variability in sensitivity.
	AOP networks	Adverse Outcome Pathway (AOP) Networks specify how biological events (effects) can lead to an adverse outcome (AO) in a qualitative way through relations of upstream and downstream key events (KEs), starting from molecular initiating events (MIEs).
Risk	Risks	Risks (health impacts) are quantified by comparing exposures and hazard characterisations at the chosen level (external or internal) via margins of exposure (MOE) or more generalised or integrated margins of exposure (IMOE). In addition, risks can be assessed from a plot of hazard characterisations vs. exposures.