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'MicroHibro': A software tool for predictive microbiology and microbial risk assessment in foods

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ABSTRACT

A tool able to quantitatively assess the fate of potential pathogenic microorganisms in foods along the food chain and their impact on public health is highly valuable for food safety decision-makers. The aim of this work was to present an overview of the Predictive Microbiology software MicroHibro, which is able to assess the evolution of potential pathogens and spoilage microorganisms along the food chain, providing estimates for the exposure level and risk associated with a food product. The application is built on an extensive Predictive Microbiology Model Data Base (PMDB) including kinetic processes like growth, inactivation, transfer as well as dose-response models. PMDB can be populated with new models by using an on-line tool in combination with a standardized method for describing Predictive Microbiology models. This enables MicroHibro to be easily updated, increasing its applicability and use. Estimation of microbial risk associated with a food product can be achieved, in MicroHibro, by describing steps in any food chain using four different microbial processes (growth, inactivation, transfer and partitioning). As a result, an estimate of the concentration and prevalence of microorganisms in the food of interest as well as attendant risk are provided. Also, MicroHibro allows comparing different predictive models and validate them by introducing user's data. In this paper, examples are provided to illustrate how predictive models can be incorporated in MicroHibro, and then, used to develop a Quantitative Microbial Risk Assessment model. The use of expert computational systems is a powerful tool for supporting food safety and quality activities by Health Authorities and the food industry. They represent a breakthrough in the assessment and management of food safety based on scientific evidence.

1. Introduction

The increasing awareness on the need of strengthening microbiological food safety is one of the major challenges for the globalized food sector (Ercsey-Ravasz et al., 2012; Fung et al., 2018). Food safety risk management has been adopted by food industries as a part of their official control system throughout the production chain (Codex Alimentarius, 2007). End-product testing based on a hazard-based approach has already been catalogued as inefficient as it does not provide in many cases quantitative information about the sanitary conditions of a given food product. Moving to a (quantitative) risk-based approach (i.e. estimation of the probability that a hazard is present in a food commodity) requires the evaluation of generated knowledge (statistical and computational methods) and interpretation of results through the development and application of resources (databases and software tools) readily available to be used by the food safety community, including risk assessors and managers, food operators or research institutions (Membré and Guillou, 2016).

Predictive food microbiology is a scientific field intended to study the microbial behaviour in food environments, including the development of mathematical models that can be deployed to perform predictions under certain specific conditions (Pérez-Rodríguez and Valero, 2013). The application of the knowledge generated in predictive microbiology has been mainly focused on the quantification of the bacterial behaviour in culture media and foods under certain environmental conditions. The kinetic parameters estimated from mathematical equations (i.e. maximum specific growth rate, lag time, inactivation rate, etc.) have been used to describe growth, inactivation, survival or probability of growth of several pathogens and/or spoilers in a wide range of foods. These outcomes are compiled in a series of published books/briefs (i.e. Brul et al., 2007; McMeekin et al.,

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1993; McKellar and Lu, 2004; Pérez-Rodríguez and Valero, 2013).

On the other hand, predictive microbiology is taking part of Quantitative Microbial Risk Assessment (QMRA) which is defined as a structured process for determining the risk associated with a pathogenic microorganism in a food. The characterization of risk typically contains both qualitative and quantitative information and is associated with a certain degree of scientific uncertainty (FAO/WHO, 2016). By increasing the knowledge on process/product/pathogen combinations and associated variability, uncertainty in achieving a food safety outcome is reduced (Zwietering, 2002). Nonetheless, the dissemination of this knowledge to food industries in order to optimize food processes and to provide assistance in decision-making processes in a short time frame is still ongoing. In this sense, the routine and successful use of mathematical models by the food operators, risk assessors and managers or educational agencies, will depend on the development of appropriate and useful applications (software packages and on-line platforms) of easy use. These are generally called *user-friendly food safety software tools* and allow different users to retrieve information in an efficient way assisting decision-making processes (McMeekin and Ross, 2002; McMeekin et al., 2006).

A wide range of food safety software tools are currently available. These can be classified as i) mathematical fitting (primary modelling) tools, ii) secondary and tertiary modelling tools, and iii) risk assessment tools. An in-depth review of the features of some of the most up to date available tools can be seen in Tenenhaus-Aziza and Ellouze (2015). However, there is still a lack of a correct understanding and interpretation of modelling outcomes by end users which limits their application in a real case scenario. To enable the application of predictive microbiology resources, users need to retrieve and exchange information such as product characteristics, storage condition, kinetic parameters and the related predicted response (Plaza-Rodríguez et al., 2018). Food safety repositories (Filter et al., 2016), harmonized formats and rules for model annotation (Plaza-Rodríguez et al., 2015, 2018) have been created in the last few years to improve information exchange between resources in the QMRA and predictive modelling domain in the so-called Risk Assessment Modelling and Knowledge Integration Platform (RAKIP) (Haberbeck et al., in press). Nowadays, food safety software tools are increasingly offering a wider range of solutions through the creation of dynamic computational environments (expert systems) which facilitate the openness, transparency and systematically reporting of the risk analysis process (FAO/WHO, 2016).

In this paper, the key features of the second version of the on-line software tool named MicroHibro developed by the University of Cordoba (Spain) will be described. Further, food safety applications with MicroHibro will be exemplified combining predictive modelling and risk assessment functions. The application MicroHibro is freely available at http://www.microhibro.com for which a previous registration is required including different user levels (students, predictive microbiology users and advanced users).

2. General concepts for predictive microbiology software design

Integration of predictive microbiology and risk assessment models into food safety software tools may be dimensioned according to the perspective of the users, being classified into three main components: mathematical, prediction and applicability.

The mathematical dimension corresponds with the screening and selection of the most suitable mathematical function(s) describing microbial observations using different mathematical approaches (e.g. regression analysis, Bayesian approach, machine learning, etc.). By analyzing the mathematical function, insight on the system can be obtained, i.e. definition of specified independent variables and the relationship between them and the response variable (i.e. deterministic models). Also, apart from the response variable such as growth, death rate, etc., other environmental variables can also be dependent on others, and this fact should be mathematically described. An example of this dependence relationship could be the pH or Aw of dry-cured meats as factors depending on time during ripening or storage. In addition, the performance of a sensitivity analysis of the model variables allows testing the effect of these variables on the final output (i.e. individual and population risk, microbial growth, death, survival, etc.). The outcome of a sensitivity analysis can assist to determine those variables exerting a higher impact on microbial behaviour (e.g. temperature over pH in a thermal process) or those ranges, in the variable, that can be used to obtain specific outcomes (e.g. growth limits for *Listeria monocytogenes* growth in RTE products). This information can be later used to optimize experimental designs or improve predictive model generation.

The prediction component of the model stands for the use of predictive models to foresee how microorganisms will behave under certain conditions. Prediction ability of models is usually evaluated by comparing models against observations obtained in independent experiments to those used for model development (Baranyi et al., 1999; Ross, 1996). This procedure is typically named as validation and may be supported by the calculation of goodness-of-fit indexes. Prediction from validated models can be used to obtain information in advance which can be used to develop preventive measures.

The two former components (i.e. mathematical function and prediction) are important from a scientific point of view, while the application component is relevant to an operational or regulatory level along the food chain. The application of predictive microbiology and QMRA models relies on the ability of making them available once they are published and validated. The integration of software engineering elements into modelling is crucial to provide an applicability dimension to predictive microbiology models.

The MicroHibro food safety software tool developed by the University of Cordoba strives to confer this character to predictive models by means of expert systems. The principal software components and interconnection between them are described in the following sections.

3. MicroHibro modules and tools

MicroHibro is divided into two specific modules. The first one, “prediction module”, is focused on providing simulations/predictions of growth and inactivation under specific conditions defined by users (Fig. 1). The predictions are based on use of mathematical models contained in a Predictive Model Data Base (PMDB) integrated in MicroHibro. Models are retrieved from scientific literature and imported to PMDB by using a standardized on-line tool based on a customized mathematical notation system (see Section 5). To do so in a systematic way, terms and definitions describing model features are presented in Table 1. This terminology has been adopted in MicroHibro to facilitate users the inclusion of model equations and their further interpretation. Predictions can be obtained for different microorganisms in different food matrices and culture media. Dynamic conditions are also considered in MicroHibro by applying the 4th order Runge Kutta algorithm for temperature (Runge, 1895).

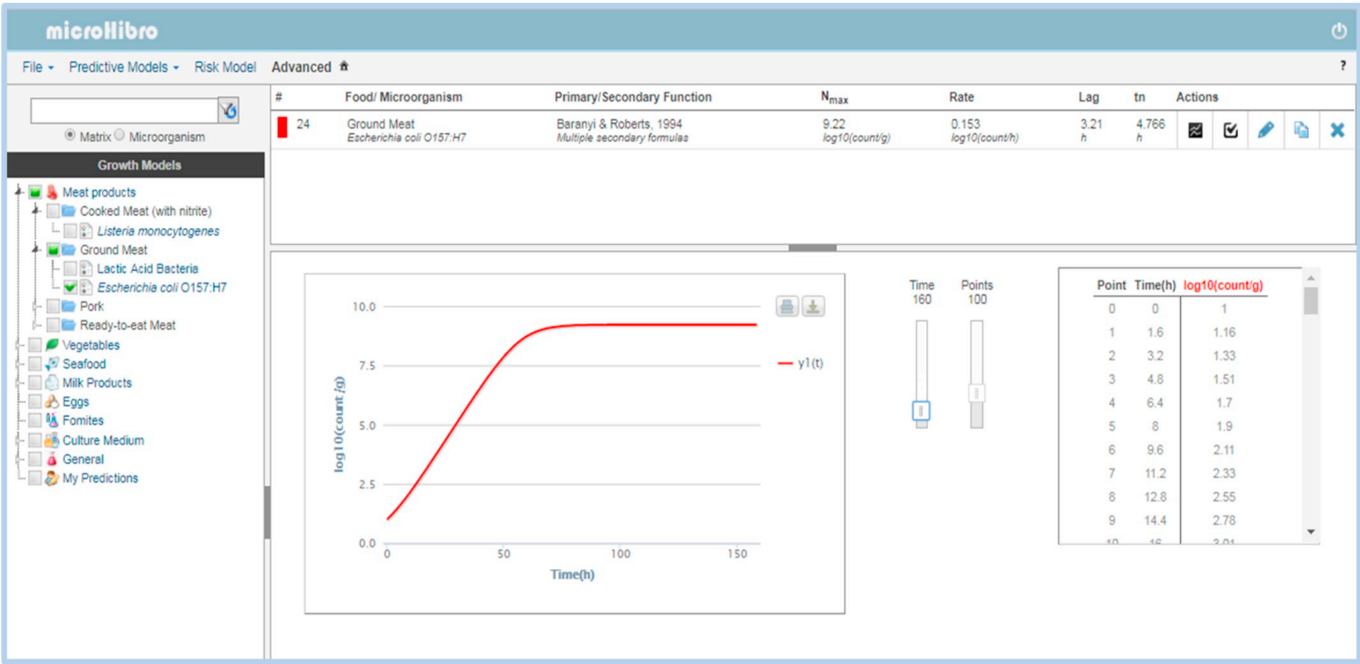

Fig. 1. Screenshot of the MicroHibro prediction module.

Table 1
Main terms and their definitions describing model features used to introduce models in the Predictive Model Data Base (PMDB).

Term	Definition	Metadata type
Default values	Those values automatically assigned by the software MicroHibro to model variables. They are set on the basis of reported values and assumptions found for these models in literature or mean values within the model domain. They are allowed to be modified by users.	Mathematical
Food matrix	Food matrix is usually referred to the food substrate on which the model data have been generated, which could correspond to raw or processed food products. As the development of models on real food matrix is laborious and time-consuming, originating in many cases heterogeneous results, predictive models are mainly based on simplified and artificial systems (microbial culture broth and agars) that are modified to include the most relevant factors to microbial responses (i.e. temperature, pH, water activity, organic acid, etc.).	Descriptive
Function coefficient	Any parameter from a mathematical function that is constant for a specific model and cannot be modified by the end-user or related to other existing functions (e.g. regression parameters in the polynomial models).	Mathematical
Mathematical function	Mathematical expression of the relationship between independent variables and a response variable. In predictive microbiology, the latter usually describes specific trait or dynamics of the microorganism (e.g. kinetic, transfer, increase/decrease, etc.).	Mathematical
Mathematical model	Mathematical representation of microbial behaviour based on a simplification of the targeted system to the most relevant variables, that can provide accurate predictions of microbial behaviour under specific prediction conditions within the model domain. Primary and secondary functions when applied separately or jointly to represent microbial response in foods are considered mathematical models.	Mathematical
Microorganism	Microscopic organism, referred to bacterium, virus or fungus which is challenged in/on food or culture media used to obtain data to generate a novel model, and/or define model parameters of an existing model, or validate a model enabling reliable model predictions.	Descriptive
Model domain	The ranges of values of prediction variables, which enable to produce reliable predictions. Model domain is usually set based on experimental data used to derive the mathematical function.	Descriptive
Model output	Results/predictions from the mathematical model obtained by modifying/introducing input values in the prediction variables.	Mathematical
Model parameter	The values, in the mathematical model, able to describe specific properties of the microbial dynamics in a food matrix or culture media. By extension, kinetic parameter also stands for dependent variables relevant for a microbial process, but not necessarily represent a kinetic process, such as initial concentration or maximum population density.	Mathematical
Model source	Reference of the study where the model was published, including all information required for its implementation and application in the software MicroHibro.	Reference
Model type	A class of mathematical function that can be applied as predictive microbiology model such as logistic, Gompertz, Baranyi, Davey, Ratkowsky, polynomial, Gamma, cardinal, etc. (McKellar and Lu, 2004).	Descriptive
Model variables	Extrinsic and intrinsic factors of foods that are addressed in mathematical models in the form of prediction variables. Variables can be modified by end-users to obtain a prediction.	Mathematical
Primary function	Mathematical function that is generated from direct observations: microbial death, growth kinetics, microbial transfer, or dose-response relationship.	Descriptive
Secondary function	Mathematical function that is built relating the estimated parameters of the primary function to environmental variables or other relevant variables to the microorganism (physiological state, stress, strain, etc.).	Descriptive
Units	Dimension of the variables to be represented in the mathematical functions.	Mathematical
Validation indexes	Statistical indexes that are used to assess the performance of models in different food matrices than those used to generate or define the predictive model.	Mathematical

PMDB models can be validated through a validation tool implemented in MicroHibro (Fig. 2). Validation is performed by means of mathematical indexes such as Accuracy factor, Bias factor, RMSE (Root Mean Square Error) and by graphical representation (Ross, 1996). Once results are analyzed, models can be labeled as "validated models" and stored as such in the PMDB. This information will be published for all users after approval by software curators. In that way, models can be continuously tested, by users, by comparing their predictions with observations from other food matrices.

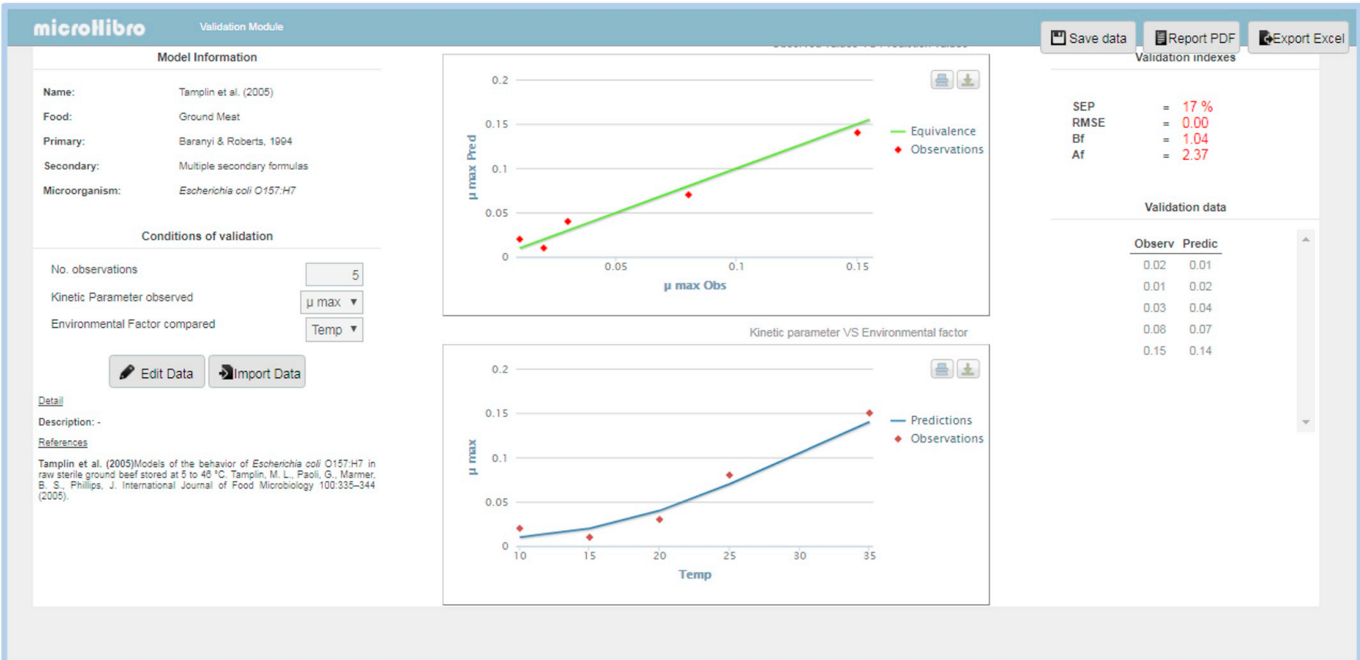

Fig. 2. Screenshot of MicroHibro validation tool to compare the predictions of models integrated in the database with real observations. Validation indexes provided: standard error of prediction (SEP), root mean square error (RMSE), bias factor (B_f), accuracy factor (A_f).

The second module consists of an object-oriented system aimed at developing QMRA models. This module, named "risk module", includes features to develop an Exposure Assessment and Hazard Characterization according to the scheme proposed by FAO/WHO (1999). The main principle applied for Exposure Assessment is that the dynamics of microorganisms along the food chain, expressed as changes in prevalence and concentration, can be described by using four basic microbial processes: reduction, increase, transfer and partitioning (Nauta, 2001). Mixing and removing are not included in MicroHibro so these microbial processes cannot be considered in the current version. The four processes can be defined by means of predictive models contained in the PMDB or probability models (i.e. stochastic models) defined by users. Output from the Exposure Assessment (i.e. prevalence and concentration) is combined with a dose-response model to estimate individual and population risk (Risk Characterization). Population risk is expressed as the individual probability of becoming infected, ill or dead after ingesting a contaminated serving, or as the number of cases per year (population risk).

Variables in QMRA models can be represented by probability distributions, including continuous and discrete distributions such as Exponential, Normal, Gamma, Uniform, Triangular or Binomial distributions. To sample values from distributions, the inverse transform sampling method is applied. Sampling is performed in a linear direction from the initial (initial concentration and prevalence) to the final food chain step, that could be, for example, the time of consumption. Thus, each iteration can be tracked through the modular risk process, assessing the changes in microbial population in each step. This feature is intended to facilitate the risk model analysis, identifying possible risk factors or variable ranges that are especially significant for risk. The number of iterations of sampling can be selected by users. Each iteration represents one execution of the set of models describing a specific food chain or process. Model executions are performed linearly and sequentially, whereby the output from a previous model is the input for the next model and so until the last model, yielding the final output that can be exposure levels (prevalence and concentration) or probability of being infected/ill/dead depending on the nature of the model designed (i.e. exposure assessment or risk assessment). For each model execution (i.e. iteration), values are sampled from those distributions used to define model variables (temperature, water activity, etc.). When a point-estimate is used instead of probability distribution, the point value selected is used for all model executions. The in-silico simulation is performed on-line in real time and calculations are processed in a separate computing server. This feature, though entailing a certain delay in reporting outputs due to the great consumption of system resources, can in turn, allow obtaining results in a very short time frame.

MicroHibro also contains a sensitivity analysis toolbox intended to analyze how sensitive an output is to any change in an input (Cullen and Frey, 1999). By doing so, the most significant risk factors and variables in the QMRA can be identified. The sensitivity analysis toolbox in MicroHibro is made up of a scatter plot representing output vs inputs and a scenario analysis where every input variable assessed is fixed during simulation allowing the other variables to vary.

Regarding the reporting systems of the software, outputs and simulation results are reported as graphs and numerical values using Google code and tools. Numerical values can be exported to MS Excel format (csv files), and graphs and predictions can be also used to generate pdf reports. Outputs from simulation of the risk model are reported in a pop-up window, and numerical values can also be exported to an MS Excel file.

4. Predictive Model Data Base (PMDB) and Food Matrix ontology for Predictive Microbiology (FMPM)

As pointed out by several authors, standardization in terminology and mathematical approaches is crucial to facilitate model application (Filter et al., 2016). To this end, the use of databases and standardized term definitions should be encouraged in order to provide an ordered structure enabling model global implementation and application. MicroHibro is based on this approach, and models are stored in the PMDB. The PMDB was designed to contain the types of models to be used in the different MicroHibro modules. Related vocabulary was defined based on the type of the mathematical function, function components (kinetic parameters, variables, output), model, food matrix, model validation and microorganism (Table 1). This enables to describe the most relevant model properties together with the metadata, which may be employed by end-users to select and use a model according to the desired application. A graphical scheme describing the workflow and

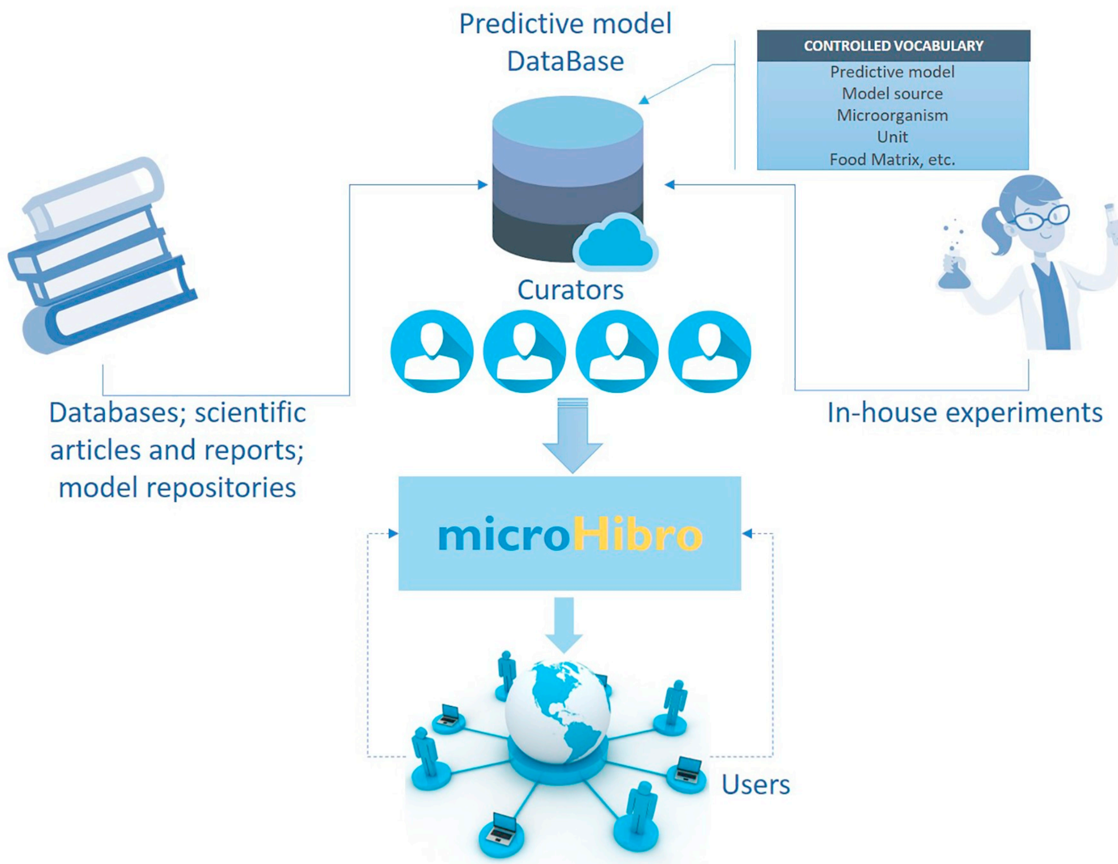

Fig. 3. MicroHibro workflow and elements of the Predictive Model Data Base (PMDB). Models taken from literature or generated from in-house experiments are used for populating the PMDB by using a controlled vocabulary. The PMDB is built in MicroHibro allowing users to access them to develop different applications based on the different MicroHibro features. Users can also provide feedback on missing models or applications, thus informing curators on the most relevant models to be integrated in the PMDB.

elements in relation to the PMDB integrated in MicroHibro is depicted in Fig. 3. Each PMDB field is linked with a specific database including a list of standardized elements that can be selected to define the model metadata. For instance, the database for environmental factors includes name, description and units; while microorganisms database includes genus, species and strains, when available. Model sources database is configured with references described by authors, title, journal, book, report, DOI (Digital Object Identifier), pages and publication year. Due to its relevance for the model selection and application process, a database for food matrix was also built based on a food matrix-related ontology designed by MicroHibro team as a standard for predictive microbiology software.

In the ontology, food matrix and culture medium lists were extracted from existing predictive microbiology data-bases (PMM Lab, ComBase, …). Then, selected items were structured using as basis the FoodEx hierarchical structure (EFSA, 2015). The food matrix nomenclature is defined according to the taxonomic classification, compositional structure and the manufacturing conditions. The food matrix names, properties (composition, additives, physicochemical parameters, manufacturing conditions, etc.) and definitions were based on suitable ontologies available in the libraries from the Open Biological and Biomedical Ontologies (BioPortal, https://bioportal.bioontology.org/), which is an on-line repository curated by the National Center of Biomedical Ontologies (NCBO): Nacional Cancer Institute, Grow Medium Ontology, and SNOMED Clinical Term. The ontology was built with the Protégé software (https://protege.stanford.edu/) and later uploaded in the BioPortal website.

The Ontology developed in this project, called Food Matrix for Predictive Microbiology (FMPM), and available at BioPortal website (https://bioportal.bioontology.org/ontologies/FMPM) was connected in real time to MicroHibro with the application of APIs and NCBO widgets provided by BioPortal. This system enabled MicroHibro to set a controlled vocabulary for food matrix, based on the ontology FMPM, so to define the field “Food Matrix” within PMDB.

Predictive Model Data Base considers two types of entries, corresponding to two types of mathematical functions, the so-called secondary and primary mathematical functions. In the MicroHibro context, primary functions refer to those generated from direct observations and can be used to describe microbial death, growth kinetics, transfer models or dose-response models. Secondary functions are built taking into account the estimated parameters of the primary function with the environmental factors. Additionally, the PMDB can incorporate nested primary-secondary functions including variables from both primary and secondary functions. Nested functions are equations including independent variables such as time and environmental factors altogether. Dose-response models correspond to mathematical functions used in the QMRA module to estimate the probability of infection or disease based on an estimated dose of the pathogen.

Mathematical functions are added to the PMDB through a standardized on-line form based on a customized mathematical notation system. Through this feature, MicroHibro can be continuously updated with new models as they become available in literature. A curator team oversees introducing, testing and verifying reliability of mathematical functions and models in PMDB, with rights to enable or disable their availability and use in the two MicroHibro modules (Fig. 3). This team is formed by scientists within the area of Predictive Microbiology and Risk Assessment who have been specifically trained in the use of the application including protocols for model definition and interpretation, together with vocabulary and metadata structure.

The models currently incorporated in the PMDB and their description can be accessed in the model navigation tree included within the "prediction module" of MicroHibro. The models' list is also available in the online tool "Open Food Safety Model Repository" (https://sites.google.com/site/openfsmr/), developed by the research group HIBRO at University of Cordoba and the German Federal Institute for Risk Assessment (BfR). This tool is a repository for predictive microbiology models included in predictive software tools, collecting metadata (microorganism, environmental conditions, microbial process, etc.) and enabling users to perform search for specific models or model conditions (Plaza-Rodríguez et al., 2015).

5. Practical examples of the use of MicroHibro

5.1. The use of MicroHibro software tool to predict microbial behaviour

As mentioned above, MicroHibro has a default models' list corresponding to the models introduced in the PMDB by the curator team; however, depending on the application, other models could be required by users. In those cases, the user's feedback is crucial to allow the curator team to integrate the needed models (taken from literature or other existing model resources) into the PMDB, making them available at MicroHibro for further application (Fig. 3). To illustrate how to integrate a model on the software database, we hypothesise that a user can be interested in evaluating the behaviour of *Escherichia coli* O157:H7 in ground beef at different temperatures. After a literature searching, a suitable model source was chosen, corresponding to Tamplin et al. (2005), that includes a growth model for *E. coli* O157:H7 in raw sterile ground beef stored at 5 to 46 °C. To obtain predictions of the microbial behaviour within this temperature range using MicroHibro, a stepwise process should be followed as described hereafter.

The first step when including a model in the software database is to define which microbial process is described by the model: growth, inactivation or transfer. After defining that a growth model will be included, information regarding the model, such as the model source, its description, the microorganism under study and the food matrix in which the microbial behaviour was modelled should be specified by the user, so the model is accurately classified and can be easily found in the prediction, validation or risk modules.

Once all the basic information is inserted, the user might proceed to define the primary function in which the model of interest was based on. In the study by Tamplin et al. (2005), the model of Baranyi and Roberts (1994) was fitted to their growth data, so this primary function must be selected in MicroHibro. Many primary functions are already available on the database, such as the Baranyi's and the Weibull model, and other functions can be included. After selecting the primary function on the software, the variables and kinetic parameters values may be defined, as well as their appropriate units. When secondary functions predicting the effect of environmental variables on kinetic parameters (lag time, growth rate, inactivation rate, etc.) are available, the secondary functions can also be introduced in MicroHibro (Square-root model, Arrhenius, Gamma concept, etc.) by using an equation editor. If any parameter cannot be described by a secondary function, it can be defined by a specific (fixed) value (e.g. lag time = 0 h). Since in our case study, growth parameters (i.e. growth rate, lag time and maximum population density) were described by secondary functions, the user may proceed to include these functions on the software. In Fig. 4, a screenshot of MicroHibro is presented showing the definition and description of variables, the model domain, references used, parameters and units for the secondary function describing the growth rate as reported by Tamplin et al. (2005).

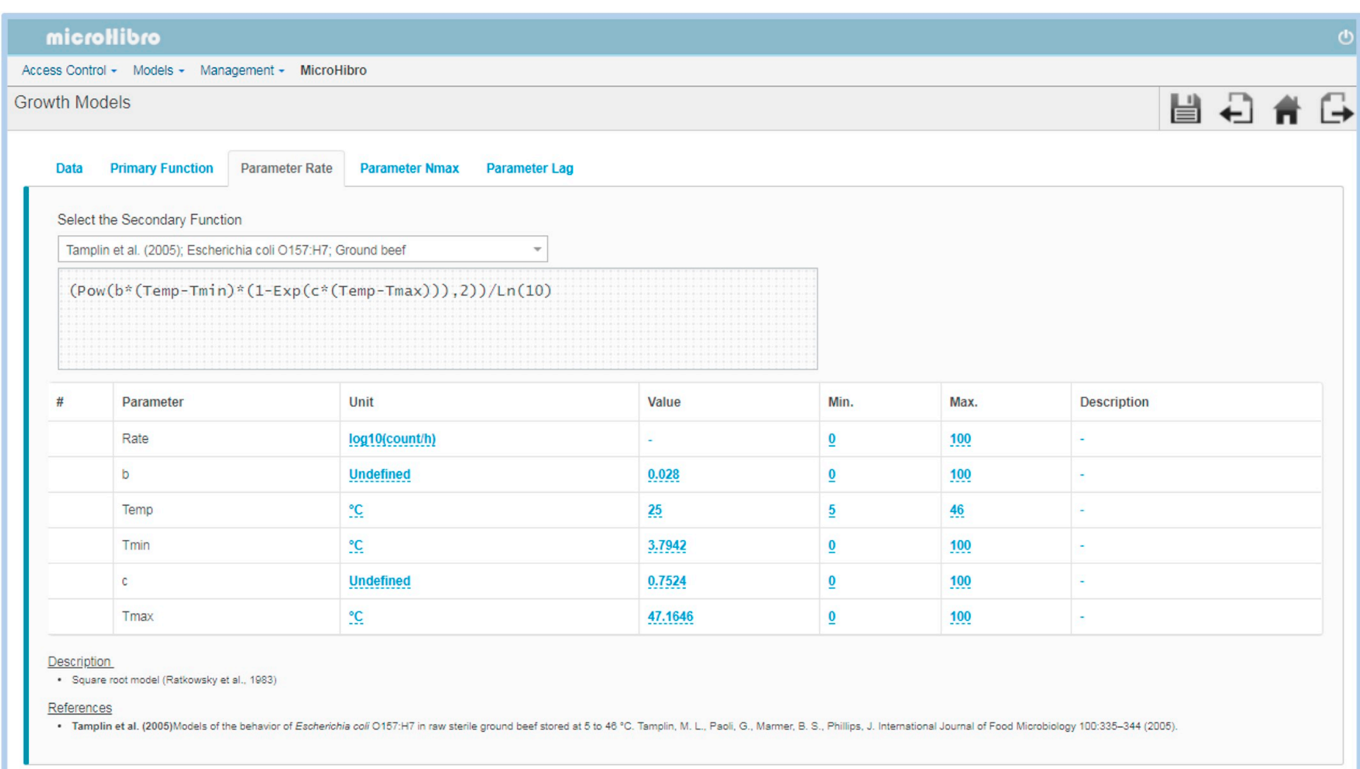

Fig. 4. Incorporation and definition of a predictive model in MicroHibro database, using a standardized on-line form. Example illustrated with the growth model by Tamplin et al. (2005) for *Escherichia coli* O157:H7 in ground beef.

The user must save all the information included and the model will be available on the database, as soon as the curator team authorizes it. To estimate microbial data-points (concentration vs time), MicroHibro combines primary and secondary functions to build the so-called tertiary model. At the computational level, the secondary functions are applied first to obtain the kinetic parameters values, and then, these estimates are input into primary functions to finally yield the extent of growth under specific conditions. Once primary and secondary functions are combined, the growth model can be used to obtain predictions without any mathematical complexity in a user-friendly interface, by informing temperature and time as variable inputs. Finally, it is important to highlight that the verification of the reliability of model predictions, according to its source, is strongly recommended. In relation to this, the validation tool in MicroHibro can be used to compare predictions from implemented models and observations obtained from external sources (e.g. in-house experiments or historical data).

5.2. The use of MicroHibro software tool to perform a QMRA

A case-study of a QMRA regarding the presence of *E. coli* O157:H7 in ground beef is presented in this section. It is important to highlight that the objective of this case-study is not to produce an accurate risk assessment as several assumptions and hypothetical values were used. Rather, the purpose is to show MicroHibro features and how it can be used to perform exposure assessments and to obtain risk estimates associated to the consumption of a food commodity contaminated with a microbial hazard. The four stages of the QMRA are briefly presented.

1) Hazard identification: The presence of *E. coli* O157:H7 in ground beef has recently led to 3 recalls in the USA (FSIS, 2018). Moreover, the consumption of ground beef contaminated with this pathogen has been linked to various foodborne outbreaks (CDC, 2007; CDC, 2014). Since *E. coli* O157:H7 is a foodborne pathogen to human health, this case-study is focused on answering the question: what is the risk of *E. coli* O157:H7 associated with the logistics of ground beef?
2) Exposure assessment: At this stage, the influence of any process or storage condition on the final distribution of bacteria in ground beef is quantified, thus the logistic chain of this food commodity should be well defined. The logistic chain evaluated in this case-study encompasses the five main steps of ground beef distribution chain after manufacturing steps at the factory (i.e. cutting, grinding and packaging), which are: 1) Storage; 2) Distribution; 3) Retailing; 4) Domestic storage; 5) Cooking. As environmental conditions and ground beef characteristics may enable *E. coli* growth, it is expected that the level of bacteria increases in all steps, excepting the cooking step. Thus, after concluding that growth may occur in steps 1, 2, 3 and 4, the deterministic model of Tamplin et al. (2005) was selected and applied to describe changes in *E. coli* O157:H7 concentration as a function of temperature (°C) and time (h). This model was already available in the software database as described in the previous example (Section 5.1). On the other hand, bacteria reduction during the cooking step was described by a stochastic model. The design of the ground beef distribution chain in MicroHibro is shown in Fig. 5.

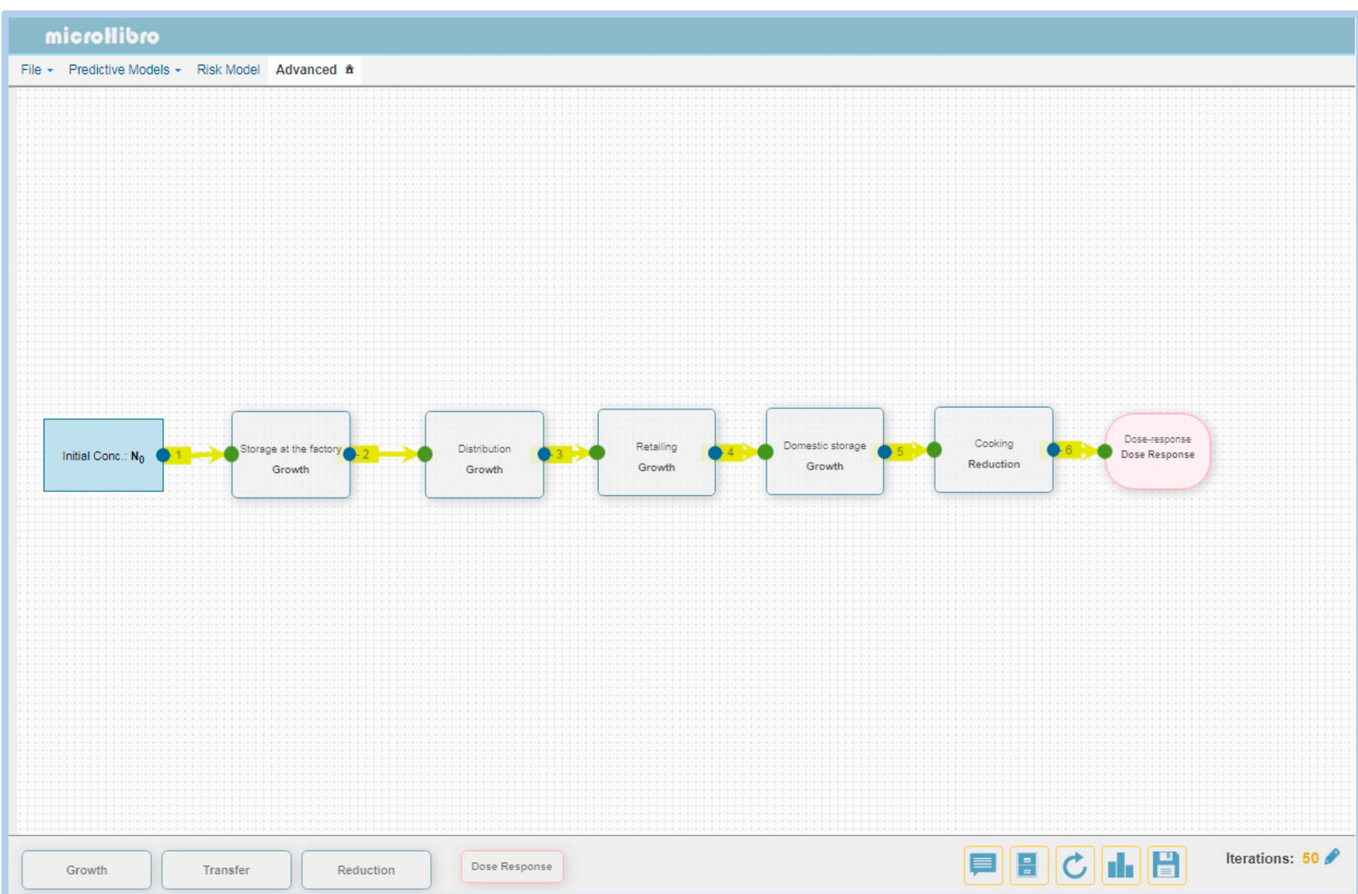

Fig. 5. Ground beef distribution chain designed in MicroHibro risk module.

The prevalence and the initial level of contamination in ground beef must be firstly defined, as well as the amount of meat entering the logistic chain. The amount of meat entering the logistic distribution chain from storage at the factory up to cooking step was assumed to be 500 g, which is representative of a pack of ground beef that can be found in retail environments. The initial level of contamination of ground beef was defined to be described by a uniform distribution, with minimum and maximum values set at 1 and 100 cfu/g, respectively (0 to 2 log cfu/g). *E. coli* O157:H7 prevalence, defined as the probability of having a 25 g sample of ground beef contaminated with the microorganism, was assumed to be equal to 100%.

The growth model input variables (i.e. temperature and time) for each step of ground beef logistic chain are shown in Table 2. According to ground beef manufacturers, the variability of temperature and duration of each step were described by a probability distribution in most cases, which are shown in Table 2. An example of an input variable definition interface in MicroHibro is shown in Fig. 6.

For the cooking step (i.e. heat treatment), the reduction of *E. coli* O157:H7 in ground beef was described by a uniform distribution with reductions varying from 0.60 to 2 log cfu/g, during a 3-min treatment at 65 °C.

Besides modelling the pathogen distribution in ground beef throughout the logistic chain, other aspects should be addressed to estimate the exposure of an individual or population to a pathogen via food. For example, the portion consumed or serving size should be estimated, as well as the extent of the population or subpopulations (e.g. the elderly, infants, healthy adults, etc.) consuming ground beef. In order to obtain risk estimates in this case-study, the serving size of ground beef was set at 50 g.

3) Hazard characterization: it is based on the relationship between the amount of ground beef consumed and the probability of occurrence of a clinical outcome (i.e. infection, illness or death), by applying a dose-response model. To the purpose of this case-study, the dose-

Table 2
Input variables values for the steps of the ground beef distribution chain involving a potential growth of *E. coli* O157:H7.

Step	Input variables[a]							
	Time (h)				Temperature (°C)			
	Max	Min	Most likely/mean	Distribution	Max	Min	Most likely/mean	Distribution
Storage at factory			12	–	9.5	2	4	Triangular
Distribution	24	2		Uniform	10	3	7	Triangular
Retailing	48	2		Uniform	9.5	4	7	Triangular
Consumption	72	3	24	Triangular	13	4	7.5	Triangular

[a] Input values are hypothetical.

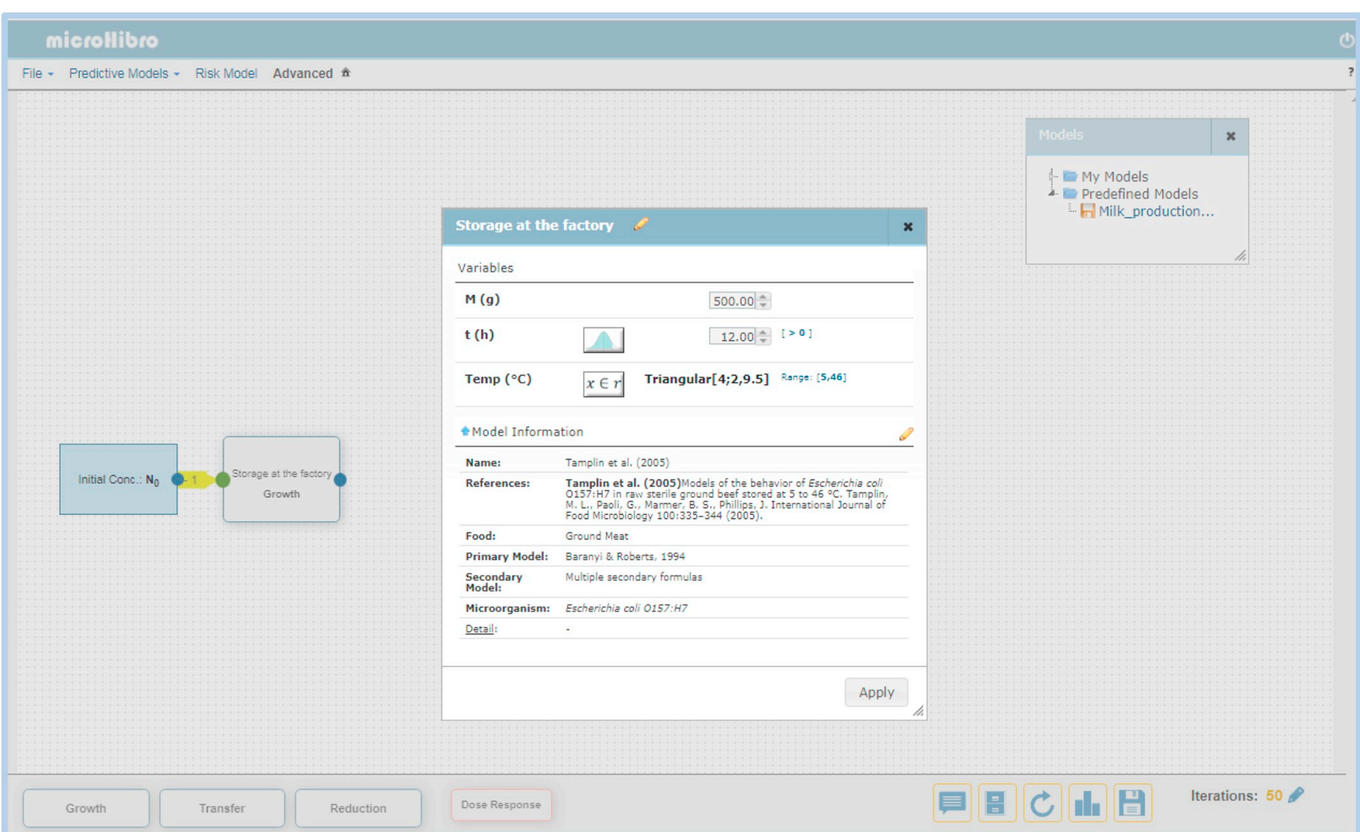

Fig. 6. Input variable definition for a ground beef pathway in MicroHibro risk module.

response model of *E. coli* O157:H7 developed by Strachan et al. (2005) was selected from PMDB. This model was developed based on outbreak data and relates the *E. coli* O157:H7 dose consumed with the probability of becoming ill, according to a Beta-Poisson model. The dose consumed (input of the dose-response model) is calculated based on the final distribution of bacteria in ground beef at the time of consumption together with the frequency of servings and the serving size.

4) Risk characterization: At this stage, the risk associated with the consumption of ground meat contaminated with *E. coli* O157:H7 is estimated by combining exposure assessment with hazard characterization. The structure of the risk model after linking the dose-response model to the exposure assessment part is shown in Fig. 5.

The stochastic risk model constructed in MicroHibro was run by using Monte Carlo simulation with 10,000 iterations. The number of iterations during simulations can also be defined by the end-users.

Simulation results are displayed by MicroHibro on a separate window. They are referred to the risk estimates and the input/output distributions reporting their main descriptive statistics (Fig. 7). The mean individual risk per serving is shown in Fig. 8, which corresponds to the output of the dose-response model (i.e. probability of becoming ill after ingestion of a contaminated serving). Additionally, users can estimate the average number of cases after ingestion of a certain number of contaminated servings in a given population.

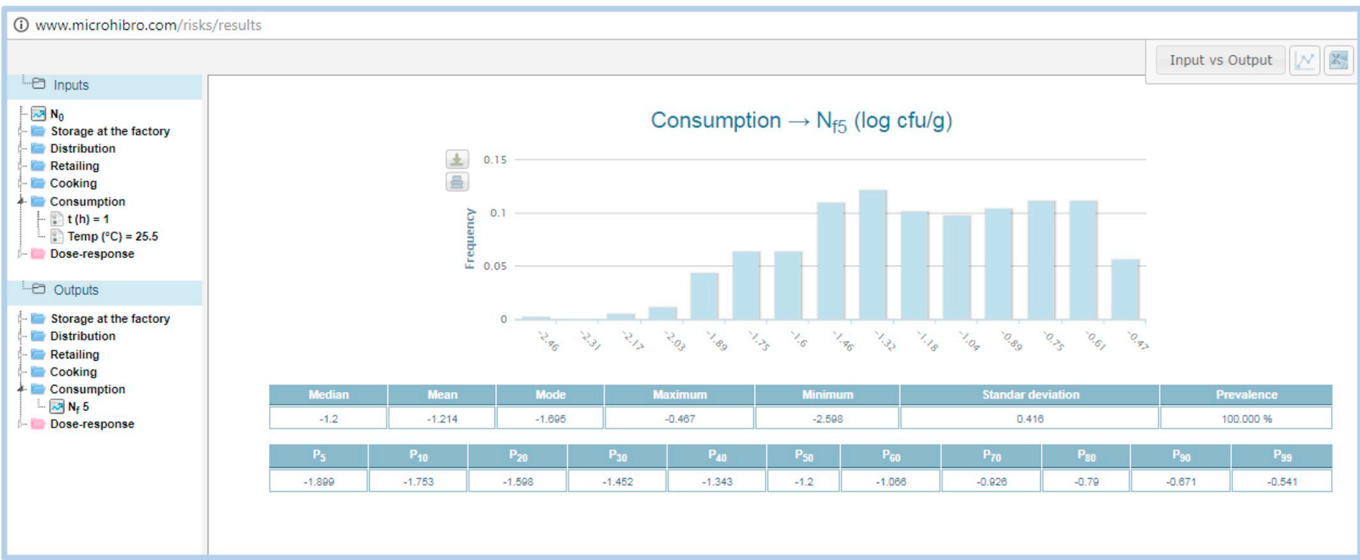

Fig. 7. Stochastic simulation outcome from MicroHibro risk module: histogram of the concentration of *E. coli* O157:H7 in ground beef at the consumption step and its descriptive statistics.

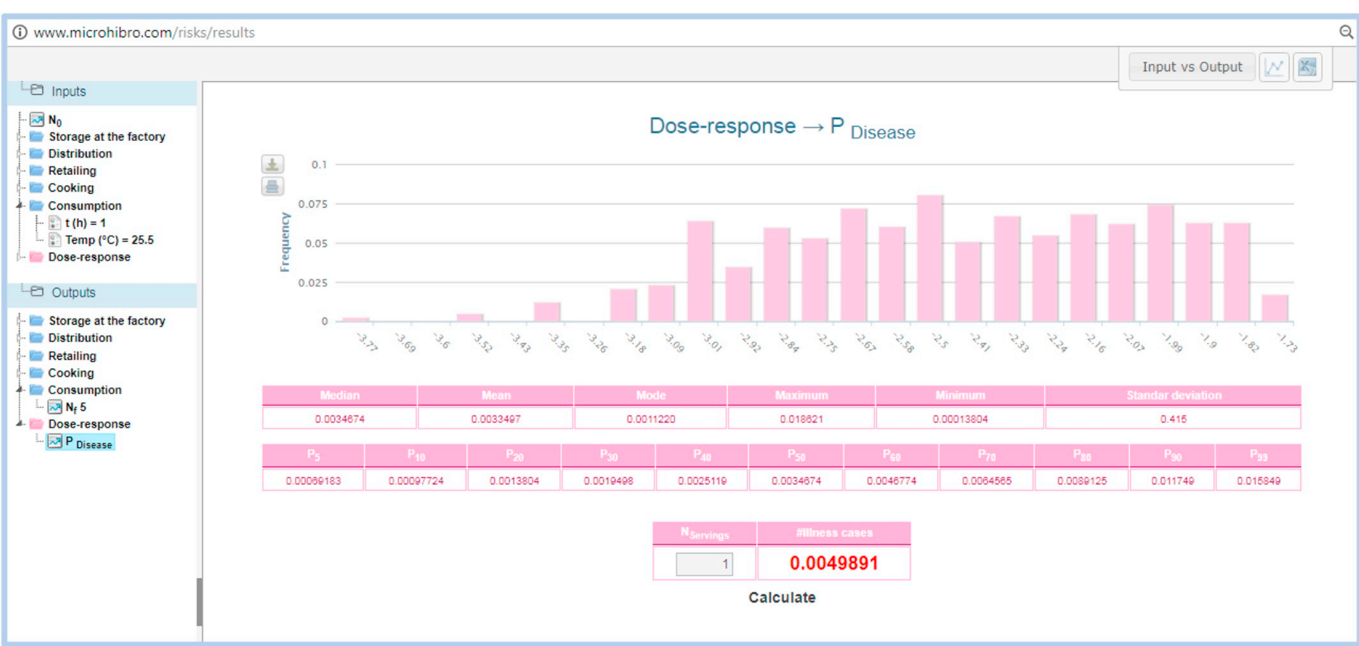

Fig. 8. Stochastic simulation outcome from MicroHibro risk module: histogram and descriptive statistics of the dose-response model output "risk of an individual getting ill after consuming ground beef contaminated with *E. coli* O157:H7" under the case-study conditions.

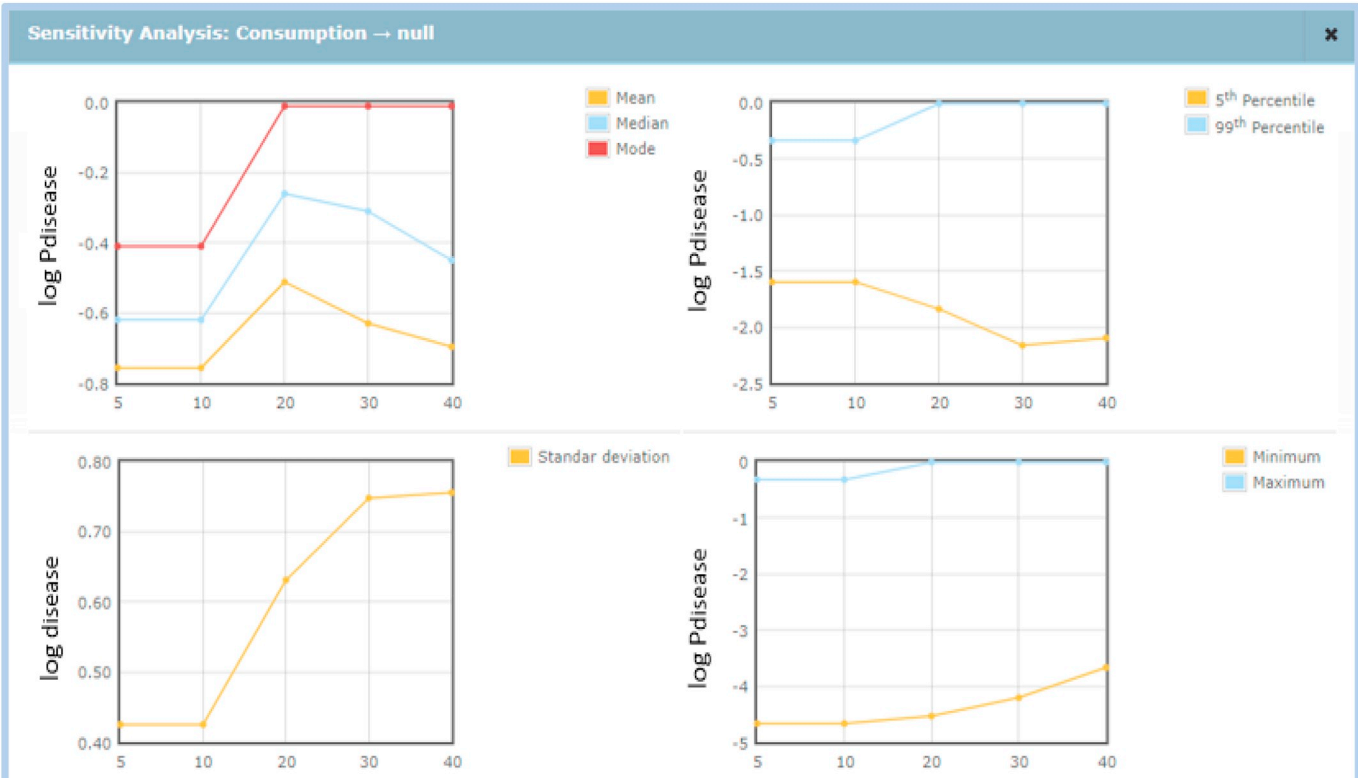

Fig. 9. Sensitivity analysis results reported by MicroHibro risk module showing the influence of the input temperature at the retailing step on the probability of illness.

Applying the sensitivity analysis toolbox, one can evaluate for instance, the effect of specific household temperatures at the consumption step on the probability of illness as shown in Fig. 9. It can be seen that storage temperatures lower than 10 °C did not increase the probability of illness, as these conditions do not generally support the growth of *E. coli* O157:H7, while the increase of household temperatures from 10 to 20 °C yielded a considerable increase in the probability of illness. This information is highly relevant for food operators and risk managers since it facilitates decision-making, being in this case the maintenance of the cold chain during distribution and refrigeration of ground beef at domestic level.

In these examples, it was demonstrated that MicroHibro is a valuable tool providing end-users with predictive models, validated in many cases, and applications in QMRA. The flexible character of the predictive model database assures the validity and use of MicroHibro for different food chain contexts and processes as well as microorganisms. This computational tool is also a relevant educational resource to convey and demonstrate concepts about QMRA, thus facilitating the development of skills in Risk Assessment for end-users in the academic and industrial sector.

6. Conclusions

Predictive microbiology has undergone significant changes in the last few years, and efforts have been mainly focused on the development of software tools that facilitate the application of predictive microbiology models by end-users. MicroHibro is a free-access predictive microbiology and QMRA tool based on a flexible structure allowing for incorporation of new models from other sources (other software, literature and existing model repository) by using a specific database and import system. Later, models can be used for different purposes, such as to predict microbial response under specific conditions or to perform a QMRA. The philosophy behind MicroHibro is that models should be accessible as soon as they are published and validated. In spite of the advances achieved by software developers in the Predictive Microbiology community, it is still necessary to reach a global agreement about standardization of model repositories, data sources and computational language in order to facilitate model exchange and use. The advances of this community driven initiative in model standardization will be crucial to face emerging challenges in the predictive microbiology field, oriented towards an improvement of prediction accuracy and model applicability, such as big data application, specially related to omics data and its integration in predictive modelling, and the use of artificial intelligent systems to support decision on microbial risk management activities.

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