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Public Health Risk-Benefit Assessment in Foods

Methodological development with application to infant milk-based diet

JURY

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Methodological development with application to infant milk-based diet

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man-imal
One world, one health
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autour du triptyque Animal-Homme-Alimentation

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« Que ton alimentation soit ta seule médecine »

“Let food be thy medicine and medicine be thy food”

Hippocrate

Preface

Do we know everything?

"Everything we know is only some kind of approximation, because we know that we do not know all the laws yet. Therefore, things must be learned only to be unlearned again or, more likely, to be corrected." - Richard P. Feynman

Do we know enough?

"We live in a society exquisitely dependent on science and technology, in which hardly anyone knows anything about science and technology" - Carl Sagan

How can we best make a decision using what we do know?

"No one knows everything. But together, we know a whole lot." - Simon Sinek

To date, scientific knowledges have evolved in various domains of "food" and "human health": microbiology, chemistry, nutrition, epidemiology, statistics, ... to such an extend that conflicting or incomplete recommendations towards consumers have been published in the previous decades. A new trend is now to consider "human diet" as a whole and to perform comprehensive risk-benefit assessment. However, "making best decisions using what we know" requires structured approach deployed within multidisciplinary teams.

The present document is a summary of a PhD project performed in Public Health and Food Safety for obtention of the degree of Doctor of Phylosophy (PhD). This project had the objective to push further the methodology of risk-benefit assessment in food by practising with an infant milk-based diet case study. Consequently, results obtained cannot be seen as definitive recommendations for infants feeding. Moreover, results related to infant milk-based diet were established using the present scientific knowledge, they will certainly have to be revisited in the future.

Table of contents

List of Figures xvii

List of Tables xix

List of Abbreviations and Acronyms xxi

Abstract 3

Résumé 4

Synthèse détaillée 5

CHAPTER 1: General Introduction and Objectives 13

1.1. Objectives 15

1.2. Outline of the thesis 15

CHAPTER 2: State of art of Risk-Benefit Assessment in Foods 21

2.1. Abstract 21

2.2. Introduction 22

2.3. Methods 24

2.4. Results 26

2.4.1. Studies of Risk-Benefit Assessment 26

2.4.1.1 History of RBA studies 27

2.4.1.2 Scientific fields of RBA studies 28

2.4.1.3 Comparison criteria used in RBA 29

2.4.1.4 Main RBA applications 32

2.4.1.5 Studies on the positive and negative health effects associated with food consumption 36

2.4.2. Methodology of Risk-Benefit Assessment 37

2.5. Conclusion 43

CHAPTER 3: Review of Risks and Benefits of Infant Milk-Based Diet 53

3.1. Abstract 53

3.2. Introduction 54

3.3. Infant milk: current lesgislation and nutritional requirements 58

3.3.1. Definitions of different infant diets 58
3.3.2. European infant formula legislation 59
3.3.3. How to fulfil infant requirements? 65
3.3.3.1 Infant nutritional needs 65
3.3.3.2 Comparison of breast milk and infant formula composition 66
3.3.3.3 Infant food intake recommendations 66
3.4. An attempt to define health, health effect, risk and benefit 67
3.5. Health effects associated with infant milk consumption 69
3.5.1. Microbiology: type of microorganisms and identification of potential adverse/beneficial health effects 69
3.5.1.1 Sources of bacteria in infant milk 69
3.5.1.2 Potential adverse and beneficial health effects 72
3.5.2. Chemistry: type of chemical hazards and identification of potential adverse health effects 75
3.5.2.1 Sources of infant milk contamination 75
3.5.2.2 Potential adverse health effects 76
3.5.3. Nutrition: relative health effects of breast milk / infant formula 80
3.6. Risk and benefit assessment of infant milk consumption 84
3.6.1. Presentation of the risk-benefit assessment approach 84
3.6.2. Studies reporting risk and/or benefit assessment of infant formula and breast milk consumption 86
3.6.2.1 Quantitative microbiological risk assessment of Cronobacter sakazakii in powdered infant formula by WHO and FAO 86
3.6.2.2 Benefit and risk assessment of breast milk consumption in Norway by VKM 87
3.6.2.3 Quantification of health effects of breastfeeding by the RIVM 88
3.6.3. Current gaps in the risk-benefit assessment of infant formula and breast milk consumption 90
3.7. Conclusion 92

CHAPTER 4: Model development 1 95

4.1. Abstract 95
4.2. Introduction 96
4.3. Model development 100
4.3.1. Model overview 101
4.3.2. Management of variability and uncertainty 102
4.3.3. Risk assessment computation 102
4.3.4. Milk intake estimation 103
4.3.5. Risk assessment of *Cronobacter sakazakii* in PIF 105
4.3.5.1 Exposure assessment of C. sakazakii 105

4.3.5.2 C.sakazakii dose-response ... *110*
4.3.5.3 Risk characterization of C. sakazakii in powder infant formula ... *110*
4.3.5.4 Conversion of risk of C.sakazakii in DALY ... *111*
4.3.6. Benefit assessment of DHA in BM and Supplemented PIF ... 114
4.3.6.1 Exposure assessment of DHA ... *114*
4.3.6.2 DHA dose-response ... *114*
4.3.6.3 Benefit characterization of DHA in supplemented powder infant formula and breast milk ... *116*
4.3.6.4 Conversion of DHA benefit in DALY ... *117*
4.3.7. Risk assessment of dl-PCB in BM and PIF ... 119
4.3.7.1 Exposure assessment of dl-PCB ... *119*
4.3.7.2 Dl-PCB dose-response ... *121*
4.4. Results ... 123
4.4.1. Main outputs of each sub-model ... 123
4.4.1.1 Intake calculation ... *123*
4.4.1.2 Microbiology ... *123*
4.4.1.3 Nutrition ... *125*
4.4.1.4 Chemistry ... *128*
4.4.2. Scenario comparison: estimation of the net health impact ... 131
4.5. Discussion ... 135
4.5.1. Towards quantitative RBA ... 136
4.5.2. Advantages and challenges of multidisciplinary RBA ... 138
4.6. Conclusion ... 142

CHAPTER 5: Model development 2 ... 147

5.1. Abstract ... 147
5.2. Introduction ... 148
5.3. Materials and methods ... 149
5.3.1. Probabilistic risk assessment framework ... 149
5.3.2. Arsenic risk assessment model development ... 152
5.3.2.1 Exposure assessment of Arsenic ... *153*
5.3.2.2 Arsenic dose-response ... *155*
5.3.2.3 Risk characterization of Arsenic ... *158*
5.3.2.4 Conversion of risk of Arsenic in DALY ... *159*
5.3.3. *Cryptosporidium* risk assessment model development ... 160
5.3.3.1 Exposure Assessment of Cryptosporidium ... *164*
5.3.3.2 Cryptosporidium dose-response ... *166*
5.3.3.3 Risk Characterization of Cryptosporidium ... *167*
5.3.3.4 Conversion of risk of due to Cryptosporidium into DALY ... *167*

5.4. Computation method considering separation of uncertainty and variability ..169
5.5. Results 169
5.5.1. Outputs of arsenic risk assessment 169
5.5.2. Outputs of *Cryptosporidium* risk assessment 172
5.5.3. Scenarios comparison: estimation of the net health impact 173
5.6. Discussion 175
5.7. Conclusion 178

CHAPTER 6: General Discussion 181

6.1. Main findings 181
6.1.1. State of art of Risk-Benefit Assessment in Foods 181
6.1.2. Review of Risks and Benefits of infant milk-based diet 183
6.1.3. Model development 1 185
6.1.4. Model development 2 186
6.1.5. Summary of all results on infant milk-based diet 187
6.1.6. PhD project outputs 189
6.2. Discussion 191
6.2.1. How to carry out a multidisciplinary Risk-Benefit Assessment considering microbiological, chemical and nutritional aspects? Is it possible to set a generic framework? .. 191
6.2.2. How to compare health impacts? Is it possible to use a common metric? 196
6.2.3. How to consider variability and uncertainty in RBA? 199
6.2.4. How to communicate to stakeholders and interpret results? 203
6.3. Conclusions 205
6.4. Perspectives 206

References 211

List of scientific publications 241

Curriculum vitae 245

List of Figures

Figure 1: Flowchart of the PhD project entitled "Public health Risk-Benefit assessment in foods: methodological development with application to infant milk-based diet" 17

Figure 2.1: Evolution of the conventional risk assessment conceptual framework toward an integrated risk-benefit paradigm, adapted from (WHO, 2017) 23

Figure 2.2: Overview of the applied data search process and the results based on PRISMA's four-phase diagram (Moher et al., 2009) 27

Figure 2.3: Classification of the 70 studies performed by year and food category 28

Figure 2.4: RBA studies performed classified by scientific fields, based on 70 studies 29

Figure 2.5: RBA studies performed classified by type of comparison, based on 70 studies 30

Figure 2.6: Illustration of DALY adapted from Tijhuis et al. (2012a) with the case of a person who falls sick and dies after a period of disability 31

Figure 2.7: Summary of Risk-Benefit Assessment methodological framework based on different literature sources 39

Figure 3.1: Worldwide exclusive breastfeeding rates at 6 months 56

Figure 3.2: Infant food diet from 0 to 3 years (Based on French nutritional recommendations (Inpes, 2004)) 58

Figure 3.3: Schematic representation of the Illness-Wellness scale, with health status and HECF, adapted from Travis and Ryan (1988) 68

Figure 4.1: Flowchart of the Risk-Benefit Assessment model 101

Figure 4.2: Cumulative distributions of IQ level in the population according to different scenarios 126

Figure 4.3: Cumulative distribution of estimated level of exposure of breast fed and formula fed infants to dl-PCB 129

Figure 4.4: Schematic representation of approach used to build dose-response in Microbiology, Nutrition and Chemistry 141

Figure 5.1: Flowchart of the proposed microbiological-chemical risk assessment model 151

Figure 5.2: Cancer treatment stages deducted from Soerjomataram et al. (2012) 159

Figure 5.3: Estimated daily exposure of infants (both genders) to Arsenic through tap water consumption (µg/kg b.w.) 170

Figure 5.4: Predicted prevalence of prepared milk bottles contaminated (%) according to different levels of *Cryptosporidium* oocysts in surface water (before treatment) 177

Figure 6.1: Summary of results obtained in the PhD project regarding infant milk-based diet 188

Figure 6.2: Outputs of the PhD project entitled “Public health Risk-Benefit assessment of food: methodological development with application to infant milk based diet” 190

Figure 6.3: Suggested framework of quantitative RBA in food..... 192

List of Tables

Table 2.1: Summary of the main results of risk-benefit assessment studies 44

Table 3.1: Synthesis of European laws regulating infant formula composition and safety indicators..... 60

Table 3.2: Energy and nutrient requirements of breastfed and formula fed infants, based on (EFSA, 2014)..... 61

Table 3.3: List of microbiological adverse health effect contributing factors identified in PIF classified by level of evidence, based on (FAO/WHO, 2004) 70

Table 3.4: Presentation of microbiological adverse health effect contributing factors and adverse health effects of infant milk..... 73

Table 3.5: Presentation of chemical adverse health effect contributing factors and health effects of infant milk 78

Table 3.6: List of identified nutritional health effects associated with breast milk compared with infant milk consumption, classified by level of evidence, based on (RIVM, 2015)..... 81

Table 3.7: Health effects of the consumption of breast milk compared with infant formula and hypothetic mechanisms..... 82

Table 4.1: Description of the five scenarios evaluated in this study 100

Table 4.2: Description of inputs used in intake calculation: infant nutritional requirements, weights and milk calorie content 104

Table 4.3: Description and distributions of inputs for quantification of the risk of *C. sakazakii* associated with powder infant formula consumption, regular and supplemented. 113

Table 4.4: Description and distributions of inputs for quantification of the potential benefit of DHA associated with supplemented powder infant formula and breast milk consumption 118

Table 4.5: Description and distributions of inputs for exposure assessment of dl-PCB associated with powder infant formula (regular and supplemented) and breast milk consumption 122

Table 4.6: Estimates of daily risk of *C. sakazakii* associated with powder infant formula consumption calculated with Equation 10 (regular and supplemented PIF) 124

Table 4.7: Estimates of number of *C. sakazakii* infection per 100 000 infants following diet 1B and 2B during 6 months (regular and supplemented PIF prepared with water at ambient temperature) 125

Table 4.8: Estimates of main outputs for quantification of benefit of DHA associated with supplemented powder infant formula and breast milk consumption 127

Table 4.9: Estimates of mean and percentiles of key outputs for exposure assessment of dl-PCB associated with powder infant formula and breast milk consumption 130

Table 4.10: Assumptions made when building the risk-benefit assessment model and their consequences on health impact estimation. 132

Table 5.1: Description and implementation of inputs for tap water intake calculation 152

Table 5.2: Description and implementation of inputs of Arsenic risk assessment 153

Table 5.3: Description and implementation of inputs of *Cryptosporidium* risk assessment 162

Table 5.4: Estimates of the Incremental Lifetime Cancer Risk (ILCR) due to arsenic exposure during the first six months of life associated with tap water consumption (used to rehydrate powder infant formula) 171

Table 5.5: Estimates of daily risk of *Cryptosporidium* associated with tap water consumption (used to rehydrate powder infant formula) during the first six months of life 173

Table 5.6: Estimates of number of illness and DALY for Arsenic and *Cryptosporidium* per 100 000 infants exposed during the first six months of life through tap water consumption (used to rehydrate powder infant formula) 174

List of Abbreviations and Acronyms

ADI	Acceptable Daily Intake
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
BM	Breast Mik
BENERIS	Benefit Risk Assessment for Food: Value-of-Information Approach
BEPRARIBEAN	BEst PRactices of Risk Benefit Analysis
BPA	BisPhenol A
BRAFO	Benefit Risk Assessment for FOod
C	Chemistry
CAC	Codex Alimentarius Commission
CDI	Chronic Daily Intake
CHD	Coronary Heart Disease
CVD	Cardio Vascular Disease
DALY	Disability Adjusted Life Years
DHA	DocosaHexaenoic Acid
dl-PCB	Dioxin-Like PolyChlorinated Biphenyls
DOHaD	Developmental Origin of Health and Disease
DTU	Technical University of Denmark
EFSA	European Food Safety Authority
EPA	EicosaPentaenoic Acid
ERB	Evaluation Risque Bénéfice
ESPGHAN	European Society for Paediatric Gastroenterology Hepatology and Nutrition
FAO	Food and Agriculture Organization of the United Nations
FSO	Food Safety Objective
HE	Health Effect
HECF	Health Effect Contributing Factor
HI	Health Impact
IAFP	International Association for Food Protection
IARC	International Agency for Research on Cancer
ICR	Incremental Cancer Risk
IF	Infant Formula
IgA	ImmunoGlobuline A

ILCR	Incremental Lifetime Cancer Risk
IQ	Intellectual Quotient
LADD	Lifetime Average Daily Dose
LOD	Limit Of Dectection
M	Microbiology
MCDA	Multi-Criteria Decision Analysis
N	Nutrition
NFA	Swedish National Food Agency
ORS	Oral rehydration Solution
PCBs	PolyChlorinated Biphenyls
PIF	Powder Infant Formula
POP	Persistent Organic Pollutants
PTWI	Provisional Tolerable Weekly Intake
QALIBRA	Quality of Life -Integrated Benefit and Risk Analysis
QALY	Quality Adjusted Life Years
RBA	Risk-Benefit Assessment
RDA	Relative Daily Allowance
RDI	Relative Daily Intake
RIVM	Netherlands National Institute for Public Health and the Environment
RR	Relative Risk
RWI	Recommended Weekly Intake
SD	Standard Deviation
SIgA	Secretoty ImmunoGlobuline A
SRA	Society for Risk Analysis
TEF	Toxic Equivalent Factors
TEQ	Toxic Equivalent Quotients
TWI	Tolerable Weekly Intake
UNICEF	United Nations Children's Fund
VKM	Norwegian Scientific Committee for Food Safety
VRM	Visual Recognition Memory
WHO	World Health Organization
WTO	World Trade Organization
YLD	Years Lived with Disability
YLL	Years of Life Lost

SUMMARY - RÉSUMÉ

Abstract

The objective of the present PhD project was to develop a conceptual and methodological framework to assess quantitatively the overall impact of food on human health, including microbiological, chemical and nutritional dimensions.

This methodology was developed using a case study on infant milk-based diet (breast milk and infant formulas) taking into account the following selected factors: *Cronobacter sakazakii*, *Cryptosporidium*, arsenic, dioxin like polychlorinated biphenyls and docosahexaenoic acid. Five probabilistic mathematical models were developed to quantify risks / benefits associated with these factors. When possible, they were harmonised using a common public health indicator, the DALY. Results were obtained by second-order Monte Carlo simulation in order to quantify separately the uncertainty and the variability.

Probabilistic techniques enabled to take into account on the one hand the biology related to variability (heterogeneity between individuals of the same population) and on the other hand the uncertainty linked to the lack of knowledge and data. In addition, separation of variability and uncertainty strengthened the evaluation by enabling a more accurate interpretation of results and by providing more comprehensive information for policy makers.

The method used in this PhD thesis can be considered as a robust basis for other case studies and can be used to continue methodological development in risk-benefit assessment. This approach is also part of a broader area: the multi-criteria decision analysis of agronomic and food systems.

Keywords: Risk-Benefit Assessment; food safety and nutrition; probabilistic techniques; infant milk.

Résumé

L'objectif de cette thèse était de développer un cadre conceptuel et méthodologique permettant d'évaluer quantitativement l'impact global de l'alimentation sur la santé des consommateurs, en prenant en compte les dimensions microbiologiques, chimiques et nutritionnelles.

Cette méthodologie a été développée à l'aide d'un cas d'étude portant sur l'alimentation des nourrissons (lait maternel et formules infantiles), incluant les facteurs suivants : *Cronobacter sakazakii*, *Cryptosporidium*, arsenic, polychlorobiphényles de type dioxine et acide docosahexaénoïque. Cinq modèles mathématiques probabilistes ont été développés pour quantifier les risques / bénéfices associés à chaque facteur. Ils ont été ensuite harmonisés, quand cela a été possible, à l'aide d'un indicateur commun de santé publique, le DALY. Les résultats ont été obtenus par simulation de Monte Carlo de second ordre afin de quantifier séparément l'incertitude et la variabilité.

Les techniques probabilistes ont permis de prendre en compte d'une part la variabilité inhérente à la biologie (hétérogénéité entre individus d'une même population) et d'autre part l'incertitude liée au manque de connaissances et de données. De plus, la séparation de la variabilité et de l'incertitude a consolidé l'évaluation, permettant une interprétation plus cohérente des résultats et donc fournissant des informations plus complètes aux décisionnaires.

La méthode mise en œuvre dans ce travail de thèse pourra servir de base pour d'autres cas d'études et pourra aussi être utilisée pour continuer le développement méthodologique de l'évaluation risque-bénéfice. Cette démarche s'inscrit dans une approche plus générale d'analyse multi-critères des systèmes agronomiques et alimentaires.

Mots clés : Évaluation Risque-Bénéfice ; sécurité des aliments et nutrition ; techniques probabilistes ; lait infantile.

Synthèse détaillée

Se nourrir est indispensable à l'homme pour survivre, permettre à son corps de se développer et maintenir ses activités. L'apport alimentaire est une source d'énergie lorsqu'il répond à des besoins précis en macro et micro nutriments, tout au long de la vie. Cependant, l'excès ou la déficience de certains nutriments, peut causer des effets néfastes pour la santé du consommateur. Cela peut aussi être le cas lors de la consommation d'aliments microbiologiquement ou chimiquement contaminés. En parallèle de ces risques, certains régimes alimentaires peuvent améliorer la qualité de vie, prévenir l'apparition de maladies voire même les traiter, apportant ainsi des bénéfices potentiels pour la santé. Pour toutes ces raisons, la consommation des aliments fait partie intégrante de la santé humaine.

Les aliments contaminés sont responsables de plus de 200 maladies différentes, ce qui entraîne chaque année dans le monde 600 millions de personnes malades, et 420 000 décès (WHO, 2015a), ce qui correspond à 33 millions d'années de vie saines perdues (DALY). Plus précisément en Europe, les aliments contaminés demeurent une préoccupation majeure de santé publique avec 310 000 cas d'infection chaque année (WHO, 2015b). De plus, dans les pays développés, les régimes alimentaires déséquilibrés ont un impact négatif considérable sur la santé humaine, avec par exemple environ 350 000 DALY chaque année aux Pays-Bas, ce qui est supérieur à l'impact des maladies liées au tabagisme (van Kreijl et al., 2006).

De façon générale, les maladies liées à l'alimentation n'affectent pas seulement la santé humaine, mais aussi les systèmes économiques, les communautés, les entreprises, les pays, les systèmes de santé, le tourisme, etc. Par ailleurs, la mondialisation du commerce alimentaire a particulièrement compliqué la sécurité sanitaire de la chaîne de production des aliments et a modifié les habitudes alimentaires des consommateurs. Ainsi, assurer une alimentation saine et sûre reste un défi majeur pour améliorer la santé publique des populations (WHO, 2013).

Jusqu'au début du 21ème siècle, les risques et bénéfices associés à la consommation d'aliments étaient évalués séparément en microbiologie, en chimie et en nutrition. Cependant, ces trois dimensions sont présentes simultanément dans l'assiette du consommateur, dans son régime alimentaire ou encore dans un même aliment. Une approche intégrative est alors nécessaire pour évaluer tous les risques et bénéfices associés à la consommation d'aliments. D'une manière plus générale, toutes les parties prenantes intéressées par le lien entre aliment et santé ont besoin d'une évaluation globale : les décideurs en santé publique doivent hiérarchiser les actions de gestion en tenant compte des effets globaux des aliments sur la santé, les nutritionnistes et les diététiciens doivent guider leurs patients dans leurs choix alimentaires et les consommateurs ont besoin de recevoir des messages clairs pour, eux aussi, faire leur choix.

L'évaluation des risques et des bénéfices liés à l'alimentation a suscité une attention particulière en Europe depuis le début du 21ème siècle. En Europe, plusieurs projets de recherche ont contribué à développer son approche, suite à une dynamique lancée par l'EFSA (EFSA, 2006; EFSA, 2010), des projets européens (BRAFO, QALIBRA, BEPRARIBEAN) qui ont posé les bases de l'évaluation risque-bénéfice (Boobis et al., 2013; Hart et al., 2010; Hoekstra et al., 2012; Tijhuis et al., 2012b; Verhagen et al., 2012b), ainsi que d'autres initiatives de recherche (Berjia, 2013; Gradowska, 2013; Sirot, 2010). Cependant, il n'existe actuellement « aucun consensus international sur les principes généraux ou les approches pour conduire l'évaluation des risques et des bénéfices des aliments et des composés alimentaires » (Eneroth and Zetterberg, 2016). L'évaluation Risque-bénéfice (ERB) est donc une discipline émergente qui nécessite de nouveaux développements méthodologiques.

Objectifs

Dans ce contexte, l'objectif de ce projet de thèse était de développer un cadre conceptuel et méthodologique permettant d'**évaluer l'impact global de l'alimentation sur la santé des consommateurs**, en prenant en compte les dimensions microbiologiques, chimiques et nutritionnelles.

Une attention particulière a été consacrée aux questions scientifiques suivantes :

- Comment réaliser une ERB **multidisciplinaire** tenant compte des aspects microbiologiques, chimiques et nutritionnels ? Est-il possible de définir une approche générique ?
- Comment **comparer les différents impacts sur la santé** du consommateur ? Est-il possible d'utiliser une "métrique" commune ?
- Comment **considérer la variabilité et l'incertitude** en ERB ?
- Comment **communiquer et interpréter** les résultats de l'ERB ?

Contenu du manuscrit

Le CHAPITRE 1 présente tout d'abord les objectifs du projet de thèse et ses principales étapes. Dans le CHAPITRE 2, un état de l'art concernant l'ERB dans les aliments est proposé pour déterminer, sur la base de la littérature existante, les approches principalement suivies jusqu'à maintenant. Plus précisément, les études actuelles portant sur des cas d'études et sur le développement méthodologique de l'ERB ont été résumés. Cette partie a permis de formaliser la méthodologie suivie en ERB et d'identifier les besoins de recherche.

Un cas d'étude modèle a ensuite été choisi pour développer davantage la méthodologie de l'ERB : l'étude du régime alimentaire des nourrissons à base de lait, incluant d'une part les formules infantiles et d'autre part le lait maternel. Il s'agissait d'un cas particulièrement intéressant car le lait représente le seul aliment des nourrissons au cours de cette première période de vie sensible et soulève de nombreuses problématiques multidisciplinaires. En effet, les premiers mois de vie sont essentiels pour le développement physiologique à court et à long terme. Les risques et les bénéfices liés à ces deux régimes alimentaires ont été étudiés dans le CHAPITRE 3, pour ses aspects

microbiologiques, chimiques et nutritionnels, afin d'identifier tous les effets potentiellement néfastes et bénéfiques sur la santé.

Ensuite, une ERB a été réalisée pour ce cas d'étude particulier relatif au lait, elle est présentée dans le CHAPITRE 4. Un modèle probabiliste et interdisciplinaire a été développé (appelé « Modèle 1 ») en prenant en compte trois facteurs de préoccupation majeure avec pour la chimie (polychlorobiphényles « dioxine-like » ou dl-PCBs), la microbiologie (*Cronobacter sakazakii*) et la nutrition (acide docosahexaénoïque ou DHA).

Puis, l'approche développée dans le modèle 1 a été réutilisée pour aborder une seconde question liée à l'alimentation des nourrissons : l'évaluation des risques liés à l'utilisation de l'eau du robinet pour la préparation des formules infantiles en France, associés à la présence potentielle de *Cryptosporidum* et d'arsenic (CHAPITRE 5).

Enfin, le dernier chapitre (CHAPITRE 6) comprend les principales conclusions du projet de thèse, une discussion sur les questions initialement soulevées : sur la méthode multidisciplinaire de l'ERB, la quantification des impacts globaux sur la santé, la prise en compte de la variabilité et de l'incertitude, ainsi que la communication et l'interprétation des résultats. Il contient également une conclusion sur la méthodologie de l'ERB et donne des perspectives de recherche future.

Principales conclusions

Le projet de thèse exposé dans ce manuscrit visait à développer un cadre conceptuel et méthodologique permettant d'évaluer l'impact global de l'alimentation sur la santé des consommateurs, en prenant en compte les dimensions microbiologiques, chimiques et nutritionnelles. Ce projet s'est focalisé sur un cas d'étude : l'évaluation des risques et des bénéfices associés au régime à base de lait des nourrissons, considérant les formules infantiles et le lait maternel. En ce qui concerne la méthodologie de l'ERB, un cadre méthodologique été suggéré (voir la discussion 6.2.1 et **Figure 6.3**), basé sur les approches principalement suivies jusqu'à maintenant et le retour d'expérience de nos deux évaluations conduites (Modèle 1 et 2). Cette méthodologie, place les décideurs au cœur de l'ERB en définissant des options de gestion ciblées dès le début afin d'évaluer

directement des scénarios d'exposition qui correspondent à une réalité potentielle pour le consommateur. Différentes stratégies d'évaluation individuelle des risques et des bénéfices ont ensuite été suggérées lors du développement des modèles 1 et 2 car il semble impossible de converger vers une seule et même approche. Puis, plusieurs approches visant à comparer les risques et bénéfices pour la santé du consommateur ont été proposées : à l'aide d'une métrique composite DALY lorsque cela était possible, ou par comparaison des niveaux d'exposition aux seuils de sécurité préventive. Bien que l'utilisation d'une seule et même métrique puisse être attractive pour comparer les différents scénarios entre eux, les résultats semblent être plus judicieusement communiqués au travers d'un tableau multicritère résumant tous les résultats à l'échelle individuelle et à l'échelle de la population. Enfin, la séparation de la variabilité et de l'incertitude a été recommandée pour fournir des résultats plus pertinents aux décideurs.

L'ERB apparaît aujourd'hui comme un outil essentiel pour fournir des recommandations complètes en matière d'alimentation et de santé humaine. C'est une approche complexe et multidisciplinaire qui s'inspire de l'évaluation traditionnelle des risques mais qui nécessite une analyse plus approfondie pour agréger tous les résultats entre eux et fournir des conclusions compréhensibles et interprétables par les diverses parties prenantes. En conséquence, l'ERB doit faire face à tous les défis de l'évaluation classique des risques / bénéfices en microbiologie, nutrition et chimie, notamment celui du manque de données (en particulier pour bâtir la relation dose-réponse) et donc de la gestion de l'incertitude. Il est aussi important de trouver des moyens appropriés pour interpréter et communiquer les résultats obtenus afin de fournir une vision générale de l'impact global sur la santé du consommateur. Enfin, comme souvent dans les disciplines intégratives (dont fait partie l'ERB au même titre que l'appréciation du risque microbiologique ou chimique), il s'agit d'un problème sans fin car les conclusions peuvent être remises en question à chaque nouvelle découverte de chaque domaine inclus dans l'évaluation.

Perspectives

Un défi majeur de l'ERB est de **faire face à la multidisciplinarité et à la multi-dimensionnalité** des questions étudiées qui conduisent à des études et à des conclusions complexes. **L'analyse de décision multicritères** peut alors s'avérer être un outil utile en

ERB afin de faciliter la communication, l'interprétation et la prise de décision. Des recherches futures pourraient être réalisées pour adapter ces méthodes plus spécifiquement à l'ERB (Ruzante et al., 2017).

Un autre défi de taille est de faire face au scepticisme face à l'utilité de l'ERB, dû à la difficulté d'interpréter et de mettre en œuvre ses résultats complexes. L'ERB a émergé pour fournir des recommandations plus réalistes et complètes pour les consommateurs en matière d'alimentation saine et sûre, et d'éviter l'émission de conclusions contradictoires des différents domaines scientifiques. Un autre défi restant non résolu est la façon dont les consommateurs vont suivre ou non au quotidien ces recommandations (van Kleef et al., 2014). Plus précisément, la communication des résultats aux utilisateurs finaux reste encore difficile et doit être ciblée, car les effets négatifs et bénéfiques affectent différemment les sous-groupes de population. De plus, l'ERB quantitative considérant la variabilité de l'individu dans la population conduira à des recommandations de plus en plus personnalisées et donc de moins en moins génériques. Il est aussi important de définir qui sont les gestionnaires : les décideurs ou les consommateurs ? Comme l'a expliqué Nauta (2015), il semble évident que les consommateurs soient directement ciblés par les études d'ERB. Par conséquent, un troisième défi sera de comprendre comment changer les habitudes et les comportements des consommateurs. Cette dernière question implique clairement des aspects sociologiques : les consommateurs ont un rôle à jouer dans le choix des aliments qu'ils consomment et leur préparation (Schmidt and Rodrick, 2003). Ainsi, il peut être recommandé pour des recherches futures, de **mener des analyses risque-bénéfice impliquant directement les consommateurs**, en les plaçant au centre de l'évaluation et non pas comme utilisateur final (Dreyer and Renn, 2013; Mikulsen and Diduck, 2016).

Enfin, en dehors des perspectives de santé, **d'autres facteurs peuvent amener à moduler les recommandations**. L'analyse des risques-bénéfices dans nos sociétés ne peut être considérée comme un processus isolé ; elle est au contraire interconnectée avec des perspectives sociétales, politiques, économiques, éthiques et environnementales. Ainsi, **l'analyse risque-bénéfice doit faire partie de la gouvernance globale de la sécurité sanitaire des aliments** (Mikulsen and Diduck, 2016). Là encore des techniques de décision multi-critères peuvent s'avérer être utiles pour y parvenir.

CHAPTER 1

General Introduction and Objectives

CHAPTER 1: **General Introduction and Objectives**

Food is a basic human necessity. Eating is essential to survive by enabling our body to grow, develop and maintain activities. Food intake can be a simple neutral source of energy when meeting precise macro and micro nutrient requirements, varying all along the life cycle. However, when not meeting precisely these requirements (deficiency or excess), food can cause adverse health effects. In case of microbiological and chemical contaminations, it can be also a cause of diseases. Paradoxically, optimised diets and consumption of certain foods are proved to improve quality of life, to prevent and treat diseases but also to bring potential health benefits. Consequently, access to sufficient, nutritious and safe food is essential to maintain health as well as having a global healthy diet throughout life.

Food safety was defined as "a reasonable certainty that no harm will result from intended uses under the anticipated conditions of consumption" (OECD, 1993) which implies that a tolerance must be considered as the zero risk might rarely be achieved (Barlow et al., 2015). On the opposite, nutrition and health claims have emerged, supporting optimised diets and potential health benefits for consumer's health (Verhagen et al., 2010).

Unsafe food is liable for more than 200 different diseases making sick 600 million person including 420 000 die every year all over the word (WHO, 2015a), representing 33 million healthy life years (DALYs). More precisely in Europe, unsafe food remains still of concern with 310 000 cases each year (WHO, 2015b). Moreover, in developed countries unhealthy diets due to unfavourable dietary composition have a huge negative impact on human health, with for instance about 350 000 DALY lost every year in Netherlands, even more than the burden associated with smoking (van Kreijl et al., 2006).

On a global basis, foodborne disease is not only affecting human health but also impose a substantial burden on economic systems, communities, businesses, countries, health-care systems, tourism, etc. Furthermore, globalisation of the food trade has made considerably more complex the food safety production chain and consumers' dietary

habits. Thus, ensuring food safety and healthy diet remains a strategic challenge all over the word to improve population public health (WHO, 2013).

In this context, quantifying the overall impact of food consumed on human health appears as a key issue to improve populations' public health. Up to the last decade, risks and benefits associated with food consumption were assessed separately with regard to the microbiology, chemistry and nutrition components. However, these three factors are often present simultaneously on consumers' plates, diets or even foods. Therefore, an integrative approach is required to balance all the risks and benefits associated with food consumption. More broadly, different stakeholders are expecting comprehensive assessment: food policy makers need to prioritise management actions considering overall effects of food consumed on health, nutritionist and dietician need to guide people in their choices and consumers need to receive clear messages, feeling more and more concerned about the effect of their diet on health. Public health recommendations in food need to be based on comprehensive, scientific and rational evaluations.

Risk-Benefit Assessment of food has gained particularly attention in Europe since the beginning of the 21st century. In Europe, several research projects have contributed to develop its approach, following the dynamic of the EFSA (EFSA, 2006; EFSA, 2010), European projects (BRAFO, QALIBRA, BEPRARIBEAN) have contributed to lay the foundation for future risk-benefit assessment studies (Hart et al., 2010; Hoekstra et al., 2012; Tijhuis et al., 2012b; Verhagen et al., 2012b), as well as complementary research initiatives (Berjia, 2013; Gradowska, 2013; Sirot, 2010). However, there is currently "no international consensus on the general principles or approaches for conducting risk-benefit assessment of foods and food components" (Eneroth and Zetterberg, 2016). Thus, RBA is still an emerging science which requires further methodological developments.

1.1. Objectives

In this context, the objective of this PhD project was to develop a conceptual and methodological framework to **assess quantitatively the overall impact of food on human health**, including microbiological, chemical and nutritional dimensions.

A particular attention has been devoted to the specific questions:

- How to carry out a **multidisciplinary RBA** considering microbiological, chemical and nutritional aspects? Is it possible to set a generic framework?
- How to **compare health impacts**? Is it possible to use a common metric?
- How to consider **variability and uncertainty** in RBA?
- How to **communicate and interpret** RBA results?

1.2. Outline of the thesis

The flowchart of our PhD project is provided in **Figure 1**.

First, the state of art regarding the RBA in food has been performed to determine, based on the literature, the current methodologies in RBA. Main applications were also summarised. This part enabled to lay down a first RBA framework and to identify needs of research in RBA.

A particular case study was selected to develop further our RBA methodology: the infant milk based diet, including breast milk on one hand and infant formula on the other hand. It was a particular case of interest as milk represents the sole diet during this critical first period of life and raised a large range of multidisciplinary safety issues. Indeed, first months of life are crucial for short and long term healthy physiological development. Risks and benefits associated with both infant milk diets were reviewed, with regard to microbiology, chemistry and nutrition to identify all potential adverse and beneficial health effects.

Then, a RBA was performed on the infant milk case study, named “model development 1”. A probabilistic and inter-disciplinary model was developed, it was limited to one key factor per domain, i.e. one in chemistry, one in microbiology and one in nutrition.

Hence, the methodology developed in model 1 was re-used for another issue linked with infant feeding: the risk assessment of tap water for infant formula preparation in France, named “Model development 2”.

Finally, the last chapter includes main findings of the thesis project and a discussion to address the questions initially raised around the multidisciplinary RBA method, the quantification of the overall health impacts, the consideration of variability and uncertainty and the interpretability as well as communication of results. It also contains a conclusion on the RBA methodology and gives directions for future research.

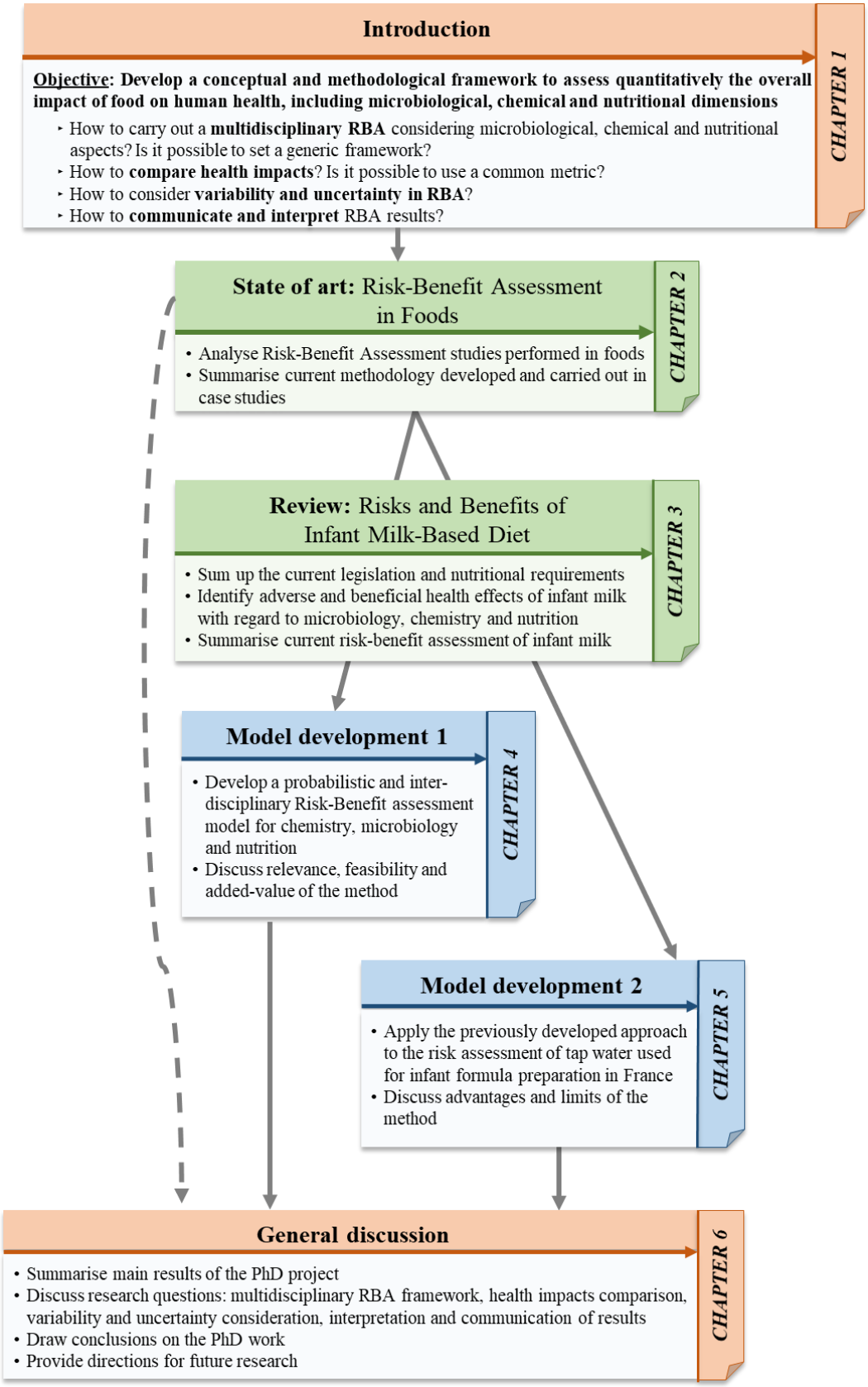

Figure 1: Flowchart of the PhD project entitled "Public health Risk-Benefit assessment in foods: methodological development with application to infant milk-based diet"

CHAPTER 2

State of art of Risk-Benefit Assessment in Foods

Redraft from:

Boué, G., Guillou, S., Antignac, J.-P., Le Bizec, B., & Membré, J.-M. (2015). Public Health Risk-benefit Assessment Associated with Food Consumption-A Review. European Journal of Nutrition & Food Safety, 5(1), 32.

CHAPTER 2: **State of art of Risk-Benefit Assessment in Foods**

The present chapter is the state of art of Risk-Benefit Assessment (RBA) in Foods. Since RBA has emerged at the beginning of the 21st century, there is currently no universal methodology. Thus, this part enabled to define a method for conducting RBA based on current trends in case studies and guidance from specific methodological projects. In addition, this exploratory work was used to identify specific needs of methodological development in RBA for our case study.

Objectives of the chapter:

- Analyse Risk-Benefit Assessment studies performed in foods,
- Summarise current methodology developed and carried out in case studies.

2.1. Abstract

Background: In the food safety field, risk assessment, including microbial and chemical components, has been applied for many years. However, a whole and integrated public health assessment also depends on the nutritional composition of food. While the fact that foods and diets can be a source of both risks and benefits now appears undisputed, carrying out a risk-benefit assessment (RBA) is still an emerging and challenging scientific subject.

Aims: The purpose of the present review was to synthesize RBA studies associated with food consumption and to summarize the current methodological options and/or tendencies carried out in this field.

Methods: The different data sources explored included around 20 accessible databases using the main terms “risk”, “benefit” and “food” as keyword enquiries in article title and

full-text. The initial research process led to 3293 screened papers, 160 of which were examined in detail.

Results: There were 126 articles dealing with RBA studies and 34 with the RBA methodological framework. Most of the available papers dealt with the comparison of nutritional beneficial effects and chemical adverse effects related to fish consumption. The majority of studies undertook a comparison of consumer exposure to risks and benefits with regard to reference safety values. However, more varied studies have emerged during the last 15 years, contributing to the diversification and the development of this issue.

Conclusions: RBA appears to be a promising scientific discipline and should be the next step in assessing the overall impact of food on health.

2.2. Introduction

Food safety management has adopted a risk-based approach in both the microbiological and chemical fields. In this context, the impact of more and more hazards associated with food consumption is evaluated by a risk assessment framework. In the nutritional field, food is recognized as having beneficial effects on health but also adverse effects. As a result, the concept of an integrated risk-benefit assessment has emerged in the last decade.

The risk can be defined as the probability that an adverse health effect affecting an organism, a system, or a sub-population will occur, as a consequence of an exposure to a hazard in food (IPCS, 2004). In contrast, the benefit is defined as the probability that a positive health effect will occur. Risk and benefit can be simultaneously related to the consumption of most foods that are commonly associated with various types of microbial (e.g. pathogens), chemical (e.g. acute toxic or endocrine-disrupting substances), and/or nutritional (e.g. saturated fatty acids) hazards, together with beneficial nutritional components (e.g. unsaturated fatty acids).

Risk-benefit assessment (RBA) falls within the concept of risk-benefit analysis, which is an integrative approach associating three interconnected and complementary parts: risk-benefit management, risk-benefit assessment, and risk-benefit communication. The

EFSA agency (EFSA, 2010) advises mirroring the traditional risk analysis process to undertake a risk-benefit analysis, while considering some differences like the addition of a benefit assessment and a risk-benefit comparison as illustrated in **Figure 2.1**.

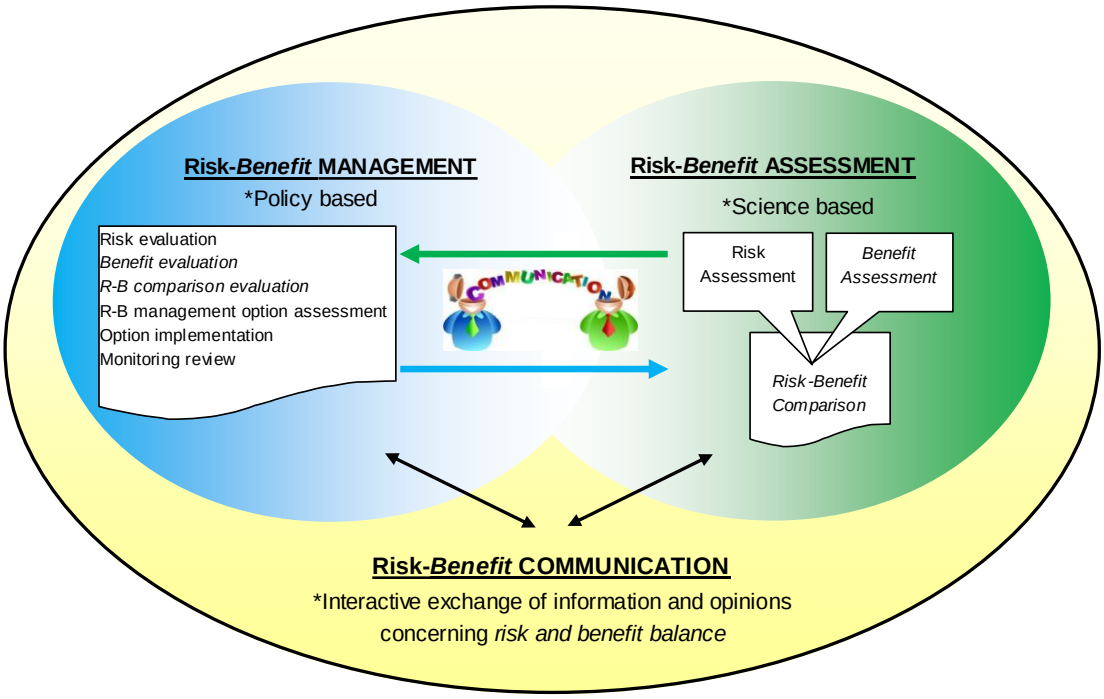

Figure 2.1: Evolution of the conventional risk assessment conceptual framework toward an integrated risk-benefit paradigm, adapted from (WHO, 2017)
The new elements are indicated in italics.

The objective of RBA is to assess risk and benefit scientifically and objectively in the same integrative methodology. Then, risk-benefit management sets up two kinds of public health action: modification of food standards, reconsidering legislation to improve the quality of food available, and establishing recommendations for consumers to change their food habits into a healthier diet and lifestyle (food choice, consumption habits and cooking practices).

Several studies of RBA have already been undertaken and methodological developments in this field were first carried out by the European Food Safety Authority (EFSA) (EFSA, 2006; EFSA, 2010) and the Netherlands National Institute for Public Health and the Environment (RIVM) (Fransen et al., 2010). Then, some European collaborative research projects have worked on the RBA framework through these programs: “Benefit Risk Assessment for Food” (BRAFO) (Hoekstra et al., 2012), “Best PRActices of RIsk-BEnefit Analysis” (BEPRARIBEAN) (Verhagen et al., 2012b), “Quality of Life –

Integrated Benefit and Risk ANalysis" (QALIBRA) (Hart et al., 2010) and "Benefit-Risk Assessment for Food: an Iterative Value-of-Information Approach" (BENERIS) (BENERIS, 2011).

In this context, RBA is becoming an established discipline. The aim of our work was to synthesize RBA studies associated with food consumption and to summarize the methodologies in a common framework.

2.3. Methods

The research of articles aimed to collect RBA studies associated with food consumption and information on the RBA methodology. We followed the PRISMA data search process advised by Moher et al. (2009) to organize the research of articles.

Databases explored included Web of Science, PubMed, MEDLINE, CABI, FSTA, ScieLO, Science Direct, EBSCO HOST, ACS Publications, Annual Reviews, edp Sciences, Endocrine Society, Cambridge Journals, NRC, Highwire Press, World Cat, Science.gov and Google Scholar. Other sources were explored like Google, citation tracking, key journal search etc.

These data searches were restricted to articles introducing RBA in terms of public health associated with food consumption in the fields of nutrition, chemistry, and microbiology. Only studies written in English or French without a publication date restriction were considered. The latest research was undertaken on 20th May 2014.

The same research was done on all the databases mentioned above. First, the search was based on the keywords "Food", "Risk* AND (Benefit* OR Beneficial*)" in the title but this did not provide all the relevant articles. Therefore, the search criteria were extended to the topic. Unfortunately, some databases did not have the option to search by topic. In this case, the nearest available option was used or, if there was none, we looked for the word "food" in the whole article. Below are the keywords used when the topic option was available, and when it was not.

When the topic option was available (e.g. for Web of Science):

- TITLE: (risk* AND (benefit* OR beneficial*)) AND TOPIC: (food)
- TITLE: ((chemi* OR toxicolo* OR microbi* OR nutrition) AND (risk* AND (benefit* OR beneficial*))) and TOPIC: (food)
- TITLE: ((risk* AND (benefit* OR beneficial*)) AND (health)) and TOPIC: (food)
- TITLE: ((risk* AND (benefit* OR beneficial*)) AND (public health)) and TOPIC: (food)
- TITLE: ((risk* AND (Benefit* OR Beneficial*) AND (review)) and TOPIC: (food)
- TITLE: ((risk* AND (benefit* OR beneficial*)) AND (state of the art)) and TOPIC: (food)

When the topic option was not available (e.g. for Science Direct):

- TITLE(risk* AND (benefit* OR beneficial*)) and FULL-TEXT(food)
- TITLE((chemi* OR toxicolo* OR microbi* OR nutrition) AND (risk* AND (benefit* OR beneficial*))) and FULL-TEXT(food)
- TITLE((risk* AND (benefit* OR beneficial*)) AND (health)) and FULL-TEXT(food)
- TITLE((risk* AND (benefit* OR beneficial*)) AND (public health)) and FULL-TEXT(food)
- TITLE((risk* AND (Benefit* OR Beneficial*) AND (review)) and FULL-TEXT(food)
- TITLE((risk* AND (benefit* OR beneficial*)) AND (state of the art)) and FULL-TEXT(food)

The article screening was carried out in three consecutive steps. The first selection of articles was based on the title accordance with the terms searched, then the abstract was explored, and finally the full article was screened. Articles that met the following criteria were selected for inclusion:

- The full article was written in English or French.
- The article was specific to the food sector.
- The main subject was a study of RBA introducing a comparison of risk and benefit or was about the methodology of RBA.
- The RBA assessment was specific to the field(s) of nutrition and/or microbiology and/or chemistry. Other subjects, like economy and sociology, were excluded.

- Reviews dealing with risks and benefits of food, like a review of the positive and adverse health effects due to the consumption of a specific food, were also selected to identify potential RBA studies.

Regarding articles dealing with the RBA methodology, the different steps recommended to undertake an RBA and the terminologies used were identified in order to summarize a common framework, which is presented in the Results section. The RBA studies identified were classified into two groups: performed and potential studies. For each study undertaken, the topic, the scientific field (microbiology, nutrition and chemistry), the type of comparison and the main results are presented in **Table 2.1**. Potential studies were investigated to compile a non-exhaustive list of future research needed in RBA.

2.4. Results

Based on the research process, 3293 papers were identified comprising 2896 peer-reviewed articles found through databases and 397 from other sources corresponding to the grey literature (mainly scientific reports and theses) or from on-line documents (website pages, electronic articles, web-seminars).The results and process are summarized in **Figure 2.2**. The screening step excluded 1819 papers by title checking and 182 by abstract reading. The screening was extensive because RBA is also an important topic in medicine, with the aim of balancing the beneficial effects of drugs against their potential adverse effects.

At the end of the query process, 160 articles were included in the review, 126 dealing with RBA studies (70 applications with recommendations and 56 studies on positive and negative health effects), and 34 with the RBA methodological framework.

2.4.1. Studies of Risk-Benefit Assessment

There were 70 articles reporting RBA applied to food. In this section, these are presented chronologically, by scientific discipline (microbiology, chemistry, and/or nutrition), by comparison criteria and by category of applications. Beside RBA studies in the strictest sense, there were also 56 studies on positive and negative health effects, which could potentially be used in RBA.

2.4.1.1 History of RBA studies

The first RBA study appeared in 1999. Since then, the number has increased gradually (**Figure 2.3**). The first case study undertaken concerned the assessment of fish consumption, which is still by far the most studied topic (70% of RBAs). Fish consumption is a well-known source of both health benefits provided by omega-3 and risks due to environmental pollutants (dioxins, PCBs and methyl mercury). These RBAs have often been conducted at the level of a specific country by food safety agencies or various scientific groups.

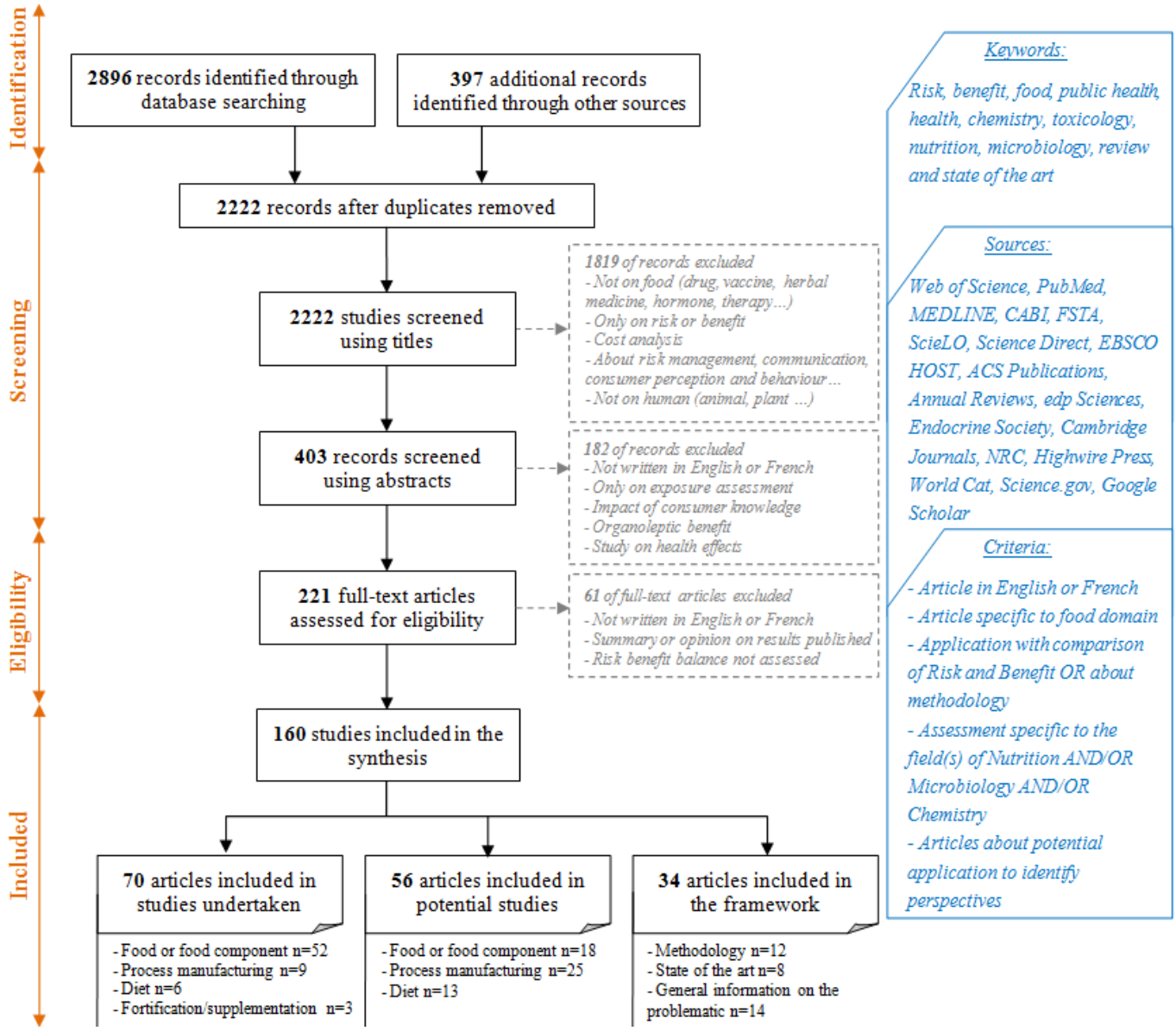

Figure 2.2: Overview of the applied data search process and the results based on PRISMA's four-phase diagram (Moher et al., 2009)

Beside RBA on fish, many other case studies have emerged: supplementation or fortification of foods, assessment of nitrates and nitrites in fruits and vegetables, food-specific molecules such as acrylamide created during the manufacturing process, water and milk treatment, replacement of sugar by intense sweeteners, consumption of trans-unsaturated fatty acids, fish cooking practices, etc. (**Figure 2.3**).

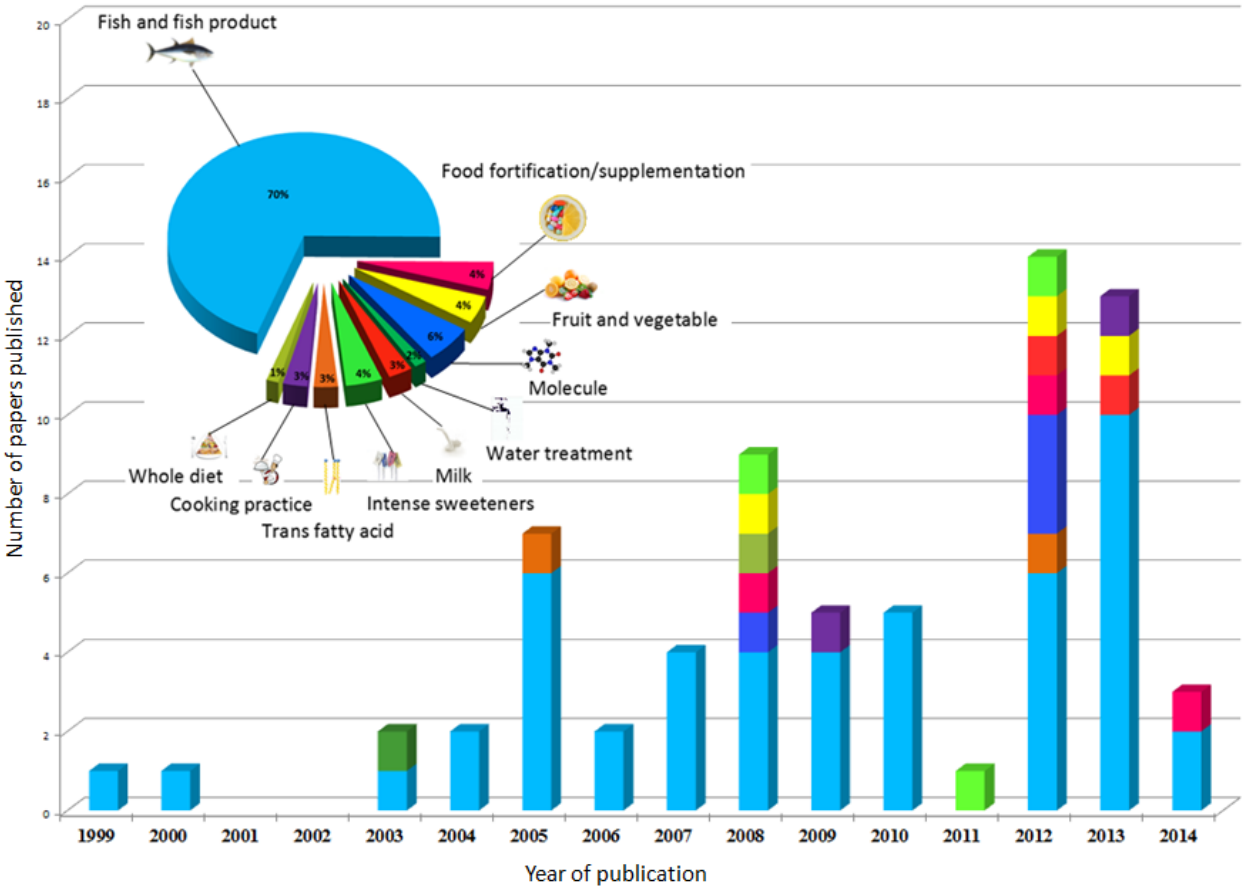

Figure 2.3: Classification of the 70 studies performed by year and food category

2.4.1.2 Scientific fields of RBA studies

All these studies fall within the fields of nutrition and/or microbiology and/or chemistry. However, only a few studies have performed an integrated approach including these three disciplines (**Figure 2.4**).

Moreover, the three available studies (ANSES, 2013b; Nesheim and Yaktine, 2007; VKM, 2013) that integrated these three disciplines compared chemical and nutritional risks-benefits using safety reference values and gave recommendations on hygiene

practices, which cannot be assimilated into a proper quantitative nutrition-chemical-microbial RBA. More generally, microbial risk is not often assessed in RBA and rarely in a quantitative way. Recently, (Berjia et al., 2012) carried out a comparison of nutritional benefits and microbiological risks associated with fish consumption.

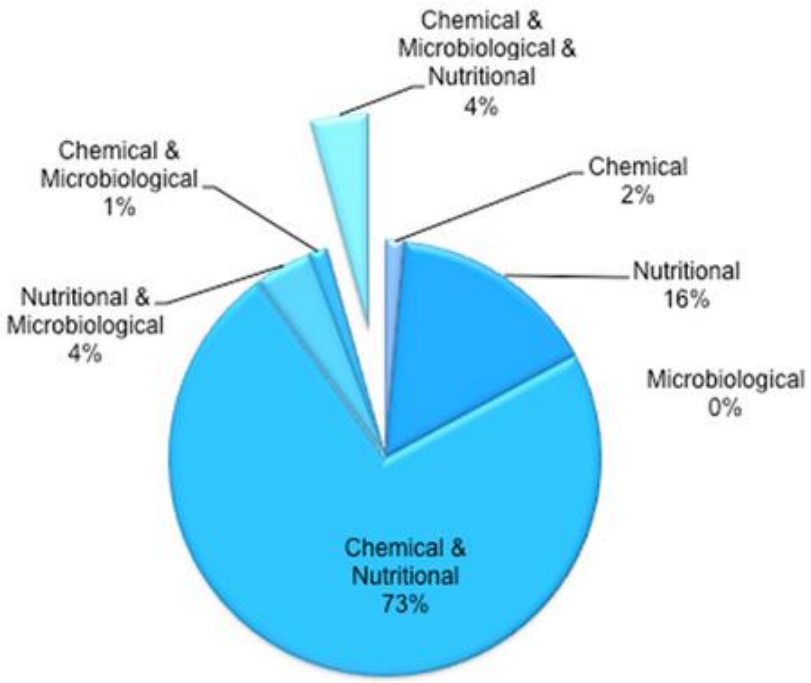

Figure 2.4: RBA studies performed classified by scientific fields, based on 70 studies

2.4.1.3 Comparison criteria used in RBA

Different criteria are used to compare risks and benefits:

1 ***Comparison of risks and benefits under constraints, based upon safety reference values***. This is a comparison of scenarios of consumer exposure. For each scenario of consumption, consumers are exposed to different risks and benefits related to the field of chemistry and/or nutrition and/or microbiology. The aim of this comparison is to set a threshold in accordance with safety levels set by food safety agencies. Regarding the risks identified, this threshold is set below the maximum levels of tolerable exposure (i.e. Acceptable Daily Intake, Tolerable Daily Intake, Upper Limit) and in agreement with nutritional intake recommendations (Recommended Daily Allowance, Estimated Average Requirement). Above this threshold,

consumers could be exposed to a risk. Then, benefits are maximized, if possible, with respect to this threshold. This comparison can be considered semi-quantitative because the RBA output is not expressed in a quantitative way (even if the assessment in chemical, microbiological or nutritional field might be quantitative). In addition, the process is likely to be iterative: RBA conclusions will be revised as often as the safety levels are reviewed. A comparison under constraints has been performed 46 times among the 70 studies (**Figure 2.5**).

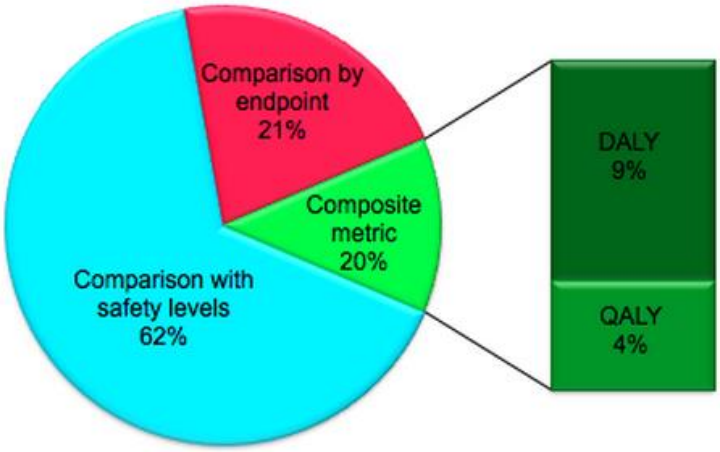

Figure 2.5: RBA studies performed classified by type of comparison, based on 70 studies

2 ***Comparison of risks and benefits based upon health endpoint***. For example, risk can be expressed as the probability of increasing the prevalence of coronary heart disease and benefit as the probability of decreasing this prevalence. It might also be expressed using the intellectual quotient (IQ) endpoint. A comparison based upon health endpoint has been performed 15 times among the 70 studies (**Figure 2.5**). Only articles that compared health endpoints one by one were included in this group.

3 ***Comparison of risks and benefits based upon a composite metric*** like the Disability Adjusted Life Years (DALY). This aims to compare quantitatively the impact of different diseases all together, contrary to the last group. It provides a comprehensive assessment of the consequences of a disease by

integrating the quality of life lost (w) after the disease onset, the duration of the disease (Years of Life with Disability, YLD) and Years of Life Lost (YLL) (Gold et al., 2002). At an individual scale, the DALY metric is calculated as indicated in Equation 2.1 and is illustrated in **Figure 2.6** by the case of a person who has fallen sick and died after a period of life with a disability.

Equation 2.1

$$DALY = w.\,YLD + YLL$$

The use of the DALY metric as a comparison criterion requires many data, which are unfortunately not always available. However, to avoid this problem, epidemiological data can be used to inform the probabilities of falling ill, dying and recovering, as was done by Hoekstra et al. (2013b) and Berjia et al. (2012). A comparison using a composite metric has been performed 9 times among the 70 studies (**Figure 2.5**).

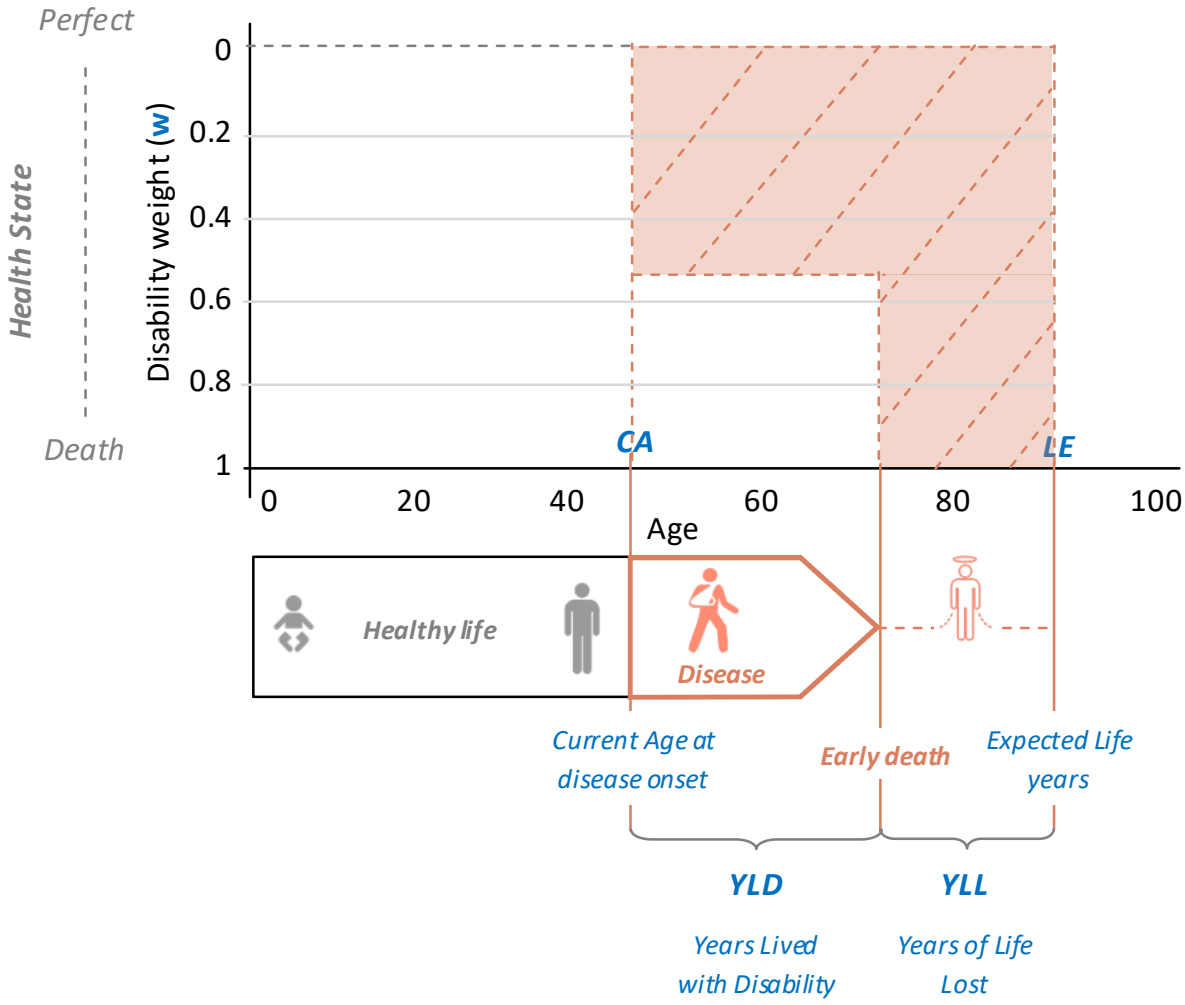

Figure 2.6: Illustration of DALY adapted from Tijhuis et al. (2012a) with the case of a person who falls sick and dies after a period of disability

2.4.1.4 Main RBA applications

Studies undertaken in recent years have resulted in progress in scientific knowledge in RBA. They have also enabled the food authorities to make recommendations on food consumption, such as the EFSA on fish consumption (2005). More generally, RBA research has led to promising applications, which can be schematically split into two categories: those leading to recommendations by food safety authorities and those leading to process and formulation design by manufacturers.

The applications are listed below. However, it is important to keep in mind that the conclusions presented here are extremely summarized, and can by no means be considered definitive statements concerning recommendations. More information on each study is provided in **Table 2.1**. However, for a comprehensive view of the study, please refer to the original paper.

- **Applications leading potentially to recommendations**
  - *Impact of a specific food on health*

The most investigated case study is fish. Fish contains docosahexaenoic (DHA) and eicosapentaenoic fatty acids (EPA) recognized for their health benefits but it is also contaminated by pollutants such as methyl mercury and dioxins, sources of adverse effects now clearly demonstrated. Fish composition is also dependent on fish species, fish feeding and place of production, which considerably influence its chemical contamination and fatty acid content. In addition, health effects vary greatly according to the subpopulation affected, which is a major issue in RBA. This topic has been investigated for 15 years and is still in progress because of its complexity. Overall, each study tackles the same subject (fish consumption) but brings new information by studying particular conditions (assimilated into co-variables in the analysis) affecting the risk-benefit assessment.

The overall recommendation is to consume two fish dishes per week, including one with fatty fish (AFSSA, 2008; ANSES, 2013b; EFSA, 2005; SACN/COT, 2004), while alternating fish species, production type (farmed or wild) and production location. The recommendation varies from strictly two portions per week of fatty fish, including ¼ of lean fish (Sirot, 2010; Sirot et al., 2012), to two to three servings per week (Becker et al.,

2007; Nesheim and Yaktine, 2007). Some studies also give specific recommendations according to the subpopulation at risk, such as women of childbearing age and children (Balshaw et al., 2012).

Other studies have compared the impact of risks and benefits on specific health endpoints and have given ranges of recommendations to minimize the risk of stroke (Bouzan et al., 2005), coronary heart disease (CHD) (König et al., 2005; Mozaffarian and Rimm, 2006), and IQ change in the newborn (FAO/WHO, 2010; Ginsberg and Toal, 2009) or stroke and fetal development disturbance (FDA, 2009).

In addition, as highlighted by Cardoso et al. (2010), the risk-benefit balance of fish consumption varies between countries. RBAs have been carried out at a country level in Norway (VKM, 2006), the Netherlands (Berjia et al., 2012; Hoekstra et al., 2013b), Poland (Usydus et al., 2008; Usydus et al., 2009), France (Guevel et al., 2008), China (Chen et al., 2014; Du et al., 2012; Gao et al., 2014; Zhang et al., 2012a; Zhang et al., 2012b), the USA (Gochfeld and Burger, 2005; Sidhu, 2003) and Bermuda (Dewailly et al., 2008). In addition, the type of fish species could change the risk-benefit balance (Cardoso et al., 2013; Dewailly et al., 2007; Gladyshev et al., 2009; Loring et al., 2010; Watzl et al., 2012). Likewise, the type of farming may have an impact (Foran et al., 2005; Hites et al., 2004). As a result of these two factors (population and fish species), some specific populations could be negatively impacted by fish consumption. For instance, the Portuguese population, which consumes about 57kg of fish per year, should favor certain fish species to limit the potential risk due to high intake (Afonso et al., 2013a; Afonso et al., 2013b; Maulvault et al., 2013). Likewise, the Inuit population should limit its fish consumption (Laird et al., 2013). Conversely, the Kahnawake community south of the St Laurence river, also high fish consumers, is not exposed to risk (Chan et al., 1999).

The complexity of the assessment of fish intake is increased by the fact that fish consumption by pregnant or lactating women or women of childbearing age could impact the newborns' neurodevelopment and thus increase or decrease their IQ (Gradowska, 2013; Leino et al., 2013; Zeilmaker et al., 2013).

Finally, a few quantitative RBAs regarding fish consumption have been performed, providing figures that enable RBA recommendations to be deciphered. For example, in the US adult population, the current fish consumption enables to gain 5000 healthy years per year per 100000 people, calculation based on the Washington state (Ponce et al.,

2000); also in US, a 50% increase in fish consumption could save 120000 years annually of perfect health for people (Cohen et al., 2005). More specifically, based on a French study on 1011 people, it was concluded that a weekly consumption of 1104g of fish could save between 97 and 285 healthy years annually (Guevel et al., 2008). This example demonstrates that a quantitative comparison of risks and benefits is more transparent and objective than a comparison under constraints.

Recommendations concerning other food categories have also been given. Although not based upon a quantitative comparison, it has been pointed out that the intake of fruits and vegetables (EFSA, 2008; Reiss et al., 2012) and soy proteins (Watzl et al., 2012) should be increased since these food categories do not expose consumers to risk. In contrast, the intake of trans fatty acids should be limited (AFSSA, 2005a; Verhagen et al., 2012a).

- *Impact of a particular type of diet on health*

The type of diet has also been studied through RBA to assess its overall impact on health.

Replacement of sugar by intense sweeteners has been judged healthy because it prevents overweight and caries (Hendriksen et al., 2011; Verhagen et al., 2012a) although risks can outweigh benefits for children who are high consumers of soft drinks with a potential risk of exceeding the acceptable limit of intense sweetener intake (Husoy et al., 2008).

Software has been developed to assess individually risk-benefit related to diet. Some programs are specific to a product, e.g. fish consumption (Domingo et al., 2007a; Domingo et al., 2007b), while others include a wide range of foods (Marti-Cid et al., 2008; TECNATOX, 2013).

❖ **Applications leading potentially to process and formulation design**

- *Impact of manufacturing process on health*

The manufacturing process is identified as a source of risks and benefits because it could introduce risk and/or benefit or modify the risk-benefit balance.

Water treatment decreases microbial contamination but introduces chemical risk at the same time. The balance has been quantitatively assessed by Havelaar et al. (2003) who demonstrated that the benefit outweighed the risk. Milk treatment is also beneficial because it decreases microbial risk in spite of biochemical reactions (Schutte et al., 2012).

RBA may be used as a tool to optimize the process line by assessing the impact of different production parameters on the risk-benefit balance. Rigaux (2013) has optimized the thermal sterilization of vegetables to maximize vitamin concentration without exposing consumers to microbial risk. Likewise, the thermal process of cookies might be optimized to enhance their antioxidant activity while limiting the formation of harmful compounds (Morales et al., 2009). The type of thermal process also has an influence on food composition and thus on the risk-benefit balance. For instance, a comparison of fish cooking processes demonstrated that grilling is healthier than boiling or roasting (Costa et al., 2013). More generally, to optimize the thermal process, it is necessary to analyze altogether the potential loss of nutritional properties, the possible formation of hazardous molecules such as acrylamide (Schutte et al., 2012; Seal et al., 2008) and benzo(a)pyrene (Verhagen et al., 2012a), and the efficiency of microbial inactivation.

- *Impact of food formulation on health*

The positive impact of **bread supplementation with folic acid** on public health has been quantitatively assessed. In the Netherlands, a small supplementation of 70 µg per 100 g of bread could save 7000 healthy years annually (Verhagen et al., 2012a) and a higher supplementation (i.e. 140 µg per 100 g of bread) could save 11812 healthy years annually (Hoekstra et al., 2008).

It has been reported that **margarine supplemented with plant sterol** could save eight healthy years per 1000 people (Hoekstra et al., 2013a).

2.4.1.5 Studies on the positive and negative health effects associated with food consumption

Besides RBA studies, there were also 56 studies on the positive and negative health effects, which could potentially be used in RBA. A list of the main subjects of interest is provided below.

First, some foods or food components have been identified as ambivalent, i.e. food for which it is not straightforward to assess whether the risk is higher than the benefit or *vice versa*. Among them, it is worth mentioning: coffee (Butt and Sultan, 2011; Ranheim and Halvorsen, 2005; Richling and Habermeyer, 2014; Ruxton, 2009; Ruxton, 2008; Taylor and Demmig-Adams, 2007), tea (Gramza-Michalowska, 2014; Schwalfenberg et al., 2013), alcohol (Ellison, 2002; Foster and Marriott, 2006; Mukamal and Rimm, 2008; Thakker, 1998), broccoli (Latte et al., 2011), meat (Biesalski, 2005; McAfee et al., 2010), chocolate (Watson, 2013), phytoestrogen (AFSSA, 2005b; Wagner et al., 2001), isoflavone (Andres et al., 2011) and nitrite/nitrate (Milkowski et al., 2010; Tang et al., 2011).

Other issues related to food agricultural practices and food manufacturing practices have been pointed out (van Boekel et al., 2010): organic food production (AFSSA, 2003), use of pesticides (Harman, 1992; Seiber James and Ragsdale Nancy, 1999), use of genetically modified organisms (AFSSA, 2004; Amin et al., 2011; Arnst, 2000; Kramkowska et al., 2013; Purchase, 2003), the thermal process (Deutsche, 2007), irradiation of food (Acheson, 2001; Ekanem et al., 2005; GAO, 2000), use of artificial sweeteners (Bukhamseen and Novotny, 2014; Gardner, 2014; Tombek, 2010), use of antimicrobials (Ilg and Kreyenschmidt, 2012), red meat cooking practices (Berjia et al., 2014), food fortification (Brzozowska, 2001), the occurrence of the Maillard reaction (Somoza, 2005), milk treatment (Claeys et al., 2013; Neaves, 2000), etc.

Finally, RBA related to diets, such as the Mediterranean diet (Brief Critical Reviews - Mediterranean Diet and Coronary Heart Disease: Are Antioxidants Critical?, 1999; Grosso et al., 2014), a raw diet (Cunningham, 2004), vegetarianism (Dagnelie, 2003; Sabate, 2001), and baby food infant formulae or breastfeeding (de Zegher et al., 2013; Fewtrell et al., 2013; Frank and Newman, 1993; Goldman et al., 2007; Harris and Highland, 1979; Mead, 2008; Serreau et al., 2011), could be of interest.

2.4.2. Methodology of Risk-Benefit Assessment

Risk-benefit assessment (RBA) is an emerging discipline and its framework is still in progress. However, important works have been carried out by European scientists to develop the RBA approach.

The search identified 34 documents related to the RBA framework. Twelve of them dealt with the methodology step by step. Among them, four papers were published by safety agencies, the EFSA (EFSA, 2006; EFSA, 2010) and RIVM (Fransen et al., 2010; Hoekstra et al., 2008), four others by the European projects BRAFO (Boobis et al., 2013; Hoekstra et al., 2012) and QALIBRA (Hart et al., 2010; Hart et al., 2013) and four by scientific researchers (Palou et al., 2009; Renwick et al., 2008; Renwick et al., 2004; van der Voet et al., 2007). The European BEPRARIBEAN project (Verhagen et al., 2012b) also contributed to developing this framework through six‘ states of the art’ in risk-benefit analysis (Kalogeras et al., 2012; Luteijn et al., 2012; Magnússon et al., 2012; Pohjola et al., 2012; Tijhuis et al., 2012a; Ueland et al., 2012b), concluded in Tijhuis et al. (2012b). Fourteen other papers added information about the framework. The International Life Sciences Institute organized a session about the risk-benefit balance of food at the North America Annual Meeting in 2013; a presentation was made about the risk-benefit analysis of food (ILSI, 2013a), another about risk and benefit for chemical contaminants (ILSI, 2013b) and a third dealt with the risk-benefit assessment of nutrient intake (ILSI, 2013c). Two other European projects, BENERIS (BENERIS, 2011; Tuomisto, 2013) and Plantlibra (Larranaga-Guetaria, 2012), addressed this issue, two thesis (Berjia, 2013; Gradowska, 2013) were published, and other scientific researchers published articles (Burlingame and Pineiro, 2007; Gold et al., 2002; Pascal, 2009; Peleg et al., 2012; Sand, 2008; Verkerk, 2010) on more specific points of the framework.

The first work on RBA methodology was carried out by the EFSA in 2006 (EFSA, 2006) followed in 2010 by their recommendations on risk-benefit analysis methodology (EFSA, 2010). In parallel, the RIVM published a decision tree (Fransen et al., 2010). Then the BRAFO working group suggested an integrative approach (Hoekstra et al., 2012), applied its methodology to case studies (Schutte et al., 2012; Verhagen et al., 2012a; Watzl et al., 2012) and published a consensus document (Boobis et al., 2013).

Other works have contributed to the RBA framework development. For example, the QALIBRA project has provided online software (Hart et al., 2010) which enables a

quantitative comparison of risk and benefit to be made based on DALY (**Equation 2.1**) and Quality Adjusted Life Years (QALY). Within the BENERIS project, an information and exchange web-platform has been created (BENERIS, 2011). The BEPRARIBEAN project has enabled good practices to be established in risk-benefit analysis (Tijhuis et al., 2012b; Verhagen et al., 2012b) within various scientific fields: Medicine (Luteijn et al., 2012), Environmental Health (Pohjola et al., 2012), Food Microbiology (Magnússon et al., 2012), Economics and Marketing-Finance (Kalogeras et al., 2012), Consumer Perception (Ueland et al., 2012b), and Food and Nutrition (Tijhuis et al., 2012a).

The RBA methodology is based on the risk assessment framework (WHO, 2017) universally applied in the fields of microbiology and chemistry, but a risk-benefit comparison step is added. The RBA framework is described below in detail and summarized in **Figure 2.7**.

First, according to the papers investigated, there is a consensus to start the RBA by a preliminary step consisting of "0. Problem definition" (EFSA, 2010; Fransen et al., 2010; Hart et al., 2010; Hoekstra et al., 2012), in order to define the case study (a food, a food compound or a diet), the (sub)population targeted, and different scenarios of consumer exposure to be assessed (reference and alternative scenarios).

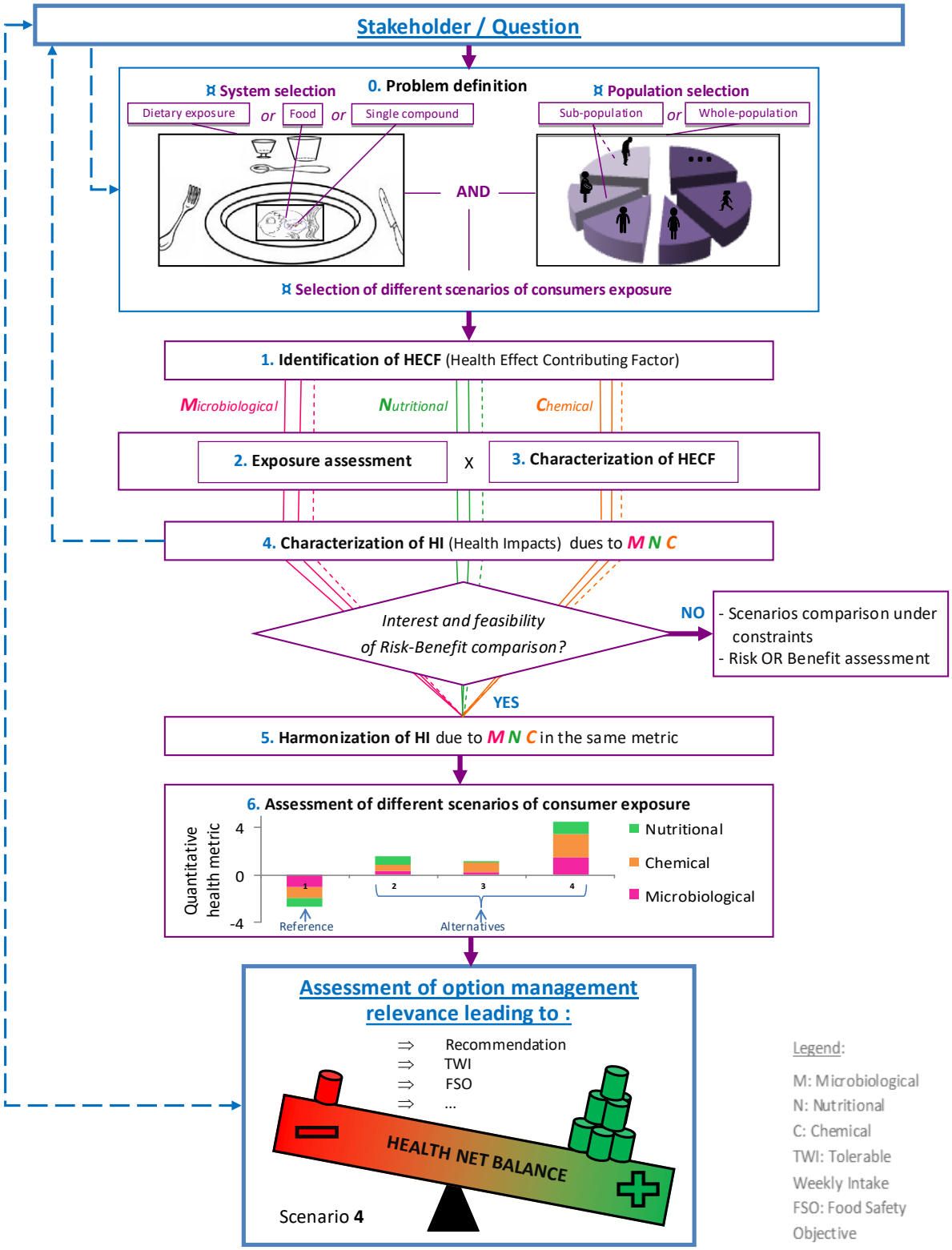

Figure 2.7: Summary of Risk-Benefit Assessment methodological framework based on different literature sources

Then, RBA mirrors a traditional risk assessment (EFSA, 2006; EFSA, 2010), which includes four steps: hazard identification, exposure assessment, hazard characterization and risk characterization (WHO). However, the terminologies used need to be adapted to integrate the benefit assessment. In fact, in a risk assessment, the term "hazard" is used to define a biological, chemical or physical agent able to cause an adverse health effect (FAO/WHO, 2008).The risk is thus "a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food" (IPCS, 2004). The term "benefit" is unanimously used to mirror the risk but we found different terms used to mirror the term "hazard": "positive effect" (Boobis et al., 2013; Hoekstra et al., 2012; Schutte et al., 2012; Verhagen et al., 2012a; Watzl et al., 2012), "benefit" (EFSA, 2006; Hoekstra et al., 2013a; Hoekstra et al., 2008),"positive health effect" (EFSA, 2010; Hellberg et al., 2012), "beneficial effect" (Tijhuis et al., 2012a), etc. Nevertheless, in the field of nutrition, the same agent could be a source of risk and benefit depending on the consumer exposure (Renwick et al., 2004). In this context, we propose to use a more general term to encompass the term hazard and its counterpart on the benefit side. We have named this term "Health Effect Contributing Factor" (HECF) and we define it as an agent able to cause an adverse or a positive health effect in the case of exposure. We chose this term because an HECF could be positive and negative, thus applicable in the nutrition field. In addition, as a positive or beneficial (health) effect is the consequence of a benefit and not its source, as a hazard is for a risk, the use of the term HECF can skirt this problem. In the same way, we have grouped together the terms risk and benefit under the expression "Health Impact" (HI), which we define as a function of the probability of an adverse or positive health effect and the severity of that effect, resulting from exposure to an HECF. A positive HI is a benefit and a negative HI is a risk. In this conceptual framework, a decrease in risk is considered a benefit and a decrease in benefit is considered a risk (**Figure 2.7**).

After defining the problem (step 0), risk and benefit are assessed in parallel in each field (nutrition, chemistry and microbiology) following the risk assessment steps. If we introduce the terminologies proposed above, we can name the next four steps as follows:

"1. Identification of HECF",

"2. Exposure assessment",

"3. Characterization of HECF" and

"4. Health impact characterization".

At any step, even if the assessment is qualitative or semi-quantitative, EFSA and BRAFO (EFSA, 2010; Hoekstra et al., 2012) advise stopping the assessment if risk outweighs benefit or *vice versa*. Yet, Berjia et al. (2012) illustrated in a cold-smoked salmon study that a quantitative comparison of risk and benefit could reverse the risk-benefit balance. However, due to a lack of data, a quantitative comparison is often not feasible. For these reasons, we suggest an alternative after step 4 (**Figure 2.7**). If the consumer is not exposed to both risk and benefit, there is no interest in performing a risk-benefit comparison, and the assessment is only performed from the risk side or from the benefit side. If the data available are too scarce to carry out a quantitative comparison, a comparison with a composite metric is not feasible but a comparison under constraints could be undertaken.

When the appropriate data are available and the risk-benefit comparison is of interest, a quantitative RBA can be performed. The assessment is extended to step "5. Harmonization of HI in the same metric" and then to step "6. Assessment of different scenarios of consumer exposure".

To move harmonization forward (step 5), there are still scientific bottlenecks. Indeed, risk assessment differs in each field because each has its own characteristics while the risk-benefit comparison aims to integrate all the results in the same metric. Performing a quantitative RBA is thus difficult due to the lack of a common unit to express the risk. Chemical risk assessment often expresses the risk as the probability of exceeding a threshold, or a safety reference value; microbiological risk assessment output is the probability of getting sick or dying from a disease; nutritional health assessment integrates two elements: deficiency or excess of a food component, and homeostasis (internal regulation to maintain a compound at a relatively constant concentration).

Finally, assessors report their conclusions to the decision-making managers who select the best scenario. At this stage, it is important to keep in mind that the best scenario is not necessarily the one corresponding to the best benefit-risk balance as the managers have to take other considerations into account, such as economic factors or food availability.

Two recent studies clearly illustrate how a quantitative RBA in the fields of microbiology/nutrition and chemistry/nutrition could be applied from step 0 to step 6 (**Figure 2.7**). They both carried out a full quantitative comparison of risks and benefits using DALY as a comparison criterion.

Berjia et al. (2012) were the first scientists to perform a quantitative RBA in the fields of microbiology and nutrition. They balanced the risk of listeriosis due to cold-smoked salmon consumption with the health benefit due to omega-3 intake. They concluded that a change in the consumption of smoked salmon from the reference scenario (women 23g/day and men 20g/day) to the alternative scenario (40g/day for adults) could save 9343 DALYs in the Danish population (5.57 million inhabitants), if the product was consumed before four weeks of storage. The sensitivity analysis highlighted that the net impact on health depends on the storage time of the product before consumption: from five weeks onwards, the net health impact is reversed and the overall effect is negative because of the increasing risk of listeriosis.

The second example of RBA was performed in the disciplines of chemistry and nutrition, which are currently those most explored. Hoekstra et al. (2013b) balanced the risk and benefit of fish consumption in Denmark. The net public health impact resulting from a change in the consumption of fish from 100g/day to 200g/day could save 2.7 DALYs per 1000 people.

2.5. Conclusion

The risk-benefit assessment discipline emerged at the beginning of the 21st century. RBA studies are intended to address various issues concerning the food supply-chain "from farm to fork". Although the first and most popular studies were related to fish consumption (48 of the 70 studies analyzed in this review), research has now diversified into a wider range of food categories such as fruits, vegetables and soy protein. The majority of RBA studies aimed to compare chemical risk with nutritional benefit (51 out of 70). The number of RBAs integrating components of nutrition, chemistry and microbiology was relatively low (3 out of 70); moreover, they were not fully quantitative but limited to a comparison under constraints (i.e. comparison of consumer exposure to reference safety levels).

Although the methodology is still in progress, these studies followed the same overall methodology based on the universal risk assessment framework (WHO) as advised by the EFSA (2010). Risks and benefits are first assessed independently and then compared with each other. This comparison can be made under constraints (46 out of 70 studies), based on health endpoints (15 out of 70) or using a composite metric such as DALY (9 out of 70). This latter metric is a practical tool to compare the effect of different diseases on health, integrating their severity and duration. To generalize further the use of a composite metric as a comparison criterion, the harmonization of scientific approaches needs to be enhanced; in particular, output risk (or benefit) assessment has to be expressed in a common unit.

To conclude, RBA is currently recognized as a scientific discipline with a wide range of applications. It is becoming a tool used in public health management, for instance in food recommendations on fish consumption (ANSES, 2013b; EFSA, 2005; FAO/WHO, 2010; VKM, 2006). It might be used in the future by food manufacturers as an aid in process and formulation design (Hoekstra et al., 2008; Schutte et al., 2012).

Table 2.1: Summary of the main results of risk-benefit assessment studies

Comparison based on*	Scientific domain**	Main results	Reference (First author, year)
• **Food component(s) is/are a source of risk(s) and benefit(s)**			
		Fish	
- Safety levels	**N/C**	Fish consumption is high in Portugal (≈57kg/year). Assessment of the three most consumed species demonstrated that its consumption should be limited to one serving/week of silver scabbardfish or three servings/week of hake or ray.	(Afonso et al., 2013b)
	N/C	A daily consumption of 160 g of fish muscle (6 species studied) does not expose consumers to risk and contributes to nutritional benefit. Consumption of liver should be avoided and a weekly consumption of L.whiffiagonis is recommended.	(Afonso et al., 2013a)
	N/C	Consumption of two portions of fish per week is recommended including one portion with a high content of EPA and DHA, but with changes in species and points of production (subgroup specifications are given).	(AFSSA, 2008)
	N/C/M	The ANSES (French Agency for Food, Environmental and Occupational Health & Safety) recommends that the general population consume 200 g/week of fish (including 100 g of fish with a high content of EPA and DHA). Specific recommendations are given for the sensitive subpopulation. It also advises specific hygiene measures.	(ANSES, 2013b)
	N/C	A list of intake recommendations is given for different subpopulations (infants, healthy adults, CHD patients and hyperglyceridemia patients) depending on fish and fish species to achieve the recommended weekly intake (RWI) without exceeding the tolerable weekly intake (TWI).	(Balshaw et al., 2012)
	N/C	Consuming fish two to three times a week decreases cardiovascular diseases, the risk of osteoporosis and fractures. Fish with up to 1 mg/kg methyl mercury should be limited to one serving per month. Pregnant or lactating women may consume one of the three weekly portions with a high omega 3 content.	(Becker et al., 2007)
	N/C	The assessment of fish consumption in eight European countries highlighted that the probability of being exposed to risk and benefit depends on the fish species. Countries with a low fish intake could be subject to small risk and benefit (Italy and the United Kingdom) or low risk but high benefit (Germany and the Netherlands) while high consumers are exposed to both (France, Spain, Portugal and Iceland).	(Cardoso et al., 2010)

	N/C	The Portuguese population exceeds the provisional tolerable weekly intake (PTWI) of methyl mercury regarding the consumption of hake, ray and silver scabbard fish without achieving the relative daily allowance (RDA) and the relative daily intake (RDI) of Selenium, EPA and DHA. They advise limiting the consumption of these three fish species to less than one meal/week.	(Cardoso et al., 2013)
	N/C	The Kahnawake community of south of the St Laurence river is not exposed to chemical risk due to fish consumption and fishing.	(Chan et al., 1999)
	N/C	Salmon and trout sold in Quebec can be regularly eaten to take advantage of nutritional benefit without exposing consumers to chemical risk (e.g. farmed Atlantic salmon can be consumed in one serving/day).	(Dewailly et al., 2007)
	N/C	43 fish species from Bermuda were analyzed and recommendations are given by subgroup. For example, women of childbearing age should not consume predatory fish while other subgroups should limit their consumption to one portion per week or month.	(Dewailly et al., 2008)
	N/C	In China, a consumption of 80 to 100 g/day of marine oily fish from the Chinese market is associated with potential nutritional benefit without exposing consumers to chemical risk.	(Du et al., 2012)
	N/C	No difference between wild and farmed fish has been identified. The advantage of farmed fish is that the contaminant level can be controlled and decreased by modification of fish feeding. A consumption of one to two portions/week is advised with restrictions for sensitive groups.	(EFSA, 2005)
	N/C	A daily consumption of Siberian grayling from Yenisei River provides the RDI of EPA but could exceed reference doses (RfD) of chromium. Concentration may vary according to month.	(Gladyshev et al., 2009)
	N	A curve of the balance of net benefit-harm is created with estimated thresholds. However, more data are required to estimate thresholds and asymptotes using this curve.	(Gochfeld and Burger, 2005)
	N/C	Wild salmon have significantly fewer chemical contaminants than farmed salmon and a higher EPA content. Farmed salmon from Europe contains a higher level of chemical contaminants than those from South and North America and a similar EPA content.	(Hites et al., 2004)
	N/C	In Canada, 35% of the Inuit population is exposed to chemical risk due to consuming fish contaminated by methyl mercury. To decrease this risk and keep the benefit, the consumption of ringed seal liver could be replaced by ringed seal meat, ringed seal blubber, beluga mukluk or Arctic char, for example.	(Laird et al., 2013)
	N/C	The Portuguese adult consumption of black scabbard fish should be limited to 90 g grilled meat and 120 g of fried meat. Edible crab brown meat should not exceed 27 g boiled meat per week and its consumption should be avoided by children and lactating or pregnant women.	(Maulvault et al., 2013)
	N/C/M	A consumption of 270 g to 340 g/week of fish is advised. Children under 12 years old and pregnant and lactating women should limit tuna consumption to150 g/week and avoid predatory fish. Other subgroups can consume more fish but they should change fish (and seafood) species. There is additional benefit by including seafood high in EPA and DHA. Microbial risk could be limited by hygiene practices during handling and cooking.	(Nesheim and Yaktine, 2007)

	N/C	Consumption of two portions/week, including one oily fish, decreases CVD risk and improves fetal development. Pregnant and lactating women should select certain fish.	(SACN/COT, 2004)
	N/C	Fish consumption has been assessed as safe in the State of Michigan. A list of the top 11 fish was established to increase benefits.	(Sidhu, 2003)
	N/C	Consumption of 181-213 g/week of certain fatty fish species and 26-72 g/week of lean fish or shellfish provides a good risk-benefit balance.	(Sirot, 2010)
	N/C	A consumption of 200 g/week of selected fatty fish and 50 g/week of lean fish maximizes benefit and minimizes risk.	(Sirot et al., 2012)
	N/C	Consumption of canned fish from the Polish market presents higher benefit than risk. Limitation depends on fish species.	(Usydus et al., 2008)
	N/C	Fish products from the Polish market vary greatly in terms of potential beneficial and adverse health effects; recommendation of quantity depends on species.	(Usydus et al., 2009)
	N/C	Consuming fish from Taihu Lake to achieve RDA of EPA and DHA does not expose consumers to chemical risk (PCBs and PBDEs).	(Zhang et al., 2012a)
	N/C	The risk-benefit ratio has been assessed for four fish species from Taihu Lake in China and for three muscles (dorsal, ventral and tail) and three viscera (heart, liver and kidney). The current Chinese fish consumption does not present a risk, except for ventral and tail consumption of top mouth cutler that should be avoided.	(Zhang et al., 2012b)]
	N/C	It is recommended that the Norwegian population increase their fish consumption to achieve two meals of fatty fish per week.	(VKM, 2006)
	N/C	Consumption of 200 g/week of farmed salmon decreases CHD incidence and increases contaminant intake but still below the PTWI.	(Watzl et al., 2012)
- Endpoint	**N/C**	Fish consumption (from one to twelve servings per week) decreases the relative risk (RR) of stroke compared with the scenario of no consumption.	(Bouzan et al., 2005)
	N/C	In Hong Kong, moderate fish consumption by pregnant women is a source of benefit for the IQ of their children with a gain of 0.79 to 5.7 points if they vary the species.	(Chen et al., 2014)
	N/C	A consumption by pregnant women of one to seven servings/week of fish (depending on fish species) decreases CHD and increases the future newborn IQ. Details are given for each subgroup and as a function of fish species.	(FAO/WHO, 2010)
	N/C	Current US fish consumption prevents 30000 deaths per year from CHD and 20000 deaths per year from stroke. Women of childbearing age should increase their fish consumption to 340 g/week to improve fetal neurodevelopment.	(FDA, 2009)

	N/C	RDI of EPA and DHA could not be achieved through farmed or wild salmon consumption without exposing consumers to carcinogenic risk. Intake recommendations are given depending on fish market location.	(Foran et al., 2005)
	N/C	The IQ gained by children during their mother's pregnancy is positive with a consumption of 175 g/week and 450 g/week of 30 fish species from Zhoushan in China; optimal weekly consumption is given for every species. Consumption of Scoliodon sorrakowah is not recommended.	(Gao et al., 2014)
	N/C	Risk and benefit due to fish consumption are assessed to optimize newborn visual recognition memory (VRM) and limit CHD. A table of intake recommendations depending on species is provided.	(Ginsberg and Toal, 2009)
	N/C	To ensure their child's IQ is more than 100 points, Finnish pregnant women should reduce their consumption of vendace by 13%, white fish by 18%, perch by 31%, and pike by 90% and increase their intake of Atlantic salmon by 2% and Baltic herring by 4%.	(Gradowska, 2013)
	N/C	A small increase in fish consumption decreases CHD mortality risk by 17% and non-fatal heart disease risk by 27%.	(König et al., 2005)
	N/C	Current fish consumption by Finnish pregnant women generates compensation in effects on infant's IQ. Fatty fish consumption creates a gain in IQ and lean fish consumption an adverse IQ effect.	(Leino et al., 2013)
	N/C	Salmon consumption presents more health benefit than risk. However, the risk-benefit balance of Arctic grayling, pike, sablefish and halibut can not be assessed because data depend on regions and studies.	(Loring et al., 2010)
	N/C	Consumption of one to two servings/week reduces CHD risk by 36% and total mortality rate by 17%. Women of childbearing age, pregnant or lactating should consume two servings/week with species restrictions.	(Mozaffarian and Rimm, 2006)
	N/C	Women's fish intake during pregnancy causes a decrease in newborn IQ for most species consumed. Risk clearly outweighs benefit (until 11 IQ points lost with swordfish), and only a few species slightly improve the IQ (+1 point for mackerel).	(Zeilmaker et al., 2013)
- DALY/QALY	**N/M**	Consumption of 40 g/day of cold-smoked salmon by the Danish population could improve population health with a potential gain of 10000 healthy years annually if the product is consumed before 4 weeks of storage.	(Berjia et al., 2012)
	N/C	In US, an increase of 50% in fish consumption by the adult population, except women of childbearing age, could save 120000 healthy years annually.	(Cohen et al., 2005)
	N/C	In France, a higher fish intake (1104 g/week) than the current consumption (334 g/week) could save between 97 and 285 healthy years based on the French study CALIPSO on 1011 people.	(Guevel et al., 2008)
	N/C	The Dutch population could improve their health with a consumption of 200 g of fish/week. On average, 2.7 healthy years per 1000 people could be gained every year compared to the current consumption.	(Hoekstra et al., 2013b)

	N/C	In Washington state, adult consumption of fish has net beneficial effects on health with a gain of approximately 5000 healthy years saved per year per 100000 people but the net health balance is negative for women of childbearing age.	(Ponce et al., 2000)
		Fruits and vegetables	
- Safety levels	**N**	Overall, consumption of 400 g of vegetable per day is a source of beneficial effects and does not expose consumers to a relevant risk due to nitrate intake.	(EFSA, 2008)
- Endpoint	**N/C**	An increase of one serving of vegetable and one of fruit per day could prevent 20000 cancer cases and create 10 cases due to pesticide consumption.	(Reiss et al., 2012)
		Soy protein	
- Safety levels	**N**	With a consumption of 25 g/day of soy protein, beneficial effects clearly outweigh the potential risk: reduction of CVD, breast and prostate cancer risk.	(Watzl et al., 2012)
		Trans fatty acids	
- Safety levels	**N**	The substitution of 5% of the energy intake from saturated fatty acids by 5% from carbohydrates brings beneficial and adverse health effects related to the same disease (CVD).	(Watzl et al., 2012)
	N	A consumption of more than 2% of trans fatty acids within the total energy food intake improves CHD risk. A suggestion of an UL of 1% of trans fatty acids within the total energy food intake and a mention of %trans fatty acids of total fatty acids on food labeling is made.	(AFSSA, 2005b)
• The manufacturing process is a source of risk(s) and benefit(s)			
		Milk treatment	
- Safety levels	**N/M**	Microbial benefit (reduction of microorganisms) from heat treatment outweighs potential risk due to the reduction of lysine and the inactivation of bioactive molecules.	(Schutte et al., 2012)
		Water treatment	
- DALY/QALY	**C/M**	Water treatment by ozonation decreases Cryptosporidium parvum infection but introduces chemical risk due to bromate. The overall health effect is a gain of one healthy year per million people annually.	(Havelaar et al., 2003)
		Vegetable transformation	
- Safety levels	**N/M**	The green bean process could be optimized to achieve the RDA without exceeding a microbial threshold of *G.stearothermophilus* by reducing waiting times and blanching duration and by increasing the sterilizing value or by decreasing the pH of the end product.	(Rigaux, 2013)

		Cookie process	
- Safety levels	**N/C**	Heat processing of cookies produces harmful compounds and modifies antioxidant activity depending on time, temperature, sugar and leavening agents. The risk-benefit ratio on compound quantity is lower at low temperature and small duration but the impact on health is not quantified.	(Morales et al., 2009)
		Fish culinary treatment	
- Safety levels	**N/C**	The comparison of three fish cooking practices (boiling, grilling and roasting) has demonstrated that grilling fish is the best fish treatment to optimize nutritional benefit and limit chemical risk with a limitation of two meals/week.	(Costa et al., 2013)
		Acrylamide formation	
- Safety levels	**N/C**	The use of sodium bicarbonate to bake products should reduce acrylamide concentration but it could cause a nutritional loss and generate other unknown molecules.	(Seal et al., 2008)
	N/C	Reduction of acrylamide in potato and cereal-based products through measures applied in production is desirable.	(Schutte et al., 2012)
		Benzo(a)pyrene formation	
- Safety levels	**C**	The use of artificial smoked flavor or industrial smoking control is beneficial to reduce the risk of benzo(a)pyrene.	(Schutte et al., 2012)
• Diet is a source of risk(s) and benefit(s)			
		Breastfeeding	
- Safety levels	**N/C/M**	Benefit associated with breastfeeding outweighs risks due to contaminants and contributes to an efficient neurodevelopment, the creation of defense against infection and the reduction of obesity risk.	(VKM, 2013)
		Replacement of sugar by intense sweetener	
- Safety levels	**N/C**	Substitution of sugar by intense sweeteners in beverages decreases sugar consumption (too high for adolescents) but acesulfame K intake becomes close to the acceptable daily intake (ADI) and benzoic acid ADI could be exceeded.	(Husoy et al., 2008)
	N/C	The substitution of sugars by low calorie sweeteners in beverages is associated with benefit: it limits caries risk, prevents overweight and chronic disease risk.	(Verhagen et al., 2012a)
	N/C	For young adults in the Netherlands, the substitution of 100% sugar by intense sweeteners in beverages is beneficial in caries prevention and body mass reduction and does not expose this population to potential risk.	(Hendriksen et al., 2011)

		Individual assessment of risk and benefit exposure	
- Safety levels	**N/C**	RIBEPEIX is software to assess risk-benefit associated with individual fish consumption according to chemical and nutritional safety reference values.	(Domingo et al., 2007a; Domingo et al., 2007b)
	N/C	RIBEFOOD is an application available online to assess individual overall diet according to safety reference values. The software guides consumers to find food substitution to improve their risk-benefit balance.	(Marti-Cid et al., 2008)
• RBA is used in food formulation			
		Margarine fortification	
- DALY/QALY	**N**	Margarine fortification with plant sterol in the Netherlands should save 8 healthy years annually per 1000 people.	(Hoekstra et al., 2013a)
		Bread supplementation	
- DALY/QALY	**N**	In the Netherlands, bread fortified with 140 µg/100 g folic acid should result in 11812 healthy years saved annually.	(Hoekstra et al., 2008)
	N	In the Netherlands, a small bread fortification of 70 µg/100 g folic acid should result in 7000 healthy years saved every year with a loss of 53 healthy years.	(Verhagen et al., 2012a)

* Risk-benefit comparisons are sorted into three groups, 'safety levels', 'endpoint' and 'DALY/QALY' which are explained in section 2.4.1.3
** N: Nutrition, C: Chemistry, M: Microbiology

CHAPTER 3

Review
Risks and Benefits of Infant Milk-Based Diet

Redraft from:

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CHAPTER 3: **Review of Risks and Benefits of Infant Milk-Based Diet**

Infant milk based-diet, including breast milk and infant formula, was selected as a case study to develop further the methodology of Risk-Benefit Assessment (RBA) in Foods. Therefore, before performing the RBA, potential risks and benefits of both infant milk diets were reviewed to identify all safety issues connected with this case study, with regard to microbiology, chemistry and nutrition. Moreover, current scientific advances in RBA dedicated to infant milk based-diet were summarysed and needs of methodological development were identified.

Objectives of the chapter:

- Sum up the current legislation and nutritional requirements,
- Identify adverse and beneficial health effects of infant milk with regard to microbiology, chemistry and nutrition,
- Summarize current risk-benefit assessment of infant milk.

3.1. Abstract

The safety and quality of infant milk, whether it is breast milk (BM) or infant formula (IF), are a major concern for parents and public health authorities. BM is recommended as the gold standard at WHO level. However, nowadays IF appears as an essential alternative in Western countries, challenging producers to optimise nutritional quality and safety of IF. The aim of the present paper is to give an overview on the assessment and comparison of risks and benefits associated with BM and IF consumption. To date, this intensively debated subject has been mainly investigated. It has been shown that both diets could be sources of beneficial health effects in terms of nutrition and also risks in

terms of chemical safety. Moreover, microbiologists have demonstrated that IF consumption can cause illness due to product contamination or inappropriate milk preparation. The paper concludes on the bottlenecks and gaps which should be investigated to further progress the quantification of the impact of early diet on infant health. Performing a multi-disciplinary risk-benefit assessment with DALY as endpoint, might be a future option to help prioritise management options.

3.2. Introduction

The first months of an infant's life are crucial for short and long term healthy physiological development (Horta et al., 2007; Horta and Victora, 2013). During this critical period baby size doubles and total brain weight triples (ANSES, 2014). To develop and thrive, infants have basic nutritional requirements which can be satisfied by consuming breast milk (BM) and/or infant formula (IF). The debate on the choice of "breast or bottle" (Wolf, 2013) has been ongoing for decades, and involves not only scientific aspects, but also societal, economical, personal/individual, if not ideological or spiritual/religious issues.

Breastfeeding is widely considered as the best adapted food for infant needs and is acknowledged to have beneficial health effects (Hörnell et al., 2013). However, nowadays the majority of infants in Western countries are formula fed by virtue of their parents' choice or due to medical circumstances. Indeed, about 2% of mothers are physiologically not able to breastfeed (Brown, 2015).

The World Health Organization (WHO) has defined different infant feeding diets (2008):

- *Exclusive breastfeeding:* infants are fed with breast milk but not with non-human milk such as formula, they might receive oral rehydration solution (ORS), drops and syrups
- *Predominant breastfeeding:* infant nourishment is predominantly composed of breast milk and certain liquids such as water, water-based drinks and fruit juice, and can also contain ORS, drops and syrups, but no milk formula

- *Partial breastfeeding or complementary feeding:* infant are fed with breast milk and other foods such as formula milk
- *Bottle feeding:* includes any food, liquid or semi-solid food, consumed with a bottle and nipple

WHO recommends to exclusively breastfeed infants under six months of age, i.e. that infants only consume BM without additional food or drink, not even water. Nevertheless, at the worldwide scale only 40% of infants are exclusively breastfed until six months of age (WHO, 2014a), however there is high variability among countries (**Figure 3.1**). In Europe, the exclusive breastfeeding rate at six months is rather low with an average of 18% (WCRF, 2009), similar to the USA rate (National Research Council, 2004). More precisely, this rate is not constant during an infant's first months of life. For instance, in France, 74% of newborns are breastfed at birth (Salanave et al., 2014), but after 48 hours, the exclusive breastfeeding rate decreases to 55.4% (Inserm, 2008) and continues to decline: 28%, 10% and 0.5% after one, three and six months, respectively (Salanave et al., 2014).

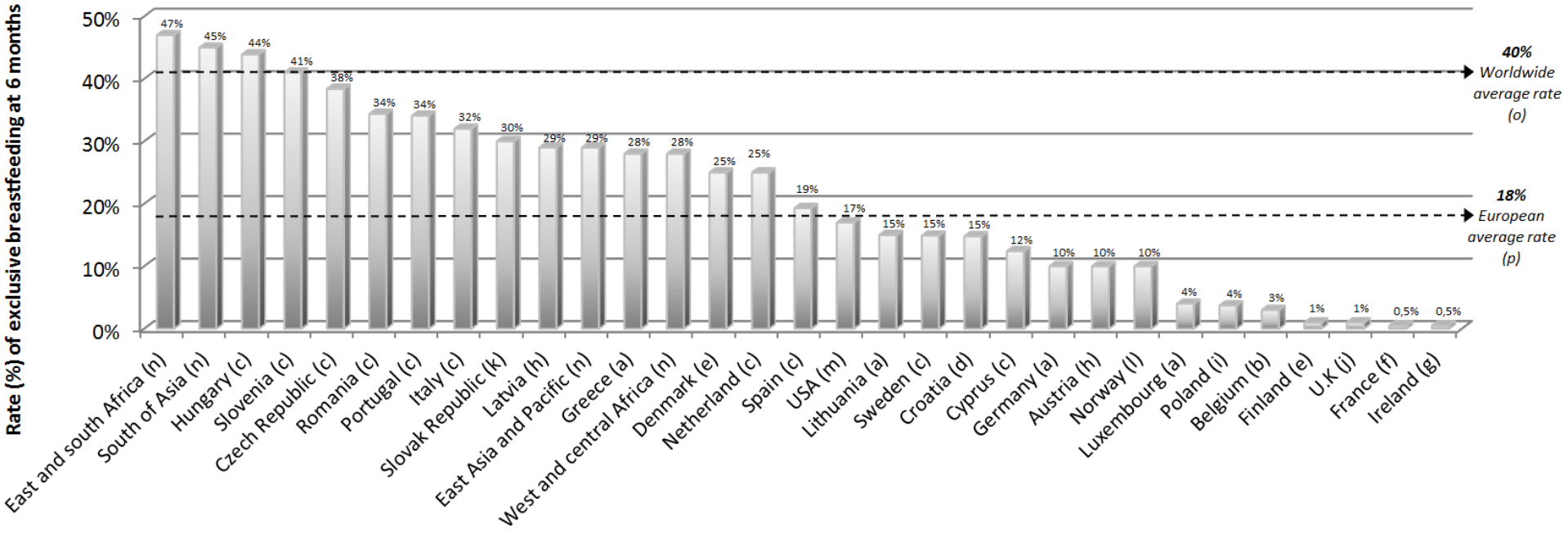

Figure 3.1: Worldwide exclusive breastfeeding rates at 6 months

Rates were extracted from different sources: a (IPA, 2003), b (Robert et al., 2014), c (OECD, 2014), d (IBFAN, 2014), e (Elmadfa, 2009), f (Salanave et al., 2014), g (Black, 2012), h (WHO, 2014b), i (Magdalena, 2013), j (HSCIC, 2012), k (Cattaneo et al., 2005), l (VKM, 2013), m (National Research Council, 2004), n (Cai et al., 2012) , o (WHO, 2014a) and p (WCRF, 2009).

Infants are more sensitive to infections during the first few months of life because their immune system is under developed and even though breastfed infants receive antibodies through breast milk they remain a particular at risk population. Sanitary issues have occurred in the last few decades due to the consumption of powder infant milk contaminated by *Cronobacter sakazakii*, causing a dozen of illnesses, with some fatalities (European Commission, 2015b). More recently, in China a scandal occurred regarding melamine adulteration that caused about 300,000 clinical cases, including six deaths (Gossner et al., 2009). Furthermore, infant health might be influenced by the consumption of milk contaminated by chemicals, whether it be BM or IF. BM might contain chemical contaminants due to a mother's exposure through food consumption, dermal contact or inhalation (e.g. persistent organic pollutants like PCBs). IF is also subject to chemical contamination due to cow's milk or even during powder processing. It could be also contaminated during the milk preparation by for instance the addition of tap water or the use of unappropriated materials (e.g. bottle containing bisphenols or phthalates).

Consequently, for years, breast milk and infant formula have captured public and scientific attention regarding, on the one hand, the BM diet and the balance of beneficial health effects with potential adverse effects due to chemical contaminants; and on the other hand, the IF diet and the assessment of its potential chemical and microbiological risks. In this context, the present review aims to i) sum up the current legislation and nutritional requirements, ii) give an overview of adverse and beneficial health effects of both diets with regard to microbiology, chemistry and nutrition fields; and then iii) summarise current advances in the risk-benefit assessment of infant milk, with a specific focus on infants from birth to six months of age from European countries.

3.3. Infant milk: current lesgislation and nutritional requirements

Infant diets have historically evolved over time and can also be differentially examined across various cultural and/or anthropological issues. The first infant food substitute was developed and commercialized in the 1860s. The Codex Alimentarius Commission (CAC) has defined the IF as a "breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding" (1981).

3.3.1. Definitions of different infant diets

Infants and young children's nutritional requirements vary from zero to three years. As described in **Figure 3.2**, infants can consume different kinds of milk: BM or IF. From a regulatory point of view, there are different formula intended for three different age categories: infants from zero to six months (also called starter or IF), from six months to one year (also called follow-on formula) and from one year to three years of age (also called growing-up formula). Additionally, the food transition that corresponds to the progressive introduction of solid food in the diet is advised to start from the age of six months by WHO (2004) and between four and six months in Europe (EFSA, 2009). This review specifically focuses on IF and BM consumed by infants from zero to six months of age, without integrating the potential consumption of solid food.

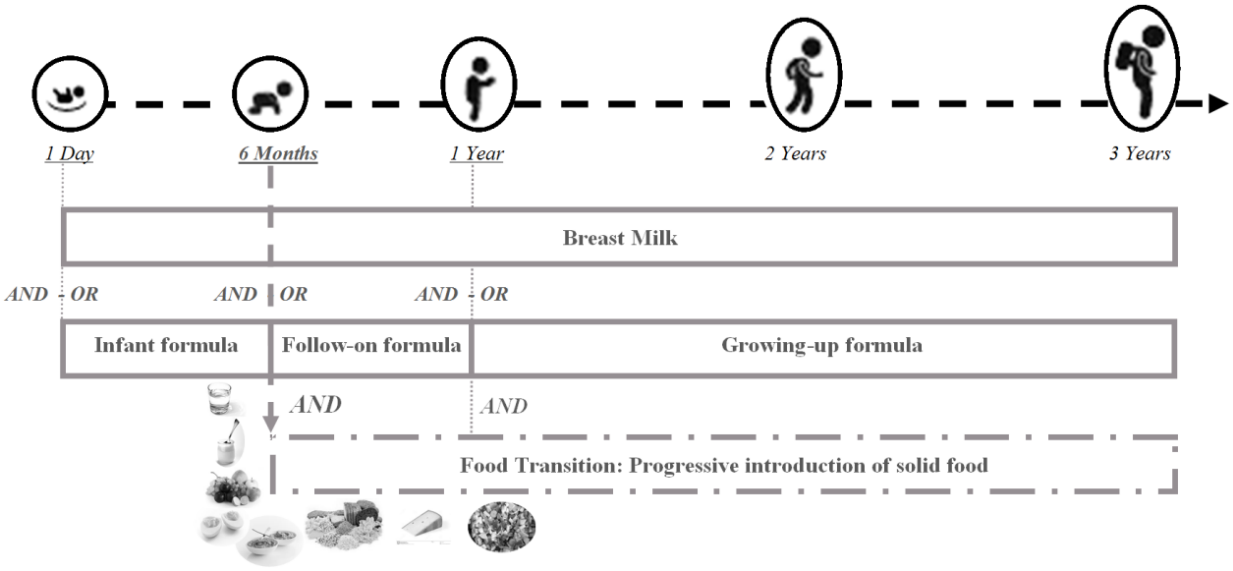

Figure 3.2: Infant food diet from 0 to 3 years (Based on French nutritional recommendations (Inpes, 2004))

3.3.2. European infant formula legislation

In Europe, IF composition has evolved over years according to the scientific progress and discoveries (National Research Council, 2004). Nowadays, even if the main ingredients are strictly regulated, there is a lot of different IF brands available on the market offering a large range of products with different protein and fat sources and novel ingredients intending to offer beneficial health effects (Tijhuis et al., 2014). For example in France in 2012 there were about 300 IF recorded (AFPA, 2012). However, the industrial's margin is thin regarding composition and safety of IF that is regulated. Indeed, at the European level, food safety is governed by the General Food Law (European Commission, 2002) and regulated "from farm to fork", i.e. including feed and primary production, food processing, storage, transport and retail sale. The laws regulating IF composition and safety, with microbiological, chemical and nutritional criteria, are presented in **Table 3.1**. The laws are based on the assessment made by the European Food Safety Authority (EFSA), the independent agency in charge of the risk assessment of food and feed. EFSA has recently published an opinion paper on IF (EFSA, 2014) updating the evaluation made by the Scientific Committee on Food (2003). The EFSA opinion (2014) suggests new reference values for nutrients (**Table 3.2**), which can be expected to be implemented at the legislation level in the near future (European Commission, 2015a). The Member States of the European Union then implement the European legislation, at their national level.

Table 3.1: Synthesis of European laws regulating infant formula composition and safety indicators

Field	Main point regulated					Legislation
Chemistry	Maximum levels for certain contaminants in infant formula: Pesticides 0.01 mg/kg for residues of individual pesticide [1] Nitrate 200 mg No_3/kg [2] Aflatoxins 0.025 µg/kg [2] Ochratoxin A 0.50 µg/kg [2] Lead 0.020 mg/kg wet weight [2] Inorganic tin 50 mg/kg wet weight [2] Benzoapyrene 1 µg/kg wet weight [2] Melamine 1 mg/kg [3] Materials in contact with foodstuffs [4,5,6]					Directive EC 2006/141[1] Regulation EC 1881/2006 [2] Regulation EC 594/2012 [3] Regulation EC 1935/2004[4] Regulation EC 10/2011[5] Regulation EC 202/2014[6]
Microbiology	Product criteria:	n*	c*	m*	M*	
	Listeria monocytogenes [7]	10	0	Abs in 25g		
	Salmonella [7]	30	0	Abs in 25g		Regulation EC 2073/2005[7]
	Cronobacter spp.[8,9]	30	0	Abs in 10g		Regulation EC 1441/2007[8]
	Enterobacteriaceae [7]	10	0	Abs in 10g		Regulation EC 365/2010[9]
	Bacillus cereus (presumption) [9]	5	1	50ufc/g	500ufc/g	
Nutrition	Composition of infant formula, minimum and maximum energy and content in: proteins, lipids, carbohydrates, mineral substances, vitamins, etc. [1,10, 11, 12] *NB: Minimum and maximum levels of nutrients are given in* ***Table 3.2****.*					Directive EC 2006/141[1] Regulation EC 1609/2006[10] Regulation EC 1243/2008[11] Directive EC 2013/46[12]

**n= number of units forming the sample; c = number of sample units giving values over m or between m and M*

References of the table: [1](European Commission, 2006a), [2](European Commission, 2006b), [3](European Commission, 2012), [4](European Commission, 2004), [5](European Commission, 2011), [6](European Commission, 2014), [7](European Commission, 2005), [8](European Commission, 2007), [9](European Commission, 2010), [10](European Commission, 2006a), [11](European Commission, 2008), [12](European Commission, 2013).

Table 3.2: Energy and nutrient requirements of breastfed and formula fed infants, based on (EFSA, 2014)

	Infant formula cow's milk based reconstituted as instructed by manufacturer		**Breast Milk**[1] Based on European data reported by EFSA (2014)	***Function*** Based on (EFSA, 2014)
	Current legislation (European Commission, 2006a)	Based on EFSA's opinion (2014)		
Energy values				
Total energy content	60 - 70 kcal/100 mL		66 ± 12 kcal/100 mL	- Needed to maintain body mass and composition and compensate growth, development and physical activity (varies among months and gender)
% Lipids energy	40 – 55 E%	39.6 – 54 E%	50 E%	
% Carbohydrates	40 – 45 E%	36 - 53.2 E%	33 – 42 E%	
% Proteins	/	7.2 – 10 E%	9 – 10 E%	
Nutrients values				
Protein*	1.8 – 3.0 g per 100 kcal	1.8 – 2.5 g per 100 kcal	1.2 – 3.2 g per 100 kcal	- Essential for normal growth for building, maintaining and repairing tissues, manufacturing enzymes, hormones, and used for energy in case of insufficiency
Fat	4.4 – 6.0 g per 100 kcal		3.7 – 9.1 g per 100 kcal	- Provide energy - Facilitate absorption of the fat-soluble vitamins A, D, E and K - Supply essential fatty acids required for normal development (brain development, healthy skin and hair, normal eye development and resistance to infection and disease): - MCFAs would increase fat absorption - LA is incorporated into skin ceramides for maintaining water permeability barrier of skin, avoid excessive trans-epidermal water loss, escort energy loss from evaporation - ALA is essential as precursor for n-3 LCPUFAs and improves retinal function in preterm infants - DHA is accumulated in large amount in the brain during first two years of life Effects on neuronal cell growth, rhodopsin function and levels of neurotransmitters Potential effects on neurodevelopment including neurological and brain function, cognition, visual function, motor skills, temperament and mental health
Trans-fatty acids	Max 3 FA%	Max 3 FA%	2 – 5 FA%	
Lauric + mystiric acids	Max 20 FA%			
Erucic acid	Max 1 FA%			
LA (18:2, n-6)	0.3 – 1.2 g per 100 kcal	0.5 – 1.2 g per 100 kcal	10 15 FA%	
ALA (18:3, n-3)	Min 0.05 per 100 kcal	0.05 – 1 g per 100 kcal	0.1 - 2.0 FA%	
Ratio LA/ALA	5 – 15			
Total n-3 LCPUFAs[2]	1 FA%			
Total n-6 LCPUFAs[2]	2 FA%			
ARA (20:4, n-6)[2]	1 FA%		0.7 - 1.1 FA%	
DHA (20:6, n-3)[2]	< total n-6 LCPUFAs	0.02 - 0.05 g per 100 kcal	0.2 - 0.5 FA%	

EPA (20:5, n-3) [2]	< DHA	< DHA		Wide variation in studies performance measure and health effects that do not allow to demonstrate a biologically effect of DHA with a convincing evidence EFSA support IF supplementation with DHA because it remains an essential structural component of the nervous tissue and the retina, it is involved in normal brain and visual development
Phospholipids[2]	2g/L			
CLA	Not permitted		0.2 – 0.6 FA%	
Carbohydrate	9 – 14 g per 100 kcal		8.2 – 10.4 g per 100 kcal	
Lactose	Min 4.5 g per 100 kcal except for "lactose-free" formula that should not exceed 0.01 g per 100 kcal			
Sucralose	Not to be added			- Used for building tissues by protein and contributing to the use of fat for "building blocks" for body components (USDA, 2009)
Fructose	Not to be added			
Glucose	Not to be added			
Maltose, maltodextrins[2]	No restriction except ≤ total carbohydrates			
Starches[2]	Max 2 g/100mL and ≤ 30% total carbohydrates			
FOS + GOS[2]	Max 0.8 g/100mL		No necessity of addition	
Minerals and trace elements				
Calcium	50 – 140 mg/100 kcal	Target 50 mg/100 kcal	31 – 46 mg/100 kcal	- Structural function as a component of the skeleton, needed for bone rigidity, strength and elasticity
Phosphorus	25 – 90 mg/100 kcal	Target 25 mg/100 kcal	28 ± 2.4 mg/100 kcal	- Act in calcium-regulating hormone and essential for numerous body functions in ion phosphate form
Ratio calcium/phosphorus	1 - 2		2	- Adequate ration needed for skeletal maintenance and growth and many cellular roles (e.g. energy production)
Magnesium	5 - 15 mg/100 kcal	Target 5 mg/100 kcal	2.3 – 9.8 mg/100 kcal	- Critical cofactor in enzyme reactions (second most abundant intracellular cation)
Sodium	20 – 60 mg/100 kcal	Target 25 mg/100 kcal	22 – 25 mg/100 kcal	- Concentration of sodium and potassium control cell membrane potentials in cell essential in neural transmission, muscle contraction, vascular tone and drive active transport for nutrients
Potassium	60 – 160 mg/100 kcal	Target 80 mg/100 kcal	80 mg/100 kcal	
Chloride	50 – 160 mg/100 kcal	Target 60 mg/100 kcal	400 mg/L	- Most abundant anion in extracellular fluid - Counterbalance intracellular negative charges provided by proteins and constituent of hydrochloric acid excreted in the gastric juice

Iron	0.3 – 1.3 mg/100 kcal	0.3 – 0.6 mg/100 kcal	0.03 – 0.06 mg/100 kcal	- Play a role in the oxygen transporting haemoglobin and myoglobin and in enzyme of metabolic pathways in the liver, brain and endocrine organs
Zinc	0.5 – 1.5 mg/100 kcal	Target 0.5 mg/100 kcal	4.11 (<1 month) up to 0.77 mg/L (>6 months)	- Involved in cell metabolism, immune function, protein synthesis, would healing, DNA synthesis and cell division
Copper	35 – 100 µg/100 kcal	Target 60 µg/100 kcal	51 - 60 µg/100 kcal	- Essential nutrient and cofactor for many proteins (e.g. enzymes involved in production of collagen and pigment, iron metabolism, immune system, heart and brain functions
Selenium	1 – 9 µg/100 kcal	Target 3 µg/100 kcal	0.46 – 12.9 µg/100 kcal	- Indispensable constituent of seleno-proteins mainly involved in redox reactions
Iodine	10 – 50 µg/100 kcal	Target 15 µg/100 kcal	5 – 15 µg/100 kcal	- Play a role in thyroid gland functioning
Chronium	Not regulated	No necessity	0.03 – 1.7 µg/100 kcal	- No convincing evidence to consider as essential nutrient
Molybdenum	Not regulated	Target 0.4 µg/100 kcal	0.72 – 0.4 µg/100 kcal	- Required as a cofactor
Manganese	1 – 100 µg/100 kcal	Target 1 µg/100 kcal	0.46 – 4.6 µg/100 kcal	- Essential dietary mineral, component of metalloenzymes
Fluoride	Max 100 µg/100 kcal	No necessity	Non detectable – 15.4 µg/100 kcal	- Decrease the risk of carries development
Vitamins				
Vitamine A	60 – 180 µg RE/100 kcal	Target 70 µg RE/100 kcal	13 µg RE/100 kcal	- Play a role in vision, maintenance of epithelial surfaces, immune competence, growth, development and reproduction
Vitamine D	1 – 2.5 µg/100 kcal	Target 2 µg/100 kcal	0.04 – 0.31 µg/100 kcal	- Key role in calcium and phosphate metabolism and essential for bone health
Vitamine E	0.5 – 5 mg α-TE/100 kcal	Target 0.6 mg α-TE/100 kcal	0.54 mg α-TE/100 kcal	- Antioxidant activity and contribute to the prevention of propagation of free radicals in various lipid structure
Vitamine K	4 – 25 µg /100 kcal	Target 1 µg/100 kcal	0.13 – 1.4 µg/100 kcal	- Needed for synthesis of various factors and proteins involved in blood coagulation
Thiamin (Vit B1)	60 – 300 µg/100 kcal	Target 40 µg/100 kcal	23 – 51 µg/100 kcal	- Coenzyme in its phosphorylated forms
Riboflavin (Vit B2)	80 – 400 µg/100 kcal	Target 60 µg/100 kcal	54 – 92 µg/100 kcal	- Precursor of two coenzymes involved in many biochemical reactions
Niacin	0.3 – 1.5 mg/100 kcal	Target 0.4 mg/100 kcal	1.8 – 2.2 mg/L	- Precursor of coenzymes that are crucial for many oxidation/reduction reactions and in catabolic and anabolic processes
Pantothenic acid	0.4 – 2 mg/100 kcal	Target 0.4 mg/100 kcal	0.38 mg /100 kcal	- Central role in a wide variety of metabolic pathways

Vitamin B6	35 – 175 µg/100 kcal	Target 20 µg/100 kcal	20 µg/100 kcal	- Play a role in metabolic reactions
Biotin	1.5 – 7.5 µg/100 kcal	1 µg/100 kcal	0.8 µg/100 kcal	- Cofactor of enzyme acethyl-CoA involved in the synthesis like fatty acids
Folate	10 -50 µg/100 kcal	Target 15 µg DFE/100 kcal	12.3 µg/100 kcal	- Essential for carbon transfer reactions
Cobalamin (Vit B12)	0.1-0.5 µg/100 kcal	Target 0.1 µg/100 kcal	/	- Required as a coenzyme
Vitamine C	10 – 30 mg /100 kcal	Target 4 mg/100 kcal	5.4 – 13.8 mg /100 kcal	Involved in biosynthesis of collagen, carnitine and catecholamines and in the metabolism of cholesterol and to bile acids
Others ingredients				
Chlorine	7 – 50 mg/100 kcal	Target 25 mg/100 kcal	25 mg/100 kcal	- Involved in the transport and metabolism of lipids
Inositol	4 – 44 mg/100 kcal	Target 4 mg/100 kcal	20 – 50 mg/100kcal	- Play a role in regulation of cell osmolality, as structural components of the developing neural system, etc.
Taurine[2]	Max 12 mg/100 kcal	No necessity	4.7 mg/100 kcal	- Involved in intestinal, hepatic function, auditory and visual development in pre-term infants
L-Carnitine	Min 1.2 mg/100 kcal		0.9 – 1.6 mg/100 kcal	- Indispensable nutrient because of temporary insufficient synthesis capacity
Nucleotides and nucleosides[2]	Max total 5 mg/100 kcal Different max for each	No necessity	Different values for each	- Involved in metabolism reactions and in the synthesis of proteins, lipids carbohydrates
Pro- and syn- biotics[2]			No necessity	- Lack of convincing evidence of beneficial health effects

In parallel, from international perspectives, the CAC established by the FAO (the Food and Agriculture Organization of the United Nations) and WHO, develops food standards that are not legally mandatory norms, but are often used by national and regional legislations. For example, in Europe, the Codex standards have often served as the basis for European legislation (Luning et al., 2006). This harmonisation facilitates the trade between countries at the global level, encouraged by the World Trade Organization (WTO, 1995; WTO, 1998). In 1981, the CAC published the food standard for IF that sets levels of nutrients (Codex Alimentarius Commission, 1981). This standard was reviewed and updated by a group of experts, namely the Committee on Nutrition of the "European Society for Paediatric Gastroenterology, HepAtology, and Nutrition" (ESPGHAN) and "the international scientific committee", their work has been published (Koletzko et al., 2005).

3.3.3. How to fulfil infant requirements?

3.3.3.1 Infant nutritional needs

Humans, and then infants, need energy to perform and regulate all biochemical processes that maintain body structures (e.g. synthesis of growing tissues) and functions (e.g. basic metabolism, thermoregulatory needs) and to perform physical activities (EFSA, 2013; FAO/WHO, 2001). The energy requirement mainly comes from carbohydrate and lipid intakes.

Infants also need macro- and micro-nutrients. Schematically, proteins help to maintain and build tissues, essential fatty acids to regulate cell membrane fluidity, water to assure the transport of nutrient and metabolic waste. Vitamins and minerals participate to all main biochemical processes. More details on the functions are provided in **Table 3.2**.

For Europe, the reference intake for energy and nutrients are listed in **Table 3.2**, using the EFSA assessment (EFSA, 2014).

The sum of these energy costs should be met by milk intake. Prediction of the energy required according to age, gender, weight and height has been estimated in the EFSA opinion on nutrient requirements and dietary intakes of infants (EFSA, 2013). Combining

these needs, it varies from zero to six months for boys between 78 and 109 kcal/kg per day and between 78 and 103 kcal/kg per day for girls.

3.3.3.2 Comparison of breast milk and infant formula composition

To comply with nutritional requirements, in terms of energy and nutrients, infants from zero to six months consume two kinds of products: BM and/or IF, whose different compositions are highlighted in **Table 3.2.** Regarding IF composition, two figures are presented, one from the current EU legislation and another one from EFSA recommendation (EFSA, 2014). Indeed, the essential IF composition for nutrients and energy content was assessed by EFSA after the publication of a systematic review by Tijhuis et al. (2014) that compared the nutrient status and health effects of different IF compositions. Nonetheless, the comparison between IF and BM cannot be based exclusively on quantitative indicators, it has to take also into account the nature and quality of nutrients. For instance, BM contains smaller micelles of casein and about 60% of soluble proteins that do not precipitate with casein; both these properties lead to a more efficient absorption of BM in the stomach compared with IF (Turck, 2010). Another difference is the lower content of Vitamin D in BM, requiring a supplementation for breastfed infants. In addition, human milk composition is not standardised and might change depending on the lactating woman. For example, fats and fatty acids in BM composition depends on the mothers food intake (VKM, 2013).

3.3.3.3 Infant food intake recommendations

Exclusive breastfeeding is recommended by the United Nations Children's Fund (UNICEF, 2014) and WHO (2014a) until six months of age, meaning that infants during this period should only consume BM and no other food, not even water. UNICEF and WHO support the fact that breastfeeding is better for child survival and health (WHO/UNICEF, 1989), particularly in countries where population has no access to safe water. The European recommendation is more nuanced and varies among countries between four and six months of exclusive breastfeeding (Yngve and Sjöström, 2001).

3.4. An attempt to define health, health effect, risk and benefit

Health is defined here as a "state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (WHO, 1948). A **health status** of an individual is the location on the "Illness-Wellness continuum" (Dever, 1997; Travis and Ryan, 1988), going from serious illness causing premature death to high level of wellness, schematised i**n Figure 3.3**. More precisely, the high level of wellness corresponds to the "optimal level of functioning or capacity in all the important dimensions of health, and from any type of illness or disease" (Goodacre et al., 2010), it is not only associated with the absence or presence of disease.

Health effect can be defined as any change in the Illness-Wellness scale of the health status represented in **Figure 3.3** resulting from the exposure to a microbiological factor (e.g. *Cronobacter sakazakii*), a chemical factor (e.g. methyl mercury) or a nutritional factor (e.g. fatty acids), named a **HECF** (Health Effect Contributing Factor) in a previous study (Boué et al., 2015). A HECF is an agent that causes a change of the health status on the "Illness-Wellness continuum" of an individual. An **adverse health effect** is a decrease of the health status in the direction of illness/premature death and a **beneficial health effect** is an increase of the health level in the direction of the high level of wellness.

Risk/benefit can be then defined as the probability of having a consequent health effect following exposure to a HECF in food.

Based on the Illness-Wellness concept, the probability of location on the wellness part of the scheme can be associated with the term **benefit** if the health effect is not linked to the absence of disease but to an improvement of the capacity to compensate for additional stress (e.g. development of the immune and digestive system or intellectual quotient improvement). Similarly, the probability of location on the illness part of the scheme can be related to **risk** associated with illness (e.g. Listeriosis, obesity or cancer). In this context, a decrease of a risk will not be considered a benefit. For example, the adjunction of a preservative in food can simultaneously introduce a chemical risk and decrease a microbiological risk; the risk of illness in this case is not located on the superior part of illness-wellness scale and therefore will not be named a benefit. In such a case, the output should be analysed in a risk-risk assessment.

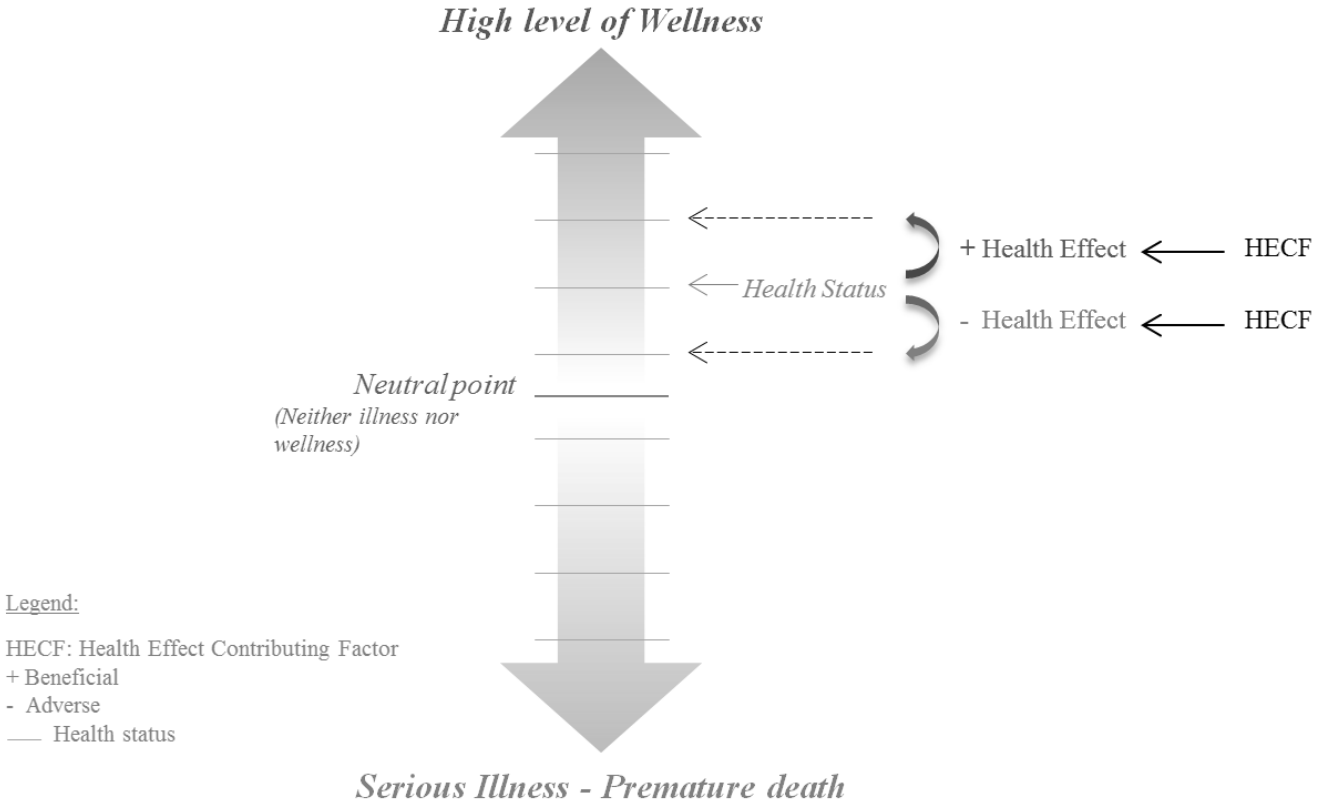

Figure 3.3: Schematic representation of the Illness-Wellness scale, with health status and HECF, adapted from Travis and Ryan (1988)

3.5. Health effects associated with infant milk consumption

Health effects associated with the consumption of breast milk and/or infant formula with regard to nutritional, microbiological and chemical components have been an important research topic from the 1990s and around which a lot of studies have been published and reviewed in the last ten years. The scientific literature on this subject is then very extensive. Consequently, this section does not aim to be exhaustive. Instead, it intends to give an overview of the main adverse/beneficial health effects associated with infant milk consumption.

3.5.1. Microbiology: type of microorganisms and identification of potential adverse/beneficial health effects

3.5.1.1 Sources of bacteria in infant milk

Intrinsic contaminants of powder infant formula: The first IF commercialised was responsible for many deaths due to microbiological contaminants of the milk or cross-contaminations during preparation. Nowadays, the ready to feed formula are safe because they are sterilized (FAO/WHO, 2004), while contrary to many beliefs, powder infant formula (PIF) might present a risk as it is not a sterile product. In PIF, microorganisms have been identified and classified (**Table 3.3**) according to the level of evidence (FAO/WHO, 2004). The level of evidence attributed to each microorganism depends on the causal relation established between PIF contaminated and cases reported of infant illness. The evidence is classified into three classes: clear evidence and causality (Grade A), causality plausible, not yet demonstrated (Grade B) and causality less plausible or not yet demonstrated (Grade C). The most incriminated bacteria are *Cronobacter sakazakii* (also named *Enterobacter sakazakii* until 2008) and *Salmonella* spp. which are also the only two microorganisms classified with clear evidence and causality. However, some grading C microorganisms have been found in PIF. More precisely, among the 18 alerts recorded on the Rapid Alert System for Food and Feed (European Commission, 2015b) between 1988 and May 2015, nine reported PIF were contaminated by *Cronobacter sakazakii*, four by *Salmonella* spp. and one by *Clostridium botulinum*. In parallel, there were infant botulism cases identified in Europe, seven in France (Brett et al., 2005) and one in UK potentially linked to PIF consumption (King et al., 2010).

Table 3.3: List of microbiological adverse health effect contributing factors identified in PIF classified by level of evidence, based on (FAO/WHO, 2004)

A. Clear evidence and causality	B. Causality plausible, not yet demonstrated	C. Causality less plausible or not yet demonstrated
Cronobacter sakazakii.*[r] *Salmonella* spp.[r]	*Pantoea agglomerans* *Escherichia vulneris* *Hafnia alvei* *Klebsiella pneumonia* *Citrobacter koseri* *Citrobacter freundii* *Klebsiella oxytoca* *Enterobacter cloacae*	*Bacillus cereus*[r] *Clostridium difficile* *Clostridium perfringens* *Clostridium botulinum* *Staphylococcus aureus* *Listeria monocytogenes*[r]

***** The risk associated with powder infant milk formula consumption was quantified by WHO (FAO/WHO, 2004; FAO/WHO, 2006; FAO/WHO, 2011; Paoli and Hartnett, 2006)
r Microorganism regulated in the PIF final product after manufacturing process or during its whole shelf life, levels are given in **Table 3.1**.

Contaminants of infant formula introduced during preparation: The list previously given only takes into account milk supply contamination whereas infants can be infected by cross-contamination occurring during preparation. Indeed, PIF preparation requires different steps with potential cross-contamination and growing phases: water addition, warm-up, storage, bottle and nipple cleaning, etc. Formula milk preparation might be contaminated by extrinsic sources like inappropriate handling or ineffective disinfection of bottle and nipple. The WHO has published a guide on PIF preparation at home (FAO/WHO, 2007a) and in a care settings (FAO/WHO, 2007b) that gives hygiene recommendations. Nevertheless these guidelines are not yet exactly applied and there are a lot of different possible scenarios, highlighting potential different points of contamination and microbial growth (Sani et al., 2013).

Inadequate handling and temperature abuse during storage might expose infants to *Bacillus cereus* toxin (Buchanan and Oni, 2012; Haughton et al., 2010; Kim et al., 2011; Shaheen et al., 2006). In a study, about 5% of bottles were contaminated by *Staphylococcus aureus* just after use (Buchanan and Oni, 2012; Redmond et al., 2009) and other *enterobacteriaceae* were identified in rehydrated powder milk (Buchanan and Oni, 2012; Sani et al., 2013). Reusing bottles also constitutes a potential source of contamination due to inefficient cleaning methods, *Staphyloccocus aureus* was detected

in 12% of unclean bottles in a UK experiment (Redmond and Griffith, 2008; Redmond and Griffith, 2009; Redmond et al., 2009) although this bacterium is classified as C by the FAO/WHO (2004) considering only intrinsic factors.

Water added to powder is a pathway of contamination. Illness due to contaminated water is responsible for about 1.7 million deaths world-wide every year with a higher prevalence in developing countries (Ashbolt, 2004; Marino, 2007; ten Veldhuis et al., 2010). Bacteria incriminated in these illnesses are *Pseudomonas aeruginosa*, *Aeromonas* spp., *Cryptosporidium parvum*, *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* O:157 (Fawell and Nieuwenhuijsen, 2003). More precisely, in France, hazards incriminated are *Campylobacter* spp., *Cryptosporidium parvum* and norovirus (ANSES, 2013a).

Havelaar et al. estimated in 2004 that the burden of disease associated with drinking water due to *Cryptosporidium parvum*, *Escherichia coli* and *Campylobacter* spp. contamination was about 60 years of perfect health lost per 1,000 people per year (Havelaar and Melse, 2003).

Pre- and pro- biotics in breast milk and powder infant formula: Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Sanders, 2008). On the other hand, a prebiotic is an ingredient, not a microorganism. It induces changes in the gastrointestinal microflora composition and/or activity that confers "benefits upon host wellbeing and health" (Gibson et al., 2004). Both, pre- and pro- biotics (named synbiotics when added conjointly) are recognised as having an impact on the infant microbiota establishment (also called the gut microbiota). The gut microbiota plays a role in different beneficial health effects highlighted by (Penders et al., 2006) such as the establishment of a barrier limiting the colonisation by pathogens, the participation in metabolic functions like the fermentation of non-digestible fibers, the salvation of energy as short-chain fatty acids, the production of vitamin K and also the stimulation of the development of the immune system. It is also involved in the reduction of infections by competitive exclusion and production of antimicrobial compounds (Fernandez et al., 2013). Those benefits are mainly associated with the presence in the gut microbiota of probiotic bacteria from species of Bifidobacteria spp. and Lactobacilli spp.; and their growth are promoted by prebiotics such as galacto-oligosaccharides and fructo-oligosaccharides transmitted through BM.

A clear difference has been shown in the composition of the gut microbiota of formula fed compared with breastfed infants, with a higher level of Bifidobacteria spp. and Lactobacilli spp. for breastfed infants and of *Clostridium difficile* for formula fed infants (Francino, 2014). The gut microbiota is considered to evolve until the age of three to five years and then would remain relatively stable, susceptible to vary if there is a bacterial infection, antibiotic treatment, surgical, lifestyle or significant change in diet (Rodríguez et al., 2015). During this first critical period the diet is a major factor that regulates its composition, given the opportunity to supplement IF in order to manipulate the microbiota profile. As a result, commercialised IF are more and more supplemented by pre- and/or pro- biotics by industrials intending to promote beneficial intestinal microbiota. However, even though the addition of pre- and/or pro- biotics in PIF has been judged to be not a "safety concern with regard to growth and adverse effects" by the Committee on Nutrition of the ESPGHAN, they could not recommend its supplementation due to a lack of convincing evidence (Braegger et al., 2011). Moreover, EFSA does not consider pre- or pro- biotics as essential in infant milk composition (EFSA, 2014).

Microorganisms associated with breast milk: BM is not sterile and can be a source of microbiological contaminations (Fernandez et al., 2013) by bacteria, viruses, fungi and parasites, depending on the associated transmission rate. Among them, *Escherichia coli*, and *Staphylococcus aureus* were identified as responsible for several infections (Jones, 2001; May, 2012) as well as *Brucella* (MacDonald, 2006), *Listeria monocytogenes* (Jones, 2001), *Streptococci* (Jones, 2001), *Salmonella* (Jones, 2001) and *Coxiella burnetti* (Jones, 2001).

3.5.1.2 Potential adverse and beneficial health effects

The main microorganisms associated with infant milk (both rehydrated IF and BM) are listed in **Table 3.4** with the sources of human exposure and potential health effects.

Table 3.4: Presentation of microbiological adverse health effect contributing factors and adverse health effects of infant milk

HECF	Characteristics		Source of human exposure	HE	Current trend	Reference
Bacillus cereus	Rod Gram + Aero-anaerobic facultative Sporulative bacteria Able to produce enterotoxins	4 to 55°C Opt 30 - 37°C PHopt 6-7	Spore in soil are presents at a level of 10^4 to 10^5 ufc/g Contamination transmitted from the soil to food through vegetable for example	Emetic and diarrheal symptoms from a contamination of 10^5 ufc/g	Third cause of food toxi-infections in France in 2008 Food process hygienic criteria to PIF production	(ANSES, 2011b)
***Bifidobacterium* spp.**	Rod Gram + Anerobic-strict Fermentative bacteria producing acetic and lactic acid, used in cheese and yogurt manufacturing	25 to 46°C Opt 37 - 41°C PHopt 6-7	Major bacteria of the gastro intestinal microflora in humans, especially those breastfed	Recognized as "GRAS" (Generally Recognized As Safe) Probiotic, prevents intestinal infections and stimulates the immune system Not considered as essential in infant formula in the EFSA opinion (2014)		(Swidan, 2010)
***Brucella* spp.**	Coccobacillus 0,5 to 0,7 µm (diam) 0,5 to 1,5 µm Gram - Aerobic-strict Zoonosis Ability to survive more than 2 months in water at 20°C, more than 8 months in manure and also in dried conditions	20 to 40°C Opt 34°C PHopt 6,6-7,4	Mammal reservoir: cattle, sheep, goats, pigs Transmitted by skin, mucous contact	At low dose: 10 to 100 ufc ingested Influenza symptom Fever Septicemia	Between 2008-2011: 20 cases reported per year in average in France. Mainly due to consumption of raw milk based product	(ANSES, 2011c)
Clostridium botulinum	Bacillus Gram + Anaerobic strict Sporulative bacteria Able to produce botulic neurotoxins	10 to 48°C Opt 35 - 40°C PHopt 4,6-9	Environment: water, soil, dust	Paralysis Digestive disturbances	2007-2009: 43 severe clinical cases including 4 infants	(ANSES, 2011g)
Cronobacter sakazakii	Rod 1 to 3 µm Gram + Facultative anaerobic	5.5 to 47°C Opt 39°C Phopt 5 to 9	Ubiquitous : water, soil, plants, dust	Meningitis, septicemia, necrotizing, enterocolitis Death	PIF disease case 1/100 000 per year (infants 1-12 months)	(ANSES, 2011d)

	Ability to form biofilms and to survive up to 2 years in dry products					
***Lactobacillus* spp.**	Bacillus Gram + Anaerobic strict Fermentative bacteria producing lactic acid, used in dairy manufacturing	Opt 35 - 40°C, up to 45°C PHopt 5,5-6	Major bacteria of the gastro intestinal microflora in humans, especially those breastfed	Recognized as "GRAS" Probiotic, prevents intestinal infections and stimulates the immune system Not considered as essential in infant formula in the EFSA opinion (2014)		(Swidan, 2010)
***Salmonella* spp.**	Bacillus Gram - Can survive in extreme conditions: in chocolate with water activity of 0.3-0.5 and at -23°C in butter	5 to 50°C Opt 35 - 37°C PHopt 7-7.5	Gastrointestinal tract of mammals Contamination is transmitted from feces to pastures, soil and water	Acute gastroenteritis, fever Death		(ANSES, 2011e)
Staphylococcus aureus	Cocci 0,5 to 1 µm Gram + Aero-anaerobic facultative Able to produce toxins Healthy carriers	6 to 48°C Opt 35 - 41°C PHopt 6-7	Ubiquitous: water, soil, dust From humans: skin, mucous, rhinopharyngitis Fridge, kitchen Human contamination through food consumption	Fever, diarrhoea, vomiting, Death	Third cause of food toxi-infections in Europe in 2009, and first cause involving dairy products (process hygienic criteria)	(ANSES, 2011f)

3.5.2. Chemistry: type of chemical hazards and identification of potential adverse health effects

Chemical contaminants of BM and IF have been investigated for decades and a large list of substances has been established (Massart et al., 2008; Sonawane, 1995). However, this list can evolve over time according to regulatory dispositions or changes in human exposure.

Most contaminants are produced by humans as a response to technological purposes (e.g. pesticides for agriculture, food packaging, paintings, fuel car, etc.) and another part results from industrial processes (e.g. waste incineration, cement manufacturing, etc.) (Cattaneo, 2013). Food is one of the main sources of human chemical contaminations (natural or synthetic) through substances found in the diet; they are present in raw materials and/or introduced during production and processing steps. The number of deaths attributable worldwide to diseases due to chemical exposure was estimated to 4.9 million per year in 2011 which corresponds to a loss of 86 million years of perfect life per year integrating the quality of life lost due to disease (Pruss-Ustun et al., 2011). This estimation was judged as an underestimation (Fulcher and Gibb, 2014) partly due to unknown dose-response relationships.

3.5.2.1 Sources of infant milk contamination

Breast milk chemical contaminations: Mothers are unavoidably exposed to environmental chemical compounds during basic activities and through different media such as food, water, air, or manufactured products (Cattaneo, 2013). The main routes of exposure are then ingestion, inhalation, and/or cutaneous contact. Some of these contaminants (the lipophilic and persistent ones) can accumulate in their fatty tissues and can be released during secretion of milk which represents one route of excretion for mammals (in addition of urine or faeces) (Marseglia et al., 2014). As a consequence, the levels of some contaminants in human milk are influenced by the number of infant breastfed. BM can also be contaminated as a consequence of other occupational and lifestyle factors (professional activity, drug usage, active or passive smoking, geographical living area, etc.). The temporal evolution of these environmental and lifestyle factors (human exposure is variable across the entire lifetime) also contributes to this complex problem, as emphasised by Solomon and Weiss (2002). Monitoring of historical persistent environmental contaminants (dioxins, PCBs, DDT, etc.) has

highlighted a significant decrease in some countries in the past decades, due to regulation decisions banning or regulating them. Conversely, other emerging substances such as food contact materials (bisphenols, phthalates) are presenting a reverse temporal trend.

Infant Formula chemical contaminations: Cow's milk can be basically contaminated in the same way as BM. The observed levels of contamination in cow milk are, however, usually lower than those reported in human milk (VKM, 2013). The existing regulatory dispositions imposed at European levels (and other countries) in the field of food safety, but also the differences in terms of diet and environmental exposure level versus volume of milk produced ratio, explains this observation. Cow milk can also contain veterinary drugs administrated to animals. On the other hand, powdered IF is obtained from milk dehydration by a high thermal process. This step first affects milk nutritional properties, destroying vitamins, minerals and amino acids like lysine which are essential for growth and development. These controlled industrial processes ensure reduced levels of most environmental chemical contaminants, even if this step may also produce harmful molecules, like those produced during the Maillard reaction. Then, PIF is rehydrated with water potentially chemically contaminated and served with a bottle and a nipple that could also contaminate milk through chemical migration from materials. Such water contaminants can have severe effects on health. Havelaar and Melse (2003) have estimated that in 2004 the worldwide burden of disease associated with drinking water contaminated with arsenic and bromate at about 64,900 years of life in perfect health lost per 1,000 people per year. Finally, the levels of some contaminants may be found more elevated in IF compared to BM. For example, contaminations by arsenic, cadmium, lead and uranium have been assessed to be higher in IF than in BM (Ljung et al., 2011).

3.5.2.2 Potential adverse health effects

The main substances investigated in the literature are presented in **Table 3.5**, with their potential health effects, following three different sections:

- Raw milk contamination concerning both IF and BM that can be contaminated by the same substances.
- IF can be contaminated by the manufacturing process steps and also by water addition in case of rehydration.
- Packaging used can contaminate IF and pumped BM.

Several classes of environmental chemical contaminants have been pointed out with regard to their role in apparition and/or development of various human health outcomes: reproductive and developmental functions, hormono-dependant cancers, immune system, and, metabolic syndrome / obesity. The recent International Agency for Research on Cancer monography on PCBs (IARC, 2015) states that "there is sufficient evidence in humans for the carcinogenicity of polychlorinated biphenyls (PCBs). PCBs cause malignant melanoma. Positive associations have been observed for non-Hodgkin lymphoma and cancer of the breast". The link between a perinatal chemical exposure and neurodevelopmental outcomes (IQ, autism, etc.) is also a very strong emerging concern. In spite of extensive scientific literature and existing evidential base, the unequivocal demonstration of causality between chemical exposure and deleterious health impact however still remains extremely challenging at the population scale in humans and animals. The cumulative and/or mixture effect of these substances is also a major issue that remains largely unknown (Pohl et al., 2004).

Another important issue to be considered is whether the relative impact is caused by in-utero versus ex-utero exposure. Indeed in-utero infant exposure to chemical contaminants (i.e. the fetal exposure during the nine months of gestation due to the mother-fetus transfer from cord blood or amniotic fluid) could have a higher impact on health than ex-utero exposure through several weeks or months of breast or IF based feeding (Pronczuk et al., 2002).

Table 3.5: Presentation of chemical adverse health effect contributing factors and health effects of infant milk

HECF	Characteristics	Source of human exposure	HE	Current trend	Reference*
Raw milk:Breast milk and infant formula					
AFM1	Mycotoxin No effect of heat treatment on AFM1 amount	Food	Carcinogenic		(Khaniki, 2007)
Brominated flame retardants	POP Persistent, Lipophilic and bio-accumulative High process resistance	Food consumed Breathing air	Toxic effects in liver, thyroid hormone, reproductive, nervous system and neurodevelopment	PBDE increase in BM in Sweden (Solomon and Weiss, 2002)	(VKM, 2013)
Cadium	Heavy Metal	Food consumed Water Active/passive smoker	Carcinogenic Impairment of kidney, skeletal and the respiratory systems		(WHO)
Dioxins	Persistent organic pollutants (POP) 17 PCDDs and PCDFs Bio-accumulated Stable until 850 to 1000°C	Food consumed (90% of exposure)	Carcinogenic Impairment of reproduction and development	Banned in many Europeans countries Decrease in BM in Sweden (Solomon and Weiss, 2002) and Netherland (WHO/UNEP)	(VKM, 2013)
Lead	Heavy Metal	Water Food consumed Dust	Impairment of infant neuronal development and hematological, gastrointestinal, cardiovascular, and renal systems		(WHO, 2014c)
Mercury	Heavy Metal Hg0 (elemental) $Hg^{2+}{}_2$ (inorganic) Hg^{2+} (organic)	Breathing air Fish or seafood consumed	Impairment of nervous, digestive immune systems, lungs, kidneys, skin and eyes		(WHO)
Organochloride pesticides	POP Lipophilic and persistent	Water Food consumed (animal fat)	Carcinogenic Impairment of liver, reproductive immune and nervous system	Decrease, banned in many EU countries (Solomon and Weiss, 2002; VKM, 2013).	(VKM, 2013)

PCBs Polychlorinated biphenyls	POP 2 categories: dioxin-like PCB-DL and non-doxin-like PCB-NDL Lipophilic, chemically stable low biodegradability	Food (90% of exposure)	Carcinogenic Impairment of reproduction, central development, nervous and immune system	Decrease in western countries	(VKM, 2013)
Process: Infant formula					
Acrylamide	Water soluble and not accumulated in food chain	Food	Carcinogenic		(VKM, 2013)
Furan	Volatile, lipophilic		Carcinogenic		(VKM, 2013)
PAHs	Organic compounds	Food Smokers	Genotoxic and carcinogenic		(VKM, 2013)
3-MCPD	Organic compounds		Carcinogenic Impairment of reproductive and immune systems		(VKM, 2013)
Packaging: Pumped breast milk and infant formula					
Bisphenol A (BPA)	Food contact materials contaminant Organic compounds Stable and resistant	Migration of BPA from baby bottle and teat Food consumed Breathing air Dust Water	Impairment of brain development, reproductive and cardio-vascular systems	Banned in France in 2015 in food contact packaging (Legifrance, 2015) but is replaced	(FDA, 2015a)
Phthalates	Food contact materials contaminant	Food consumed Drinks Breathing air Dust	Impairment of liver, kidney and reproductive functions, endocrine system		(VKM, 2013)

3.5.3. Nutrition: relative health effects of breast milk / infant formula

The approach undertaken in nutrition to identify health effects associated with BM and IF consumption is different from those used in microbiology and chemistry. Indeed, health effects are identified by comparison of health status of breastfed infants with those who are formula fed (epidemiological studies). Generally, in epidemiological studies enable to link specific health effects to diets (e.g. BM vs IF) but they cannot always back to the causing agent in the food element involved in the health effect. The health effects associated with BM compared with IF consumption have been classified into two categories by WHO: short and long term effects. In the short term, BM consumption could reduce the prevalence of gastro intestinal infection and respiratory tract infection (Horta and Victora, 2013) and in the long term it could decrease the prevalence of obesity, blood pressure, total cholesterol, type 2 diabetes and increases infant intellectual quotient (Horta et al., 2007). Recently, the National Institute for Public Health and the Environment of Netherlands (RIVM, 2015) have undertaken a systematic literature review and have integrated the new findings to identify the list of health effects, associated with both diets, and classify them according to their grade of evidence (**Table 3.6**). Each grade of evidence depends on the kind of study investigated: the number and type of studies (randomised controlled trial, prospective cohort, case-control studies), result consistency, study quality, biological gradient, experimental evidence, biological plausibility, etc. The evidence is classified in five grades: Convincing, Probable, Possible, Insufficient, No evidence and Conflicting for health effects investigated in different studies of sufficient power that demonstrated an opposite effect. Main health effects and hypothetical mechanisms were summarised in **Table 3.7**.

Table 3.6: List of identified nutritional health effects associated with breast milk compared with infant milk consumption, classified by level of evidence, based on (RIVM, 2015)

1.Convincing	2.Probable	3.Possible	4.Insufficient	5. Conflicting	6. No evidence
↓[s] Gastro intestinal infection	↓[s] Asthma	↓[s] Childhood cancer	↓[l] Adult cancer	Ø↓ Atopic disease	↓ Multiple sclerosis
↓[s] Otitis media	Ø Cardiovascular disease	Ø Helicobacter pylori infection	Ø Dental caries	Ø↓ Coeliac disease	
↓[s] Respiratory tract infection	↓[s] Crohn's disease	↑[s+l] Intellectual and motor development	↓[s] Fever	Ø↓↑ Eczema	
	↓[s] Inflammatory bowel disease	↓[s] Leukemia	Ø Growth in 1st year of life	Ø↓ Jaundice	
	↓[l] Obesity	↓[s] Sudden infant death syndrome	↓ Haemophilus influenza	Ø↓ Lung function	
	↓[s] Ulcerative colitis	↓[l] Type 1 diabetes	↓ Hodgkin lymphoma		
	↓[s] Wheezing	↓[l] Type 2 diabetes	↓[l] Lynphoma		
			↓ Neonatal weight loss		
			↓ Pyloric stenosis		
			↓[s] Urinary tract infections		

Health effects that are found to decrease by the breastfeeding diet are specified with an arrow going down ↓; those that are found to increase with an arrow going up ↑; when there is no effect demonstrated of both diets this sign is written: Ø. Then, it is specified if the health effect occurs at short term, during childhood, with the letter [s]; and at long term with the letter [l].

Table 3.7: Health effects of the consumption of breast milk compared with infant formula and hypothetic mechanisms

HE	Consumption of breast milk compared with infant formula		Hypothesis of mechanism	
Obesity	Lower prevalence of overweight and obesity during childhood and/or adult life Obesity prevalence is reduced by 4% per month of exclusive breastfeeding Obesity is one of the main risk factors involved in chronic diseases such as diabetes, cardiovascular disease and cancer Pooled odd-ration 0.76 (0.71;0.81)	(Hörnell et al., 2013) (Harder et al., 2005) (Horta et al., 2007)	Suckling allows the child to learn to control his diet. Breastfeeding would regulate leptin concentration which is a hormone that regulates appetite. Consumption of infant formula would result in a higher insulin concentration in blood that stimulates fat deposition in tissues	(Hörnell et al., 2013)
Respiratory tract infection (RTI)	Breastfed infants for at least 4 months reduce their risk by 72% to be hospitalised due to respiratory disease In average, the protection associated with BF is about 30% for morbidity, 50% for hospitalisation and 60% for mortality. Relative Risk 0.35 (0.09;1.36)	(Ip et al., 2007) (Horta and Victora, 2013)	BM oligosaccharides could prevent RTI by inhibiting the adherence of pathogens to mucosa (adherence competition) BM reduces risk of under nutrition which is a factor that increases infection risk.	(Horta and Victora, 2013)
Gastro Intestinal Infection	BM for at least 6 months' decreases by 80-90% mortality and hospital admission rate due to diarrhea symptom. Relative Risk 0.67 (0.46;0.97)	(Ip et al., 2007)	Same hypothesis as RTI. Lactoferrin contained in BM may reduce inflammatory response and destroy pathogens. Breastfed infants are less exposed to pathogens than formula fed infants.	(Horta and Victora, 2013)
Growth	Food intake should fulfill energy and all essential nutrients requirements. Malnutrition causes diseases due to an inadequate food intake in term of quality and/or quantity. For example, an excessive food intake causes obesity that increase cardiovascular diseases and diabetes risk.	(FAO/WHO, 2001)	Metabolism reactions associated with food consumed are at present well known.	(Berdanier et al., 2013)
Neurodevelopment IQ	The Intellectual Quotient is an indicator of neurodevelopment. Breastfed infants were associated with an increase of 5.9 IQ points (-1.0 to +12.8).	(Kramer et al., 2008)	Hypotheses have been made and are still controversial. DHA transmitted by BM could be linked with brain structural changes (e.g. volume and white matter) through the FADS2 gene regulation.	(Horta and Victora, 2013)

Finally, BM is also recognized as having immunological properties associated with certain identified components of the milk (VKM, 2013). These properties have not been fully demonstrated yet. An infant's mother transmits immunological components through BM such as: antibodies, lactoferrin, α-lactalbumine, lysozyme, carbohydrate components, fats and fatty acids, cytokines, hormones, growth factors, immune cells, prebiotics and probiotics. It is not transposable to IF consumption because cows' milk composition is different. For example, cow's milk contains ten times less lactoferrin than BM. Some BM's components protect infants against microbial infection:

- Antibodies protect infants from infectious diseases and act in the mucosal microbiota development. Among them immunoglobulin A (IgA) and secretory IgA named SIgA are well known.
- Lactoferin is a bactericidal BM protein which has antiviral effects. This protein survives in the gut and acts as an intestinal barrier.
- α-Lactalbumine is an antimicrobial efficient against bacteria, fungi and also malignant cells.
- Lysosyme is an anti-microbial component against gram positive and negative bacteria and against viruses.
- Carbohydrate components act as competitive components, binding to the mucosal surface that avoids bacteria adhesion. It has been demonstrated to protect infants against *E.coli, Campylobacter* spp., *S. pneumonia* and *V. cholerae*.

3.6. Risk and benefit assessment of infant milk consumption

A set of beneficial and adverse health effects associated with BM and IF consumption have already been identified in the nutrition, microbiology and chemistry fields. Then, ideally, to estimate the overall impact of the infant diet on health, a multidisciplinary approach comparing all risks and benefits is required. However, so far, such risk-benefit, or risk-risk assessment of infant milk (both BM and IF) has not yet been carried out. Nevertheless, three recent scientific studies have paved the way towards a comprehensive and multidisciplinary assessment.

3.6.1. Presentation of the risk-benefit assessment approach

The risk-benefit assessment (RBA) is the scientific evaluation of known or potential health effects resulting from human exposure to a factor contributing to health effects in food (adapted from the WHO definition of the risk assessment (WHO)). It is one of the three interconnected parts of the risk-benefit analysis that includes also the risk-benefit communication and management. The objective of the risk-benefit management is to set up public health actions which are based on the RBA results, to improve the level of health of the population.

The RBA is not the sum of a risk assessment and a benefit assessment. It is a more complex approach that includes a comparison of the evaluated risks and benefits (Tijhuis et al., 2012b). This comparison introduces the notion of scenarios: reference (or baseline) and alternatives. The baseline scenario is the current exposure of consumers or zero exposure. This scenario serves as a reference for the first RBA. Alternative scenarios are hypothetical consumer exposures which are used to test the levels of exposure that are likely to improve public health. These alternative scenarios are designed to target potential management options. Although management options are designed by managers and not by assessors, the RBA assessment is done in close collaboration between them and the assessment is oriented towards targeted and initially defined management options.

The approach undertaken to compare risks and benefits from different fields is detailed in the review of RBA associated with food consumption (Boué et al., 2015) and generally follows these steps:

0 – Problem definition

1 – Identification of Health Effect Contributing Factor (HECF)

2 – Exposure assessment

3 – Characterisation of HECF

4 – Health impact (HI) characterisation

5 – Harmonisation of HI

6 – Assessment of different scenarios of consumer exposure

The main case study in RBA concerned the assessment of fish consumption with more than 33 papers published on this issue, as detailed in Boué et al. (2015). Different beneficial and adverse health effects were associated with fish consumption (e.g. neurodevelopment, cancer, Listeriosis, coronary heart disease, etc.) and have been linked to nutritional (DHA and EPA), chemical (methyl mercury and dioxins) and microbiological (Listeria monocytogenes) HECF. The main recommendation resulting from these assessments was to consume two fish dishes per week, including one with fatty fish and alternating fish species, type of production and location of production (ANSES, 2013b; FAO/WHO, 2010). Other assessments were conducted for specific countries (Norway, Netherlands, Poland, France, China, USA, Portugal and Bermuda), different fish species or type of farming. Finally two studies have illustrated how a multidisciplinary and quantitative RBA could be conducted using the Disability Adjusted Life Year (DALY, number of years of life lost in a perfect health state (Gold et al., 2002)) as a single public health indicator. Berjia et al. (2012) have performed a RBA in the fields of microbiology and nutrition balancing the beneficial health effect due to omega-3 intake with the risk of listeriosis due to cold-smoked salmon consumption; and Hoekstra et al. (2013b) in the fields of chemistry and nutrition balancing the risks and benefits of fish consumption, in Denmark and the Netherlands.

3.6.2. Studies reporting risk and/or benefit assessment of infant formula and breast milk consumption

3.6.2.1 Quantitative microbiological risk assessment of Cronobacter sakazakii in powdered infant formula by WHO and FAO

The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) performed a quantitative microbiological risk assessment of *Cronobacter sakazakii* in powdered infant formula (FAO/WHO, 2004; FAO/WHO, 2006; Paoli and Hartnett, 2006) and developed an interactive website (FAO/WHO, 2011). Different scenarios of milk preparation and sampling plans were evaluated regarding the potential intrinsic contamination of manufactured PIF with *Cronobacter sakazakii*. The following sets of scenarios of milk preparation were assessed:

- Room ambient temperature during preparation: cool, warm and very warm.
- Water temperature of reconstitution: 10°, 20°, 30°, 40°, 50°, 60° and 70°C.
- Cooling step: by refrigeration at 4°C or holding at room temperature.
- Re-warm action: no re-warm or re-warm with a bottle warmer.
- Feeding duration: short or long period.

The model developed enables an estimate of the risk of illness associated with each scenario of preparation and the potential number of illnesses per million infants per day. The output is expressed as a relative risk which is the risk estimated for a given scenario divided by the risk of a baseline scenario (specified for each five sets of scenario listed above). This expression allows analysis of an increase or decrease of the risk compared with the baseline scenario. The main conclusion was that the risk is lower if milk is reconstituted with water at 60° and 70°C and higher if milk is reconstituted with water at 40° and 50°C. Based on scenario analysis, one of the main potential management options was the recommendation of reconstitution of the milk by adding water at 60 to 70°C to the powder. This quantitative microbiological assessment represents a key progress toward the comprehensive and multidisciplinary assessment of infant milk consumption. Indeed, the individual risk assessment of *Cronobacter sakazakii* has been undertaken from the 'Identification of Health Effect Contributing Factor' (step 1 in RBA presented before) up to the 'Health impact characterisation' (step 4 in RBA); that gives the substantive information to assess the main concern identified from the microbiology field in this complex issue.

3.6.2.2 Benefit and risk assessment of breast milk consumption in Norway by VKM

The Norwegian Scientific Committee for Food Safety (VKM) undertook a benefit and risk assessment of BM for infant health in Norway (VKM, 2013). This population is particularly of interest because it is among the highest breastfeeding rates compared with European rates, with about 84% of infants exclusively breastfed at zero month, 10% at six months and 80% partially breastfed at six months. The objective of the study was to assess risks and benefits of BM consumption in Norway considering the current level of contaminants. The alternative of breastfeeding, IF, was discussed for comparison and it was out of scope to perform an integrative RBA of both diets.

The approach carried out in the study was to identify, the main adverse and beneficial health effects of BM consumption and to compare, using health outcomes, the grade of evidence attributing to each health effect. Beneficial health effects associated with breastfeeding were challenged by chemical contamination of BM. For example, nutritional epidemiological studies have highlighted that the improvement of infant neurodevelopment supported by breastfeeding was impaired by persistent organic pollutants (POPs) contaminating BM. A grade of evidence was associated with each one, based on the WCRF guidance report (2007). Grades of evidence were classified as followed: Grade 1 - Convincing (high), Grade 2 – Probable (moderate), Grade 3 – Limited suggestive (low), Grade 4 – Limited no conclusion (insufficient). Each grade of evidence depends on the studies used: type and number of studies, result homogeneity, study quality, biological gradient, experiment evidence and biological plausibility. For chemicals, the exposure of the Norwegian infants was estimated by calculating the level of contaminants in BM in Norway and the daily intake of BM by infants. This exposure was compared with safety reference values, when available, like tolerable intakes defined by WHO or JECFA and it was combined with studies on the potential health effect to estimate the grade of evidence attributed. Based on this approach, the main findings were:

- **Neurodevelopment:** evidence is convincing that breastfeeding improves the infant neurodevelopment whereas the risk associated with POPs exposure is judged “limited suggestive” and to mercury “limited and no conclusion”.
- **Immune response-associated disease:** evidence is convincing that BM protects infants against infections as long as they are breastfed. The evidence of negative effect on vaccine antibody titer, middle ear infections, thymus weight, asthma and

wheezing associated with POPs contaminations is "limited and inconclusive" (based on studies of other countries that are 3 to 100 times more exposed to POPs).

- **Growth, overweight and obesity:** evidence is convincing that BM protects infants against obesity and being overweight in childhood. The evidence of negative effects of POPs are "limited and inconclusive" (based on studies with populations with higher level of chemical contamination).

VKM concluded that infants currently breastfed (exclusively or partially) in Norway up to six or 12 months of age, have nutritional beneficial health effects that outweigh the risk of impaired neurodevelopment, reduced resistance to infection, overweight and obesity associated with chemical contaminants considering the current level of contaminants (n.b. those considered in the study) in BM. This semi-quantitative risk and benefit assessment constitutes another key progress toward the comprehensive and multidisciplinary assessment of infant milk consumption. It gives a valuable summary of the literature regarding potential risks and benefits associated with both diets mainly from the nutritional and chemical sides. Through this report, a list of Health Effect Contributing Factors associated with the consumption of both infant milk has been identified (step 1 in RBA presented before), the exposure of the Norwegian population has been evaluated (step 2 in RBA) and materials to advance on the characterisation of health effect contributing factors were given (step 3 in RBA); it contributes to the first steps of the RBA of both diets in chemistry and nutrition.

3.6.2.3 Quantification of health effects of breastfeeding by the RIVM

The National Institute for Public Health and the Environment (RIVM) quantified the relative health effects of breastfeeding both for the mothers and their infants at first in 2005 (Büchner et al., 2007). These relative health effects were based on relative risks and odd ratios estimated through nutritional epidemiological studies comparing the occurrence of various health effects in the population of breastfed infants versus the formula fed population. It combined at the same time the potential adverse and beneficial health effects.

The current rate of mothers partially breastfeeding for six months or more, with no distinction between exclusive and mixed lactation, is about 35% for the Dutch population. The remaining partially breastfeeding five (3%), four (4%), three (8%), two (9%°) or one

month (19%) or did not breastfeed at all (22%) (Büchner et al., 2007). This assessment has been refined in 2007 (Van Rossum et al., 2005) integrating new data and also the health care cost of diseases for different potential policy scenarios depending on the rate of breastfed infants and breastfeeding duration. The economic gain associated with the decrease of diagnostic and treatment of diseases enabled by breastfeeding was balanced with the health gain associated with each scenario. These scenarios were:

- Current situation: Reference scenario
- All infants are formula fed during six months
- All infants are breastfed during six months
- All infants are breastfed one month longer than the current situation
- Infants breastfeeding less than three months are breastfeed up to three months
- Etc.

To compare these scenarios together and integrate different health effects, the incidence of each disease was converted into the same indicator DALY. The output of this model represents the overall burden of disease. The best scenario in terms of health and cost saved was the third one, i.e. all infants are breastfed during six months. This scenario was associated with 28 DALYs saved/year per 1,000 newborns and 205 euros gained per newborn, representing 50 million euros saved on health care costs annually; this result integrates mother and infant health effects. This gain is mainly associated with the reduction of the incidence of infants' asthma and mothers' rheumatic arthritis.

All the results presented above were obtained by data gathering, statistical analysis and modelling. This approach enables to rank different management options by comparing health effects; it enables also to estimate the health gain expected per euro spent for each intervention. This comparison is made possible because risks and benefits were converted into the same public health measure, specifically DALY. This indicator is valuable for policy maker to compare different interventions, to support preventive measures and to set policy objectives. This is illustrated in the RIVM report (Büchner et al., 2007) with the successful implementation of the "Masterplan" in the Netherlands. This plan was implemented in 2002 to extend the duration of breastfeeding with the certification of hospitals that implement the "Ten steps to successful breastfeeding" established by WHO and UNICEF (1989), the training of medical staff and the development of a communication plan. Five years later, the duration of breastfeeding has notably increased

and a gain of 0.002 DALY and 20 euros per newborn were estimated. The new objective of the second phase of the Masterplan was to reach 85% of mothers initiating breastfeeding with 60% continuing until one month, and 25% until six months. This would result in a gain of 0.006 DALY and 50 euros per newborn. This quantitative risk and benefit assessment also constitutes a key progress toward the comprehensive and multidisciplinary assessment of infant milk consumption. RIVM performed the individual risk-benefit assessment of BM consumption, from the nutritional side, until the harmonisation of health impacts (step 5 in RBA presented before); it gives results, data and methodological considerations from the nutritional field for the assessment of the BM diet.

In light of these three main studies and the current advances published in the literature, a multidisciplinary assessment of both infant diets integrating adverse and beneficial health effects would be required: an integrated risk-benefit assessment. The use of a public health measure such as DALY would allow a comparison of different potential management options, targeted by scenario analysis, and thus give a decision tool for policy makers.

3.6.3. Current gaps in the risk-benefit assessment of infant formula and breast milk consumption

Despite the studies described above on PIF (FAO/WHO, 2004; FAO/WHO, 2006; FAO/WHO, 2011; Paoli and Hartnett, 2006) and breastfeeding nutritional values (Büchner et al., 2007; Van Rossum et al., 2005), there are still health effects which have not yet been quantitatively assessed. For example, in microbiology the effect of different potential contaminants, and route of contamination, remains unquantified. The intrinsic contamination of PIF by *Salmonella* spp. could be of interest because this microorganism was judged responsible with a clear evidence and causality for infant illness following formula milk consumption (FAO/WHO, 2004). Although the intrinsic contamination of IF is limited thanks to the regulation, the rate of compliance with regulatory levels depends also on sampling plans. Nevertheless, there is at present no study assessing the impact of regulatory levels and sampling plan on infant illness due to formula contaminated consumption. Other sources of contamination than the milk itself have not been considered either. Indeed, there are no assessments of potential cross-contaminations during milk preparation while there are rehydrated formula contaminated

with different bacteria such as *Bacillus cereus* (Buchanan and Oni, 2012; Haughton et al., 2010; Kim et al., 2011; Shaheen et al., 2006), and even BM with *Staphylococcus aureus* for example. In addition, the water used to rehydrate the powder formula is also a potential source of contamination not yet assessed. All these contaminations might evolve according to different scenarios of preparation. Regarding the chemical component, the current major concerns are referred to the endocrine disrupting effects following a perinatal exposure. In line with the Developmental Origin of Health and Disease (DOHaD) and early programming concepts, this challenge is imposing new integrated approaches and new metrics for investigating and assessing the “real” overall and long term impact consecutive to the early consumption of milk (BM or IF) during the first months of life. Finally, in nutrition the main health effects were quantified but not compared with microbiological and chemical contaminants whereas nutritional factors mainly led to beneficial health effects or a decrease of risks and the others to an increase of risks. An overall RBA approach is necessary to quantify the health effect of BM and IF consumption integrating microbiological, nutritional and chemical factors.

Beside these scientific gaps, it would also be interesting to investigate furthermore the consequence of current diets and potential alternatives in infant milk feeding by scenario analysis, as encouraged in risk-benefit assessment, (see RBA methodology, steps 1 to 6, above). This comprehensive approach, from hazard identification to DALY calculation, would be valuable when assessing infant milk consumption risk and benefit. This will enable further progress on the evaluation of potential management options such as recommendations by the policy makers on the "ideal" duration of exclusive breastfeeding and the food intake for lactating mothers (depending on diet, country, etc.), a guidance on practices of milk preparation and packaging choice (bottle, nipple, etc.) or regulation of final manufactory controls of PIF production (sampling plan, frequency, method, criteria, etc.).

3.7. Conclusion

Infant food intake during the first months of life, whether it be breast milk or infant formula, affects their health status during the short and long term. Different beneficial and adverse health effects have been linked to both diets in the fields of nutrition, chemistry and microbiology. The main risks and benefits have been assessed individually and even partially compared. However, an integrative and quantitative approach would be required to compare all risks and benefits and to assess different scenarios of consumption and milk preparation. In addition, other complex issues remain unassessed, like guidance on practices of milk preparation and packaging choice or regulation of final manufactory controls of PIF production for chemical and microbiological hazards. The use of a public health measure such as DALY or other quantitative metrics to compare different outcomes related to different scientific fields appears to be valuable to compare results from the three fields and also, to enable policy makers to compare different potential interventions or, to underpin preventive actions.

CHAPTER 4

Model development 1

Redraft from:

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CHAPTER 4: **Model development 1**

A first RBA was performed on the infant milk case study, following the approach defined in CHAPTER 2. It was named "Model development 1". This model was inter-disciplinary considering one factor of major concern in microbiology, chemistry and nutrition. The selection of factors was based on the review done in CHAPTER 3: *Cronobacter sakazakii*, dl-PCB and DHA. Moreover, Model 1 was probabilistic with separated variability and uncertainty as both CHAPTERS 3 and 4 have highlighted a need of methodological development in this mathematical domain. Five different scenarios of consumer's exposure were assessed: six months of breastfeeding versus powder infant formula feeding, with the option of supplemented infant formula in fatty acids and the addition of water at ambient temperature or boiled.

Objectives of the chapter:

- Develop a probabilistic and inter-disciplinary Risk-Benefit assessment model for chemistry, microbiology and nutrition,
- Discuss relevance, feasibility and added-value of the method.

4.1. Abstract

A probabilistic and inter-disciplinary risk-benefit assessment (RBA) model integrating microbiological, nutritional and chemical components was developed for infant milk, with the objective of predicting the health impact of different scenarios of consumption. Infant feeding is a particular concern of interest in RBA as breast milk and powder infant formula have both been associated with risks and benefits related to chemicals, bacteria and nutrients, hence the model considers these three facets. *Cronobacter sakazakii*, dioxin-like polychlorinated biphenyls (dl-PCB) and docosahexaenoic acid (DHA) were three risk/benefit factors selected as key issues in microbiology, chemistry and nutrition,

respectively. The present model was probabilistic with variability and uncertainty separated using a second-order Monte Carlo simulation process. In this study, advantages and limitations of undertaking probabilistic and inter-disciplinary RBA are discussed. In particular, the probabilistic technique was found to be powerful in dealing with missing data and to translate assumptions into quantitative inputs while taking uncertainty into account. In addition, separation of variability and uncertainty strengthened the interpretation of the model outputs by enabling better consideration and distinction of natural heterogeneity from lack of knowledge. Inter-disciplinary RBA is necessary to give more structured conclusions and avoid contradictory messages to policy makers and also to consumers, leading to more decisive food recommendations. This assessment provides a conceptual development of the RBA methodology and is a robust basis on which to build up-on

4.2. Introduction

The microbiological and chemical safety of food consumed, in addition to its nutritional composition, plays a crucial role in influencing human health in the short and long term. This complex balance of multi-disciplinary factors has caught the attention of the scientific community and food safety agencies since the beginning of the twenty first century. The first issue tackled in risk-benefit assessment (RBA) was related to fish consumption (Cohen et al., 2005; FAO/WHO, 2010; Hoekstra et al., 2013b; Ponce et al., 2000). This later case study has also enabled to consolidate the methodology carried out in RBA (Boué et al., 2015). Only a few risk-benefit assessment studies have been provided which include altogether microbiological, chemical and nutritional risk/benefit factors; and generally they have been carried in a semi-quantitative (rather than quantitative) and deterministic (rather than probabilistic) manner. The relevance, feasibility and added-value of using in RBA probabilistic approaches including the separation of variability and uncertainty of inputs has not yet been fully investigated (Tijhuis et al., 2012a). The aim of the present study was to develop a probabilistic and inter-disciplinary RBA model in food. The model was applied to the assessment of breast milk and powder infant milk consumption.

The infant milk diet during the first months of life (either breast milk or powder infant formula), is an interesting case-study for the development of the RBA methodology as it

is of concern during the first months when diet is only composed of milk while the body is in development and the immune system is not yet prepared to defend itself. Breast milk is the main diet recommended for the first six months of life (WHO, 2014a) whereas powder infant formula is the most common alternative taken in western countries (Cattaneo et al., 2005). Both diets have been associated with microbiological, chemical and nutritional risks and benefits (Boué et al., 2016; Meltzer et al., 2016). In this context, where a diet is associated with several food safety issues related to different scientific fields, an integrative RBA is required to estimate the potential overall health impact. To date, three main studies have already contributed to address this complex issue (Büchner et al., 2007; FAO/WHO, 2006; Meltzer et al., 2016) but none of them undertook an inter-disciplinary RBA.

The model was developed for the French population of infants from birth to six months of age and used data preferably from France or when not available from Western countries. It was decided to select only one factor from microbiology, one from nutrition and one from chemistry, to focus on the conceptual development of the RBA methodology. However, additional factors may need to be added in the future for a more comprehensive assessment. This study does not claim to estimate the final health impact of different infant feeding strategies but aims to illustrate the usefulness of probabilistic and inter-disciplinary RBA in food.

From the microbiological point of view, powder infant formula (PIF) is not a sterile product and can contain pathogenic bacteria like *Cronobacter sakazakii* and *Salmonella* spp. which were most often incriminated in outbreaks (FAO/WHO, 2006). The milk preparation can be a source of cross-contaminations due to inadequate handling or ineffective cleaning of the bottle and nipple; most frequently involved bacteria were: *Bacillus cereus* (Buchanan and Oni, 2012; Shaheen et al., 2006), *Staphylococcus aureus* (Buchanan and Oni, 2012; Redmond et al., 2009) and other *enterobacteriaceae* (Buchanan and Oni, 2012; Sani et al., 2013). The addition of water is also a possible pathway of contamination that might bring parasites like *Cryptosporidum parvum* (Pouillot et al., 2004), viruses like norovirus (ANSES, 2013a) and bacteria like *Pseudomonas aeruginosa*, *Aeromonas* spp., *Cryptosporidium parvum*, *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* O:157 (Fawell and Nieuwenhuijsen, 2003). Breastmilk (BM) is neither a sterile product and can contain on the one hand adverse bacteria: *Escherichia coli* and *Staphylococcus aureus* (Jones, 2001; May, 2012) as well

as *Brucella* (MacDonald, 2006), *Listeria monocytogenes* (Jones, 2001), *Streptococci* (Jones, 2001), *Salmonella* (Jones, 2001) *and Coxiella burnetti* (Jones, 2001). On the other hand, BM is a source of pre- / pro- biotics that have been associated with beneficial health effect which are now included into some PIF formulations, intending to reproduce BM composition. Consequently, there is a large number of bacteria that should be integrated into a comprehensive analysis, introduced through different pathways, with a level varying according to environmental conditions during storage and preparation. *Cronobacter sakazakii* was selected in the model developed due to the clear evidence of presence in PIF and causality of infant infection (FAO/WHO, 2004; FAO/WHO, 2006). The microbiological risk assessment associated with this pathogen has already been undertaken (FAO/WHO, 2006; Paoli and Hartnett, 2006) but has not been compared with other health impacts.

From the chemical side, various chemicals may be present in both types of milks. Lipophilic and persistent organic pollutants that are stored in fatty tissues can be present in BM (Massart et al., 2008; Sonawane, 1995), coming from different sources of exposure: inhalation, ingestion and dermal contact (Cattaneo, 2013). Up until now, polychlorinated biphenyls (PCBs), organochloride pesticides, dioxins and brominated flame retardants were of major concern (Meltzer et al., 2016) as well as heavy metals (e.g. cadmium, lead and mercury)(WHO) and mycotoxins (e.g. aflatoxin M1 (Khaniki, 2007)). PIF is also subject to these same contaminants but at a lower level since the bovine diet, patterns and sources of exposure and volumes of production are different. However, the PIF preparation can also bring other chemical contaminants through the manufacturing process (Meltzer et al., 2016) (e.g. acrylamide, furan, PAHs and 3-MCPD), the water addition (Villanueva et al., 2014) (e.g. disinfection by-products, heavy metals, organochloride pesticides, etc.) and/or contact material migrations (bisphenol A and phthalates)(Meltzer et al., 2016). Consequently, there is also in chemistry a large number of chemicals coming through different sources of exposure that should be included in a comprehensive analysis. Chemicals mentioned here were found to be implicated in apparition and/or development of various human health outcomes (Boué et al., 2016; Meltzer et al., 2016): reproductive and developmental functions, hormono-dependant cancers, immune system, and, metabolic syndrome / obesity. However, the link between exposure to specific chemicals and adverse effects in humans remains challenging regarding cumulative and/or mixture effects (Pohl et al., 2004). Therefore, different levels

of evidence were associated with most of chemicals and health effects to characterise the strength of the link between exposure and outcome. Among chemicals of interest in our case study, dioxin-like polychlorinated biphenyls (dl-PCB), accumulated in breast milk and at a lower level in PIF, were selected considering a still relevant concern for this particular subpopulation in spite of the global decreasing trend observed in terms of human environmental exposure (Llobet et al., 2008). Indeed, the recent International Agency for Research on Cancer monography on PCBs (IARC, 2015) has judged that "there is *sufficient evidence* in humans for the carcinogenicity of polychlorinated biphenyls (PCBs). PCBs cause malignant melanoma. Positive associations have been observed for non-Hodgkin lymphoma and cancer of the breast".

In parallel, in the domain of nutrition BM has been associated with different beneficial health effects by epidemiological studies (Victora et al., 2016). The main effects identified were the decrease of gastro intestinal and respiratory tract infections in the short term (Horta and Victora, 2013) and in the long term, the decrease of obesity, type-2 diabetes and the improvement of cognitive development (Horta et al., 2007). Furthermore, some immunological properties are transmitted to the infant through BM (Meltzer et al., 2016). However, the levels of evidence associated with these different outcomes have evolved over years of research (Büchner et al., 2007; Hörnell et al., 2013; Horta et al., 2007; Horta and Victora, 2013; Meltzer et al., 2016; RIVM, 2015; Van Rossum et al., 2005; Victora et al., 2016). To date, the protective effect of breast milk consumption (compared with infant formula) against gastrointestinal infections, respiratory tract infections and otitis media remains convincing as well as the potential effect on cognitive development even if the latter might have a modest effect (Büchner et al., 2007). Other endpoints were found to have a lower level of evidence or were judged as conflicting due to contradictory results (RIVM, 2015). For the present study it was decided to focus on a case where the health effect was linked to a specific nutrient to allow a comparison with microbiology and chemistry. That criterion has substantially decreased the list since most of endpoints were found through epidemiological studies without either identifying biological mechanisms or the specific nutrients involved. Indeed, only cognitive development in the short and long term (Kuratko et al., 2013; Weiser et al., 2016) was linked to a specific nutrient: docosahexaenoic acid (DHA). The evidence that infants consuming breast milk have a better neurodevelopment was judged convincing (RIVM, 2015) up to recently (Victora et al., 2016). Even if this endpoint might

not have the greatest impact on infant health, it is particularly of interest for the development of the RBA methodology as this case allows to have an approach from a specific factor (DHA) to a particular health effect (cognitive development).

4.3. Model development

Five scenarios corresponding to six months of a particular diet were chosen (**Table 4.1**); they were named scenarios 1A, 1B, 2A, 2B and 3 for three different kinds of milk (1, 2 and 3) and two options of preparation (A and B). Scenario 1 corresponded to exclusive PIF consumption. Scenario 2 corresponded to supplemented PIF with fatty acids (DHA). For both scenarios two options of milk preparation were included. Preparation A followed the WHO recommendation (WHO, 2007b), the addition of boiled water to the powder followed by a half-an-hour cooling and consumption within two hours. Preparation B corresponded to the addition of water at ambient temperature and consumption of the milk within two hours. Scenario 3 corresponded to six months of exclusive breast milk consumption.

Table 4.1: Description of the five scenarios evaluated in this study

	Milk			**Preparation**		
	Regular powder infant formula (PIF)	**PIF supplemented in DHA**	**Breast milk**	**Preparation A[a]:**	**Preparation B[b]:**	**No preparation:**
Scenario 1A: (reference)	X			X		
Scenario 1B:	X				X	
Scenario 2A:		X		X		
Scenario 2B:		X			X	
Scenario 3:			X			X

[a]Preparation A: WHO recommendation (WHO, 2007b): water boiled at minimum 70°C, milk cooled 30 min at ambient temperature, consumption within 2h.

[b]Preparation B: Water at ambient temperature, consumption within 2h.

4.3.1. Model overview

The RBA was carried out in 5 steps following the traditional risk assessment approach detailed in Boué et al. (2015). More precisely, the RBA model developed was divided into several modules (Nauta, 2001), as illustrated in **Figure 4.1**. Modules 1 to 5 represent the main steps of the RBA and one sub-model was developed for each field. All calculations were done for one infant of reference for each gender i (boy and girl) and for each age in month j (from 1 to 6 months of age). This approach was used to consider the specific variability of each month of age and gender.

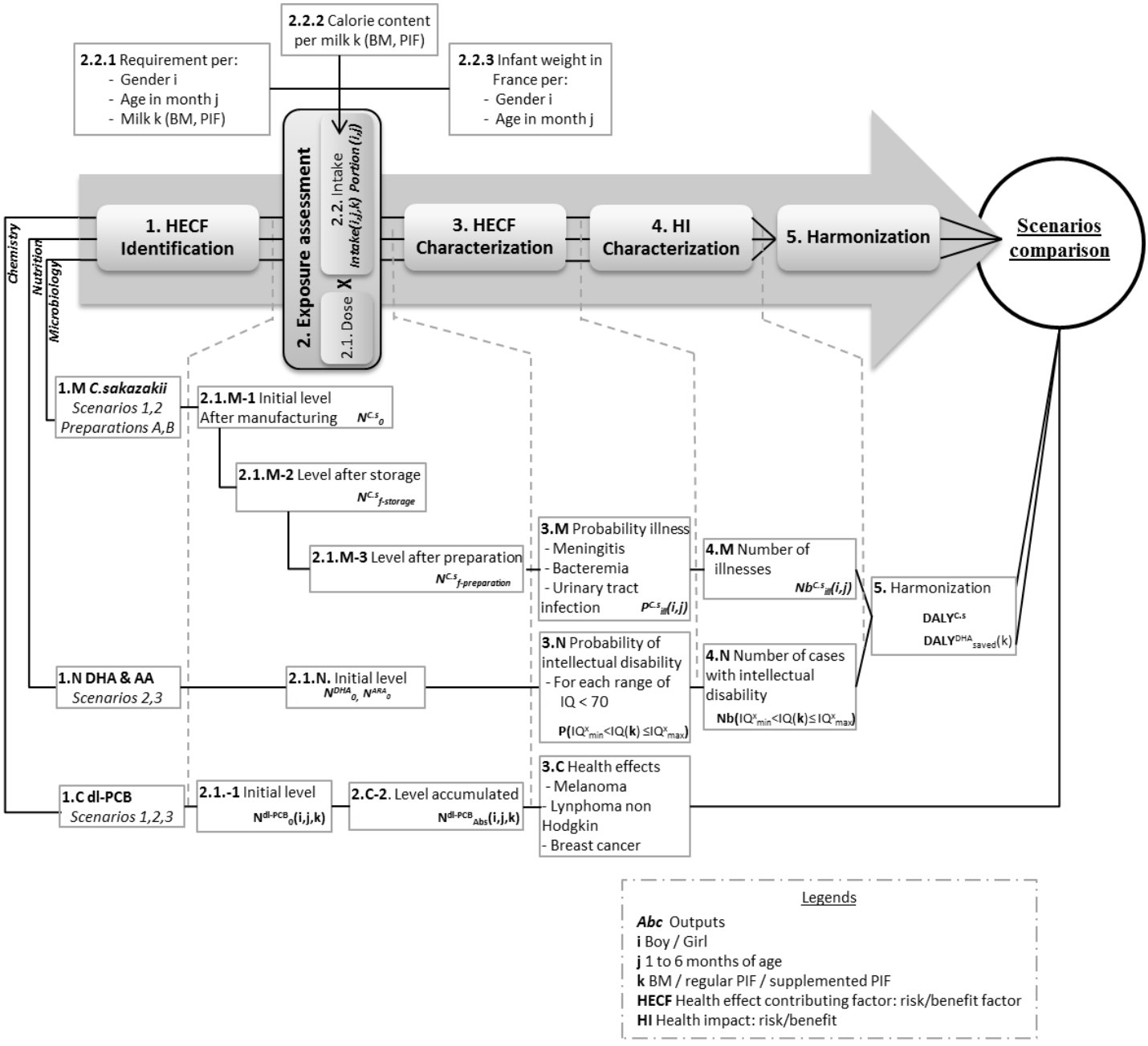

Figure 4.1: Flowchart of the Risk-Benefit Assessment model

4.3.2. Management of variability and uncertainty

To consider the variability of individuals in the population due to the natural heterogeneity, the final risk and benefit cannot be predicted using only deterministic values. Indeed, we can predict different levels of risk/benefit in the population according to different levels of food intake by individuals or according to the natural diversity of hazard levels in food for instance. Probabilistic processes were used to capture this variability and to estimate risks and benefits with a distribution reflecting possible range of results in the population. Nevertheless, it is also necessary to consider the element of accuracy of the model, i.e. the uncertainty that refers directly to the lack of knowledge. Contrary to variability, uncertainty can be reduced by integration of additional data or knowledge (Nauta, 2002). Assumptions made for both kinds of inputs (uncertain and variable) were explained when used in the model development and summarised in the discussion section for a transparent RBA (Nauta, 2000). On top of that, variability and uncertainty were separated to help policy makers to take informed decisions by providing greater confidence for results interpretation and by identifying need of data collection (Cummins, 2016).

4.3.3. Risk assessment computation

The model was implemented in Excel 2010 using the @Risk software (version 6.3.1) to carry out second order Monte Carlo simulations. In the variability dimension, 10 000 iterations were generated and 100 in the uncertainty dimension, both using the Latin Hypercube Sample method (Mokhtari and Frey, 2005). One iteration in the variability dimension represents one infant; hence different values obtained in this dimension reflect different possibilities according to the natural heterogeneity: different infant weights, intakes of PIF or levels of each factor in PIF for instance. More precisely in the microbiological model it represents one daily feed of one infant whereas in nutritional and chemical models it represents one infant for six months. On top of that, the uncertainty referring to the lack of knowledge in inputs and parameters is considered. Hereafter, if not mentioned otherwise, model outputs are summarized by their mean, median and 90^{th} percentile values.

Convergence of the model was checked by running the model three times independently: three sets of 100 iterations in the uncertainty dimension were generated. Uncertainty interval bounds did not vary significantly. Outputs were reported with significant number of digits accordingly to the degree of accuracy obtained.

4.3.4. Milk intake estimation

The milk intake was calculated for each gender (i), age in month (j), and type of milk consumed (k) (**Equation 4.1**). Nutritional requirements are different for breast fed and formula fed infants as the digestive processes differ (Butte, 2005). Calorie contents of PIF varying in the French market were implemented with a uniform distribution between the minimum and maximum values found. For BM, European data reported in the EFSA report (2014) were also implemented with a uniform distribution.

Equation 4.1

$$\text{Intake}(i,j,k) = \frac{\text{N\_Req}(i,j,k)\ .\ 100}{\text{calorie}(k)}$$

Where:

Intake(i,j,k): Daily intake of milk per gender i, age j and milk k (mL/kg b.w. per day),
i: Gender = Boy / Girl,
j: Age = 1 to 6 months of age,
k: BM / regular PIF / supplemented PIF,
N_Req(i,j,k): Daily nutritional needs (kcal/kg b.w. per day) (Butte, 2005),
Calorie (k): Calorie content of milk k (kcal/100 mL),
100: Conversion factor.

Infant weights are gender and age dependent. French data (Scherdel et al., 2015) were implemented with a cumulative distribution that represents the variability among the population. More particularly for PIF, the portion of powder taken from a box to prepare a bottle is estimated with **Equation 4.2**. It took into account the number of daily feeds that was set at 6, 5 and 4 for infants aged 0 to 2, 2 to 4 and 4 to 6 months respectively, based on French products recommendation. The percentage of dilution of PIF with water, named Rdil, was implemented with a uniform distribution between minimum and

maximum found on the French market. Inputs used to estimate the intake of milk and portions of PIF are summarised in **Table 4.2**.

Equation 4.2

$$\text{Portion(i,j)} = \frac{\text{Intake(i,j,k=PIF)} \,.\, \text{Rdil} \,.\, \text{Weight(i,j)}}{\text{Feed(j)}}$$

Where:

Portion(i,j): Quantity of PIF per bottle of milk prepared (g/feed),
Intake(i,j,k=PIF): Daily intake of PIF per gender i and age j (mL/kg b.w. per day),
Rdil: Rate of dilution of PIF with water,
Weight(i,j): Weight of infant of gender i and age j (kg),
Feed(j): Number of daily feeds.

Table 4.2: Description of inputs used in intake calculation (Equation 4.1 and Equation 4.2): infant nutritional requirements, weights and milk calorie content

		Age (j) (month)	Nutritional requirement N_Req(i,j,k)[a] (kcal/kg per day)		Category
			Breast milk	Infant formula	
Gender (i)	Boy	1	99	117	Deterministic values
		2	95	108	
		3	90	101	
		4	80	89	
		5	79	87	
		6	79	85	
	Girl	1	106	122	
		2	98	111	
		3	91	100	
		4	79	86	
		5	79	85	
		6	78	83	
Calorie content (kcal/100mL)			Uniform(54; 78)[b]	Uniform(66; 69)[c]	Variability
Rdil			Percentage of dilution of the PIF	Uniform(12.5; 14%)	Variability

[a] Butte (2005), [b] EFSA (2014), [c] French products.
Column "category" indicates the kind of dispersion that reflects the distribution of inputs: variability or uncertainty.

4.3.5. Risk assessment of *Cronobacter sakazakii* in PIF

C. sakazakii is the main bacteria found in PIF (Iversen and Forsythe, 2003) that can potentially lead to meningitis, bacteremia and urinary tract infection (Reij et al., 2009). It was not associated with BM (Cossey et al., 2011; Jones, 2001) , as a consequence, scenario 3 was assumed to be no risk with no variability and no uncertainty. For scenarios 1 and 2, two kinds of preparations were evaluated. The model has been developed mainly based on the work done by the FAO/WHO (FAO/WHO, 2006) with data adapted for France when possible, inputs are summarised in **Table 4.3**.

4.3.5.1 Exposure assessment of C. sakazakii

The exposure assessment step aimed to estimate the level of bacteria which infants are exposed to. Consequently, it includes the intake of milk estimated previously (Module 2.2 in **Figure 4.1**) as well as the calculation of the dose of *C. sakazakii* in milk at the time of consumption.

First the initial level of *C. sakazakii* in a box of PIF, after manufacturing, $N^{C.s}_0$, was implemented by bootstrapping data reported in FAO/WHO (2006) which are based on more than 29 000 samples collected from 12 industries and 7 publications. These data are the most extensive available at the moment in the literature and are in line with new data found (Jongenburger et al., 2011). However, it should be noted that cells are assumed in the present model to be homogeneously distributed in PIF which is not the case when contaminations occur, for instance after mixing, *via* air or filler heads. This assumption can lead to under-estimation of the risk but this phenomenon is under investigation (Jongenburger et al., 2012) and there is still not enough data to characterise adequately the heterogeneous distribution of bacteria.

After the step of manufacturing, boxes of PIF are stored during a certain period of time, called $t_{storage}$, including the transportation, the time spent in the supermarket and at home before use. This period was set at 30 days in the FAO-WHO model (FAO/WHO, 2006) and the option to choose 0, 30, 100 and 365 days was available in the online model (Paoli and Hartnett, 2006). A distribution was implemented in the present model to take into account the variability of different practices in the population. It was assumed that the mean storage duration which is uncertain, mean_ $t_{storage}$, would be between 10 and 30 days

(1 month). The storage duration was represented with a log-normal distribution to give more weight at the first month (Vose, 2008) after manufacturing and a truncation was applied at shelf life of 365 days. More precisely, it was implemented in @Risk with a log-normal distribution (Vose, 2008): LogNorm(mean_ $t_{storage}$; 100) with a mean of mean_ $t_{storage}$,and a standard deviation of 100 days. During this period of storage the level of *C. sakazakii* decreases in the box due to dehydration conditions (**Equation 4.3**).

Equation 4.3

$$\text{Log}\,(N^{C.s}_{f\text{-}storage}) = N^{C.s}_{0} - t_{storage} \,.\, Dr$$

Where:

$N^{C.s}_{f\text{-}storage}$: Level of C. *sakazakii*, after storage (cfu/g),

$N^{C.s}_{0}$: Level of C. *sakazakii*, after manufacturing (cfu/g),

$t_{storage}$: Duration of storage of a box of PIF between manufacturing and its use (day),

Dr: Decline rate of C. *sakazakii* in box of PIF (log cfu/day)(FAO/WHO, 2006).

At the time of preparation, a portion of powder is taken from the box, portion(i,j). This portion is potentially contaminated in *C. sakazakii* at the level $N^{C.s}_{f\text{-}storage}$. The level of bacteria in the portion taken, $N^{C.s}_{Portion}(i,j)$, follows a Binomial distribution according to the partitioning laws (Nauta, 2005) as cells are assumed to be homogeneously distributed. This distribution was approximated with a Poisson distribution to avoid numerical problems (Mendenhall et al., 1990) due to a very low level of *C. sakazakii* in PIF.

Equation 4.4

$$N^{C.s}_{Portion}(i,j) \sim \text{Poisson}\,(N^{C.s}_{f\text{-}storage} \,.\, Portion(i,j))$$

Where:

$N^{C.s}_{Portion}(i,j)$: Level of C. *sakazakii*, in a bottle of PIF (cfu/feed),

Portion(i,j,k = PIF): Daily intake of milk, estimated with **Equation 4.2** (mL/kg b.w. per day).

Then, the addition of water to the powder can influence the level of bacteria by changing temperature conditions inducing potential growth and/or inactivation. In conditions of non-equilibrium between the ambient temperature and the water temperature, the milk temperature $T_{milk}(t)$, changes following **Equation 4.5**.

Equation 4.5

$$T_{milk}(t) = T_{amb} + (T_0 - T_{amb}) \cdot \exp(-\beta \cdot t)$$

Where:

$T_{milk}(t)$: Temperature of milk at time t (°C),
t: Time (min),
T_{amb}: Ambient temperature (°C),
T_0: Temperature of the water added to PIF (°C),
β: Parameter of temperature change (FAO/WHO, 2006).

The ambient temperature was assumed to be 19°C during cold months and between 20°C and 30°C during warm months. To take into account temperature variability during the year the ambient temperature was implemented with a uniform distribution between summer and winter temperatures. The initial milk temperature was set at ambient temperature for preparation B and at 70°C for Preparation A.

The calculation of the level of *C. sakazakii* ends when the duration reaches the time of the complete consumption of milk, $t_{f\text{-}cons}$ in minutes. In absence of data this input was implemented in the uncertainty dimension with a uniform distribution including the cooling stage and the minimal duration of milk consumption set at 15 minutes. For both preparations a limit of 2 hours of consumption was defined, preceded by half an hour of cooling for Preparation A.

According to different scenarios, the milk temperature can induce either bacterial inactivation or growth or both. Temperature profile was approximated by successive constant temperature steps of Δt (Δt is set here at 1 minute). At each step s, $T_{milk}(s)$ was compared with the maximum temperature where growth is expected (T_{max}) to determine if there is growth ($T_{milk}(s) < T_{max}$) or inactivation ($T_{milk}(s) \geq T_{max}$). This value is variable among the different strains of *C. sakazakii* (Kandhai et al., 2009). However, there is still not enough data to include strain variability in the analysis so a worst case strategy was adopted as already done in a previous assessment (Paoli and Hartnett, 2006). The most thermotolerant strain, the strain 607, was taken into account in calculation.

The inactivation of *C. sakazakii* is calculated with a specific decimal reduction time, D(s), estimated with **Equation 4.6** and the growth is estimated with a specific growth rate μ(s) (**Equation 4.7**).

Equation 4.6

$$D(s) = 10^{(\log(Dref) - ((Tmilk(s) - Tref) / z))}$$

Equation 4.7

$$\mu(s) = \begin{cases} \mu_{opt}\left((T_{milk}(s) - T_{min}) / (T_{opt} - T_{max})\right)^2 & \text{if } T_{min} < T(s) \leq T_{opt} \\ \mu_{opt}\left((T_{milk}(s) - T_{max}) / (T_{max} - T_{opt})\right)^2 & \text{if } T_{opt} \leq T(s) < T_{max} \\ 0 & \text{if } T(s) < T_{min} \text{ or } T(s) \geq T_{max} \end{cases}$$

Where:

D(s): Specific decimal reduction time of *C. sakazakii* at step s (min),

S: Step used for calculation in dynamic condition (min),

D_{ref}: Reference decimal reduction at steps of *C. sakazakii* (min) (FAO/WHO, 2006),

$T_{milk}(s)$: Milk temperature at step s (°C),

T_{ref}: Reference temperature of *C. sakazakii* (°C),

z: Thermal resistance constant of *C. sakazakii* (FAO/WHO, 2006) (°C),

μ(s): Growth rate of *C. sakazaki* at step s (min^{-1}),

μ_{opt}: Optimal growth rate of *C. sakazakii* (min^{-1}) (Kandhai et al., 2009),

T_{min} and T_{max}: Minimum and maximum temperatures of growth of *C. sakazakii* (°C) (Kandhai et al., 2009).

To estimate the time of adaptation before growth, namely the lag phase, under changing temperature condition, the "work to be done" approach was used (Baranyi and Roberts, 1994; Koutsoumanis, 2001). This approach assumes that before growth, cells of bacteria need to adapt to environmental conditions (e.g. temperature). This adaptation called a "work" (Swinnen et al., 2004) requires a certain amount of time which is equivalent to the product of lag time and growth rate must be done. In practice, this work to be done, w(s), is estimated at each step s and compared with the sum of works done during previous steps (from 1 up to s-1). When the sum of works done is higher than the work to be done, w(s), the lag phase is finished (Daelman et al., 2013) (**Equation 4.8**). Iteration process to calculate the remaining work to be done, w'(s), is as follows:

Equation 4.8

$$
\begin{cases}
\text{For } s = 1 \\
w'(s) = w(s) \\
\text{For } s = 2 \text{ to } t_{f\text{-}cons}/\Delta t \text{ (rounded up to the nearest whole number):} \\
\lambda(s) = (b\,(T_{milk}(s)-T_{min})\,.\,(1 - \exp(c\,.\,(T_{milk}(s)-T_{max}))))^{-2} \\
w(s) = \lambda(s)\,.\,\mu(s) \\
w\_done(s) = \Delta t\,.\,\mu(s) \\
w'(s) = w(s) - \sum_{l=1}^{s-1} w\_done(l)
\end{cases}
$$

Where:

$\lambda(s)$: Lag phase at step s (min),

b and c: Constants (Kandhai et al., 2009),

w(s): "Work to be done" before growth given temperature at step s,

w_done(s): Work done during the previous step s (from 1 up to s-1),

Δt: Step of 1 minute used in the calculation,

$t_{f\text{-}cons}$: Time to consume a bottle, it includes the time of preparation and consumption (min).

The level of *C. sakazakii* is estimated at each step s as follows:

Equation 4.9

$$
\begin{cases}
\text{For } s = 1 \\
\quad N^{C.s}_{f\text{-}preparation}(s=1) = N^{C.s}_{Portion}(i,j) \\
\text{For } s = 2 \text{ to } t_{f\text{-}cons}/\Delta t \text{ (rounded up to the nearest whole number)} \\
N^{C.s}_{f\text{-}preparation}(s) = \begin{cases}
N^{C.s}_{f\text{-}preparation}(s\text{-}1)\,.\,10^{-\Delta t/D(s)} & \text{if } T_{milk}(s) \geq T_{max} \\
N^{C.s}_{f\text{-}preparation}(s\text{-}1)\,.\,\exp(\mu(s)\,.\,\Delta t) & \text{if } T_{min}<T_{milk}(s)<T_{max} \text{ and } w'(s)<0 \\
N^{C.s}_{f\text{-}preparation}(s\text{-}1) & \text{if } T_{milk}(s) \leq T_{min} \text{ or } w'(s) \geq 0
\end{cases}
\end{cases}
$$

Where:

$N^{C.s}_{f\text{-}preparation}(s)$ and $N^{C.s}_{f\text{-}preparation}(s\text{-}1)$: Levels of *C. sakazakii* at steps s and s-1 (cfu/feed),

w'(s): Remaining "work to be done", it is corrected for the work that has been done during the previous steps from 1 up to s-1.

4.3.5.2 C.sakazakii dose-response

The probability of illness associated with *C. sakazakii* was calculated with an exponential dose-response model. The dose-response parameter (r) used in this model, was estimated between 10^{-5} and 10^{-10} (FAO/WHO, 2006) and implemented with a uniform distribution of the logs representing the uncertainty. This parameter was not considered as specific to infant age. The daily probability of illness, $P^{C.s}_{ill,}$, was estimated with **Equation 4.10** (Havelaar and Zwietering, 2004).

Equation 4.10

$$P^{C.s}_{ill}(i,j) = Feed(j) \,.\, (1- \exp(-r \,.\, N^{C.s}_{f\text{-}preparation}(t_{f\text{-}cons} \,/\, \Delta t)))$$

Where:

$P^{C.s}_{ill}(i,j)$: Probability of illness per day for an infant of gender i and age j,

Feed(j): Number of daily feeds,

r: Exponential dose-response parameter (FAO/WHO, 2006),

$N^{C.s}_{f\text{-}preparation}(t_{f\text{-}cons}/\Delta t)$: Level of *C. sakazakii* at the end of consumption (cfu/feed).

4.3.5.3 Risk characterization of C. sakazakii in powder infant formula

The expected daily number of illnesses $Nb^{C.s}_{ill}(i,j)$ per gender i and age in month j was estimated at the population level for 100 000 infants. Mean daily probability of illness, $mean\_P^{C.s}_{ill}(i,j)$, was multiplied 100 000 times which was equivalent to a sum of 100 000 $P^{C.s}_{ill}(i,j)$ randomly sampled from the variability dimension. Thus, the expected number of cases integrates the variability of the population and is reported with uncertainty intervals. The expected number of illnesses of each gender and age in months, $Nb^{C.s}_{ill}(i,j)$, was multiplied by 30 days to get a monthly estimation and then a sum over genders and ages in months was used to get an estimation for six months of exposure (**Equation 4.11**).

Equation 4.11

$$Nb^{C.s}{}_{ill}(i,j) = mean\_P^{C.s}{}_{ill}(i,j) \,.\, 100\,000 \,.\, p(i) \,.\, 30$$

Where:

$Nb^{C.s}{}_{ill}(i,j)$: Expected number of illnesses per month of each gender i and age j,

$Mean\_P^{C.s}{}_{ill}(i,j)$: Mean Probability of illness per day for gender i and age j,

p(i): Percentage of boy and girl in France,

100 000: Number of infants considered in the calculation,

30: Number of days set per month.

Then the total number of infant illnesses is estimated for each scenario following six months of milk consumption with the sum of the number of cases estimated for each of the six months for both genders.

4.3.5.4 Conversion of risk of C.sakazakii in DALY

The global burden of disease was estimated in DALY, the Disability Adjusted Life Year (Gold et al., 2002) with **Equation 4.12** for each disease associated with *C. sakazakii* infection: meningitis, bacteraemia and urinary tract infection. The proportion of infants illnesses per disease, p(d), was estimated by Reij et al. (2009).

Equation 4.12

$$DALY^{C.s} = \sum_{d=1}^{3} \sum_{i=1}^{2} \sum_{j=1}^{6} Nb^{C.s}{}_{ill}(i,j) \,.\, p(d) \,.\, [p_{die}(d) \,.\, LE(i) + p_{seq}(d) \,.\, w_{seq}(d) \,.\, LE(i) + p_{rec}(d) \,.\, t_{ill}(d) \,.\, w_{rec}(d)]$$

Where:

$DALY^{C.s.}$: DALY per 100 000 infants for six months of exposure associated with *C. sakazakii* infection,

d: Disease = meningitis / bacteraemia / urinary tract infection,

p(d): Proportion of infants sick of the disease d based on Reij et al. (2009),

$p_{die}(d)$, $p_{seq}(d)$ and $p_{rec}(d)$: Percentages of person who die, has sequellae and recover when falling sick of disease d (Reij et al., 2009),

$w_{seq}(d)$ and $w_{rec}(d)$: Disability weights of person sick who has sequelae or who recovers (Reij et al., 2009),

LE(i): Remaining life expectancy of each gender,

$t_{ill}(d)$: Duration of illness when recover (Reij et al., 2009),

p_{girl} and p_{boy}: Percentages of girl and boy in France (INSEE, 2015),

LE_{girl} and LE_{boy}: Life expectancy of girls and boys tabulated for France (United Nations, 2013).

Table 4.3: Description and distributions of inputs for quantification of the risk of *C. sakazakii* associated with powder infant formula consumption, regular and supplemented *(Equations 4.3 to 4.12.)*

Input	Description	Model implementation	Unit	Reference	Category
$N^{C.s}_0$	Initial Level of *Cronobacter sakazakii* in box of PIF, after manufacturing	Bootstrap	log cfu/g	FAO/WHO (2006)	Variability
Mean_$t_{storage}$	Mean duration of storage of a box between manufacturing and its use to prepare a bottle	Uniform (10 ; 30)	day	Assumption	Uncertainty
$t_{storage}$	Storage duration of a box between manufacturing and its use to prepare a bottle	LogNormal (Mean_$t_{storage}$; 100)	day	Assumption based on FAO/WHO (2006)	Variability
Dr	Decline rate of *Cronobacter sakazakii* in PIF	0.001	log units/day	FAO/WHO (2006)	D
T_o	Initial milk temperature	Preparation A: 70°C Preparation B: T_{amb}	°C	Set according to scenarios	D
β	Cooling parameter	0.1	/	Estimated based on a figure in FAO/WHO (2006)	D
Dref	Decimal reduction time at reference temperature	9.6	min	Reported from FAO/WHO (2006)	D
Tref	Reference temperature	58	°C		D
z	z-value	5.6	°C		D
T_{amb}	Ambient temperature	Uniform(19 ; uniform(20 ; 30))	°C	Assumption	Variability
T_{min}	Minimum temperature of growth	2.5	°C		D
T_{opt}	Optimum temperature of growth	37	°C		D
T_{max}	Maximum temperature of growth	49	°C		D
$t_{f\text{-}cons}$	Duration between milk preparation and the end of consumption	Preparation A Uniform (0.75 ; 2.5) . 60 Preparation B Uniform(0.25 ; 2) . 60	min	Assumption	Variability
μ_{opt}	Growth rate	0.039	min-1	Kandhai et al. (2009)	D
b	Lag model parameter	0.023	/		D
c	Lag model parameter	0.645	/		D
r	Dose-response parameter	$10^{Uniform\ (-10\ ;\ -5)}$	/	FAO/WHO (2006)	Uncertainty
For each disease d = meningitis / bacteraemia / urinary tract infection:					
p(d)	Rate of cases of a particular disease d		%	Values for each disease provided in Reij et al. (2009)	D
p_{die}**(d)**	Rate of death per illness	Values reported for each disease	%		D
p_{Seq}**(d)**	Rate of illnesses with sequaellae		%		D
p_{Rec}**(d)**	Rate of cases that recover		%		D

$w_{Seq}(d)$	Disability weight when sequellae	Uniform(0.4 ; 0.8)	%		Uncertainty
$w_{Req}(d)$	Disability weight when recovering	Values reported for each disease	%		D
$t_{Ill}(d)$	Years of life live with the disease		Year		D

Column "category" indicated the kind of dispersion that reflects the distribution of inputs; variability or uncertainty. When none is considered, a deterministic value is used D.

4.3.6. Benefit assessment of DHA in BM and Supplemented PIF

The fatty acid composition of BM is variable and depends on the mothers' diet (Yuhas et al., 2006). It is also variable for PIF as the regulation gives maximum limits for some fatty acids (including DHA)(Codex Alimentarius Commission, 1981). On the French market, around one third of the products available are supplemented in DHA and also in arachidonic acid (ARA)(Briend et al., 2014). **Table 4.4** summarises inputs of the nutritional model.

4.3.6.1 Exposure assessment of DHA

The level of DHA and ARA in BM in France was reported in Bernard et al. (2015), data were fitted with a Normal distribution.

4.3.6.2 DHA dose-response

The health effect associated with DHA is the potential improvement of the cognitive development measured through different Intellectual Quotient (IQ) tests that were mostly developed in US and Britain. At the world level, national differences were observed regarding the mean IQ of the population (Lynn and Vanhanen, 2002; Lynn and Vanhanen, 2006), they were compared with the Greenwich scale corresponding to the British IQ which followed a normal distribution with a mean of 100 and standard deviation of 15. The IQ result cannot be seen as a linear link between IQ and the level of intelligence but only as a rank between people. For a standard distribution of IQ, the lower ranges of IQ, below 70, have been associated with intellectual disability (INSERM, 2016; Salomon et

al., 2015). The WHO estimated the disability weights associated with four low ranges: mild 69-50, moderate 49-35, severe 34-20 and profound < 20 (Salomon et al., 2015).

At the level of the French population, the current mean IQ was found between 98 and 101 (Christainsen, 2013). A uniform distribution was used to catch this uncertainty. This mean IQ was associated with the scenario of non-supplemented PIF feeding (Scenario 1) since the majority of French infants are fed with non-supplemented PIF (Salanave et al., 2014). This baseline scenario was compared with the two other ones to estimate the potential benefit of breastfeeding and supplemented PIF. The benefit was introduced in our study at its higher level to estimate the "best case" in the same way of a "worst case" approach with a risk assessment. The dose-response of BM consumption (scenario 3) was constructed based on the study from Gustafsson et al. (2004). This study investigated the IQ of infants at five years regarding the duration of BM consumption in a week, d_{BF}, the ratio of DHA and ARA in BM and the duration of gestation. IQ was predicted with a multi regression including these variables. The mean IQ of breastfed infants, scenario 3, was predicted by including levels of DHA and ARA in France with **Equation 4.13**. The dose-response of supplemented PIF (scenario 2) was deduced from the study from Birch et al. (2007). In this study, the IQ of infants fed with supplemented PIF, regular PIF and BM were compared. An improvement of 6.5 IQ points, Δ, was found as compared with the control population consuming regular PIF. This shift has been added at the mean IQ of the baseline population to simulate the same increase (**Equation 4.13**). IQ was implemented with a normal distribution with the mean and a standard deviation of 15 (Neisser et al., 1996) (**Equation 4.14**) representing variability between individuals.

Equation 4.13

$$IQ_{mean}(k) = \begin{cases} \text{Uniform}(98\ ;\ 101) & \text{if } k = \text{regular PIF} \\ IQ_{mean}(k=\text{regular PIF}) + a\ .\ N^{DHA}{}_0\ /\ N^{ARA}{}_0 + b\ .\ d_{BF} & \text{if } k = \text{BM} \\ IQ_{mean}(k=\text{regular PIF}) + \Delta & \text{if } k = \text{supplemented PIF} \end{cases}$$

Equation 4.14

$$IQ(k) \sim \text{Normal}\ (IQ_{mean}(k)\ ;\ SD)$$

Where:

IQmean(k): Mean IQ of the population consuming milk k,

a and b: Dose-response parameters (Gustafsson et al., 2004),

NDHA0, NARA0: Levels of DHA and ARA in BM (Bernard et al., 2015),

dBF: Duration of breast milk consumption set at 26 weeks,

Δ: Increase of the mean IQ when breastfed compared with formula-fed (Birch et al., 2007),

IQ(k): IQ distribution of the population consuming milk k,

SD: Standard deviation set at 15 (Neisser et al., 1996).

4.3.6.3 Benefit characterization of DHA in supplemented powder infant formula and breast milk

The health effect of DHA on the cognition development is measured by a change of IQ level. This improvement can be translated as a potential decrease of the number of infants in the population with intellectual disability (IQ<70). Indeed, knowing the distribution of IQ at the population level, the probability for an infant of having a particular IQ can be estimated for each type of milk. This probability was calculated for each IQ range x associated with different levels of burden of disease (mild 69-50, moderate 49-35, severe 34-20 and profound <20) (**Equation 4.15**).

Equation 4.15

$$P(IQ^{x}_{min} < IQ(k) \leq IQ^{x}_{max}) = F(IQ(k) < IQ^{x}_{max}) - F(IQ(k) \leq IQ^{x}_{min})$$

Equation 4.16

$$Nb(IQ^{x}_{min} < IQ(k) \leq IQ^{x}_{max}) = 100\,000 \,.\, P(IQ^{x}_{min} < IQ(k) \leq IQ^{x}_{max})$$

Where:

$P(IQ^{x}_{min} < IQ(k) \leq IQ^{x}_{max})$: Probability of having an IQ between IQ^{x}_{min} and IQ^{x}_{max} when consuming milk k,

$Nb(IQ^{x}_{min} < IQ(k) \leq IQ^{x}_{max})$: Number of infants having an IQ between IQ^{x}_{min} and IQ^{x}_{max}, when consuming milk k,

IQ^{x}_{min} and IQ^{x}_{max}: Minimum and maximum of IQ of range x,

$F(IQ(k) < IQ^{x}_{max})$ and $F(IQ(k) \leq IQ^{x}_{min})$: Cumulative probability of having an IQ lower than IQ^{x}_{min} and IQ^{x}_{max} respectively,

100 000: Number of infants considered in the calculation.

Then at the population level, for 100 000 infants the number of infants per IQ range can be estimated for each milk with **Equation 4.16**.

4.3.6.4 Conversion of DHA benefit in DALY

The benefit associated with fatty acids can be estimated by comparison with the baseline scenario of regular PIF (scenario 1). So, the net health impact would be represented by those who have shifted a range x of intellectual disability to a higher level. This benefit was converted in DALY. A triangular distribution was used to implement the disability weights w^x of each IQ range x to take into account the uncertainty (Salomon et al., 2015). The burden of disease was calculated with **Equation 4.17**. Here, the life expectancy, LE, is a mean of LE(i) of each gender weighted by the proportion of girls and boys in the population.

Equation 4.17

$$DALY^{DHA}(k) = Nb\sum_{x=1}^{4} \left(Nb(IQ^x_{min} < IQ(k) \leq IQ^x_{max}) \,.\, w^x.\, LE\right)$$

Where:

$DALY^{DHA}(k)$: DALY per 100 000 infants associated with intellectual disability when consuming milk k,

X: Category of disability = mild 69-50 / moderate 49-35 / severe 34-20 / profound <20,

$Nb(IQ^x_{min} < IQ(k) \leq IQ^x_{max})$: Number of infants having an IQ between IQ^x_{min} and IQ^x_{max}, when consuming milk k,

w^x: Disability weight of the range x of intellectual disability (Salomon et al., 2015).

Finally, the number of DALY potentially saved by six months of BF or six months of supplemented PIF consumption can be estimated by subtraction of the burden of disease of each scenario with the baseline of regular PIF consumption (**Equation 4.18**).

Equation 4.18

$$DALY^{DHA}{}_{saved}(k) = DALY^{DHA}(k=\text{regular PIF}) - DALY^{DHA}(k)$$

Where:

$DALY^{DHA}{}_{saved}(k)$: DALY saved when consuming milk k, compared with k=regular PIF.

Table 4.4: Description and distributions of inputs for quantification of the potential benefit of DHA associated with supplemented powder infant formula and breast milk consumption *(Equations 4.13 to 4.18)*

Input	Description	Model implementation	Unit	Reference	Category
N^{DHA}_0**(BM)**	Initial level of DHA in BM	Normal($mean_{DHA}$; sd_{DHA})	mg/100ml	Bernard et al. (2015)	Variability
$mean_{DHA}$	Mean level of DHA in French BM	0.64	% total fatty acids		D
sd_{DHA}	Standard deviation	0.19	/		D
N^{ARA}_0**(BM)**	Initial level of ARA in BM	Normal($mean_{ARA}$; sd_{ARA})	mg/100ml	Bernard et al. (2015)	Variability
$Mean_{ARA}$	Mean level of ARA in French BM	0.86	% total fatty acids		D
sd_{ARA}	Standard deviation	0.15	/		d
IQmean	Mean IQ in France	Uniform(98 ; 101)	/	Christainsen (2013)	Uncertainty
SD	Standard deviation of the IQ	15	/	Neisser et al. (1996)	D
a	Multi regression parameter of DHA/AA	0.510	/	Gustafsson et al. (2004)	D
b	Multi regression parameter of the duration of breastfeeding	0.528	/		D
d_{BF}	Duration of breastfeeding	26	week		D
Δ	Potential improvement of IQ of infants breastfed with supplemented IF	6.5		Birch et al. (2007)	D
For each range x of IQ					
$w_{IQ(69-50)}$	Mild disability weight for IQ 69-50	Triangular(0.026 ; 0.043 ; 0.064)	/	Salomon et al. (2015)	Uncertainty
$w_{IQ(49-35)}$	Moderate disability weight for IQ 49-35	Triangular(0.066 ; 0.100 ; 0.142)	/		Uncertainty
$w_{IQ34-20)}$	Severe disability weight for IQ 34-20	Triangular(0.107 ; 0.160 ; 0.226)	/		Uncertainty
$w_{IQ<20)}$	Profound disability weight for IQ<20	Triangular(0.133 ; 0.200 ; 0.283)	/		Uncertainty

Column "category" indicated the kind of dispersion that reflects the distribution of inputs; variability or uncertainty. When none is considered, a deterministic value is used D.

4.3.7. Risk assessment of dl-PCB in BM and PIF

Breast milk is a route of exposure to chemicals for infants as various classes of contaminants are accumulated in maternal fatty tissues and released during lactation. It is particularly the case for persistent organic pollutants (POPs) in general and for dioxin-like polychlorinated (dl-PCB) in particular, still present in the environment and in humans despite their ban in the early nineties and despite a global decreasing trend in terms of environmental exposure. PIF are not exempt from these chemicals but the shorter accumulation times and the higher milk production volumes lead to very significantly lower levels in bovine than in human milk. Some industrial processes also lead to decreased levels especially when a delipidation step is introduced.

Dioxin-like PCB (dl-PCB) encompasses twelve chemicals exhibiting similar structure and toxicity as dioxins. They are highly lipophilic and can be stored during several years in the body due to their long half-life (Grandjean et al., 2008; Ogura, 2004). Among the health effects associated with these chemicals, melanoma, non Hodgkin lymphoma and breast cancer were pointed out, with significant evidence in humans (IARC, 2015). **Table 4.5** summarises the inputs used to develop our chemical model.

4.3.7.1 Exposure assessment of dl-PCB

Exposure assessment for PIF has been calculated in two steps with **Equation 4.19**: first the level of dl-PCB in PIF was collected and then the fraction absorbed in the infant body was estimated. Initial level of dl-PCB, $N^{dl\text{-}PCB}{}_0(k = PIF)$, was based on European data (including French)(EFSA, 2012). A uniform distribution was implemented between half of the limit of detection and the maximum level detected (medium bound approach). A part of the ingested dl-PCB is excreted whereas the other one is absorbed and stored (Ulaszewska et al., 2011). The absorption in infants' body is potentially different among infants but was often reported between 85% and 100% with more frequent values at 95% (Abraham et al., 1996; Dahl et al., 1995; Moser and McLachlan, 2002; Schlummer et al., 1998; Tanabe et al., 1981; Ulaszewska et al., 2011). To take into account the uncertainty of this input, a triangular distribution was implemented.

Equation 4.19

$$N^{dl\text{-}PCB}{}_{Abs}(k = PIF) = N^{dl\text{-}PCB}{}_0(k = PIF) \,.\, AF \,.\, Intake(i,j,k = PIF)$$

Where:

$N^{dl\text{-}PCB}_{Abs}$(i, j, k = PIF): Level of exposure in dl-PCB of formula fed infants, of gender i and age j (pg/kg b.w. per day),

$N^{dl\text{-}PCB}_{0}$(k = PIF): Level of dl-PCB in PIF (pg/mL),

AF: Percentage of absorption of dl-PCB in infant,

Intake(i,j,k = PIF): Daily intake of milk, estimated with **Equation 4.1** (mL/kg b.w. per day).

Exposure assessment for BM has been calculated in three steps: first the level of dl-PCB in BM is estimated in "toxic equivalent" (TEQ) with **Equation 4.20**, then the depuration phenomena is considered to estimate decrease of contaminants in BM during the lactation period and finally the fraction absorbed in infant body (**Equation 4.20**). The levels of dl-PCB found in BM in France were collected from research studies (Focant et al., 2013) as well as a biomonitoring report (2007). For each dl-PCB congener a distribution was reconstituted based on the reported percentiles. It was then implemented with a cumulative distribution given the concentration of each of the twelve congeners, [c]. The initial levels of dl-PCB in BM ($N^{dl\text{-}PCB}_{0}$(k=BM)), was classically estimated in "toxic equivalent" (TEQ) by weighing the concentration of each congener with its specific toxic equivalent factors (TEF) (van den Berg et al., 2006), then expressed per unit of lipid contained in BM [Fat] (**Equation 4.20**). This last parameter was estimated on the basis of French data that were fitted as a normal distribution (Antignac JP et al., 2016).

Equation 4.20

$$N^{dl\text{-}PCB}_{0}(k = BM) = \sum_{c=1}^{12} TEF(c)\,.\,[c]\,.\,[Fat]$$

Where:

$N^{dl\text{-}PCB}_{0}$(k = BM): Level of dl-PCB in BM (pg/mL),

TEF(c): Toxicological equivalent factor of the congener c (van den Berg et al., 2006),

[c]: Concentration of each congener of dl-PCB (pg/g lipids) (Focant et al., 2013; INVS, 2007),

[Fat]: Concentration in fatty acids in BM (g lipids/mL).

In addition it needs to be taken into account that levels of chemicals in breast milk could decrease according to the lactation duration due to a depuration phenomenon, in average this decrease is around 0.17% per week (Ulaszewska et al., 2012). Nevertheless this

depuration phenomenon remains discussed (Hooper et al., 2007; LaKind et al., 2009) so a uniform distribution was implemented between 0 up to 1.7% per week. The daily exposure of breastfed infants was thus estimated with **Equation 4.21** adapted from Ulaszewska et al. (2011).

Equation 4.21

$$N^{\text{dl-PCB}}{}_{\text{Abs}}(i,j,k = BM) = N^{\text{dl-PCB}}{}_{0} \,.\, (k = BM) \,.\, AF \,.\, \text{Intake}(i,j,k = BM) \,.\, \int_{0}^{4j} e^{-y \,\cdot\, w} .\ dw \,/\, 7$$

Where:

$N^{\text{dl-PCB}}{}_{\text{Abs}}(i,j,k)$: Level of exposure in dl-PCB of infants, of gender i, age j and milk k (pg/kg b.w. per day),

W: Number of week,

Y: Weekly decrease of dl-PCB of lactating women,

7: Number of days per week,

$(\int_{0}^{j} e^{-y.w}.\ dw)$ was integrated as (1/-y . (exp(y . j) - 1)).

4.3.7.2 Dl-PCB dose-response

Chronic exposure to dl-PCB were found to be associated with a risk of breast cancer, melanoma, and non Hodgkin lymphoma (2015). As persistent chemicals, they are progressively eliminated from the body over time so "the accumulated concentration should be considered (body burden) rather than the daily exposure" (Béchaux et al., 2014) but kinetic dietary exposure models are under development. In addition, available data regarding the dose-response relationship of dl-PCB in relation to cancer did not appear sufficiently clear and robust to be implemented in our integrative model. Indeed, the current risk assessment approach in chemistry is to compare exposure levels with safety reference values as done by Béchaux et al. (2014) which corresponds to a "non-effective" level integrating safety margins whereas in RBA "effective" levels are needed to allow prediction of the number of cases. This limitation is due to the lack of clear established dose-responses. Nonetheless this is progressively evolving and a framework has been recently developed to build probabilistic dose-responses (Chiu and Slob, 2015) and a guidance was established to help better consideration of uncertainty in dose-response (IPCS, 2014).

In this context, our work regarding the chemical component was stopped at the exposure assessment stage; development of chemical dose-response was out of scope of the present paper. In addition, RBA is already a complex analysis integrating a lot of sources of uncertainty due to its multidisciplinary so it would be better to consider only strong points in the assessment (i.e. dose-response, endpoint, inputs…). A more advanced model will be required to tackle and overcome the major challenges and current front of sciences that are associated with the very complex multiple chemical exposure and late effect consecutive to early exposure issues.

Table 4.5: Description and distributions of inputs for exposure assessment of dl-PCB associated with powder infant formula (regular and supplemented) and breast milk consumption *(Equations 4.19 to 4.21)*

Input	Description	Model implementation	Unit	Reference	Category
$\mathbf{N^{dl\text{-}PCB}_0(k}$ **PIF)** =	Concentration of dl-PCB in PIF	Uniform(LOD/2,Max)	pg/g wet weight	European data (including French) from EFSA (2012).	Variability
AF	Absorption factor	Triangular(85; 95 ; 100)	%	Collected in the literature (Abraham et al., 1996; Dahl et al., 1995; Moser and McLachlan, 2002; Schlummer et al., 1998; Tanabe et al., 1981; Ulaszewska et al., 2011)	Uncertainty
TEF(c)	Toxicological equivalent factor of the congener c	Value for each congener	/	Data collected in van den Berg et al. (2006)	D
[c]	Concentration in each congener of dl-PCB in BM	Cumulative distribution	pg/g lipids	Data collected in Focant et al. (2013) and INVS (2007)	Variability
[Fat]	Concentration in fatty acids in BM	Normal(3.0166 ; 1.0203)	g lipids / ml	French data fitted	Variability
y	Decrease factor of BM depuration	Uniform(0 ; 0.017)	/	Ulaszewska et al. (2012)	Uncertainty

Column "category" indicated the kind of dispersion that reflects the distribution of inputs; variability or uncertainty. When none is considered, a deterministic value is used D.

4.4. Results

4.4.1. Main outputs of each sub-model

4.4.1.1 Intake calculation

The daily intakes for each considered consumption scenario were estimated for different ages in months and for both genders. Values obtained are reported into **Appendix Table 4.11**.

4.4.1.2 Microbiology

The level of *C. sakazakii* in PIF at the time of manufacturing was initially varying from -5.2 up to -2.8 log cfu/g (FAO/WHO, 2006) due to natural heterogeneity between batches. Then during powder storage, a potential decrease was estimated due to dehydration conditions, thus the level of *C. sakazakii* in PIF was estimated to vary after storage from -5.4 up to -2.9 log cfu/g in the variability dimension. Based on this level, the prevalence of bottles of PIF contaminated in *C. sakazakii* was estimated. It varied with the initial level of bacteria in the box of PIF, the portion of powder taken from the box, itself varying according to infant age, weight, gender and the calorie content of PIF. The scenario of PIF hydrated with boiled water at a minimum of 70°C (preparation 1A and 2A) was followed by a full inactivation of the level of *C. sakazakii*. Indeed, this bacteria has a low thermal resistance (Edelson-Mammel and Buchanan, 2004; Iversen and Forsythe, 2003; Osaili and Forsythe, 2009) and even the most resistant strain has a D-value as low as 3.9 seconds at 70°C (Edelson-Mammel and Buchanan, 2004).

The other scenario with water added at ambient temperature could be subject to growth. However, for all simulations, the lag phase was higher than the 2 hours of consumption set in this scenario so no growth occurred, as found previously (Kandhai et al., 2009).

At this stage, only preparation B gave a potential exposure to risk of *C. sakazakii*. The daily probabilities of illness are reported in **Table 4.6** per gender and age in months. While the differences between age and gender are small (all mean values ca 10-10), the variability within a specific combination of age and gender is larger (5^{th} percentile at 0, 99.9^{th} percentile ca $10^{-7)}$. This variation reflects the consideration of different levels of PIF contamination and different levels of intake. A sensitivity analysis is required to

explain precisely this source of variation. The addition of uncertainty to the model gives the precision of this result for the 5th and 99.9th percentiles: 0 [0 ; 0] up to 1.7 10^{-7} [9.0 10^{-10} ; 3.1 10^{-5}]. The uncertainty of this output might be due to the r parameter uncertainty, which is large.

Table 4.6: Estimates of daily risk of *C. sakazakii* associated with powder infant formula consumption calculated with **Equation 4.10** (regular and supplemented PIF)

			Powder infant formula[a]				
			Mean	**5%**	**50%**	**95%**	**99.9%**
Probability of illness per day	**Girl Age in month**	1	8.3 10^{-10} [4.5 10^{-12}; 1.5 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.7 10^{-07} [9.0 10^{-10}; 3.1 10^{-5}]
		2	9.5 10^{-10} [5.1 10^{-12}; 1.7 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.7 10^{-07} [9.0 10^{-10}; 3.1 10^{-5}]
		3	10 10^{-10} [5.4 10^{-12}; 1.8 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.5 10^{-07} [7.5 10^{-10}; 2.6 10^{-5}]
		4	9.9 10^{-10} [5.3 10^{-12}; 1.8 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.5 10^{-07} [7.5 10^{-10}; 2.6 10^{-5}]
		5	11 10^{-10} [5.9 10^{-12}; 1.9 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.2 10^{-07} [6.0 10^{-10}; 2.1 10^{-5}]
		6	11 10^{-10} [6.0 10^{-12}; 2.0 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.2 10^{-07} [6.0 10^{-10}; 2.1 10^{-5}]
Scenarios 1 and 2, Preparation B $P^{C.s}_{ill}(j)$	**Boy Age in month**	1	8.6 10^{-10} [4.6 10^{-12}; 1.5 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.7 10^{-07} [9.0 10^{-10}; 3.1 10^{-5}]
		2	9.9 10^{-10} [5.4 10^{-12}; 1.8 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.7 10^{-07} [9.0 10^{-10}; 3.1 10^{-5}]
		3	11 10^{-10} [5.9 10^{-12}; 1.9 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.5 10^{-07} [7.5 10^{-10}; 2.6 10^{-5}]
		4	11 10^{-10} [5.8 10^{-12}; 1.9 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.5 10^{-07} [7.5 10^{-10}; 2.6 10^{-5}]
		5	12 10^{-10} [6.4 10^{-12}; 2.1 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.2 10^{-07} [6.0 10^{-10}; 2.1 10^{-5}]
		6	13 10^{-10} [6.8 10^{-12}; 2.3 10^{-7}]	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	1.2 10^{-07} [6.0 10^{-10}; 2.1 10^{-5}]

[a] Percentiles given represent the variability of outputs, mean values are given with their uncertainty interval, when available 90% confident interval is given in square brackets, with the lower bound at 5% and the upper bound at 95%.

Example: The mean daily risk of infection in the population is estimated at 8.3 10-10 for girls during the first month of life. Fifty percent of the population (girls from birth to 1 month of age) has an estimated risk of 0 and 0.1 percent has an estimated risk ≥ 1.7 10-7.

NB: Preparation A with boiled water lead to a full inactivation of *C. sakazakii* so probability of illness, number of illness and DALY are equal to zero. Scenario 3 of BM consumption is not assessed for *C. sakazakii* as it is not found in BM.

A mean of 0.1 cases of infection per 100 000 infants for six months of exposure was found, varying from 0.0007 and 3 cases when considering the uncertainty (**Table 4.7**). Finally, the mean burden of disease associated with 100 000 infants following the diet 1B

and 2B during 6 months was estimated at 6 DALY on average, ranging from 0.03 up to 130 when integrating the uncertainty.

Table 4.7: Estimates of number of *C. sakazakii* infection per 100 000 infants following diet 1B and 2B during 6 months calculated with *Equation 4.11* and *Equation 4.12* (regular and supplemented PIF prepared with water at ambient temperature)

Subpopulation		Mean risk per day Scenarios 1 and 2, Preparation B Mean_$P^{C.s}_{ill}$(j)	Predicted number of cases for 100 000 infants for 6 months $Nb^{C.s}_{ill}$	DALY of exposure $DALY^{C.s}$
Girl Age in month	1	$8.3\ 10^{-10}$ [$4.5\ 10^{-12}$; $1.5\ 10^{-7}$]		
	2	$9.5\ 10^{-10}$ [$5.1\ 10^{-12}$; $1.7\ 10^{-7}$]		
	3	$10\ 10^{-10}$ [$5.4\ 10^{-12}$; $1.8\ 10^{-7}$]		
	4	$9.9\ 10^{-10}$ [$5.3\ 10^{-12}$; $1.8\ 10^{-7}$]		
	5	$11\ 10^{-10}$ [$5.9\ 10^{-12}$; $1.9\ 10^{-7}$]		
	6	$11\ 10^{-10}$ [$6.0\ 10^{-12}$; $2.0\ 10^{-7}$]		
			0.1 [0.0007 ; 3]	6 [0.03 ; 130]
Boy Age in month	1	$8.6\ 10^{-10}$ [$4.6\ 10^{-12}$; $1.5\ 10^{-7}$]		
	2	$9.9\ 10^{-10}$ [$5.4\ 10^{-12}$; $1.8\ 10^{-7}$]		
	3	$11\ 10^{-10}$ [$5.9\ 10^{-12}$; $1.9\ 10^{-7}$]		
	4	$11\ 10^{-10}$ [$5.8\ 10^{-12}$; $1.9\ 10^{-7}$]		
	5	$12\ 10^{-10}$ [$6.4\ 10^{-12}$; $2.1\ 10^{-7}$]		
	6	$13\ 10^{-10}$ [$6.8\ 10^{-12}$; $2.3\ 10^{-7}$]		

Confident interval is given in square brackets, with the lower bound at 5% and the upper bound at 95%.

NB: Preparation A with boiled water lead to a full inactivation of *C. sakazakii* so probability of illness, number of illness and DALY are equal to zero. Scenario 3 of BM consumption is not assessed for *C. sakazakii* as it is not found in BM.

4.4.1.3 Nutrition

In the nutritional model, the first step was to build the IQ curve of the population that was associated with the consumption of regular PIF. Based on this scenario, the IQ curves of the two other scenarios were predicted integrating the potential benefit that would be expected for these diets. As a result, the IQ curve of these two last scenarios was shifted towards a higher IQ, decreasing at the same time the proportion of the population with intellectual disability, IQ < 70. Curves were represented in **Figure 4.2** for each scenario; the variability among the population can be seen following the shapes of the curves whereas the uncertainty associated with this prediction can be seen with the thickness of each group of curves. Considering 100 000 infants, 2 460 were on average suffering from

intellectual disability in the baseline scenario of infant fed with regular PIF when considering all ranges of intellectual disability (see **Table 4.8** for results per IQ range). This figure decreases at 820 in scenario 2 (supplemented PIF) and at 190 in scenario 3 (breast milk). The uncertainty around the IQ was relatively low as the three curves are well distinguished.

Then, the number of cases is converted in DALY to estimate the potential burden of disease saved with scenarios 2 and 3. At this stage, the source of uncertainty around the disability weights for each IQ range x had a high impact on the output that obscures the distinction on the effect of both scenarios.

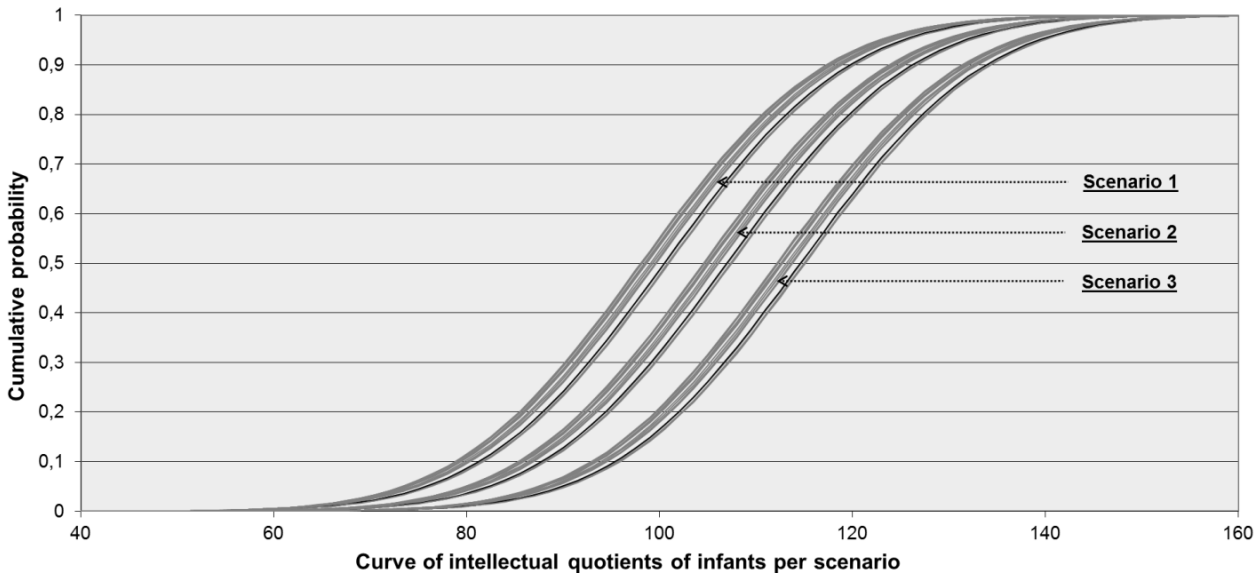

Figure 4.2: Cumulative distributions of IQ level in the population according to different scenarios

(Based on estimates reported in **Table 4.8** and obtained with Equation 4.14). Each curve represents the variability of individuals in the population for one dimension of uncertainty. For each scenario, different curves obtained highlight the influence of uncertainty on this output. For each percentile of variability (y values) the x values of the corresponding points on the different curves corresponds to the estimate IQ level in the uncertainty dimension.

Example: If we look at IQ of 100: for scenario 1 an estimate of about 50% of individuals in the population has an IQ ≤100, for scenario 2 it is about 40% and for scenario 3 it is about 20%. Uncertainty associated with these results can be appreciated by the dispersion of all curves associated with each scenario.

Table 4.8: Estimates of main outputs for quantification of benefit of DHA associated with supplemented powder infant formula and breast milk consumption, calculated with *Equations 4.15 to 4.18*

		Powder Infant Formula Scenario 1	Supplemented Powder Infant Formula Scenario 2	Breast milk Scenario 3			
					Percentile[b]		
		Mean[a]	Mean[a]	Mean	5%	50%	95%
$P(IQ^x_{min}<IQ(k)<IQ^x_{max})$							
IQ ranges	**<70**	**2.5 [1.9;3.1] E-02**	**8.2 [6.4;10.1] E-03**	**1.9 [1.4;2.4] E-03**	**1.8 [1.3;2.3] E-03**	**1.9 [1.4;4.2] E-03**	**2.0 [1.4;2.5] E-03**
	50-69	2.4 [1.9;3.0] E-02	8.1 [6.3;10] E-03	1.9 [1.4;2.4] E-03	1.8 [1.3;2.3] E-03	1.9 [1.4;2.4] E-03	2.0 [1.4;2.5] E-03
	35-49	4.7 [3.4;6.5] E-04	9.3 [6.5;13] E-05	12 [7.4;16] E-06	12 [7.4;16] E-06	12 [7.4;16] E-06	12 [7.9;17] E-06
	20-34	8.5 [5.6;13] E-06	1.1 [0.7;1.7] E-06	8.5 [4.9;13] E-08	7.7 [4.5;12] E-08	8.5 [4.9;13] E-08	9.2 [5.3;14] E-08
	<20	5.8 [3.5;9.4] E-08	4.9 [2.9;8.3] E-09	2.3 [1.2;3.8] E-10	2.1 [1.1;3.4] E-10	2.3 [1.2;3.8] E-10	2.6 [1.3;4.1] E-10
$Nb(IQ^x_{min}< IQ(k) < IQ^x_{max})$							
IQ ranges	**<70**	**2461 [1986;3028]**	**819 [639;1044]**	**189 [137;240]**			
	50-69	2413 [1951;2962]	810 [632;1031]	188 [136;238]			
	35-49	47 [34;65]	9 [7;13]	1 [1;2]			
	20-34	1 [1;1]	0[0;0]	0 [0;0]			
	<20	0 [0;0]	0 [0;0]	0 [0;0]			
$DALY^{DHA}(k)$		9131 [6228;12354]	2965 [1981;4313]	696 [432;963]			
$DALY^{DHA}_{saved}(k)$		/	6047 [4190;8427]	8770 [5886;11390]			

[a] Expressed only by mean because there is no variability dimension for powder infant formula (all inputs are deterministic or uncertain).

[b] Percentiles given represent the variability of outputs, mean values are given with their uncertainty interval, when available 90% confident interval is given in square brackets, with the lower bound at 5% and the upper bound at 95%.

4.4.1.4 Chemistry

The exposure assessment of breastfed and formula fed infants to dl-PCB demonstrated a significant discrepancy between these two populations (**Figure 4.3**). Indeed, formula fed infants had a daily exposure ranging from 0.5 up to 3 pg/kg b.w. whereas breastfed infants are exposed to doses ranging from 11 to 77 pg/kg b.w. A safety reference level was set at 2 pg/bw per day (JECFA, 2001) but it is currently being re-assessed by the European food safety agency (EFSA, 2015b). In addition, the exceedance of this value has to be primarily interpreted as a necessity to do further research on the potential health impact of a chronic exposure at early stages to health status later in life. Exposure levels of each scenario are detailed per age and gender in **Table 4.9**.

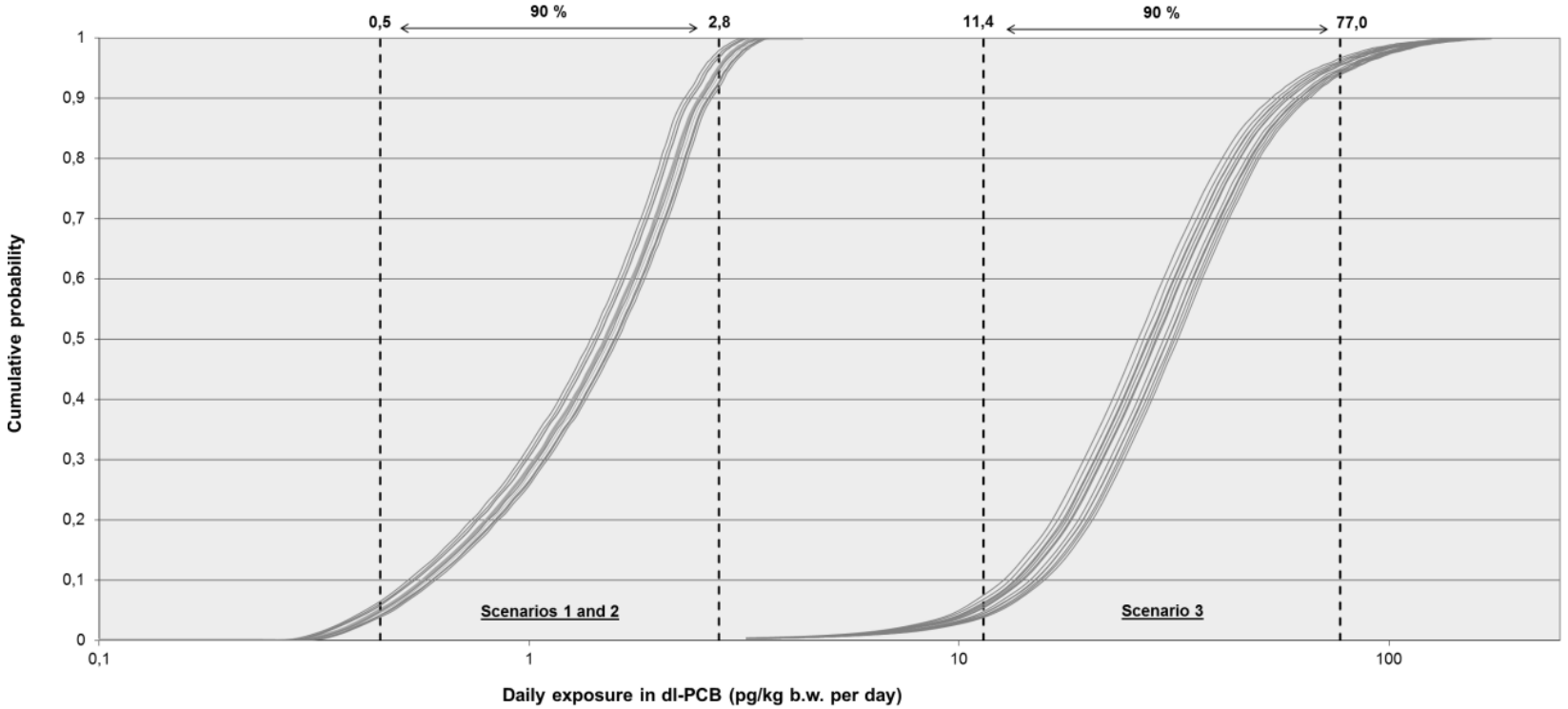

Figure 4.3: Cumulative distribution of estimated level of exposure of breast fed and formula fed infants to dl-PCB

(Based on estimates reported in **Table 4.9** and obtained with *Equation 4.19* and *Equation 4.21*). Each curve represents the variability of individuals in the population for one dimension of uncertainty. For each scenario, different curves obtained highlight the influence of uncertainty on this output. For each percentile of variability (y values) the x values of the corresponding points on the different curves corresponds to the estimate level of exposure in the uncertainty dimension. Note that the daily exposure in dl-PCB is shown on a logarithmic scale.

Example: If we look at a daily exposure of 1 pg/kg b.w.: for scenario 1 and 2 an estimate of about 30% of individuals in the population as an exposure ≤1 pg/kg b.w, and for scenario 3 100% of individuals in the population has an exposure ≥1 pg/kg b.w. If we look at a daily exposure of 11.4 pg/kg b.w.: for scenario 1 and 2 an estimate of 100% of individuals in the population as an exposure ≤11.4 pg/kg b.w, and for scenario 3 about 5% of individuals in the population has an exposure ≤11.4 pg/kg b.w.

Table 4.9: Estimates of mean and percentiles of key outputs for exposure assessment of dl-PCB associated with powder infant formula and breast milk consumption

			Breast milk				Powder Infant Formula			
				Percentile [c]				Percentile [c]		
			Mean	5%	50%	95%	Mean	5%	50%	95%
Level in milk[a] (pg/mL milk)			0.261	0.089	0.226	0.568	0.011	0.003	0.011	0.019
Exposure (i,j,k)[b] (ml/kg b.w. per day)	**Girl Age in month**	1	40 [38 ; 42]	14 [13 ; 14]	35 [33 ; 36]	89 [84 ; 93]	1.9 [1.8 ; 2.0]	0.6 [0.5 ; 0.6]	1.9 [1.8 ; 2.0]	3.2 [3.1 ; 3.4]
		2	38 [36 ; 40]	13 [12 ; 13]	33 [31; 34]	83 [79 ; 87]	1.7 [1.7 ; 1.8]	0.5 [0.5 ; 0.5]	1.7 [1.7 ; 1.8]	2.9 [2.8 ; 3.1]
		3	36 [34 ; 38]	12 [11 ; 13]	31 [29 ; 33]	79 [74 ; 84]	1.6 [1.5 ; 1.6]	0.5 [0.4 ; 0.5]	1.6 [1.5 ; 1.6]	2.6 [2.5 ; 2.8]
		4	32 [29 ; 34]	11 [10 ; 11]	27 [25 ; 29]	70 [65 ; 75]	1.3 [1.3 ; 1.4]	0.4 [0.4 ; 0.4]	1.3 [1.3 ; 1.4]	2.2 [2.2 ; 2.4]
		5	28 [11 ; 71]	11 [10 ; 12]	28 [25 ; 30]	71 [65 ; 77]	1.3 [1.3 ; 1.4]	0.4 [0.4 ; 0.4]	1.3 [1.3 ; 1.4]	2.3 [2.2 ; 2.4]
		6	33 [29 ; 35]	11 [10 ; 12]	28 [25 ; 30]	71 [64 ; 78]	1.3 [1.2 ; 1.4]	0.4 [0.4 ; 0.4]	1.3 [1.2 ; 1.4]	2.2 [2.1 ; 2.3]
	Boy Age in month	1	38 [36 ; 39]	13 [12 ; 13]	32 [31 ; 34]	83 [79 ; 87]	1.8 [1.7 ; 1.9]	0.5 [0.5 ; 0.6]	1.8 [1.7 ; 1.9]	3.1 [3.0 ; 3.3]
		2	37 [35 ; 39]	12 [12 ; 13]	32 [30 ; 33]	81 [76 ; 85]	1.7 [1.6 ; 1.8]	0.5 [0.5 ; 0.5]	1.7 [1.6 ; 1.8]	2.9 [2.7 ; 3.0]
		3	35 [33 ; 38]	12 [11 ; 13]	30 [28 ; 32]	78 [73 ; 83]	1.6 [1.5 ; 1.7]	0.5 [0.5 ; 0.5]	1.6 [1.5 ; 1.7]	2.7 [2.6 ; 2.8]
		4	28 [11 ; 70]	11 [10 ; 12]	28 [26 ; 30]	70 [65 ; 75]	1.4 [1.3 ; 1.5]	0.4 [0.4 ; 0.4]	1.4 [1.3 ; 1.5]	2.4 [2.3 ; 2.5]
		5	28 [11 ; 71]	11 [10 ; 12]	28 [25 ; 30]	71 [65 ; 77]	1.4 [1.3 ; 1.4]	0.4 [0.4 ; 0.4]	1.4 [1.3 ; 1.4]	2.3 [2.2 ; 2.4]
		6	33 [30 ; 36]	11 [10 ; 12]	28 [25 ; 31]	73 [65 ; 79]	1.3 [1.3 ; 1.4]	0.4 [0.4 ; 0.4]	1.3 [1.3 ; 1.4]	2.2 [2.2 ; 2.4]

[a] Levels in breast milk are estimated with Equation 4.20.

[b] Exposure are estimated with Equation 4.21 for breast milk and Equation 4.19 for powder infant formula.

[c] Percentiles given represent the variability of outputs, mean values are given with their uncertainty interval, when available 90% confident interval is given in square brackets, with the lower bound at 5% and the upper bound at 95%.

Example: The mean daily exposure to dl-PCB in the population is estimated at 40 [38;42] ml/kg b.w. per day for girls during the first months of life. Fifty percent of the population (girls from birth to 1 month of age) has an estimated exposure ≥35 [33 ; 36] and five percent has an estimated exposure ≥89 [84 ; 93].

4.4.2. Scenario comparison: estimation of the net health impact

A prerequisite to the comparison of scenarios is the conversion of all considered health impacts in a same unit. In the present case, health impacts were converted in DALY for microbiology and nutrition and compared with the reference values for chemistry. The scenario of regular PIF prepared with boiled water (1A) was seen as a reference point for the comparison, it is not associated with a microbiological risk nor a nutritional benefit so it is set at 0 DALY with no uncertainty.

First of all, regarding to microbiology, Preparation A with boiled water was found to be efficient to protect formula fed infants against *C. sakazakii* contrary to Preparation B with water at ambient temperature that was associated with a mean burden of disease of 6 [0.003; 130] DALY for 100 000 infants following this diet during 6 months (**Table 4.7**). As a result, scenario 1B was seen as potentially worse than 1A. With regard to nutritional benefit, supplemented PIF (scenario2) was associated with a mean of 6 050 [4 190;8 430] DALY saved (vs regular PIF) for 100 000 infants (**Table 4.8**). BM (scenario 3) could not be distinguished from scenario 2 as the uncertainty is too high: mean value of 8 770 [5 890;11 390] DALY saved (vs regular PIF) for 100 000 infants (**Table 4.9**). However, it is important to keep in mind that the nutritional benefit was assessed using maximum dose-responses (Birch et al., 2007; Gustafsson et al., 2004), an over-estimation of DALY is possible. This result could be consolidated by integrating other available dose-responses (Campoy et al., 2012). Regarding chemistry, dl-PCB could remain an issue for breastfed infants (scenario 3) (**Figure 4.3**), even if the assessment stopped at the exposure level.

Table 4.10: Assumptions made when building the risk-benefit assessment model and their consequences on health impact estimation.

A "+" means that the estimated health balance might be more beneficial (i.e. more benefit or smaller risks than estimated) whereas a "-" means that the estimated health balance might be worse (larger risks or smaller benefits than expected). A "+/-" means that we do not know the direction (Hoekstra et al., 2012). Multiple signs is used to highlight if the balance is likely (+ or – or +/-) or definitively (++ or -- or ++/--) under/over -estimated or unknown.

Assumptions generating uncertainties	Information to support assumption	Impact on the estimated health balance	
➢ **Uncertainties affecting problem formulation**			
- Assume 6 months of exclusive feeding for reference and alternative intake scenarios	Comparison of "exclusive" intake scenarios allows having a clearer view on the impact of each diet on health.	We know that infant diet during first 6 months of life is often composed of both milks.	+/-
➢ **Uncertainties affecting risk/benefit factor(s) and health endpoint(s) identification**			
- Selection of 3 factors: DHA, *C. sakazakii* and dl-PCB.	One factor per scientific field was selected to work on RBA methodological development. They were selected according to their relevance for the case study (see Introduction).	Both milks were linked with other nutrients and contaminants which must be integrated in the analysis (see Introduction).	++/--
- DHA was linked with cognitive development, *C.sakazakii* with meningitis, urinary tract infection and bacteremia and dl-PCB with non-Hodgkin lymphoma, breast cancer and melanoma.	The most obvious endpoints were selected according to the literature (FAO/WHO, 2004; FAO/WHO, 2006; Horta and Victora, 2013; IARC, 2015; Meltzer et al., 2016; Reij et al., 2009; RIVM, 2015; Victora et al., 2016; Weiser et al., 2016).	Factors selected could be linked with other health endpoints already identified or still unknown.	+/-
➢ **Uncertainties affecting intake assessment**			
- Levels of *C. sakazakii* reported in FAO/WHO (2006) were assumed representative for France.	Data used were collected in 12 different industries in different countries representing more than 29 000 samples collected.	French data are not expected to be very different from the large range of values included.	+/--
- *C. sakazakii* is supposed homogeneously distributed in PIF.	This assumption is based on the assumption made by FAO/WHO (2006) and Reij et al. (2009).	Possible ways of PIF contaminations suggest a non-homogeneous distribution of bacteria in PIF (Jongenburger et al., 2011; Jongenburger et al., 2012).	-
- Measurements of dl-PCB below the limit of detection were replaced by half of this limit.	This approach is commonly used in chemistry to avoid under-estimation due to limits of analytical methods.	This assumption implies that PIF is never considered as non-contaminated.	+

- Levels of dl-PCB collected in 2007 from French data were still considered valid.	The set of data considered (Focant et al., 2013; INVS, 2007) from a national study was judged as the most suitable to represent French variability.	Levels of dl-PCB are expected to decrease in the future due to their prohibition (Malisch and Kotz, 2014).	+
- Milk intake were estimated with dietary recommendations reported in Butte (2005).	This table gives recommendations according to gender, age and kind of milk consumed. It is in accordance with general French recommendations.	Real intakes could be slightly higher or lower than nutritional recommendation.	+/-
- Level of prenatal exposure (during pregnancy) to dl-PCB is not considered.	The aim of the model was to compare different diets for the first six months of life whatever the past or future exposure.	A high level of dl-PCB in breastmilk is certainly associated with a high level of exposure during pregnancy which would increase the chemical risk.	-
➢ **Uncertainties affecting dose/response relationships**			
- The higher dose-response associated with DHA was taken.	The aim of the present paper was to work on methodological development. A best-case approach was used to see first if benefit could be relevant or not.	The estimated benefit is the highest expected then the health balance should be lower. A meta-analysis must be done to refine this dose-response.	--
- Infection to *C. sakazakii* was estimated assuming an independence of infection from one day to the other.	Infection to *C. sakazakii* is an acute health effect.	If an infant is infected we can imagine that more preventive measures will be put in place for the following feeds.	+
➢ **Uncertainties affecting conversion to a common health currency: DALY**			
- Age of onset of *C. sakazakii* infections (from 0 up to 6 months of age) were not integrated, the life expectancy at birth was used.	The potential 1 to 6 months lost when infection lead to death were not subtracted to the life expectancy as this approximation would be smaller than the precision of the DALY estimation.	The DALY could be overestimated to 1 to 6 months representing an over 0.08 up to 0.5 DALY per case.	+
- Disease severity, durations and mortality rates of *C. sakazakii* infections were assumed similar in France than in the Netherlands (Reij et al., 2009).	No French data has been found and data from Netherlands have been assumed transposable.	French disability weights could be different if health states are perceived differently and disease duration and mortality rates could be different according to health care systems but the direction of the change is unknown.	+/-
➢ **Uncertainties in the probabilistic treatment of uncertainties**			
- Choice of a uniform distribution to represent uncertainties of *C.sakazakii* dose-response parameter.	It was implemented as already done in FAO/WHO (2006) with 10^Uniform (-10 ; -5).	No more information on this dose-response parameter then difficult to assess the change in the health balance output.	+/-

-	Choice of a uniform distribution to represent uncertainties of the mean IQ in France.	A uniform distribution was implemented between the lower and higher values reported by Christainsen (2013).	No more information on mean IQ in France.	+/-
-	Choice of triangular distributions to represent uncertainties of ranges of intellectual disability weights.	For each range of intellectual disability (mild, moderate, severe and profound) a triangular distribution was used with the 95% confidence interval giving the minimum and maximum and the mean giving the most likely value.	No more information on ranges of intellectual disability weights in France.	+/-
-	Choice of a triangular distribution to represent uncertainties of dl-PCB absorption factor.	A triangular was used with 100% as the maximum, 95% as the most likely values and 85% as a minimum value (both based on values collected).	Absorption factor if probably variable and uncertain. In absence of clear data, those available were used to characterise the uncertainty.	+
-	Choice of a uniform distribution to represent uncertainties of the decrease factor of dl-PCB due to breast milk depuration.	A uniform distribution was implemented between the lower (0: no depuration) and higher values reported (0.017).	No more information on depuration factors.	+
-	Uncertainty introduced by the number of simulations and iterations selected.	Number of iterations was tried with 100 000 and number of simulation with 100. For all main outputs, three repetitions were run with different collections of inputs (uncertain and variable), it did not show a major change.	Limited computation machine capacity has limited number of iterations and simulations and it is not clear in which direction could change the health balance.	+/-

4.5. Discussion

The aim of the study was to develop a probabilistic and inter-disciplinary risk-benefit assessment model to investigate the conceptual development of the RBA methodology, its relevance, feasibility and added value. The supporting application was focused on breast milk and powder infant formula consumption, considering simultaneously microbiological (*C. sakazakii*), nutritional (DHA) and chemical (dl-PCB) factors. Five scenarios of infant milk consumption were assessed and compared: six months of breastfeeding versus powder infant formula feeding, with the option of supplemented infant formula in fatty acids and the addition of water at ambient temperature or boiled. In addition to the inter-disciplinary approach, the originality of the strategy used in the present RBA model was the use of probabilistic techniques as well as the consideration and separation of variability and uncertainty.

The health impacts associated with microbiological and nutritional factors were compared using a common metric, the DALY, but the potential chemical risk associated with PIF and BM consumption could not be characterised due to limitations in the dose-response establishment. When considering microbiological and nutritional factors, among the scenarios defined, the non-supplemented infant formula prepared with ambient temperature water had the worst health impact (6 [0.03; 130] mean DALY lost per 100 000 infants for 6 months of exposure). On the opposite supplemented PIF (including both preparation A and B) and BM seemed to have a better health balance; even if we cannot make any definitive conclusion without considering further study on DHA dose-responses. Regarding the chemical risk assessment, levels of exposure to dl-PCB were compared for breast fed and formula fed infants. The obtained results have shown a clear higher level of exposure for breast fed (mean of 35 pg/kg b.w. per day) compared with formula fed infants (mean of 1.5 pg/kg b.w. per day). Moreover, the consideration of variability in the exposure assessment has pointed out a wide range of levels of exposure of French breastfed infants, ranging from 11 up to 77 pg/kg b.w. per day (5^{th} and 95^{th} percentiles in the variability dimension). However, due to the lack of a well-established and documented quantitative dose-response relationship for dl-PCB the assessment has been stopped at the stage of exposure for the chemical part. Indeed, the link between early chemical exposure in life with risk of disease later in life has not yet been clearly elucidated (Vaiserman, 2014).

The DALY is a common metric which has been chosen here to compare risks and benefits due to its usefulness in public health when thinking at the population level. However, when used in RBA the DALY indicator has limitations in informing individual health status as it is more of a population health indicator. In the present study, as per definition, the DALY simultaneously integrates the prevalence and the severity of diseases. When expressed at the population level in DALY per 100 000 infants, the nutritional benefit outweighed the microbiological risk. The nutritional benefit was a decrease of intellectual disability, a small improvement of quality of life. Whereas in microbiology, the number of estimated cases were extremely low but could lead to severe disease and even death. As a result, when expressed at the individual scale in DALY per case, the health impact is higher for the microbiological risk than for the nutritional benefit (60 lost versus 4 saved DALY, mean values calculated using **Table 4.7** and **Table 4.8** by dividing the mean number of DALY lost/saved by the mean number of illness/cases saved). Regarding the different severity and prevalence for both outcomes, as well as the different stages of life that can be affected, the DALY has some limitations to inform consumers in making informed choices. Indeed, the consumer may feel concerned mostly regarding information about the severity of the health effect and age of onset.

4.5.1. Towards quantitative RBA

Food RBAs are generally complex, with a lot of beneficial and adverse effects, but also a lot of routes of exposure. Developing a quantitative and modular framework enables the development of the assessment step by step.

In the present quantitative risk-benefit assessment we have tried to go as much as possible up to the risk-benefit comparison, to provide a “best estimation of the health impact” as recommended by Berjia et al. (2012). In the tiered approach suggested by EFSA (2010) and the BRAFO project (Hoekstra et al., 2012), the assessment would have been stopped at an earlier stage: the scenario “supplemented PIF prepared with boiled water” would have been chosen as better option (potential nutritional benefit, no microbiological risk and levels of exposure to dl-PCB lower than in breast milk). The advantage of carrying out the quantitative assessment up to the health impact for all the scenarios is to provide a comprehensive set of information (all scenarios are assessed) to policy makers to

underpin their decision. That enables also to operate in the risk-based food decision-making paradigm.

Most of the quantitative RBAs, developed so far, have been based on a deterministic approach (Berjia et al., 2012; Cohen et al., 2005; Guevel et al., 2008), i.e. with worse-case point estimates as inputs. It requires less data and less computational processing than a probabilistic approach but it leads to an output which is not realistic when the model contains many inputs (Cummins, 2016). Moreover, in this study, the probabilistic model was particularly valuable because there were inputs of which the values were not accurately known, they were then implemented in the model with a large uncertainty interval; uncertainty and variability separation was then essential to compare scenarios while visualizing the impact of uncertainty. More generally, this probabilistic approach enabled an assessment of variability and the tail of distributions without making any worse-case assumption. However, when variability and uncertainty are separated, outputs are represented by distributions and not by a deterministic value. They can be summarised in tables but also in graphs which may be easier to interpret and can be an efficient tool to visualize the output (see **Figure 4.2** and **Figure 4.3**).

Results of the RBA must be considered in the context of assumptions used to build the model (Boobis et al., 2013) and uncertainty generated. The use of subjective assumptions regarding data selection, model simplification as well as scenarios definition; is an inherent part of the development of simulations models (Cummins et al., 2010) but they are often associated with considerable uncertainty which represents the lack of knowledge. In absence of data or knowledge, assumptions were made to allow quantification of risks and benefits. When data or knowledge was available to describe assumptions we have quantified the associated uncertainty using second order Monte Carlo simulations. Nonetheless, some sources of uncertainty were identified but not quantified due to a lack of data/knowledge and even those quantified remains uncertain. To help better consideration of all sources of uncertainty, they have been qualitatively reviewed in **Table 4.10** to highlight the consequence of their use on the overall health balance estimated (Hoekstra et al., 2012).

However, the list of potential sources of uncertainty could be very long when developing a RBA model so we have reported in **Table 4.10** only those considered as potentially not

impacting the final conclusion, some were neglected when judged as non-impacting the final results (e.g. D and z values on microbiological risk).

Complementary to these uncertainty sources, accounting for probability of causation of each health effect would be useful to integrate the degree of "biological knowledge of the day" (Hill, 1965). A "weight of evidence" could be associated with each pair of agent/ health effect according to the current scientific knowledge. This probability of causation could be estimated with expert elicitation for instance, as done by Trasande et al. (2016) to evaluate the attributed burden of disease associated with several endocrine-disrupting chemicals in Europe. The same approach could be applied in RBA to account for differential consideration of uncertainty for each chemical, microbiological and nutritional assessment.

4.5.2. Advantages and challenges of multidisciplinary RBA

Multidisciplinary RBA aims to evaluate all potential agents present in food prone to induce health effects to consumers whether they are from microbiology, chemistry or nutrition origin; the focus is put on consumer health. This holistic approach aims to help policy makers to improve public health by making more informed decisions (EFSA, 2010; Verhagen et al., 2012a). Indeed, to weigh the human health risks against the benefits is essential to take more complete decision closer to reality. RBA is thus a guidance tool of public health management. In addition, it gives more structured conclusions and avoids contradictory messages for policy makers but also for consumers when leading to food recommendations. As a matter of fact, consumers are confused if they are advised for instance to avoid a certain food due to chemical contaminations and on the other hand to consume the same food to reach potential benefits. They need to receive only one final message which includes an evaluation of the overall health balance. However, this balance is more complex than a simple juxtaposition of both risks and benefits assessments and policy makers require scientific structured evaluations. Multidisciplinary RBA is needed to avoid contradictory messages and to improve policy maker's decisions quality as well as consumers trust.

Coping with multidisciplinary RBA might be a challenge. Indeed, each discipline has its own method to carry out a risk assessment but the whole risk-benefit assessment needs to be conducted with a harmonized approach to compare risks and benefits together. In practice, an individual assessment in each discipline is first conducted and then risks and benefits estimated are harmonised into a common metric to estimate the overall health impact (EFSA, 2010). To date, the DALY (or QALY) was used in RBA to compare risks and benefits leading to different health outcomes (Berjia et al., 2012; Cohen et al., 2005; Guevel et al., 2008; Hoekstra et al., 2013b; Ponce et al., 2000). However, this metric can be used only once the number of cases per outcome has been estimated; therefore, risks and benefits must be all expressed with the same level of information whatever the scientific field. Nevertheless, this harmonization of approach might face some difficulties due to methodological differences of each field. In microbiology, quantitative farm-to-fork risk assessment has been developed since the nineties, the methodology (Commission, 1999) is well shared among the scientific community (academics and authorities), a relatively large amount of data is available and probabilistic models are mostly developed to take biological variability into account (Membré and Guillou, 2016). Biological mechanisms are often known and allow developing mathematical dose-responses characterizing the hazard (**Figure 4.4**). A different approach is used in nutrition as both over and under consumption of nutrients can be associated with particular risks, thus an adequate intake of nutrients needs to be defined with an upper and lower limit of intake, based on the traditional nutritional risk assessment method (WHO, 2006). A top-down analysis is also often used when the consumption of some nutrients (or food) is correlated with beneficial or adverse health effects through epidemiological research showing an association between an intake and a particular health effect. Consequently, dose-responses are constructed differently compared to microbiology; both approaches are summarised in **Figure 4.4**. Epidemiological studies do not give the biological mechanism but define an association between an exposure and a health state; the number of cases per outcome is then estimated. For example, the health effects of breastfeeding were quantified in DALY for infants and mother through epidemiological studies (Van Rossum et al., 2005). Epidemiological research is used in chemistry as well. The main issue when using epidemiological studies is that "causality" is assessed by observing health trends in the population according to specific profiles of exposure, hence chemical, nutritional and microbiological effects can all have a bearing on the same health endpoint. In addition when confounding factors and interactions between factors are not fully

considered, it could lead to a wrong association between the risk or benefit factor and the health endpoint. With regard to chemistry, a major current challenge is associated with the long term impact of a low dose and multiple chronic exposure, making the chemical risk quantification more complex than the microbiological case (van Kreijl et al., 2006). Additionally, the fact that contaminants are potentially bio-accumulated through different routes of exposure makes dose-responses multifaceted and difficult to estimate, especially when interested in the first window of life that could alter health effects later in life. Nowadays, the chemical risk assessment methodology is mostly based on a comparison of human levels of exposure with safety reference values, established mostly through animal experimentations (IPCS, 2009). To be implemented in RBA, this approach must be adapted to estimate "effective" doses level (limits giving risks or benefits) instead of "ineffective" dose level (safety limits allowing to avoid risk). Thus, a major challenge for the RBA is to integrate a chemical risk assessment using dose-responses instead of safety reference values which is not new in this field but currently under development. Indeed, some chemical dose-responses have been determined for particular issues, for example Hoekstra et al. (2013b) have estimated the potential variation of newborn IQ according to the mothers' intake in methyl mercury during pregnancy due to fish consumption. When dose-responses are not available, a comparison under constraints, of profiles of exposure with safety reference values, still remains a possibility to compare different scenarios of food consumption but it does not allow completion of the RBA with the estimation of the whole health impact.

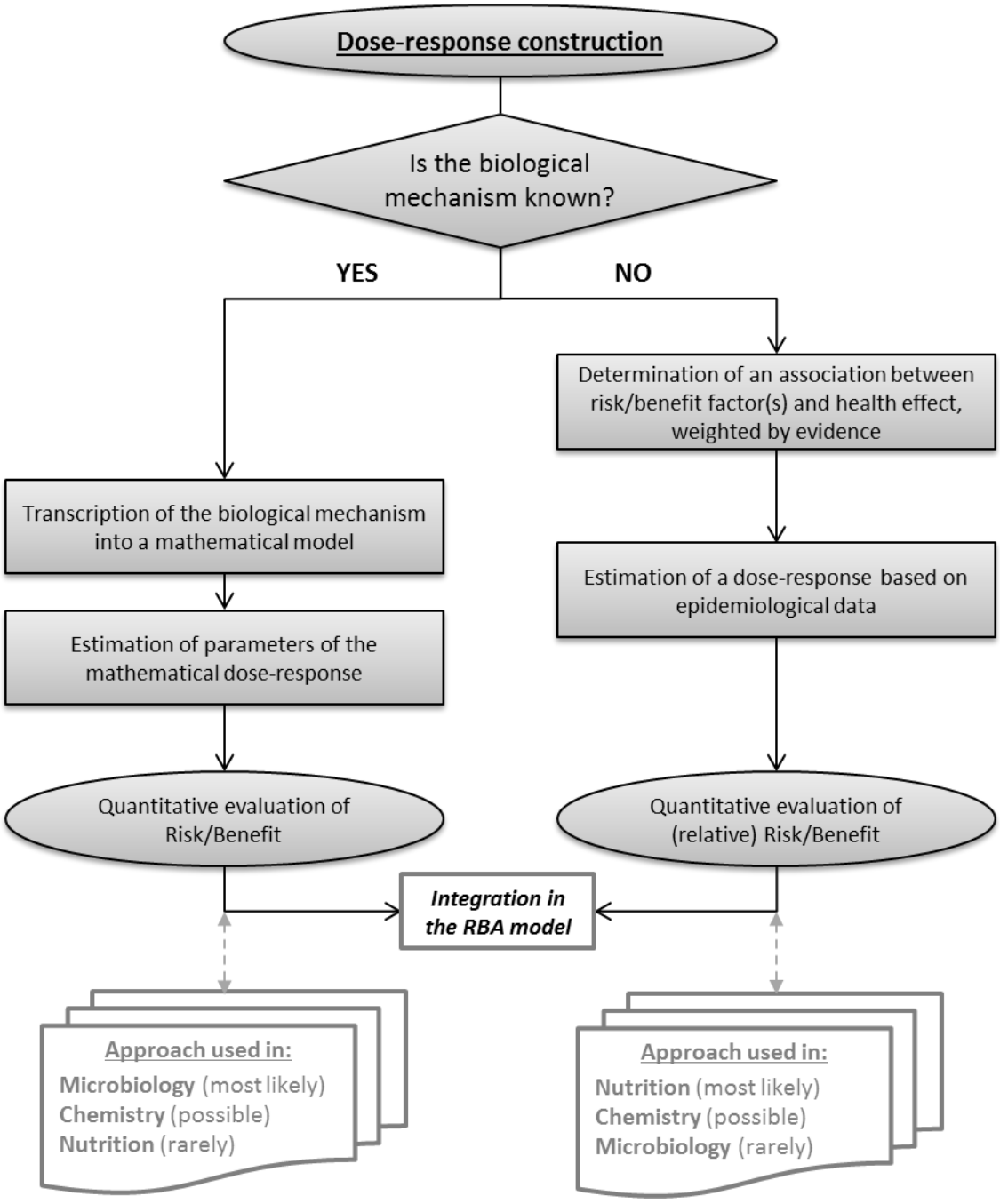

Figure 4.4: Schematic representation of approach used to build dose-response in Microbiology, Nutrition and Chemistry

4.6. Conclusion

The model developed was not meant to address all the health effects and contributing factors associated with infant feeding, but allowed for the conceptual development of the RBA methodology, and can be considered as a robust basis to build upon. The present study is to date the first probabilistic and inter-disciplinary risk-benefit assessment performed for a food type integrating microbiological, nutritional and chemical components. The case study has shown that a probabilistic approach is essential to tackle variability and uncertainty inherent in biology. Separation of variability and uncertainty based on a second order Monte Carlo analysis has strengthened the assessment and made its interpretation easier. The main advantage of interdisciplinary assessment was to provide more structured and comprehensive conclusions to policy makers and also to avoid contradiction messages when leading to food recommendation for the consumer. Nevertheless, inter-disciplinary RBA requires more time, data but also human resources with a broader panel of skills than mono-disciplinary assessment. It has to be set within a multi-partner (at least multi-team) and should be implemented where there is a balance to be ascertained between negating risk/benefit health factors.

Appendix Table 4.11: Summary of key outputs for estimation of infant intake in breast milk and powder infant formula (regular and supplemented)

Gender (i)		Age (j) (month)	Weight (i,j)[a] (kg)			Intake (i,j,k)[a] (mL/kg b.w. per day)						Portion (i,j)[a] of powder infant formula (g/feed)		
						Breast milk			Infant formula					
			Percentile[d]			Percentile[d]			Percentile[d]			Percentile[d]		
			5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
Gender (i)	Girl	1	2.8	3.7	4.5	140	160	190	175	180	185	11	15	19
		2	3.4	4.5	5.5	130	150	180	160	165	170	12	16	20
		3	4.1	5.2	6.4	120	140	165	145	150	150	16	20	25
		4	4.7	5.8	7.2	105	120	145	125	125	130	16	20	24
		5	5.2	6.5	7.8	105	120	145	125	125	130	22	27	33
		6	5.7	7.0	8.4	100	120	140	120	125	125	23	28	34
	Boy	1	2.9	3.9	4.9	130	150	180	170	175	175	11	15	19
		2	3.6	4.8	6.0	125	145	172	155	160	165	13	17	21
		3	4.5	5.6	6.9	120	135	165	145	150	155	17	22	27
		4	5.1	6.4	7.6	105	120	145	130	130	135	17	22	27
		5	5.8	7.0	8.4	105	120	145	125	130	130	24	30	36
		6	6.3	7.6	9.0	105	120	145	125	125	130	26	32	38

[a] Infant weights from Scherdel et al. (2015) ,

[b] Intakes are estimated with Equation 4.1,

[c] Portions of PIF consumed per feed are estimated with Equation 4.2,

[d] Percentiles given represents the variability of outputs.

CHAPTER 5

Model development 2

Redraft from:

Boué G, Wasiewska L, Cummins E, Antignac J-P, Le Bizec B, Guillou S, Membré J-M.

(2017). Risk Assessment of Arsenic and *Cryptosporidium* in Tap Water used for Preparation of Infant Formula, France.

Water Research (Submitted)

CHAPTER 5: **Model development 2**

The RBA approach developed in model 1 (CHAPTER 4) was re-used for another issue linked with infant feeding: the risk assessment of tap water for infant formula preparation in France. This model was inter-disciplinary considering one factor from microbiology and one from chemistry: *Cryptosporidium* and arsenic, respectively. Two scenarios of milk preparation were considered: with un-boiled or boiled tap water. Model 2 was also probabilistic with separated variability and uncertainty.

Objectives of the chapter:

- Apply the previously developed approach to tap water used for infant formula preparation in France,
- Discuss advantages and limits of the method.

5.1. Abstract

The aim of the present study was to quantify the risk of using tap water in France for preparation of infant formula, during the first six months of life. Although not sterile and possibly contaminated by microbiological and chemical hazards, tap water is used in France to reconstitute powder infant formula.

Cryptosporidium and arsenic were selected as hazards of greatest concern in microbiology and chemistry, respectively. A probabilistic model was developed using French (when available) and European (alternatively) data. Second order Monte Carlo simulation was used to separate uncertainty and variability of inputs. Outputs were expressed at the individual level as probability of illness and at the population level, using the DALY indicator (Disability Adjusted Life Year). Two scenarios of milk preparation were considered: with un-boiled or boiled tap water.

Consuming infant formula rehydrated with un-boiled tap water during the first six months of life led to a total of 2 250 DALY per 100 000 infants (90% uncertainty interval [960; 7 650]) for *Cryptosporidium* due to diarrhea, and 1 DALY [0.4; 2] for arsenic due to lung and bladder cancer. For the entire population, boiling water would suppress the risk from *Cryptosporidium*. In contrast, the cancer risk was low at the population level but rather elevated for the tail of the exposure distribution in arsenic. A stringent monitoring of tap water supply points should be continued. These risk assessment results could help public health authorities in future recommendations.

5.2. Introduction

Infants are one of the most vulnerable group to food-borne hazards, as their immune system is still not developed in comparison to adults. During the first six months of life, Powder Infant Formulae (PIF) are food predominantly consumed by infants in France whereas breast milk consumption remains marginal (Salanave et al., 2014). Before use, PIF needs to be reconstituted with water and tap water can be used in most of Western countries including France (ANSES, 2013c; FDA, 2015b). More precisely, the French food safety agency recommends to use cold tap water which run for few seconds before filling the bottle and to clean the tap head regularly.

The quality of tap water is variable among different supply points and depends on several factors including weather conditions, agriculture or industrial practices, natural soil composition and industrial processes (Moxey, 2012). In France, the quality of tap water is monitored on a daily basis and actions are taken in case of any failure (ARS, 2014). However, its consumption has caused outbreaks due to microbiological hazards and has increased levels of chemicals exposure (Beaudeau et al., 2008; Beaudeau et al., 2014; Le Bot et al., 2016). In this context, consumers in France are still concerned about possible health risks associated with tap water consumption (Boué et al., 2016; Doria, 2006; Sofres, 2014).

The present paper focuses on one microbiological and one chemical hazard which were identified of greatest concern in France: *Cryptosporidium* and arsenic. On one hand, *Cryptosporidium* has caused several waterborne outbreaks (Dalle et al., 2003; Gallay et al., 2006; Therre, 2008). It is a parasite spread by fecal contamination which is resistant to chlorination during water treatment (LeChevallier and Au, 2004). *Cryptosporidium* is

a parasite producing oocysts spread by fecal contamination by infested hosts. Despite *Cryptosporidium* cannot reproduce outside the host, its cysts can survive and remain virulent for a long time in the environment (RIVM, 1999). Infants are one of the most susceptible groups to *Cryptosporidium* infections (DuPont et al., 1995) leading to watery diarrhea, stomach cramps or pain, nausea, vomiting and even death (CDC, 2015). On the other hand, tap water was also found to be a predominant source of exposure to inorganic arsenic (ANSES, 2011h; INVS, 2008) which is a natural element of the Earth crust and can be widely found in the environment due to natural processes or industrial activities (IARC, 2012; WHO, 2016). The level in tap water is variable according to different regions due to the geographical features (INVS, 2011). This chemical has been classified as "proved to be carcinogenic to human" (IARC, 2012) and was judged to be of particular concern for public health in tap water in France as potentially being accountable for bladder and lung cancer (INVS, 2008).

To date, risk assessments have been carried out for drinking tap water consumption of adults or infants' populations but none of them were performed in the context of infant formula consumption. Most often, hazards were assessed individually per scientific field: either in chemistry (Le Bot et al., 2016) or in microbiology (Cummins et al., 2010; Razzolini et al., 2016; Sato et al., 2013; Schijven et al., 2011; Xiao et al., 2013; Xiao et al., 2012). Only Havelaar et al. (2003) have considered microbiological and chemical contaminants altogether in the same risk assessment study, their study was focused on the Dutch population.

In this context, the aim of the present study was to quantify the microbiological and chemical risks associated with the use of tap water in France for preparation of infant formula, during the first six months of life.

5.3. Materials and methods

5.3.1. Probabilistic risk assessment framework

Considering *Cryptosporidium* on the one hand and arsenic on the other hand, this risk assessment falls into the scope of risk-risk assessment, itself belonging to risk-benefit assessment (Boué et al., 2015; EFSA, 2010; Watzl et al., 2012). In practice, the framework applied in this study followed the recent approach developed by Boué et al.

(2017a) that includes problem formulation, scenario generation, mathematical model development for each hazard, and then comparison of risks according to various scenarios.

More specifically, two scenarios of powder infant formula preparation were assessed. The reference scenario (Scenario 1) corresponds to the use of boiled tap water. It corresponds to the general WHO recommendation for PIF preparation worldwide (WHO, 2007a) and it is also in line with the FDA advice (FDA, 2015b). In contrast, the French food safety agency advices to use boiled water only when travelling abroad and in case of no access to suitable drinking or bottled water (ANSES, 2013c). The second scenario (Scenario 2) hence considers the direct use of cold water from the tap. Both scenarios were assessed for each particular preparation, using boiled or un-boiled during the first six months of life.

Regarding the mathematical model, it was divided into several modules (Nauta, 2001) to facilitate calculations and understanding (**Figure 5.1**). The model was probabilistic to take into account variability (reflecting heterogeneity of individuals in the population) and uncertainty (referring to the lack of knowledge). Variability and uncertainty were separated to facilitate model interpretation. All calculations were done for both scenarios and for one infant of reference for each gender i (boy and girl) and for each age in month j (from 1 to 6 months of age) to consider the specific variability among age and gender.

Outputs were expressed at two levels. Firstly, individual probabilities of illness were reported to highlight natural differences among infants, due to different levels of infant milk intake (different with age, gender and weight) as well as the natural diversity of hazards levels in tap water. For each risk predicted, an uncertainty interval was given representing the lack of knowledge of implemented inputs and parameters (e.g. not sufficient number of data, simplifications, assumptions or parameters estimation). Secondly, the whole health impact was estimated at the population level for 100 000 infants with the DALY indicator (Disability Adjusted Life Year). This indicator was built by integrating the number of illnesses in the whole infant population.

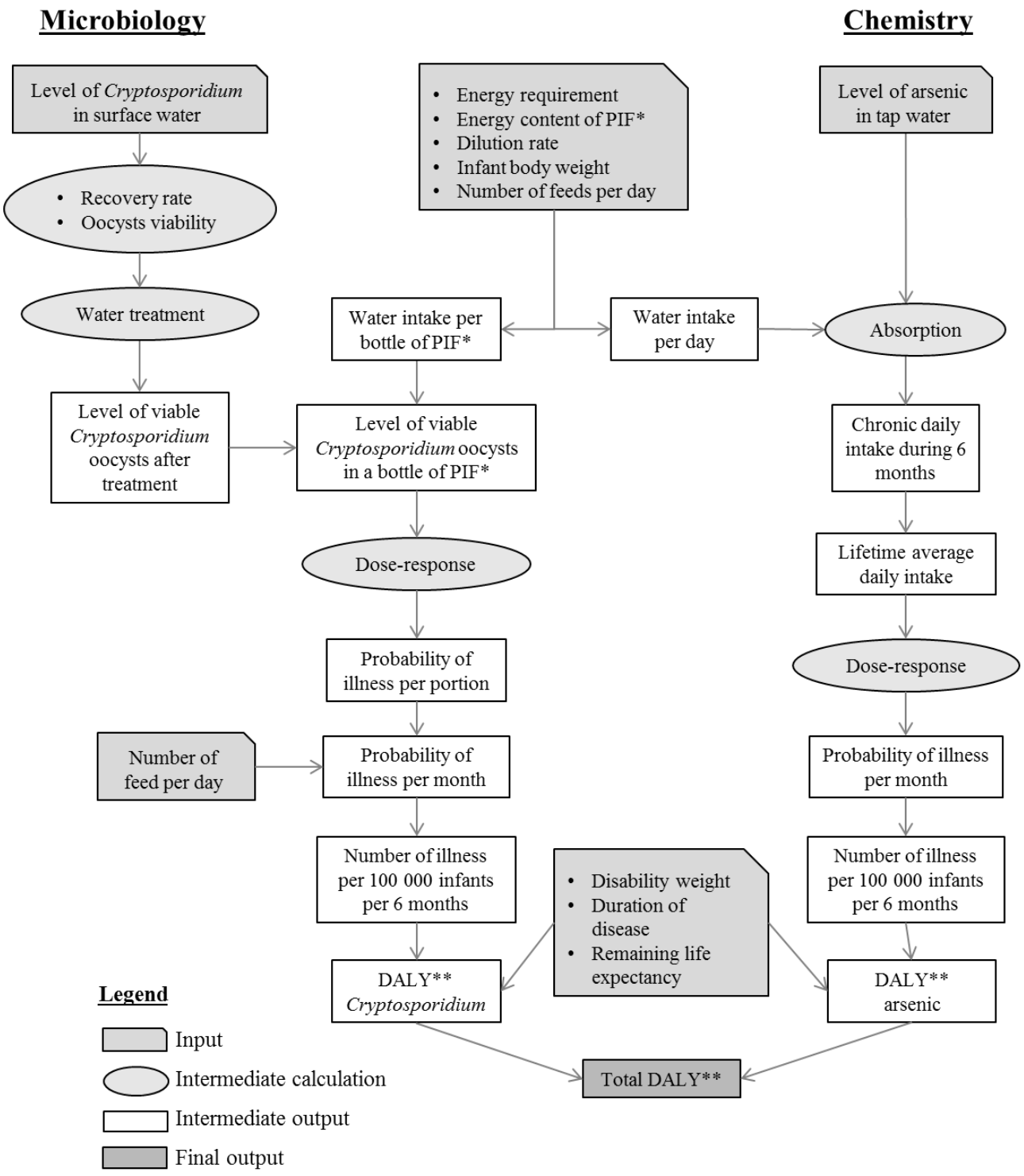

Figure 5.1: Flowchart of the proposed microbiological-chemical risk assessment model

*PIF: Powder Infant Formula, **DALY: Disability Adjusted Life Years

5.3.2. Arsenic risk assessment model development

Water was found to be a predominant source of exposure to inorganic form of arsenic both for adults and children. In France, the level of arsenic in tap water is variable among regions, due to the geographical features (INVS, 2011). Current water treatment methods applied and boiling tap water cannot remove arsenic (CDC, 2015). As a result, both scenarios, using boiled and un-boiled water, referred to the same risk assessment process. All inputs introduced to develop the risk assessment model are summarized in **Table 5.1**.

Table 5.1: Description and implementation of inputs for tap water intake calculation *(Equations 1 and 2)*

Input	Description	Model implementation	Unit	Reference	Category
$N_{Req}(i,j)$	Daily nutritional requirement	From 1 to 6 months of age: Girl (117, 108, 101, 89, 87, 85) Boy (122, 111, 100, 86, 85, 83)	kcal/kg b.w. per day	(Butte, 2005)	Deterministic
Calorie(PIF)	Calorie content of PIF	Uniform(66; 69)	kcal/100 mL	Product information collected on French market	Variability
R_{dil}	Part of PIF in reconstituted milk	Uniform(12.5; 14)	%		Variability
BW(i,j)	Body weight	Cumulative distribution	kg	(Scherdel et al., 2015)	Variability
$N_{feed}(j)$	Number of feeds per day for both genders	For each range of age: 0 – 2 months: 6 2 – 4 months: 5 4 – 6 months: 4	/	Product information collected on French market	Deterministic

5.3.2.1 Exposure assessment of Arsenic

Infant tap water intake calculation was based on milk intake estimation used in Boué et al. (2017a). It has been estimated for each gender i (boys and girls) and each age in months' j (from one to six months of age) per day per kg body weight (**Equation 5.1**) using infant nutritional requirements (Butte, 2005). PIF energy content and part of the powder in reconstituted PIF were based on French products information and implemented with a uniform distribution using the lowest and the highest values found. Inputs used to estimate tap water intake are summarized in **Table 5.2**.

Equation 5.1

$$V_{day}(i,j) = \frac{N_{req}(i,j) \times 100 \times (1 - R_{dil})}{Calorie(PIF)}$$

Where:

$V_{day}(i,j)$: Daily intake of tap water per gender i and age j (mL/kg bw per day),

i: Gender = Boy / Girl,

j: Age = 1 to 6 months of age,

$N_{Req}(i,j)$: Daily nutritional needs (kcal/kg b.w. per day),

R_{dil}: Part of PIF in reconstituted milk,

Calorie(PIF): Calorie content of PIF (kcal/100 mL).

Table 5.2: Description and implementation of inputs of Arsenic risk assessment *(Equations Equation 5.1 and Equation 5.2)*

Input	Description	Model implementation	Unit	Reference	Category
➢ **Exposure calculation**					
N^{Ar}_0	Concentration of arsenic in tap water	Cumulative distribution based on reported values: Min: 0.1 P5: 0.1 P25: 0.1 P50: Pert(0.2; 0.2; 0.3) P75: Pert(0.3; 0.5; 0.8) P95: Pert(1.5; 2.1;3.7) Max: 8.9	µg/L	Values collected in (Le Bot et al., 2016)	Variability Deterministic Deterministic Deterministic Uncertainty Uncertainty Uncertainty Deterministic
Abs^{Ar}	Absorption of arsenic	100	%	Assumption based on the literature	Deterministic
ED	Exposure duration	6 months = 180 days	day	Set by scenarios definition	Deterministic

AT	Average time	70 years = 25 550 days	day	Assumption based on the literature	Deterministic
➢ **Dose-response calculation**					
α(c=lung)	α value for lung cancer	0.0154	/	Equation deduced from (FDA, 2016)	Deterministic
α(c=bladder)	α value for bladder cancer	0.0323	/		Deterministic
β(c=lung)	β value for lung cancer	1.7142	/		Deterministic
β(c=bladder)	β value for bladder cancer	0.0545	/		Deterministic
ε(c=lung)	Error value for lung cancer	Normal(0;0.107)	/		Uncertainty
ε(c=bladder)	Error value for bladder cancer	Normal(0;0.2565)	/		Uncertainty
➢ **DALY calculation**					
p(i)	Ratio of boys and girls in France	51.09 | 48.91	%	Values collected in (INSEE, 2014)	Deterministic
$Prev^{Ar}_{survive}(i,c)$	Prevalence of surviving cancer disease after 5 years for each cancer and gender: boy | girl	Lung: 16 | 20 Bladder:55 | 49	%	Values collected in (INCa, 2015)	Deterministic
$LE^{Ar}(i,c)$	Remaining Life Expectancy for each cancer and gender: boy | girl	Lung: 68 | 63 Bladder:76 | 83	year	Values collected in (INSEE, 2014)	Deterministic
L(c,s)	Average duration of each cancer stage s for each cancer: lung | bladder:				
s = diagnosis and initial treatment		0.5 | 0.33	Year		Deterministic
s = control cure		6 | 4	Year		Deterministic
s = control death		0.267 | 1.867	Year		Deterministic
s = metastatic/pre-terminal phase		0.25 | 0.25	Year		Deterministic
s = terminal phase with medications		0.083 | 0.083	Year		Deterministic
w(c,s)	Disability weight of each cancer: lung | bladder: and stage s			Values collected in (Soerjomataram et al., 2012)	
s = diagnosis and initial treatment		0.72 | 0.27	/		Deterministic
s = control		0.47 | 0.18	/		Deterministic
s = metastatic/pre-terminal phase		0.91 | 0.64	/		Deterministic
s = terminal phase with medications		0.93 | 0.93	/		Deterministic

The level of arsenic in tap water in France is variable. It has been recently reported by Le Bot et al. (2016). Reported percentiles have been implemented with a cumulative distribution and values below the detection limit (LOD=0.2 µg/L) were assigned half of the limit according to a medium bound approach (LOD/2=0.1 µg/L).

Moreover, the absorption rate must be considered since part of ingested chemicals are excreted. Arsenic present in tap water is almost solely inorganic which has a bioavailability rate between 90-100% (FDA, 2016; Hrudey, 1995; Meacher et al., 2002; Mondal and Polya, 2008; Zheng et al., 2002). The most often reported value from the literature, 100%, was used. Exposure to arsenic was then calculated with **Equation 5.2**.

Equation 5.2

$$N^{Ar}(i,j) = \frac{V_{day}(i,j) \times N^{Ar}{}_{0}}{1000} \times Abs^{Ar}$$

Where:

$N^{Ar}(i, j)$: Daily exposure to arsenic (µg/kg bw per day),
$N^{Ar}{}_{0}$: Concentration of arsenic in tap water (µg/L),
Abs^{Ar}: Absorption of arsenic (%).

5.3.2.2Arsenic dose-response

Chronic exposure to arsenic was linked with potential lung, bladder and skin cancers (IARC, 2012; INVS, 2008; WHO, 2016). Even though a strong correlation between arsenic and skin cancer has been established for decades (Shannon and Strayer, 1989), it is a multi-causal disease (Martinez et al., 2011) which cannot only be associated with arsenic exposure and can lead to numerous endpoints (skin lesions, pigmentation changes, skin cancer). Therefore, only bladder and lung cancers were included in this assessment. More precisely, the exposure during the first months of life might contribute to increase cancer risk later in life (FDA, 2016). Until now, other arsenic risk assessments in tap water were performed for drinking water and stopped at the estimation of levels of exposure (Le Bot et al., 2016). The present model was based on another full risk assessment that was performed for arsenic for rice, cereal and other sources (FDA, 2016; Shibata et al., 2016), estimating an Incremental Cancer Risk (ICR) due to a specific period

of exposure. This ICR represents part of the cancer risk which can be attributed to the first window of exposure in life. Its calculation is based on the chronic daily intake (CDI) (Shibata et al., 2016) during the first six months of life (**Equation 5.3**).

Equation 5.3

$$\mathrm{CDI}^{\mathrm{Ar}}(\mathrm{i}) = \frac{1}{6}\sum_{\mathrm{j}=1}^{6} \mathrm{N}^{\mathrm{Ar}}(\mathrm{i},\mathrm{j})$$

Where:

$\mathrm{CDI}^{\mathrm{Ar}}(\mathrm{i})$: Average chronic daily intake to Arsenic (µg/kg bw per day).

It was then converted into lifetime average daily dose (LADD) with **Equation 5.4** by considering this first exposure regarding the whole chronic exposure (AT) duration, commonly set at 70 years (25 550 days) in chemical risk assessments (ATSDR, 2005; Shen et al., 2014; Shibata et al., 2016).

Equation 5.4

$$\mathrm{LADD}^{\mathrm{Ar}}(\mathrm{i}) = \mathrm{CDI}^{\mathrm{Ar}}(\mathrm{i}) \times \frac{\mathrm{ED}}{\mathrm{AT}}$$

Where:

$\mathrm{LADD}^{\mathrm{Ar}}(\mathrm{i})$: Lifetime average daily dose (µg/kg bw per day),
ED: Exposure duration (6 months = 180 days) (day),
AT: Average time (70 years = 25 550 days) (day).

Dose-responses for both cancers were deduced from those reported by FDA (2016) which were based on the studies of Chen et al. (2010a) and Chen et al. (2010b). Data were extracted from graphs using the online application WebPlotDigitizer 3.10 (WebPlotDigitizer, 2016) and were transformed with the natural logarithm to obtain a linear shape. A dose-response equation was deduced from this transformed curve and an error (ε) was added to integrate uncertainty bounds. It follows a Normal distribution centered at 0 with a standard deviation (SD) of Δ/1.96 (based on the confidence intervals for a regression coefficient that is described with the equation Δ = SD × 1.96; 1.96

represents 95% confidence interval) where Δ is the mean difference between the predicted curve and its uncertainty bounds.

The obtained dose-responses were used to calculate the Lifetime Cancer Risk (LCR) frequency for both cancers (**Equation 5.5**).

Equation 5.5

$$\ln\left(\mathrm{LCR}^{\mathrm{Ar}}(\mathrm{i},\mathrm{c})\right) = \alpha(\mathrm{c}) \times \mathrm{LADD}^{\mathrm{Ar}}(\mathrm{i}) + \beta(\mathrm{c}) + \varepsilon(\mathrm{c})$$

Where:

$LCR^{Ar}(i,c)$: Lifetime cancer risk frequency after being exposed to arsenic,

c: Bladder cancer / lung cancer,

α(c): Model parameter from linear regression,

β(c): Model parameter from linear regression,

ε(c): Error of the model (describes uncertainty).

Subsequently, Incremental Lifetime Cancer Risk (ILCR) was calculated. It was defined as the cancer risk due to the exposure to arsenic. It was estimated by subtraction of LCR^{Ar}_0 (when exposure to arsenic is equal 0 µg/day per bw) from LCR^{Ar} at the LADD considered (**Equation 5.6**).

Equation 5.6

$$\mathrm{ILCR}^{\mathrm{Ar}}(\mathrm{i},\mathrm{c}) = \frac{\left(\mathrm{LCR}^{\mathrm{Ar}}(\mathrm{i},\mathrm{c}) - \mathrm{LCR}^{\mathrm{Ar}}{}_0(\mathrm{c})\right)}{100}$$

Where:

$ILCR^{Ar}(i,c)$: Incremental life time cancer risk due to arsenic exposure during the first 6 months of life,

$LCR^{Ar}_0(c)$: Lifetime cancer risk frequency when no exposure to arsenic ($LADD^{Ar}$=0 µg/kg bw per day) (%).

5.3.2.3 Risk characterization of Arsenic

Number of additional cases of bladder and lung cancer due to the first six months of exposure, were estimated per gender and per 100 000 infants (**Equation 5.7**), integrating the variability of the population by using the mean ILCR obtained.

Equation 5.7

$$N^{Ar}_{ill}(i,c) = 100\,000 \times p(i) \times mean\_ILCR^{Ar}(i,c)$$

Where:

$N^{Ar}_{ill}(i,c)$: Number of cancer cases per 100 000 infants exposed during first six months of life,

$p(i)$: Ratio of boys and girls in France (%),

$Mean\_ILCR^{Ar}(i,c)$: Mean incremental life time cancer risk due to arsenic exposure during the first six months of age.

Among estimated cases, number of cured patients (**Equation 5.8**) was deduced using prevalence of surviving from each cancer (INCa, 2015; Jakobsen et al., 2016).

Equation 5.8

$$N^{Ar}_{cure}(i,c) = N^{Ar}_{ill}(i,c) \times Prev^{Ar}_{survive}(i,c)$$

Where:

$N^{Ar}_{cure}(i,c)$: Number of cured cancer cases,

$Prev^{Ar}_{survive}(i,c)$: Prevalence of surviving cancer disease after 5 years (%).

Then, the number of possible deaths was estimated using the number of cancer cases and those cured (**Equation 5.9**).

Equation 5.9

$$N^{Ar}_{death}(i,c) = N^{Ar}_{ill}(i,c) - N^{Ar}_{cure}(i,c)$$

Where:

$N^{Ar}_{death}(i,c)$: Number of death cancer cases.

5.3.2.4 Conversion of risk of Arsenic in DALY

DALY calculation estimates years of life lost and lived with a disease (**Equation 5.10**).

Equation 5.10

$$DALY^{Ar} = \sum_{i,c} YLL^{Ar}(i,c) + YLD^{Ar}(i,c)$$

Where:

$DALY^{Ar}$: DALYs lost due to a cancer disease,

YLL^{Ar} (i,c): Years of life lost due to a premature death caused by cancer disease for each gender,

YLD^{Ar} (i,c): Years of life lived with a disability caused by cancer disease for each gender.

Two situations were considered for each cancer cured or died patient (Mathers et al., 1999). It was also assumed that there were no sequelae after the patient had been cured as done in Xiao et al. (2012). Available data did not allow to integrate more details. The cancer treatment was divided into 4 stages (**Figure 5.2**).

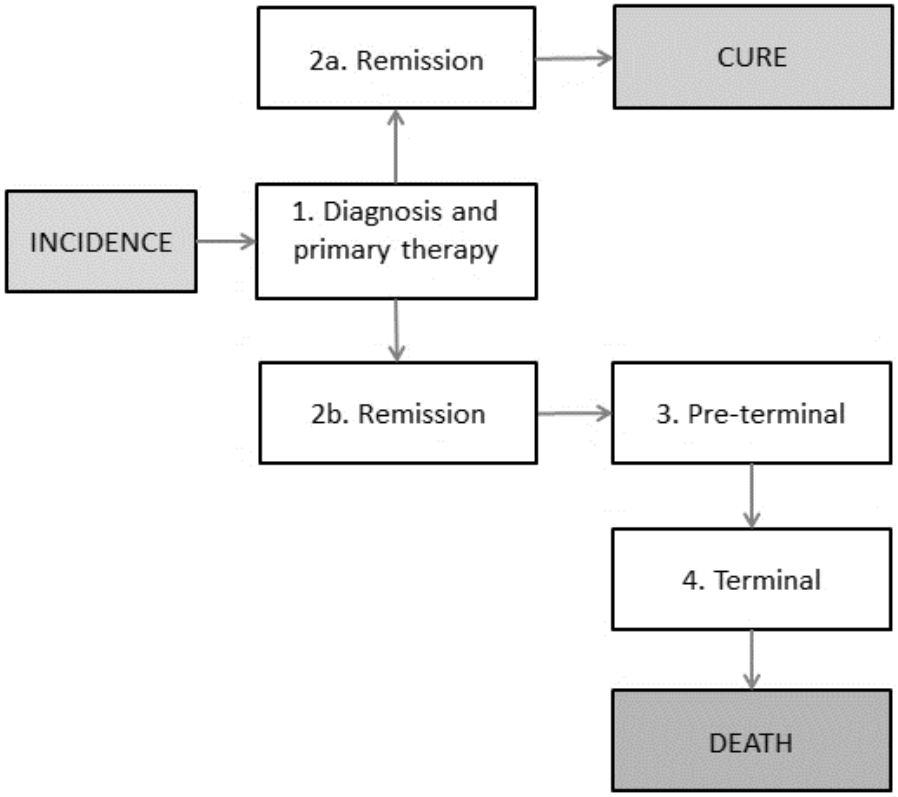

Figure 5.2: Cancer treatment stages deducted from Soerjomataram et al. (2012)

YLL (**Equation 5.11**) and YLD (**Equation 5.12**) were estimated with the average duration of each cancer stage and disability weights obtained from Soerjomataram et al. (2012). The remaining life expectancy (LE) was deduced from French statistics regarding age of new cancer specific incidences (INCa, 2015) and life expectancy at this age (INSEE, 2014). Firstly, the probability of getting cancer at each age was estimated using the number of cancer incidences in France per age. Secondly, a cumulative distribution was applied to select the age of incidence using a given probability. The duration of cancer disease before death was added to estimate the age of death. Finally, the remaining life expectancy for a given age was based on recent data for French population (INSEE, 2014).

Equation 5.11

$$\mathrm{YLL}^{\mathrm{Ar}}(\mathrm{i},\mathrm{c}) = \mathrm{N}^{\mathrm{Ar}}{}_{\mathrm{death}}(\mathrm{c}) \times \mathrm{LE}(\mathrm{i},\mathrm{c})$$

Where:

LE(i,c): Remaining life expectancy (year).

Equation 5.12

$$\mathrm{YLD}^{\mathrm{Ar}}(\mathrm{i},\mathrm{c}) = \sum_{\mathrm{s}} [\mathrm{N}^{\mathrm{Ar}}{}_{\mathrm{cure}}(\mathrm{i},\mathrm{c}) \times \mathrm{w}(\mathrm{c},\mathrm{s}) \times \mathrm{L}(\mathrm{c},\mathrm{s}) + \mathrm{N}^{\mathrm{Ar}}{}_{\mathrm{death}}(\mathrm{i},\mathrm{c}) \times \mathrm{w}(\mathrm{c},\mathrm{s}) \times \mathrm{L}(\mathrm{c},\mathrm{s})]$$

Where:

s: Cancer treatment stage: 1/2a/2b/3/4, see Figure 5.2,
L(c,s): Average duration of cancer stage,
w(c,s): Disability weight for both cancers (c) and cancer stages (s).

5.3.3. *Cryptosporidium* risk assessment model development

Water is a predominant source of infections with *Cryptosporidium* (CDC, 2015) and most of outbreaks in France have been reported in the age group 0-4 (ANOFEL, 2010).

However, this parasite is sensitive to heat treatment as for instance only 1 minute at 72.4˚C can lead to the whole inactivation of oocysts (Fayer, 1994). Thus, it was assumed that boiling water for infant milk preparation, corresponding to Scenario 1, ensures complete inactivation of oocysts, leading to zero risk of infection. All inputs introduced below are summarized in **Table 5.3**.

Table 5.3: Description and implementation of inputs of *Cryptosporidium* risk assessment

Input	Description	Model implementation	Unit	Reference	Category
➢ **Exposure calculation**					
N^{Cr}_0	Level of detected *Cryptosporidium* oocysts in surface water	DiscreteUniform(a×pa, b×pb, c×pc, d×pd, e×pe, f×pf, g×pg, h×ph) a=Triangular(0.5, 3.0, 30.5), pa= Binomial(1, 7/13) b=Triangular(0.5, 0.6, 2.5), pa= Binomial(1, 6/12) c=Triangular (0.5, 1.4, 9.0), pa= Binomial(1, 5/13) d=Triangular (0.5, 1.1, 13.1), pa= Binomial(1, 21/39) e=Triangular (0.5, 0.5, 3.0), pa= Binomial(1, 12/32) f=Triangular (0.5, 0.5, 4.6), pa= Binomial(1, 12/39) g=Triangular (0.5, 3.2, 20.0), pa= Binomial(1,4/7) h=Triangular (0.5, 35.9, 245.4), pa= Binomial(1,7/7) With a=Joinville,b=Orly, c=Ivry, d=Tolbiac, e=Alma, f=Garigliano, g=Suresnes, h=Clichy.	oocysts/ 10 L	Values collected in (Mons et al., 2009)	Variability
Rr	Recovery rate	Beta(α=2.64; β=3.64)	%	(Pouillot et al., 2004)	Uncertainty
Ov	Part of viable oocysts	Beta(α=2.6; β=3.4)	%	(Pouillot et al., 2004)	Uncertainty
➢ **Water treatment calculation**					
Tr_{sec}	Reduction of oocysts during secondary water treatment	(1-Sed) × (1-Fred)	% reduction	(Cummins et al., 2010).	Variability
Sed	Sedimentation	$1\text{-}10^{-(\text{Triangular (0.5; 1; 2)})}$	% reduction		Variability
F_{red}	Filtration reduction	$1\text{-}10^{-f(Crun, Frun)}$	% reduction		Variability
C_{run}	Coagulation run	Discrete ([C_{opt}, C_{sub}, C_{fail}],[C_{prob_opt}, C_{prob_sub}, C_{prob_fail}])			Variability
C_{opt}	Coagulation opt	0	Log reduction in efficiency		Deterministic
C_{sub}	Coagulation subopt	Triangular (0; 2.1; 2.1)			Variability
C_{fail}	Coagulation failure	Uniform (4.2; 4.4)			Variability
C_{prob_opt}	Probability of optimal coagulation	0.99	Probability		Deterministic
C_{prob_sub}	Probability of sub-optimal coagulation	0.005	Probability		Deterministic

C_{prob_fail}	Probability of fail coagulation	0.005	Probability		Deterministic
F_{run}	Filtration run	Discrete ([F_{opt}; F_{fail}],[F_{prob_opt};F_{prob_fail}])			Variability
F_{opt}	Dual media Filtration opt	Weibull (α=4.47; β=5.31; Shift0-1.06)	log reduction		Variability
F_{fail}	Dual media Filtration fail	$Fopt_{Dual\text{-}media}$-Trianglular (0;0;1.4227)	log reduction		Variability
F_{prob_opt}	Filtration opt	Uniform (0.95; 0.97)	Probability		Uncertainty
F_{prob_fail}	Filtration failure	1 - Fopt	Probability		Uncertainty
➢ **Dose-response calculation**					
r	Dose-response parameter	0.354	/	(Pouillot et al., 2004)	Deterministic
➢ **DALY calculation**					
$Prev^{Cr}_{death}$	Prevalence of death due to diarrhea in immunocompromised population – infants	RiskPert(0.06;0.12;0.2)	%	Xiao et al. (2012)	Uncertainty
LE(i)	Remaining life expectancy of boy | girl at birth	78.1 | 84.7	Year	(INSEE, 2014)	Deterministic
w^{Cr}	Disability weight of diarrhea	RiskPert(0.036; 0.061; 0.093)	/	(Salomon et al., 2015)	Uncertainty
L^{Cr}	Average duration of diarrhea	Triangular (0.0055; 0.0329; 0.0822)	Year	(ANSES, 2011a; DuPont et al., 1995; Health Canada, 2013)	Uncertainty

5.3.3.1 Exposure Assessment of Cryptosporidium

The daily tap water intake of formula fed infants (**Equation 5.1**) has been converted into intake per bottle of milk (**Equation 5.13**), integrating French infant weights (Scherdel et al., 2015) implemented with a cumulative distribution and the daily number of feeds approximated from products recommendations (**Table 5.1**).

Equation 5.13

$$V_{bottle}(i,j) = \frac{V_{day}(i,j) \times BW(i,j)}{N_{feed}(j) \times 1000}$$

Where:

$V_{bottle}(i,j)$: Intake of tap water per prepared bottle of infant formula (L/bottle of PIF),
$BW(i,j)$: Infant body weight (kg),
$N_{feed}(j)$: Number of feeds per day.

The level of *Cryptosporidium* in tap water in France was based on the level found in surface water, corrected with the effect of water treatment. Initial levels of oocyst N^{Cr}_0, were reported for different water treatment plants around Paris (Mons et al., 2009). Levels found in contaminated samples collected in each plant were implemented with a Triangular distribution and weighted with a binomial distribution of the prevalence of contaminated samples. The level in surface water, N^{Cr}_0, used in the model was collected with a Discrete Uniform distribution of each plant level, considering equiprobability between towns.

The enumeration of reported levels of *Cryptosporidium* was made using a procedure adopted from the U.S. EPA method 1623 for the detection of *Cryptosporidium* and *Giardia* in water (Mons et al., 2009). This method is likely to underestimate the number of parasites present in water, therefore the level was corrected (**Equation 5.14**) with a recovery rate (Rr). It was implemented with a negative binomial distribution having a mean of 0.41 and a standard deviation 0.18 (Pouillot et al., 2004).

Equation 5.14

$$N^{Cr}_{true} = \left[N^{Cr}_0 + Negbin\left(N^{Cr}_0 + 1\ ;\ Rr\right)\right]$$

Where:

N^{Cr}_{true}: True level of *Cryptosporidium* oocysts in surface water [oocysts/10 L],

N^{Cr}_{0}: Level of detected *Cryptosporidium* oocysts in surface water [oocysts/10L],

Rr: Recovery rate.

Afterwards, the level of oocysts in tap water was calculated by including an estimation of the effect of water treatment, adapted from Cummins et al. (2010) for France, as done in Xiao et al. (2012) for China. Three different levels of treatment can be applied. The primary treatment aims to remove big elements, like sticks, stones or debris from water and it is not likely to change the level of *Cryptosporidium*. The secondary treatment removes remaining debris and most of the contaminants, including oocysts, by sedimentation, coagulation, flocculation and filtration. The tertiary treatment is a water disinfection deactivating viable oocysts, using for instance ozonation or UV. Chlorination is commonly applied in France but it is highly resistant to chlorine (WHO, 2011) so it was not considered further.

Based on expert's information (personal communication), the following water treatment steps were considered in the model: coagulation, sedimentation, dual media filtration and ozonation. They were implemented using calculations of Cummins et al. (2010).

During secondary treatment (coagulation, sedimentation and filtration), oocyst reduction is implemented with a probability of "optimal", "suboptimal" or "failed" coagulation and filtration, as massive outbreaks were reported due to water treatment failures (Mackenzie et al., 1994). In addition, it was considered that not all oocysts are viable, and then capable of causing an infection. The viability was included in the model after secondary and before tertiary treatment using values from Pouillot et al. (2004). Overall, the level of viable oocysts after water treatment was estimated using **Equation 5.15** (Cummins et al., 2010; Xiao et al., 2012).

Equation 5.15

$$N^{Cr}_{treat} = \frac{N^{Cr}_{true} \times (1 - Tr_{sec}) \times (1 - Tr_{tert}) \times Ov}{10}$$

Where:

N^{Cr}_{treat}: Number of *Cryptosporidium* in tap water, after surface water treatment [oocysts/L],

Tr_{sec}: Reduction of oocysts during secondary water treatment [%],

Tr_{tert}: Reduction of oocysts during tertiary water treatment [%],
Ov: Viability of oocysts.

Finally, the number of oocysts per bottle of PIF was estimated using the number of oocysts after water treatment and the tap water intake per bottle of PIF (**Equation 5.16**).

Equation 5.16

$$N^{Cr}{}_{bottle}(i,j) = Poisson(N^{Cr}{}_{treat} \times V_{bottle}(i,j))$$

Where:

$N^{Cr}{}_{bottle}(i,j)$: Number of oocysts per bottle of PIF [oocysts/bottle of PIF],
$V_{bottle}(i,j)$: Intake of tap water per prepared bottle of PIF (L/bottle of PIF).

5.3.3.2 Cryptosporidium dose-response

Cryptosporidium infection leads mainly to watery diarrhea and may also include stomach cramps, nausea, vomiting (CDC, 2015). Diarrhea was defined as the main adverse effect, potentially leading to death, especially for infants as they are a vulnerable group (DuPont et al., 1995).

To estimate the probability of illness due to *Cryptosporidium* per bottle of infant formula, an exponential dose-response model was applied (DuPont et al., 1995), as already done in several risk assessments of *Cryptosporidium* (Cummins et al., 2010; Pouillot et al., 2004; Xiao et al., 2012). For the considered population (infant only group) it was assumed that each infection would result in a disease (Pouillot et al., 2004).The probability of illness per day was estimated based on the probability of illness per bottle of PIF and the daily number of feed as explained in Havelaar and Zwietering (2004) (**Equation 5.17**). The r parameter applied for the dose-response was estimated for immunodeficient population, as infants were considered to belong to that group (Pouillot et al., 2004). This r value was higher than the value used for infants and adults in (Razzolini et al., 2016) due to higher sensitivity levels observed in developed countries compared with developing ones.

Equation 5.17

$$P^{Cr}{}_{ill}(i,j) = 1 - \left(e^{-r \times N^{Cr}{}_{bottle}(i,j)}\right)^{N_{feed}(j)}$$

Where:

$P^{Cr}_{ill}(i,j)$: Daily probability of illness,

r: Dose-response parameter.

5.3.3.3 Risk Characterization of Cryptosporidium

The number of illnesses per day for 100 000 infants was estimated for each gender and age in months, using the mean probability of illness. Indeed, multiplying the mean daily probability of illness, $mean\_P^{C.r}_{ill}(i,j)$ by 100 000 is equivalent to summing 100 000 different $P^{C.r}_{ill}(i,j)$ randomly taken in the variability dimension. A monthly estimation was obtained by multiplying it with 30 days (**Equation 5.18**). Consequently, the expected number of cases integrates the variability of the population.

Equation 5.18

$$Nb^{C.r}_{ill}(i,j) = mean\_P^{C.s}_{ill}(i,j) \times 100\,000 \times p(i)\,.\,30$$

Where:

$Mean\_P^{C.r}_{ill}(i,j)$: Mean Probability of illness per day for gender i and age j, obtained when using Equation 5.17,

100 000: Number of infants considered in the calculation,

p(i): Ratio of boys and girls in France (%),

30: Number of days set per month.

The number of death was calculated using the prevalence of fatal cases in the immunodeficient group given by Xiao et al. (2012), **Equation 5.19**.

Equation 5.19

$$Nb^{Cr}_{death}(i,j) = Nb^{Cr}_{ill}(i,j) \times Prev^{Cr}_{death}$$

Where:

$Nb^{Cr}_{death}(i,i)$: Number of deaths per 100 000 babies per day for gender i and age j,

$Prev^{Cr}_{death}$: Prevalence of death due to diarrhea in immunocompromised population – infants.

5.3.3.4 Conversion of risk of due to Cryptosporidium into DALY

DALY represents years of life lost due to premature death (YLL) and years of life lost due to life with disability (YLD), **Equation 5.20**.

Equation 5.20

$$\mathrm{DALY}^{\mathrm{Cr}} = \sum_{i=1}^{2}\sum_{j=1}^{6} \mathrm{YLL}^{\mathrm{Cr}}(\mathrm{i},\mathrm{j}) + \mathrm{YLD}^{\mathrm{Cr}}(\mathrm{i},\mathrm{j})$$

Where:

$\mathrm{DALY}^{\mathrm{Cr}}$: DALY lost due *cryptosporidiosis*,

$\mathrm{YLL}^{\mathrm{Cr}}$ (i,j): Years of life lost due to premature death caused by diarrhea,

$\mathrm{YLD}^{\mathrm{Cr}}$ (i,j): Years of life lived with disability caused by diarrhea.

Two options were implemented in the model to describe the possible outcome of disease – full recovery or death. Sequelae were not considered, as previously done in RIVM (1999), Xiao et al. (2012), and Razzolini et al. (2016) because it is not likely to occur after diarrhea. DALY was estimated with **Equation 5.21** and **Equation 5.22**.

Equation 5.21

$$\mathrm{YLL}^{\mathrm{Cr}}(\mathrm{i},\mathrm{j}) = \mathrm{Nb}^{\mathrm{Cr}}{}_{\mathrm{death}}(\mathrm{i},\mathrm{j}) \times \mathrm{LE}^{\mathrm{Cr}}(\mathrm{i})$$

Where:

$\mathrm{LE}^{\mathrm{Cr}}$(i): Remaining life expectancy of boy | girl at birth.

Equation 5.22

$$\mathrm{YLD}(\mathrm{i},\mathrm{j}) = \left(\mathrm{Nb}^{\mathrm{Cr}}{}_{\mathrm{ill}}(\mathrm{i},\mathrm{j}) - \mathrm{Nb}^{\mathrm{Cr}}{}_{\mathrm{death}}(\mathrm{i},\mathrm{j})\right) \times \mathrm{w}^{\mathrm{Cr}} \times \mathrm{L}^{\mathrm{Cr}}$$

Where:

w^{Cr}: Disability weight of diarrhea,

L^{Cr}: Average duration of diarrhea.

5.4. Computation method considering separation of uncertainty and variability

As described above, each input was assigned as being variable, uncertain or deterministic (always the same value, **Table 5.1**, **Table 5.2** and **Table 5.3**). The model was implemented in Excel 2010 @Risk version 6.3.1. (Microsoft Excel 2010) and second order Monte Carlo simulations were used to separate uncertainty and variability. A total of 1 000 000 iterations and 100 simulations were performed, both using the Latin Hypercube Sampling method (Mokhtari and Frey, 2005). One iteration represents one variability realization, while one simulation represents one uncertainty realization.

Outputs were reported with their mean and percentile values obtained in the variability dimension. For each reported value, in the uncertainty dimension, median and the 90% confidence interval were collected. Each value was rounded according to the degree of accuracy obtained. To determine the number of significant digits, the model was run three times independently with three sets of 100 iterations in the uncertainty dimension. That enabled also to check the convergence of the model and the stability of the uncertainty interval bounds.

At the end, the predicted number of cases due to 6 months of exposure to arsenic and *Cryptosporidium* for 100 000 infants were estimated using the mean probability of illness which reflects the variability among infants. Then, the burden of disease was estimated in DALY (Gold et al., 2002) at the population level using the predicted number of cases and parameters of the burden of disease.

5.5. Results

5.5.1. Outputs of arsenic risk assessment

The arsenic risk assessment model developed in this study enabled estimation of the incremental bladder and lung cancer risk due to the consumption of infant formula prepared with tap water during the first six months of exposure (the same for both scenarios, i.e. with boiled or un-boiled tap water). The model encompassed the estimation of the level of arsenic in tap water, daily infant exposure level, incremental bladder and

lung cancer risk, and eventually number of DALY. It included heterogeneity of inputs due to different water supply points and various water intake levels among infants, in addition to uncertainty in initial level of arsenic and dose-response estimation.

The mean level of arsenic in tap water in France was estimated to be 0.7 [90% uncertainty confidence interval: 0.6; 0.8] µg/L, it varied among supply points between 0.1 [0.1; 0.1] µg/L (5^{th} percentile) and 2.3 [1.8; 3.1] µg/L (95^{th} percentile) (data not shown); these levels were below the maximum level advised in WHO Guidelines (<10 µg/L) set in the French regulation (Arrêté du Ministère de la Santé et des Solidarités).

The daily exposure of infants (both genders mixed) to arsenic through tap water consumption varied between 0.01 µg/kg bw (5^{th} percentile) and 0.35 µg/kg bw (95^{th} percentile) with a low effect of uncertainty on values from the 5^{th} percentile up to the median as illustrated in **Figure 5.3** (grey uncertainty realizations curves gathered into a narrow bundle), but a higher uncertainty from the median to the 95^{th} percentiles.

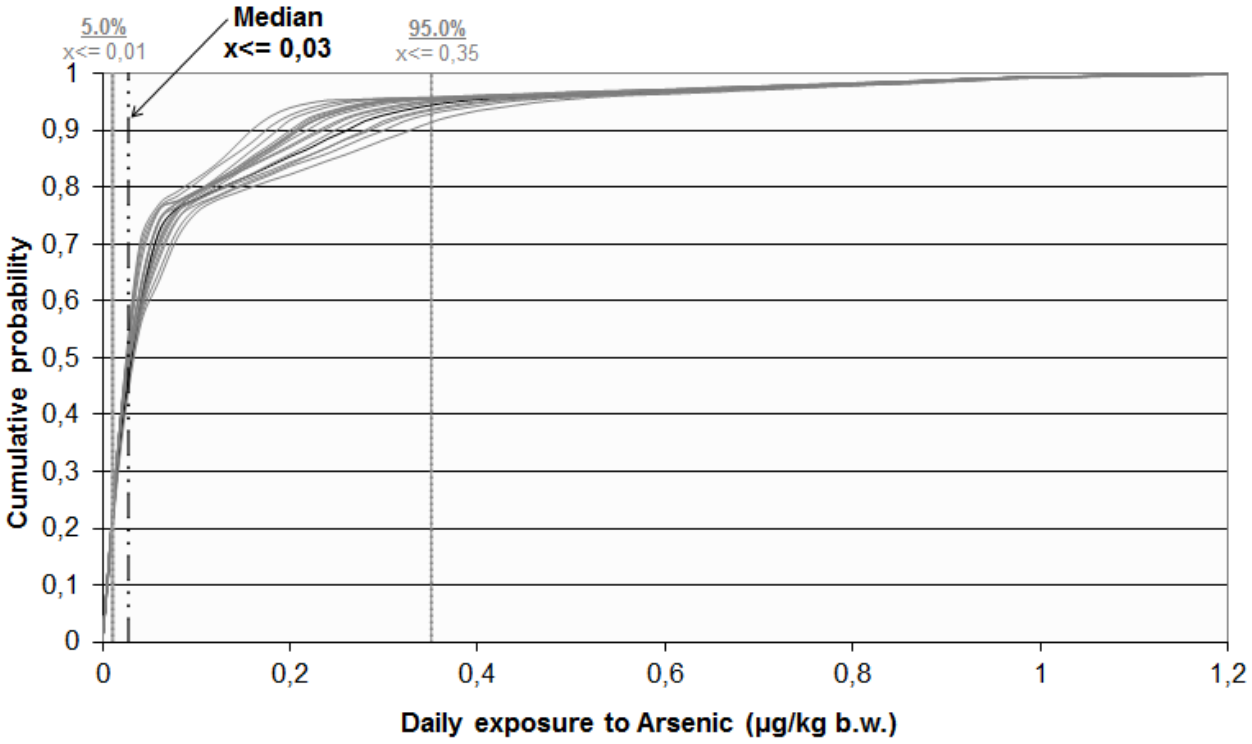

Figure 5.3: Estimated daily exposure of infants (both genders) to Arsenic through tap water consumption (µg/kg b.w.)

At the individual level, the cancer risk due to arsenic exposure was highly variable among infants (**Table 5.4**). The mean probability of illness expressed as an Incremental Lifetime Cancer Risk (ILCR) was higher for lung cancer (5.4 90% CI: [4.3; 6.7] × 10^{-7}) than for bladder cancer (2.1 [1.4; 3.6] × 10^{-7}); it was similar for both genders. The risk varied mainly in the variability dimension going from 7.4 [6.2; 8.9] × 10^{-8} (5^{th} percentile) up to 1.7 [1.3; 2.4] × 10^{-6} (95^{th} percentile) and from 3.0 [2.0; 4.5] × 10^{-8} (5^{th} percentile) up to 7.0 [4.4; 11.7] × 10^{-7} (95^{th} percentile) for lung and bladder cancer, respectively. The uncertainty associated with these results (as indicated by the confidence interval bounds) was small compared with variation in the variability dimension, leading to a variability / uncertainty ratio of around 5 in case of bladder cancer (ca 50 for lung cancer).

Table 5.4: Estimates of the Incremental Lifetime Cancer Risk (ILCR) due to arsenic exposure during the first six months of life associated with tap water consumption (used to rehydrate powder infant formula)

			Scenarios 1 and 2			
			Mean	**Percentiles [a]**		
				5%	**50%**	**95%**
Incremental Lifetime Cancer Risk	**Lung cancer**	**Girl [b]**	5.4 [4.3; 6.7] 10^{-7}	7.4 [6.2; 8.9] 10^{-8}	1.6 [1.3; 2.0] 10^{-7}	1.7 [1.3; 2.4] 10^{-6}
		Boy	5.4 [4.3; 6.7] 10^{-7}	7.4 [6.2; 8.9] 10^{-8}	1.6 [1.3; 2.0] 10^{-7}	1.7 [1.3; 2.4] 10^{-6}
ILCR	**Bladder cancer**	**Girl**	2.1 [1.4; 3.6] 10^{-7}	3.0 [2.0; 4.5] 10^{-8}	6.5 [4.4; 9.7] 10^{-8}	7.0 [4.4; 11.7] 10^{-7}
		Boy	2.1 [1.4; 3.6] 10^{-7}	3.0 [2.0; 4.5] 10^{-8}	6.5 [4.4; 9.7] 10^{-8}	7.0 [4.4; 11.7] 10^{-7}

[a] Percentiles given represent the variability of outputs, mean values are given with their uncertainty interval, when available 90% confident interval is given in square brackets, with the lower bound at 5% and the upper bound at 95%.

[b] Illustration: The mean Incremental Lifetime lung Cancer Risk in the population is estimated at 5.4 10^{-07} for girls due to the first six month of life exposure. Fifty percent of the population (girls) has an estimated risk of 1.6 10^{-07} and five percent has an estimated risk ≥ 1.7 10^{-06}.

When integrating into the population of infants, the number of expected cases per six months, was 0.05 (90% CI: [0.04; 0.07]) and 0.02 ([0.01; 0.04]) cases per 100 000 infants, for lung and bladder cancer, respectively. These values represented 1 [0.4; 2] DALY in total for both cancers (**Table 5.6**).

5.5.2. Outputs of *Cryptosporidium* risk assessment

The *Cryptosporidium* risk assessment model developed in this study enabled the estimation of the oocyst level after water treatment, the individual daily risk of illness and the DALY in case of using un-boiled tap water (scenario 2) for preparation of infant formula during the first six months of life (no risk assumed for boiled water, scenario 1). It included heterogeneity of inputs due to various levels of oocysts in surface water, plant treatment efficiencies and water intake among infants. Uncertainty in estimation of oocyst recovery rate, viable oocyst prevalence and probability of water treatment failure was also taken into account in the model.

The level of *Cryptosporidium* in surface water varied between 0 oocyst/10L and 245 oocysts/10L (median value of 3 oocysts/10L) with a mean of 1.10 [0.32; 3.91] $\times 10^{-4}$ oocysts/10L. After water treatment, this level was estimated to decrease to a value varying in the variability dimension from 2.9 [0.9; 10.8] $\times 10^{-12}$ (5^{th} percentile) up to 5.0 [1.5; 18.4] $\times 10^{-5}$ (95^{th} percentile, data not shown).

At the individual level, the mean daily probability of illness of formula-fed infants varied mainly between 0.9 and 1.5 $\times 10^{-5}$ according to genders and age in months (**Table 5.5**). More precisely, the individual mean probability of illness varied considerably in the variability dimension with more than 95% of infants having zero risk of *cryptosporidiosis* and 0.0001% infants having a risk higher than 7.6 [7.6; 9.4] $\times 10^{-1}$ (value for girl during 1^{st} month of age, see **Table 5.5** for all results). The uncertainty associated with the individual probability of illness was also important as confidence intervals varied by a factor of ten (**Table 5.5**).

Table 5.5: Estimates of daily risk of *Cryptosporidium* associated with tap water consumption (used to rehydrate powder infant formula) during the first six months of life

			Scenario 1				
				Percentiles [a]			
			Mean	5%	50%	95%	99.9999%
Probability of illness per day $P^{C.s}_{ill}(j)$	**Girl Age in month**	**1** [b]	1.1 [0.4; 4.0] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	8.8 [8.8; 8.8] 10^{-01}
		2	1.0 [0.2; 4.2] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	8.8 [8.8; 8.8] 10^{-01}
		3	1.2 [0.3; 5.9] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	8.3 [8.3; 9.7] 10^{-01}
		4	1.4 [0.3; 4.8] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	8.3 [8.3; 9.7] 10^{-01}
		5	1.4 [0.3; 6.1] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	7.6 [7.6; 9.4] 10^{-01}
		6	1.3 [0.3; 7.0] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	7.6 [7.6; 9.4] 10^{-01}
	Boy Age in month	**1**	0.9 [0.3; 3.8] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	8.8 [8.8; 9.4] 10^{-01}
		2	1.1 [0.3; 4.4] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	8.8 [8.8; 9.9] 10^{-01}
		3	1.1 [0.2; 4.7] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	8.3 [8.3; 9.7] 10^{-01}
		4	1.2 [0.3; 5.3] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	8.3 [8.3; 8.3] 10^{-01}
		5	1.4 [0.4; 6.7] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	7.6 [7.6; 9.4] 10^{-01}
		6	1.5 [0.4; 6.8] 10^{-05}	0 [0 ; 0]	0 [0 ; 0]	0 [0 ; 0]	7.6 [7.6; 9.9] 10^{-01}

[a] Percentiles given represent the variability of outputs, mean values are given with their uncertainty interval, when available 90% confident interval is given in square brackets, with the lower bound at 5% and the upper bound at 95%.

[b] Illustration: The mean daily risk of infection in the population due to infant formula consumption prepared with tap water is estimated at 1.1 10^{-5} for girls during the first month of life. Ninety-five percent of the population (girls from birth to 1 month of age) has an estimated risk of 0 and 0.0001 percent has an estimated risk ≥ 8.8 10^{-01}.

When integrating to the whole population of infants, the number of expected cases per six months was estimated to be 209 [71; 707] per 100 000 infants, which represented 2 250 [960; 7 650] DALY.

5.5.3. Scenarios comparison: estimation of the net health impact

Two scenarios of infant formula preparation were assessed and compared: the use of boiled (Scenario 1) and un-boiled (Scenario 2) tap water, corresponding to the French (ANSES, 2013) and the worldwide (WHO, 2007; FDA, 2015) recommendations, respectively.

Scenario 1 included only the arsenic risk as *Cryptosporidium* risk was negligible with boiled water. The health impact was around 0.07 cancer case for 100 000 infants following this scenario during six months (result deduced from **Table 5.6**), representing

around 1 DALY. With regard to scenario 2, the health impact was around 0.07 [0.05; 0.1] cancer cases and 209 [71; 707] *cryptosporidiosis* cases for 100 000 infants (**Table 5.6**), representing a total of 2 251 [961; 7 652] DALY.

Table 5.6: Estimates of number of illness and DALY for Arsenic and *Cryptosporidium* per 100 000 infants exposed during the first six months of life through tap water consumption (used to rehydrate powder infant formula)

Health effect	**Health impact** for 100 000 infants consuming un-boiled tap water during the first six months of life (used to rehydrate powder infant formula) **Scenario 2**		
	Number of illnesses	**Number of death**	**DALY**
Diarrhea (*Cryptosporidium*)	209 [71; 707][a]	28 [12; 94]	2250 [960; 7650]
Lung cancer (Arsenic)	0.05 [0.04; 0.07]	0.04 [0.03; 0.06]	0.9 [0.4; 1.6]
Bladder cancer (Arsenic)	0.02 [0.01; 0.04]	0.01 [0.007; 0.02]	0.1 [0.04; 0.3]

[a] Confident interval is given in square brackets, with the lower bound at 5% and the upper bound at 95%.

5.6. Discussion

The aim of the study was to quantify the risk of using tap water in France for preparation of powder infant formula, during the first six months of life. Arsenic and *Cryptosporidium* were selected as hazards of highest concern. First, a probabilistic risk assessment model was developed for Arsenic and *Cryptosporidium* independently and then outputs were converted into the DALY common metric.

In chemistry, it is the first complete risk assessment of arsenic in tap water going up to the risk-of-cancer and DALY estimation, the other studies have stopped at the exposure level. The estimated daily exposure of infants varied between 0.01 and 0.35 µg/kg b.w. (5^{th} and 95^{th} percentiles), indicating the variability of infants' exposure to arsenic. Consequently, the estimated risks of bladder and lung cancers associated with arsenic varied among infants. However, even higher estimated levels of exposure were below the acceptable risk level set by WHO for additional cancer cases (set at 10^{-5}, i.e. one additional cancer case in 100 000 inhabitants) and below the acceptable risk range introduced for drinking water by EPA (range of 10^{-4} - 10^{-6}) (Fewtrell and Bartram, 2001). Once converted into DALY, the risk was far below the burden of disease due to all cancers in France (from all sources) which amounted to 1 355 000 DALY in 2004 (John and Ross, 2010).

In microbiology, several *Cryptosporidium* risk assessments were carried out for drinking tap water (not for infant formula consumption) and results can be compared as intakes are similar (daily tap water intake of 400-800mL for formula-fed infants compared with 500mL of drinking water for children and 1-2L for adults). Moreover, most studies have estimated the risk for immunocompromised people, a rather similar sensitive population to infants. The risk assessment model presented in this study was based on previously developed calculations (Cummins et al., 2010; Pouillot et al., 2004; Xiao et al., 2012), but updated with the most recent French data, or European ones when necessary. The model started with the level of oocysts found in surface water in France and included water treatment efficiency. This methodology is similar to the one carried out by Cummins et al. (2010) and Xiao et al. (2012), but more comprehensive than the one performed by Pouillot et al. (2004) as it included recent levels of oocysts from surface water and the efficiency of water treatment. Additionally, in our study, probabilities of illness were converted into DALY while Cummins et al. (2010) work ended at the risk level estimate.

Overall, that meant a risk assessment framework similar to the one performed by Xiao, An et al. (2012) for China. The estimated mean daily probability of illness of formula-fed infants using un-boiled tap water was in line with results of immunocompromised people from Cummins et al. (2010) and Xiao et al. (2012). Nevertheless, it is lower than values reported in Pouillot et al. (2004), this can be explained by different levels of oocysts considered after water-treatment. Indeed, in the present model based on collected French data, oocyst levels decreased after treatment to 10^{-12} - 10^{-5} oocyst/L which is considerably lower than levels reported by Pouillot et al. (2004): 0-10 oocyst/L.

The separation of variability and uncertainty has not often been done in water risk assessment studies (only by Pouillot et al. (2004)) although it is well acknowledged that allows a better interpretation of model outcomes by providing more comprehensive and enhanced information. It helps also policy makers to adopt more secure decisions. It was particularly essential in the present study to pinpoint that the chemical model was mainly driven by variability and the microbiological model by both uncertainty and variability. Indeed, the risk associated with *Cryptosporidium* was variable due to different levels of oocysts in surface water and various degrees of water treatment failures, and uncertain due to lack of information on recovery rates, prevalence of viable oocysts and probability of water-treatment failures. Results reported in the variability dimension indicated that the vast majority of infants (more than 95%) was not concerned with *Cryptosporidium* risk as they were not even exposed to the hazard. The prevalence of contaminated infant formula bottles is directly linked with the level of oocysts in surface water (before treatment) as illustrated in supplement materials (**Figure 5.4**), obtained by running the model for different initial levels of oocysts). Altogether, these results highlighted that current water treatment in France might not be sufficiently efficient in a case of high oocyst contamination levels. It is important to continue monitoring tap water quality regarding *Cryptosporidium* contamination and working on surface water treatment efficiency.

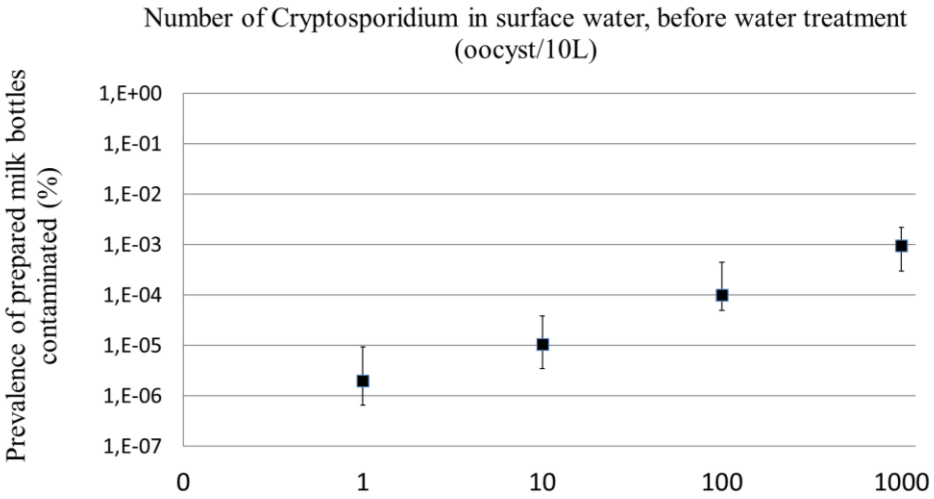

Figure 5.4: Predicted prevalence of prepared milk bottles contaminated (%) according to different levels of *Cryptosporidium* oocysts in surface water (before treatment)

For PIF reconstituted with boiled water, the microbiological risk is assumed to be zero as *Cryptosporidium* is sensitive to heat treatment. Regarding the risk of developing lung and bladder cancer later in life due to arsenic exposure, at the population level, we can conclude that using tap water in France does not appear to be of public health concern as the risk is small compared with prevalence of cancers in France (less than 10^{-6} even for high levels of exposure in some regions). However, at the individual level, the variability analysis revealed that the risk might not be seen so negligible in all cases: it varied among infants and for those exposed to high level of arsenic, the consequence can be severe (cancer); monitoring of supply points should also be continued for chemicals.

DALY has been used to compare microbiological and chemical outputs. This composite metric is valuable at the population level to compare different scenarios involving risks and benefits linked with different health effects. It has been widely used in risk and risk-benefit assessment (EFSA, 2010; Havelaar and Melse, 2003; Watzl et al., 2012) as it enables an estimate of the overall health impact. In the present study, at the population level, the scenario using boiled tap water does not seem to be of concern either for *Cryptosporidium* or arsenic: less than 1 [0.4; 2] DALY for the population of 100 000 infants. In contrast, the scenario using un-boiled tap water is of concern, at least for individuals exposed to the highest oocyst concentration: 2 251 [961; 7 652] DALY attributed to less than 0.001% of 100 000 infants means about 22 DALY per infant, i.e a certain premature death for those infants. More generally, the DALY metric has the advantage of making comparison between risks associated with various hazards possible,

but has the limitation of hiding behind a single digit two distinct phenomena, namely the number of at-risk individuals and the severities of illness. Thus, to keep all relevant information, it is worthwhile to report results in both formats, individual risk and DALY, this suggestion is consistent with conclusions reported in recent risk-benefit assessment studies (Berjia et al., 2012; Boué et al., 2017a).

5.7. Conclusion

This study is to date the first probabilistic and inter-disciplinary microbiological-chemical risk assessment of tap water for preparation of infant formula. Results of this study could help policy makers to make future recommendations. Moreover, both models developed can now be refined as soon as new French data are published, or even applied to other countries by populating the inputs with appropriate data. In addition, the framework deployed in this study could be applied to other contaminants in water. More broadly, it contributes to the development of the risk-benefit assessment approach by developing another microbiological-chemical risk assessment.

CHAPTER 6

General Discussion

CHAPTER 6: **General Discussion**

This last chapter summarises the main results obtained during this PhD project in terms of RBA methodology and regarding the infant milk-based diet case study. Then, a discussion addresses the specific research questions raised in the introduction. Finally, a conclusion is drawn and perspectives for further research are provided.

Objectives of the chapter:

- Summarise main results of the PhD project,
- Discuss research questions: multidisciplinary RBA framework, health impacts comparison, variability and uncertainty consideration, interpretation and communication of results,
- Draw conclusions on the PhD work,
- Provide directions for future research.

6.1. Main findings

6.1.1. State of art of Risk-Benefit Assessment in Foods

The state of art of Risk-Benefit Assessment (RBA) in food was carried out in CHAPTER 2 in order to synthesise RBA studies already performed and to summarise the current methodological options and/or tendencies in this field.

The use of generic terms was suggested to overcome the diversity of terms used in microbiology, chemistry and nutrition. The term "**Health Effect Contributing Factor" (HECF)** was introduced to define *an agent able to cause an adverse or a positive health effect in case of exposure*, this term encompasses the term hazard and its counterpart on the benefit side. Similarly, the terms risk and benefit were grouped under the expression **"Health impact" (HI)** defined as a *function of the probability of an adverse or positive health effect and the severity of that effect, resulting from exposure to an HECF*.

Although, RBA is still an emerging science, **a general frame was built**, based on methodologies developed in European projects (EFSA, BRAFO, QALIBRA, BEPRARIBEAN and BENERIS) and in few existing case studies. In this suggested frame, and in accordance with previous projects, RBA starts with the *definition of the case study* (a diet, a food or a food compound), the (sub)population targeted, and different scenarios of consumer exposure to be assessed (reference and alternative scenarios). Then, an *individual assessment is conducted in microbiology, chemistry and nutrition* to estimate all risks and benefits for the different scenarios of consumer exposure. It follows traditional steps of risk assessment as advised by EFSA (2006) and adapted to RBA language (Step 1: Identification of HECF, Step 2: Exposure assessment, Step 3: Characterisation of HECF, Step 4: Health impact characterisation). A *harmonisation of health impacts* into a common metric is then needed to compare all risks and benefits together and to predict the overall health impact.

So far, less than 100 studies have been carried out within the food safety area, and the main topic of interest has been the RBA of fish consumption integrating nutritional compounds (e.g. fatty acids DHA and EPA), chemicals (e.g. Methylmercury, dioxins and PCB) as well as occasionally microbiological hazards (e.g. *Listeria monocytogenes*). Few studies have also assessed risks and benefits associated with other topics such as the use of intense sweeteners, consumption of fruits and vegetables, different cooking practices, food fortification, etc. As a general observation, risks and benefits have been compared using three different ways. The most common comparison option has been based on a comparison of consumer levels of exposure with regard to safety reference levels such as TWI (Tolerable Weekly Intake) in chemistry and RDI (Recommended Daily Intake) in nutrition. Another option has been to compare the change in endpoint trends like the increase of number of deaths due to a risk with the decrease of number of deaths thanks to a benefit. Alternatively, the use of a composite metric like the DALY (Disability-Adjusted-Life-Year) has been shown to convert all risks and benefits into a same metric and then to provide a comprehensive assessment of the consequences of a disease by integrating the quality of life lost and premature death. However, the use of DALY to express RBA result requires a finalised assessment of each risk and benefit (up to the prediction of the number of cases) which is not always possible due to missing data or knowledge.

The state of art has highlighted the still “under construction” and front of science status of the RBA discipline.

6.1.2. Review of Risks and Benefits of infant milk-based diet

During the PhD poject, a specific case study was selected to develop further the RBA methodology: the infant milk-based diet. In, CHAPTER 3 potential risks and benefits associated with infant milk consumption (breast milk and infant formula) with regards to microbiology, chemistry and nutrition, were reviewed.

Infant milk-based diet is of major concern in public health as first months of an infant’s life affects health status during short and long term (Horta et al., 2007; Horta and Victora, 2013). During the first six months of life, infant diet can be mainly based on two different kinds of foods: breast milk or infant formula. Although, breast milk is recommended at the worldwide level (WHO, 2014a), formulas remain predominant infant food consumed in Western countries (Cattaneo, 2013; Salanave et al., 2014). Various beneficial and adverse health effects related to microbiological, chemical and nutritional factors have been associated with both diets. In this context, the objective of this chapter was to review potential health risks and benefits associated with both milk-based diets.

From the nutritional point of view, both diets enable to fulfil basics nutritional requirements (Butte, 2005) in terms of energy, macro and micro nutrients. Breast milk can additionally transmit immunological properties to infants (Meltzer et al., 2016) and has been associated with several health effects at short term (decrease of gastro intestinal and respiratory tract infections) and long term (decrease of obesity, type-2 diabetes and improvement of cognitive development). These associations have been characterised with different levels of evidence which have evolved over years of research (Büchner et al., 2007; Hörnell et al., 2013; Horta et al., 2007; Horta and Victora, 2013; Meltzer et al., 2016; RIVM, 2015; Van Rossum et al., 2005; Victora et al., 2016). Infant formula composition continues to evolve by attempting to replicate breast milk composition (Tijhuis et al., 2014).

On the other hand, BM is also a source of lipophilic and persistent organic pollutants that are stored in human fatty tissues (Massart et al., 2008; Sonawane, 1995) after mother exposure through inhalation, ingestion and dermal contact (Cattaneo, 2013). More precisely, polychlorinated biphenyls (PCB), organochloride pesticides, dioxins and

brominated flame retardants are of major concern (Meltzer et al., 2016) as well as heavy metals (e.g. cadmium, lead and mercury)(WHO) and mycotoxins (e.g. aflatoxin M1 (Khaniki, 2007)). In comparison, PIF is subject at a lower level to these same contaminants but can deliver additional contaminants originated from manufacturing process (e.g. acrylamide, furan, PAHs and 3-MCPD) (Meltzer et al., 2016), brought by water addition (Villanueva et al., 2014) (e.g. disinfection by-products, heavy metals, organochloride pesticides, etc.) and/or contact material migrations (bisphenol A and phthalates) (Meltzer et al., 2016). The main human health outcomes possibly associated with these exposures are linked to reproductive and developmental functions, hormono-dependant cancers, immune system, and, metabolic syndrome/obesity.

Finally, both types of milks are not microbiologically safe. PIF may lead to infant outbreaks due to powder contaminations in *Cronobacter sakazakii* and *Salmonella* spp. (FAO/WHO, 2006). Moreover, cross-contaminations may occur with inadequate handling or ineffective cleaning of the bottle and nipple addition (e.g. *Bacillus cereus* (Buchanan and Oni, 2012; Shaheen et al., 2006), *Staphylococcus aureus* and other *enterobacteriaceae*). The addition of water to the powder is also susceptible to bring parasites like *Cryptosporidum parvum* (Pouillot et al., 2004), viruses like norovirus (ANSES, 2013a) and bacteria like *Pseudomonas aeruginosa*, *Aeromonas* spp., *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* O:157 (Fawell and Nieuwenhuijsen, 2003). BM can also be a vector of adverse bacteria such as *Staphylococcus aureus* (Jones, 2001; May, 2012), *Brucella* (MacDonald, 2006), *Listeria monocytogenes* (Jones, 2001), *Streptococci* (Jones, 2001), *Salmonella* (Jones, 2001) *and Coxiella burnetti* (Jones, 2001). However, BM is also a potential source of pre- / pro-biotics associated with beneficial health effects.

Consequently, both diets raise chemical, microbiological and nutritional issues and must be considered with a comprehensive assessment to enable policy makers to compare potential interventions or, to underpin preventive actions. At this stage, only three studies have contributed to address this multidisciplinary and complex issue: the quantitative microbiological risk assessment of *Cronobacter sakazakii* in PIF by the WHO and FAO (2006), the assessment of benefits and risks of infant milk consumption in Norway by VKM (Meltzer et al., 2016) and the quantification of health effects of breastfeeding by the RIVM (Büchner et al., 2007; RIVM, 2015).

6.1.3. Model development 1

Based on the current RBA framework established in CHAPTER 2 and the case study described in CHAPTER 3, a first multidisciplinary RBA was performed to further develop the RBA methodology (CHAPTER 4). More precisely, the objective was to develop a probabilistic and inter-disciplinary risk-benefit assessment model to investigate the conceptual development of the RBA methodology, its relevance, feasibility and added-value. This model was called "Model 1".

Model 1 considered one selected factor from microbiology, chemistry and nutrition: namely *Cronobacter sakazakii*, dl-PCB and DHA, respectively. Five different scenarios of consumer's exposure were assessed: six months of breastfeeding versus powder infant formula feeding, with the option of supplemented infant formula in fatty acids and the addition of ambient temperature or boiled water.

A probabilistic second order model, which separated variability and uncertainty, was developed and implemented by Monte Carlo simulation. Data were collected from the scientific literature, reports of food safety agencies and expert elicitations. Model outputs were expressed in the same DALY metric (Disability Adjusted Life Year) for nutrition and microbiology to compare health impacts whereas in chemistry only levels of exposure were estimated due to a lack of a clear established dose-response.

In this first model where only three factors where considered, six months of consumption of supplemented infant formula prepared with boiled water, appeared as the preferable option to avoid the microbiological risk associated with *Cronobacter sakazakii* and to decrease the burden of disease due to intellectual disability while minimising the exposure to dl-PCB.

The study pointed out the necessity to undertake a probabilistic approach in RBA to consider natural inter-individual variability. Additionally, the separation of variability and uncertainty strengthened the analysis by enabling to interpret better the outputs. However, this first piece of work also highlighted that multidisciplinary RBA was a complex process as each scientific discipline uses different approaches.

6.1.4. Model development 2

The RBA approach developed with model 1 (CHAPTER 4) was then re-used in CHAPTER 5 to assess another issue linked to the infant milk-based diet: the use of tap water to prepare powder infant milk, this latter was identified as a chemical-microbiological issue through the review of risks and benefits associated with infant milk consumption (CHAPTER 3). This model was called “Model 2”.

Powder infant formula, the most consumed food by infants in France, needs to be reconstituted with water before consumption. The use of tap water is permissible (according to the French food safety agency) with the caveat that it is not sterile and may be a vector of chemical and microbiological contaminants. The aim of the study was to develop a microbiological-chemical risk assessment model to quantify the risk associated with the use of tap water in France for preparation of infant formula (during the first six months of life). This risk-risk assessment falls into the scope of a risk-benefit assessment as it requires a multidisciplinary and comprehensive approach.

Cryptosporidium and arsenic were selected as hazards of greatest concern in microbiology and chemistry, respectively. The risk assessment model considered separately uncertainty and variability, as in Model 1, was built using French data (or European ones alternatively). Outputs were expressed firstly at the individual level, as probability of illness, and then, at the population level, by using the DALY metric. Two scenarios of milk preparation were considered: the use of boiled and un-boiled tap water.

On the basis of this model, consuming infant formula rehydrated with un-boiled tap water during the first six months of life led to 6 000 DALY per 100 000 infants (90% uncertainty interval [1 500; 12 000]) for *Cryptosporidium* due to diarrhoea and 2 DALY [1.6; 2.3] for arsenic due to lung and bladder cancers. For the whole infant population, boiling water would suppress the risk from *Cryptosporidium*. In contrast, the cancer risk, although low at the population level, was rather high for infants having a high level of exposure to arsenic. For those, it might be possible to decrease the risk by changing of tap water supply point (hopefully in the same geographical area, otherwise, this management option is not enough realistic to be pushed forward).

This developed model could help authorities to quantify the risk associated with tap water for preparation of infant formula and to make some recommendations. On top of that,

Model 2 enabled to test the RBA methodology developed within the first study (Model 1 construction). Separation of variability and uncertainty, once again strengthened the interpretation of model outcomes by providing more comprehensive and enhanced information. The DALY metric was tested a second time and was still found to be valuable at the population level. However, it was advised to report results in both formats, i.e. individual risk and DALY, to keep all relevant pieces of information.

6.1.5. Summary of all results on infant milk-based diet

To sum up results obtained with models 1 and 2, a multi-criteria summary table was created (**Figure 6.1**). This figure must not be interpreted independently of assumptions made in each model development. Furthermore, only the four most relevant scenarios are presented in the table while models 1 and 2, altogether, included 10 different options (5 for Model 1 x 2 for Model 2). A colour code is used to help in reading the table: grey means the absence of a specific risk or benefit for a scenario, green a benefit, and, red a risk. Risks and benefits are reported with two levels of intensity to highlight differences among scenarios, nonetheless, it is important to keep in mind that level of intensity does not stand for a quantitative indication of the health impact magnitude.

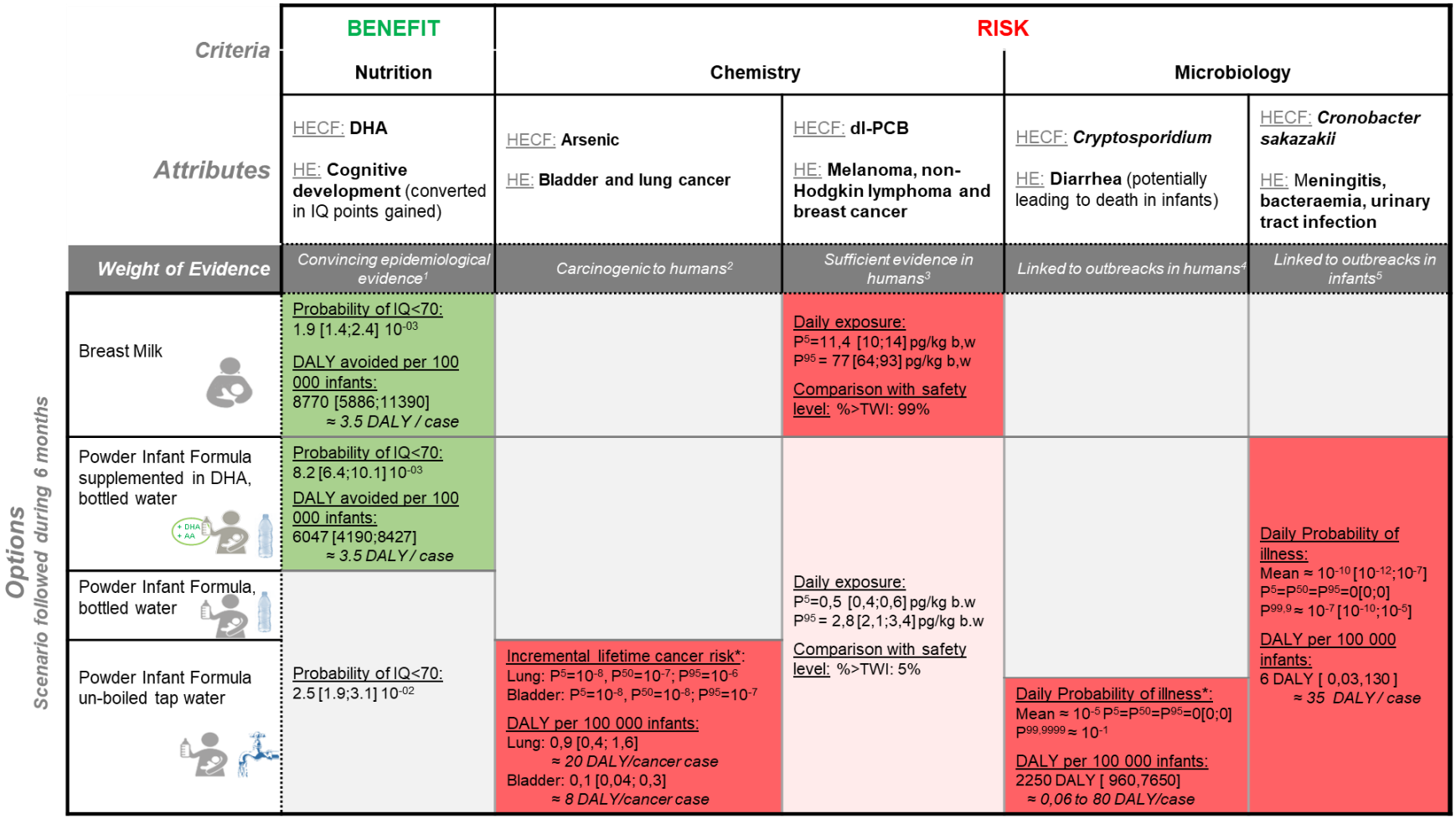

Figure 6.1: Summary of results obtained in the PhD project regarding infant milk-based diet

HE=Health Effect, HECF=Health Effect Contributing Factor;
**Uncertainty intervals were not reported to simplify reading when they had a smaller effect than variability*
[1](Kuratko et al., 2013; Weiser et al., 2016) [2](IARC, 2012) [3](IARC, 2015) [4](Therre, 2008) [5](FAO/WHO, 2006)

6.1.6. PhD project outputs

The PhD project workflow has been filled out in **Figure 6.2** to highlight project outputs in terms of publication in international peer-reviewed journals and communications at conferences.

The state of art of Risk-Benefit Assessment in food, CHAPTER 2, was published in Boué et al. (2015) and presented at the Q-Safe conference in Malta in March 2015 (poster) and at the EFSA conference in October 2015 at Milan in Italy (poster). It was also presented at a workshop on RBA organised by NFA in September 2016 at Uppsala, Sweden (introduction talk). Beside these presentations in international forums, outputs from CHAPTER 2 were presented at the scientific days of the doctorate school Venam in 2014 and in 2016 (updated version) at Angers and Nantes respectively, France (oral communications).

The review on the risks and benefits associated with the case study: infant milk consumption (breast milk and infant formula), CHAPTER 3, was published in Boué et al. (2016).

Results from Model 1 developed on the case study of infant milk-based diet reported in CHAPTER 4, was published in Boué et al. (2017a) and presented at the SRA conference in 2015 at Arlington in USA and the FoodSim conference in 2016 at Ghent in Belgium (both oral communications).

Results from Model 2, focused on water, reported in CHAPTER 5, were presented at the IAFP conference in 2017 at Brussels in Belgium and the Q-Safe conference in 2017 at Syros in Greece (both oral communications), they have been also submitted to Water Research Journal (Boué et al., 2017b).

Finally, main outputs from the PhD thesis work, captured in CHAPTER 6 were presented at a workshop on RBA organised by DTU in May 2017 at Copenhagen, Denmark (oral communication).

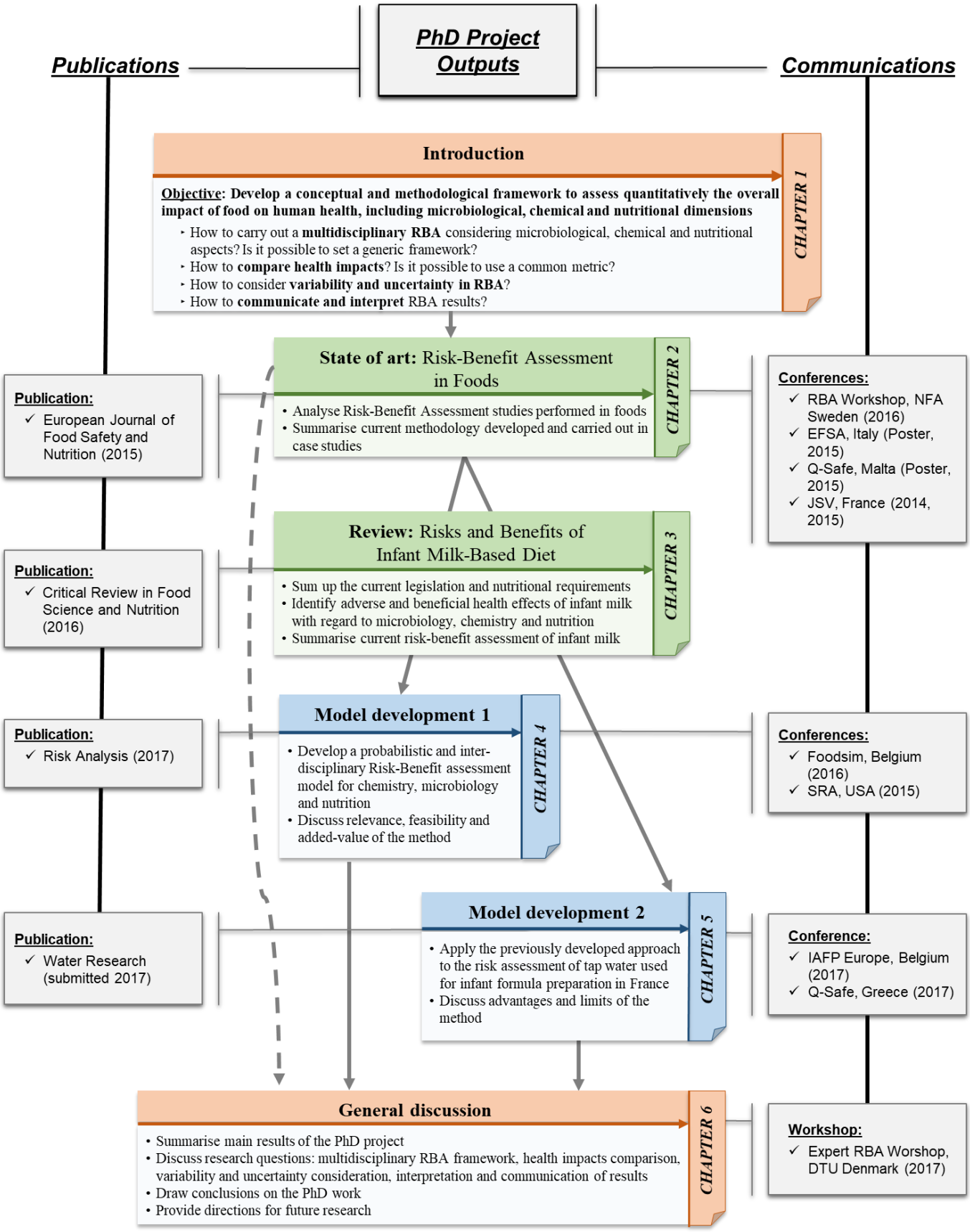

Figure 6.2: Outputs of the PhD project entitled "Public Health Risk-Benefit Assessment of Foods: methodological development with application to infant milk based diet"

6.2. Discussion

The aim of the PhD project was to develop a conceptual and methodological framework to assess quantitatively the overall impact of food on human health, including microbiological, chemical and nutritional dimensions. A particular attention has been devoted to four specific questions discussed below.

6.2.1. How to carry out a multidisciplinary Risk-Benefit Assessment considering microbiological, chemical and nutritional aspects? Is it possible to set a generic framework?

There is currently "no international consensus on the general principles or approaches for conducting risk-benefit assessment of foods and food components" (Eneroth and Zetterberg, 2016). Nevertheless, several studies have contributed to RBA methodological developments with in particular the EFSA contributions (EFSA, 2006; EFSA, 2010) and the BRAFO, BEPRARIBEAN and QALIBRA European projects (Boobis et al., 2013; Hart et al., 2010; Hoekstra et al., 2012; Tijhuis et al., 2012a; Verhagen et al., 2012b). However, RBA in general and RBA methodological developments in particular, are still high in the agenda of different European countries, with several workshops organised recently by NFA in Sweden (Eneroth et al., 2017) and DTU in Denmark (DTU, 2017).

In our PhD project, a first generic framework was summarised from the state of art of RBA in food, based on current trends and adapted with suggested terms. It proposed a seven-steps approach, mostly inspired from traditional microbiological risk assessment (Codex Alimentarius Commission, 1999):

- Step 0 - Problem definition (population and scenarios definition),
- Step 1 - Identification of HECF,
- Step 2 - Exposure assessment,
- Step 3 - Characterisation of HECF,
- Step 4 - Health impact characterisation,
- Step 5 - Harmonisation of HI in the same metric,
- Step 6 - Assessment of different scenarios of consumer's exposure.

This framework is presented below in **Figure 6.3**, it is updated from what was published in 2015, thanks to lessons learned during development of Models 1 and 2.

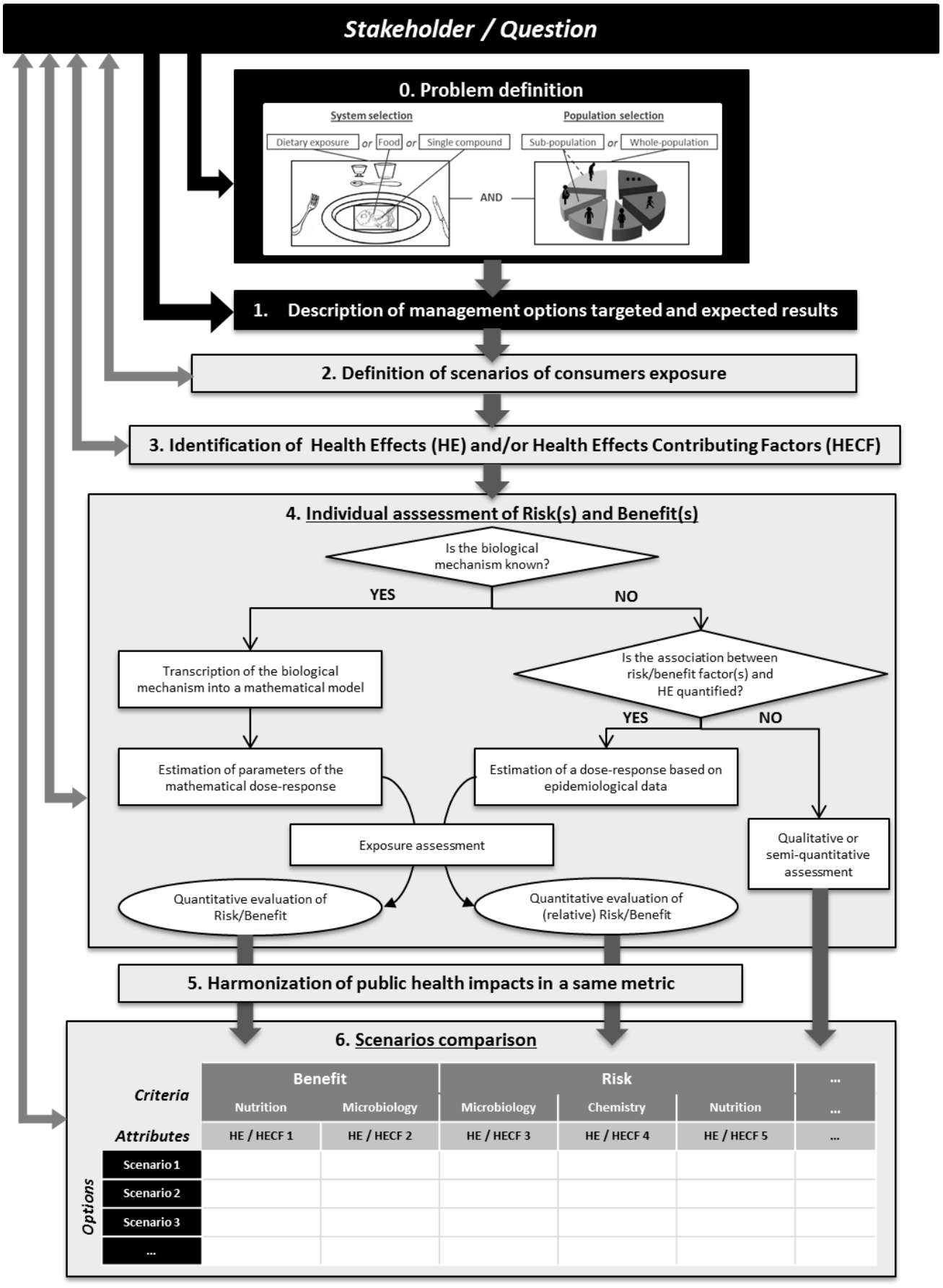

Figure 6.3: Suggested framework of quantitative RBA in food

A step of "**description of management options targeted and expected results**" by RBA managers has been added to the previous suggestion (**Figure 2.7**) at the beginning of the assessment. Indeed, RBA is a complex process, time and resources consuming, it has to be conducted only for specific scenarios, with particular results in mind and then stay fit-for-purpose. In the fish case study, for instance, conducting a RBA for different levels of intake (e.g. comparison of 1, 3 and 10 serving a week) is probably not a relevant series of scenarios to suggest as it does not take into account potential substitutions (what consumer eats when not eating fish?). Nonetheless, it is important to reinforce at this point that substitution is not often taken into account in RBA (DTU, 2017). For instance, in fish RBA, health balance was provided for various intake levels without considering what consumers are eating when not eating fish. Providing partial results might led to confusing messages to non-scientific people as it raises questions without bringing solutions, contributing to the loss of trust. Whereas, when managements options are considered since the beginning of the assessment such as in RIVM studies assessing different duration of breastfeeding (Büchner et al., 2007; Van Rossum et al., 2005), conclusions can be drawn by assessors and directly transmitted to stakeholders without requiring another RBA for potential substitutions. In addition, to facilitate decision-making process, expected results, and their formats, must be defined at an early stage to ensure that development frameworks enabled to provide what stakeholders expect. More precisely, according to different context of applications, different formats of results could be of interest: results reported at an individual, sub-population or population level, health status reported as probability of illness, mortality rates, age of disease onset, quality of life gain/lost, DALY, etc. For both models developed (CHAPTER 4 and 5), it was decided to report results at an individual scale with probability of health effect onset and at a population level in DALY (when possible).

Subsequently, a crucial second step is to **translate management options into scenarios of consumer's exposure**. A reference (or baseline) is often proposed, corresponding to a scenario of reference which might be the current exposure of consumers or zero exposure; and alternative scenarios which are hypothetical consumer exposures (Hoekstra et al., 2012). However, what people eat is guided by dietary "behaviour" which fall under sociological aspects and consumers can hardly be controlled with an optimal food frequency intake. We can also assume that RBA messages can provoke binary reactions instead of perfect adjustment to intake recommendations. Thus, another option is to assess

extreme scenarios to see first what would be the maximum magnitude of health impact. This strategy was followed in the two models developed here. It brings the advantage of assessing first whether there is significant difference between extreme scenarios, and then, if there is, opens the possibility to refine the RBA.

Moreover, RBA is always multidisciplinary and has to consider microbiological, chemical and nutritional sciences altogether in a same analysis even if each one has its own risk assessment approach. It appeared through Model 1 development that the **individual risk and benefit assessments had to be adapted** to the three scientific disciplines. Indeed, in microbiology, biological mechanisms of infection are often known and mathematical dose-responses are commonly used to model the probability of illness according to different levels of exposure (Commission, 1999). Whereas in nutrition a window of exposure is targeted as both under and over exposure are associated with a risk (Palou et al., 2009). In addition, epidemiological studies are often used to link a level of intake with an endpoint, without systematically knowing the biological mechanism involved. Both "risk-based approaches", which are used in nutrition and microbiology, can also be used in chemistry (IPCS, 2009) but the most common situation is an "hazard-based approach" which simply evaluates the presence of a potential harmful agent in food and compares human levels of exposure with safety reference values established through animal experimentations. In such case, the association between the hazard and the health effect is not quantified with a dose-response so the risk cannot be quantitatively estimated in term of probability of illness. Hence, individual risk/benefit assessment depends on dose-response building which itself depends on the availability of data and knowledge on the biological mechanism of the health effect. In addition, the HECF of a specified HE is not always clearly identified. For instance, the causal relationship between *C. sakazakii* and meningitis is established. In contrast, the HECF reducing obesity risk while breast milk consumption has not been yet clearly identified.

As a result, individual steps of risk or benefit assessment have been replaced in **Figure 6.3** by two different options: in presence or absence of biological mechanism, with a classical dose-response and with an epidemiological relation, respectively. A way out has been added in the case of no quantified relation between the risk/benefit factor and the HE. That means overall that it is more appropriate to start identifying health effects (HE) rather than associated factors (HECF). At this stage, we advise to pursue up to the "best estimation of the health impact", when possible (**Figure 6.3**), as recommended by Berjia

et al. (2012) and not to stop the assessment as soon as a conclusion can be drawn as advised in the BRAFO and EFSA tiered approach (EFSA, 2010; Hoekstra et al., 2012).

Subsequently, **results are harmonised into a common metric** when possible (e.g. DALY) and **scenarios are compared** with a table summarising results. In the present updated framework, scenarios are compared with a multi-criteria table (see example in **Figure 6.1**) instead of a unique DALY comparison because it is not always possible to end up individual assessments with a probability of health effect, required to estimate a DALY. That was the case in this work with the dl-PCB assessment. Moreover, it was found to be a more complete and efficient way of communication results, as discuss below in 6.2.3. Next, this table can be analysed by experts and policy makers to select the most valuable options.

6.2.2. How to compare health impacts? Is it possible to use a common metric?

RBA aims to estimate the overall impact of food consumed on health for different scenarios of consumer's exposure. Implicitly, this aims to find means of improving **"health"**. However, "health" is not a simple biological parameter which might be measured as weight or length; it is a concept with several dimensions which cannot be measured (Thacker et al., 2006).

As discussed in CHAPTER 2, health can be defined in RBA as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (WHO, 1948). In that respect, improving health corresponds to an increase of the "level of functioning or capacity in all the important dimensions of health, and from any type of illness or disease" (Goodacre et al., 2010). Thus, an "adverse health effect" can be seen as a decrease of the health level in the direction of illness/premature death whereas a "beneficial health effect" corresponds to an increase of the health level toward a high level of wellness. Consequently, risks and benefits are functions of probabilities of consequent adverse or beneficial health effects resulting from exposure to different factors in food (CHAPTER 2). In other words, it represents a chance to have a health effect and does not transcript the magnitude of the change in terms of health levels which might be called an "health impact". RBA considers several health effects in the same analysis which need to be quantified to enable comparison of different scenarios of consumer's exposure.

Until now, three different strategies have been used in RBA to compare risks and benefits. The most common strategy has been to **compare levels of consumer exposure with regard to safety reference levels** such as TWI (Tolerable Weekly Intake) in chemistry and RDI (Recommended Daily Intake) in nutrition. A limit to this approach based on exposure assessment is to give the same importance to all potential health effects without considering the associated severity. Indeed, it ignores health effects magnitudes or impacts and assumes equivalent impact of all factors. In addition, the exceedance (or non-achievement) of a preventive level does not ensure a potential health effect.

Another option has been to **compare change in endpoint trends** one by one like the increase of number of deaths due to a risk with the decrease of number of deaths thanks to a benefit but it is limited to similar endpoints and often cannot enable to compare all

endpoints considered in the case study. For instance, for fish RBA, a joined work of FAO and WHO have compared on one hand the potential change in mortality due to CHD and on the other hand the change in children IQ, both due to fish content in methylmercury and DHA (FAO/WHO, 2010). Similarly, mortality rates might be compared for different health effects but diseases onsets rarely occur in the same conditions of age, duration, disability and fatality. For example, a death due to a CHD can be compared with a death of cancer in theory but in practice both events are associated with different loss of years of life and different loss of quality of life before death.

For a complete comparison of risks and benefits, it seems essential to consider both the quality and quantity of life lost. The **use of a composite metric like the DALY** provides a comprehensive assessment of the consequences of a disease by integrating the quality of life lost and premature death. It has been used only in few RBA studies: 9 out of 70 RBA performed (Berjia et al., 2012; Cohen et al., 2005; Guevel et al., 2008; Hoekstra et al., 2013b; Ponce et al., 2000), probably because it requires a finalised assessment of each risk and benefit (up to the prediction of the number of cases) which is not always possible due to missing data. In addition, it implies making several assumptions with regard to each health effect (age of disease onset, duration, disability weights and years of life lost). In public health, the use of DALY might be very attractive for managers to compare and rank several public health measures and risk management options as it integrates the whole complexity of the RBA issue within a simple figure. However, it might be not a relevant tool to communicate results to the consumers, as it is complex and somewhat too much "integrative". As illustrated through the two models developed in our PhD project this indicator can guide policy makers to prioritise actions but cannot inform properly consumers in making informed choices as it integrates simultaneously the prevalence and the severity of diseases whereas each consumer has its own level of prevalence/severity acceptance.

Debate on the best way to compare health impacts in RBA will probably never ends as it belongs with the general **debate on the measurement of health** itself. There is no consensus on the best way to measure health (Thacker et al., 2006), it is an indirect measurement of a selection of indicators representing "the conception of health" (McDowell, 2006). As a result, according to the scope of the RBA and more specifically the context and culture of each population of interest (highly specific per country) "health" can be conceptualised with various indicators. For instance, more attention can

be paid to: infants or adults, to less expensive or innovative alternatives, to easily or quickly implementable measures; etc. It highlights once again the necessity to perform fit-for-purpose RBA anticipating potential public health measures and communication result formats before conducting RBA.

Another complementary issue to be considered is that RBA involves various individual risks and benefits assessments for which the current **scientific weight of evidence** might be different (Dorne et al., 2016). This weight of evidence reflects the degree of current scientific knowledge regarding the association between a certain diet or food or component consumption and the occurrence of a certain health effects, the "biological knowledge of the day" (Hill, 1965). For instance, health effects for which the biological mechanism is proved in humans will be associated with a stronger level of evidence compared to another one for which the link is only suspected in animals. Consequently, it is important to report at least this information with results description. It is particularly of interest for endpoints involving a delay between exposure and health effect as they often imply multi causal factors. It is even possible to integrate quantitatively this information by considering a "probability of causation" based on experts elicitations as done by Trasande et al. (2016).

To conclude, the overall impact of food consumed on health appears too complex to be summarised within a unique common endpoint. There is no universal indicator which might be appropriate to all cases, it needs to be defined taking into account each RBA context. The DALY is still the most interesting available measure to provide an indication regarding the impact on quality and duration of life at the population scale. Nevertheless, we can advise to provide jointly to the DALY, individual outputs to get a more complete view of results.

6.2.3. How to consider variability and uncertainty in RBA?

RBA models are built to support decision making in food safety and nutrition in order to improve human health. However, it is impossible to represent the observed reality in its totality. Given that all models are wrong, the objective is to deliver useful models providing "a simplified representation of reality" (Zwietering, 2009). The main challenge is to find a "correct" model which is a compromise between a simple and an over-complex model.

To conceptualise the reality, the phenomenon of **variability** must be considered. Indeed, we cannot ignore that there is a natural heterogeneity among individuals in terms of food diet, hazards exposure, disease susceptibility, etc. Moreover, the variety of the food chain enables to provide consumers with foods with unlimited list of products with various nutritional, microbiological and chemical qualities. It is therefore obvious that RBA cannot consider only the average population and the mean diet (or food) but must cover, as much as possible, all different possibilities.

On top of that, another phenomenon must be considered: the **uncertainty** associated with the RBA model development. Uncertainty is produced by assumptions and approximations made all along the RBA model development due to a lack of knowledge, data or potential error of the model. This level of uncertainty directly influences the level of confidence that decision makers can have regarding predicted risks and benefits. It is particularly of interest in RBA as we aim to estimate an overall health impact considering several risks and benefits with multiply sources of uncertainty in comparison with an individual risk assessment. Despite all potential sources of uncertainty reported, a final outcome (or few) are reported to characterise the health impact. Policy makers need to know if different options can lead to different health impacts or if their associated levels of uncertainty cannot make possible to distinguish them.

Characterisation of uncertainty and variability is part of the assessment while resolving the impact of both on decisions is part the management. More precisely, "uncertainty forces decision makers to judge how probable it is that risks (and benefits) will be overestimated or underestimated for every member of the exposed population, whereas variability forces them to cope with the certainty that different individuals will be subjected to risks both above and below any reference point one chooses" (Council, 1994). In this context, managers have the complex task to take stochastic results of risk

assessment and adapt to discrete decisions while considering both uncertainty and variability (Buchanan and Appel, 2010; Zwietering, 2015).

In traditional risk assessment, uncertainty and variability consideration is advised by international organisations (WHO, 1997). Variability must be characterised as much as possible whereas uncertainty must be reduced as much as possible or at least described. There is an increasing interest on uncertainty characterisation that must be part of the risk assessment and communicated to risk manager to give a transparent view of results obtained (Barlow et al., 2015). To date several approaches can be followed (ANSES, 2016a; ANSES, 2016b; EFSA, 2016). A current trend in risk assessment and more and more in RBA is to consider both phenomena, uncertainty and variability, with probabilistic approaches. Indeed, the use of probabilistic distributions allows to implement inputs with a range of values while deterministic one does not.

RBA must also consider both uncertainty and variability. More and more in RBA studies, **stochastic approaches** have been used to **consider quantitatively the variability of inputs** by integrating for instance variables profiles of nutrients and chemicals in food (Afonso et al., 2016; Afonso et al., 2015; Becker et al., 2007; Cardoso et al., 2015; Cardoso et al., 2013; Dewailly et al., 2008; FDA, 2009; GAO et al., 2015; Glynn et al., 2013; Groth, 2017; Hendriksen et al., 2011; Hoekstra et al., 2013a; Hoekstra et al., 2013b; Husoy et al., 2008; Laird et al., 2013; Matos et al., 2015; Maulvault et al., 2013; Reyes, 2016; SACN/COT, 2004; Schutte et al., 2012; Seal et al., 2008; Zeilmaker et al., 2013). It is also recommended to include "an appropriate" **expression of uncertainty** in the BRAFO tiered approach (Hoekstra et al., 2012) with at least a **qualitative** assessment of all different sources which can be easily communicated through a table listing sources of uncertainty with potential magnitude and direction of influence, as done in (Hoekstra et al., 2013b). Another option is to **quantitatively** assess sources of uncertainty using probability distributions to explore the impact on final outputs. However, the quantitative uncertainty analysis is never fully exhaustive so it must be performed beside a qualitative one which provides complementary information (Hoekstra et al., 2012). An alternative is to quantify most important sources of uncertainty and to provide at the same time a qualitative assessment of all sources to ensure a comprehensive uncertainty analysis, as done in this present work (CHAPTER 4).

When uncertainty and variability are both quantified with probabilistic methods, they can be separated to help policy makers to take informed decisions by providing greater confidence for results interpretation and by identifying need of data collection; that can be done by running **second order Monte Carlo simulations** (Cummins, 2016).

Subsequently, **sensitivity analysis techniques** can be used to determine which inputs influence the most the model outputs and to provide more valuable information for management option guidance. Different methods are available in the literature for running a sensitivity analysis (Frey et al., 2003; Frey et al., 2004; Frey and Patil, 2002) even in the case of second order risk assessment (Busschaert et al., 2011; Mokhtari and Frey, 2005; Pouillot and Delignette-Muller, 2010; Roelofs and Kennedy, 2011). However, these sensitivity analyses were performed on risk assessment not on risk benefit assessment, i.e. with only one model and one main result. In RBA, it is necessary first to **define at which level the sensitivity analysis has to be conducted**. It can be done for the output of each individual risk or benefit assessment model or on the final output of the RBA when all outputs are converted into DALY. This choice depends on what we are expecting from the sensitivity analysis: support model understanding, check the model validity, or, find optimums for management options. It seems that an **individual sensitivity analysis for each risk and benefit assessment** might be more efficient to understand and interpret each model. Then, when outputs are converted into DALY, another analysis can be conducted on this latter part of aggregation.

In this work, we did not perform any sensitivity analysis (that will be definitively done shortly). Nonetheless, in RBA, only few studies have included a sensitivity analysis: six in more than 100 RBA performed. Berjia et al. (2012) analysed sensitivity of the model by moving one variable at a time, Gradowska (2013) used Bayesian Belief Network technique, Leino et al. (2013) studied levels of correlation between inputs and outputs and Rigaux (2013) estimated Sobol indices of variable parameters for different uncertainty realisations. Two other studies have conducted a scenario analysis: FDA (2009) has analysed the effect of different levels of methylmercury exposure in fish on the output, as also done by Ponce et al. (2000). Sensitivity analysis is rare in RBA and any of the six followed the same approach which makes the RBA discipline still “under construction”. Nevertheless, when variability and uncertainty are quantified separately (2^{nd} order RBA) it would be interesting, for model output interpretation, to perform the sensitivity analysis first in the variability dimension with uncertainty inputs blocked at

their most likely values, and then do the opposite: uncertainty dimension analysis in absence of variability. This is the path that we plan to take to consolidate results obtained in this work.

To conclude, quantitative RBA is a tool for decision support in public health, particularly as far as food safety and nutrition are concerned. It involves complex issues requiring different individual risks and benefits assessment from microbiology, chemistry and nutrition. Thus, adapted method from the traditional risk assessment domain are needed to conceptualise the impact of food consumed on health. It also requires to consider uncertainty and variability related to model development. The use of probabilistic methods such as 2nd order Monte Carlo techniques and sensitivity analysis results in higher accuracy of model outputs and then in a more robust assessment.

6.2.4. How to communicate to stakeholders and interpret results?

Communication and interpretation of results in RBA is a crucial last step without which the model is useless. It is not directly part of the assessment itself but an interconnected part of the whole Risk-Benefit analysis.

Communication in RBA must give a transparent overview of final outputs (i.e. of the individual variability within the population, associated with their uncertainty) as well as model boundaries, assumptions and associated sources of uncertainties. In addition, RBA contains several individual risk and benefit assessments and often a final output aggregating all results with a common metric (e.g. DALY). On the top of that, in some cases, due to data and knowledge limitations, individual assessments are stopped at the exposure assessment step, as that was the case here with the dl-PCB risk assessment (Model 1). Consequently, there are numerous and different pieces of information to communicate at the end of a RBA, multi-criteria matrix seems valuable tool to do so (see example in **Figure 6.1**). This table must be gathered with a table of uncertainty analysis as advised by Hoekstra et al. (2012) and done in Model 1.

Subsequently, **interpretation of results** consists in making sense of data reported. According to different stakeholders concerned, same results can be interpreted differently. Indeed, public health authorities might be more interested in the overall burden of disease at the population scale whereas consumers might be more concerned about their individual risks and benefits. At the individual scale, people are more worried about "external factors over which they have little or no control" such as pesticide residues or bacteria than about "putting on weight themselves" (EFSA, 2015a) even though obesity has a higher burden of disease than food safety issues (van Kreijl et al., 2006). Results interpretation is therefore interconnected with **risk/benefit perception and trust**. This fact supports once again a close collaboration between stakeholders and assessors to conduct a fit-for-purpose risk-benefit analysis.

To conclude, both communication and interpretation of results requires a deeper understanding of stakeholder's risk-benefit perception (Ueland et al., 2012a). Indeed, how people perceive individual risks and benefits will determine how they interpret RBA results and how they will change their behaviours. This is a crucial point to effectively communicate. As a consequence, risk-benefit assessors "should not only communicate

about risks (and benefits) identified in the risk assessment, but also address the factors that influence risk perception" (Tabachnikoff, 2017).

6.3. Conclusions

The present PhD project aimed to develop a conceptual and methodological framework to assess quantitatively the overall impact of food on human health, including microbiological, chemical and nutritional dimensions. This thesis was centered around a case study of major public health concern: the assessment of risks and benefits (RBA) associated with infant milk-based diet, considering breast milk and infant formula diets. Available approaches of RBA were first reviewed (CHAPTER 2) as well as risks and benefits associated with infant milk consumption (CHAPTER 3). Then two models were developed with the case study to investigate new methodological development in RBA (CHAPTERS 4 and CHAPTER 5).

Regarding RBA methodology, an updated framework was suggested (see discussion 6.2.1) based on current trends and lessons learned from our model development (Model 1 and 2); it placed policy makers at the heart of the RBA by defining management options targeted and expected results at the beginning of the RBA to develop directly fit-for purpose scenarios of consumer's exposure. Different ways of conducting the different (microbiological, nutritional, chemical) risks and benefits assessment were suggested as it seemed impossible to converge toward a unique approach. Likewise, different ways of comparing health impacts were carried out: using preventive safety levels for each endpoint, or, using the composite DALY metric. Next, separation of variability and uncertainty was recommended to provide more accurate results to policy makers. Finally, a multi-criteria table was built to incorporate in a transparent manner the various outputs of the RBA.

RBA appears as an essential tool to provide comprehensive recommendations in food and human health. It is also a complex and multidisciplinary approach which is inspired from traditional risk assessment but requires a more-in-depth analysis to aggregate all results. Consequently, it has to face first all challenges from risk and benefit assessment in microbiology, nutrition and chemistry such as the lack of data (in particular the absence of established dose-responses). Secondly, it has to find ways to interpret and communicate all results together in order to provide an overall health impact. In addition, it is a never ending issue as conclusions can be updated with new findings in all domains included in the assessment.

6.4. Perspectives

A main challenge in RBA is to **face the multi-disciplinary and multi-dimensionality** of issues which leads to complex studies and difficult to interpret conclusions with multiple conflicting criteria in decision making. **Multi-criteria decision analysis** (MCDA) might be a technique to facilitate communication, interpretation and decision-making in RBA as suggested recently by Ruzante et al. (2017). Future research could be then focused on developing such generic MCDA/RBA method.

Another challenge is to **face the scepticism regarding RBA usefulness** due to perceived difficulties of interpreting and implementing its complex results. RBA has emerged to provide more realistic and comprehensive recommendations for consumers in food safety and nutrition and to avoid contradictive conclusions among different scientific fields. Beside, another question remaining unsolved is how consumers may respond to RBA recommendations (van Kleef et al., 2014). More precisely, it is not well known how to communicate effectively to end-users as expected adverse and beneficial health effects affect differently each sub-population group. Quantitative RBA, considering explicitely population variability, lead more and more to individual and personalised recommendations. Consequently, future Risk-Benefit assessments are expected to be not only interconnected with management and communication parts but to work in complete collaboration with them (Cummins, 2017). In such a case, it needs to be clear on who is the manager: is it the policy maker or the consumer? Nevertheless, as explained by Nauta (2015), it seems obvious that consumer's behaviours are targeted by RBA studies. Consequently, a third challenge would be to influence consumer's attitudes and behaviours. This later issue clearly involves sociologic aspects: consumers have a role to play in making informed decisions regarding food choices, handled and preparation (Schmidt and Rodrick, 2003). Thus, it can be recommended for future research to **conduct consumers participatory risk-benefit analysis,** placing consumers in the centre of the evaluation instead of final end users (Dreyer and Renn, 2013; Mikulsen and Diduck, 2016).

Finally, outside a health perspective, **other factors can completely change recommendations** made to (or more likely made by) consumers. Risk-Benefit analysis cannot be seen as an isolated process, it has to be interconnected with societal, political, economic, ethical and environmental perspectives. **Risk-benefit analysis must be part**

of the global food safety governance (Mikulsen and Diduck, 2016) and here again, multi-criteria decision analysis might be a tool to explore furthermore how health effect could be integrated in a broader decision process.

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List of scientific publications

List of scientific publications

❖ Papers published in peer reviewed journals

[1] - *Public health Risk-Benefit Assessment associated with food consumption – A review*

Authors: Boué, G., S. Guillou, J. P. Antignac, B. Le Bizec and J.-M. Membré

Journal: European Journal of Nutrition & Food Safety, 2015, 5(1), 32.

[2] - *Public health risks and benefits associated with breast milk and infant formula consumption*

Authors: Boué, G., E. Cummins, S. Guillou, J. P. Antignac, B. Le Bizec and J.-M. Membré.

Journal: Critical Reviews in Food Science and Nutrition, Accepted February 2016, DOI: 10.1080/10408398.2016.1138101

[3] - *Development and application of a probabilistic risk-benefit assessment model for infant feeding integrating microbiological, nutritional and chemical components*

Authors: Boué, G., E. Cummins, S. Guillou, J. P. Antignac, B. Le Bizec and J.-M. Membré.

Journal: Risk Analysis, Accepted February 2017, DOI: 10.1111/risa.12792

[4] - *Risk Assessment of Arsenic and Cryptosporidium in Tap Water used for Preparation of Infant Formula, France*

Authors: Boué, G., L. Waziewska, E. Cummins, J. P. Antignac, B. Le Bizec, S. Guillou and J.-M. Membré.

Journal: Water Research, submitted March 2017

❖ Book Chapter published

[5] - *Quantitative Tools for Sustainable Food and Energy in the food chain.* *Chapter 6 : Quantitative Microbial Risk Assessment during food processing*

Authors: J-.M. Membré and G. Boué

Editor: V. Valdramidis, E., Cummin, J. V Impe (Eds)

Publisher : Eurosis Publisher

❖ Oral communications at international conferences

- ***Society for Risk Analysis, SRA Annual meeting, December 2015***
  Place: Arlington, USA
  Title: Development of an integrated risk-benefit assessment model to evaluate the health impact of breast milk and infant formula diets
  Authors: G. Boué, E. Cummins, S. Guillou, J-.P Antignac, B. Le Bizec and J-.M Membré

- ***FoodSim, April 2016***
  Place: Gent, Belgique
  Title: Second order Monte Carlo simulation to characterize the health impact of different infant feeding strategies
  Authors: G. Boué, E. Cummins, S. Guillou, J-.P Antignac, B. Le Bizec and J-.M Membré

- ***IAFP Europe, March 2017***
  Place: Brussels, Belgium
  Title: Is it safe to use tap water to prepare infant formula in France?
  Authors: G. Boué, L. Wasiewska, E. Cummins, S. Guillou, J-.P Antignac, B. Le Bizec and J-.M Membré

- ***Q-Safe International Conference, April 2017***
  Place: Syros, Greece
  Title: Probabilistic risk assessment of tap water for preparation of powder infant formula in France
  Authors: G. Boué, L. Wasiewska, J-.P Antignac, E. Cummins, S. Guillou, B. Le Bizec and J-.M Membré

❖ Invitation in working groups with oral presentation

- ***ANSES hearing, July 2015***
  Place: Paris, France
  Title: Risk-benefit assessment in food, presentation of thesis work
  Authors: G. Boué

- ***Nordic risk-benefit assessment workshop, organized by NFA, Septembre 2016***

  Place: Uppsala, Sweden

  Title: Previous risk-benefit assessments of foods in Europe

  Authors: G. Boué

- ***Expert workshop on Risk-Benefit Assessment, organized by DTU, May 2017***

  Place: Copenhague, Denmark

  Title: Risk-Benefit Assessment methodological development based on an infant milk diet case study

  Authors: G. Boué

❖ Posters presentation at international conferences

- ***Introductive conference at the Erasmus training + Q-Safe, Quantitative Tools for Sustainable Food and Energy in the food chain, March 2015***

  Place: Malte

  Title: Risk-Benefit Assessment in Food

  Authors: G. Boué, S. Guillou, J-.P Antignac, B. Le Bizec and J-.M Membré

- ***EFSA conference, Shaping the future of food safety, together, Octobre 2015***

  Place: Milan, Italy

  Title: Food Risk-Benefit Assessment: An Emerging Scientific Discipline

  Authors: G. Boué, E. Cummins, S. Guillou, J-.P Antignac, B. Le Bizec and J-.M Membré

❖ Oral communications at national conferences

- ***Journées scientifiques de l'école doctorale Venam, Décembre 2014***

  Place: Nantes, France

  Title: State of the art of public health risk-benefit assessment associated with food consumption integrating microbiological, chemical and nutritional components

  Authors: G. Boué, S. Guillou, J-.P Antignac, B. Le Bizec and J-.M Membré

- ***Journées scientifiques de l'école doctorale Venam, Novembre 2016***

  Place: Angers, France

  Title: L'évaluation intégrée des risques et bénéfices associés à l'alimentation : bilan des dernières avancées scientifiques

  Authors: G. Boué, S. Guillou, J-.P Antignac, B. Le Bizec and J-.M Membré

Curriculum vitae

Géraldine Boué was born in Toulouse in France on the 29th of January 1990. In 2008 she finished high school (A-level in Scientific series) and has integrated the French "classes préparatoires aux grandes écoles" in BCPST (Biology, mathematics, physics, chemistry and earth sciences) at Lycée Pierre de Fermat in Toulouse. After these two years of preparatory courses, she entered Oniris, the Nantes-Atlantic National College of Veterinary Medicine, Food Science and Engineering.

She graduated as Master in food science and engineering in 2013 at Oniris. During her Master, she worked in apprenticeship within the company "Les Œufs Geslin", in R&D and Quality departments. During the final year project, she implemented her engineering skills on extending the shelf life of pasteurised egg products. Her Master was on management and optimization of industrial systems of food processing including the following topics: food science and technology (microbiology, biochemistry, food and biology engineering), food process technology (automatic, refrigeration engineering, packaging, unit operations engineering), specialisation in applied statistics (data mining, sensory analysis, chemometrics, sensometrics, software utilization: Matlab and R), economic, social and management sciences (management, human resources), foreign languages and communication skills.

Then, she started the present PhD project in October 2014 at Secalim in Nantes, a research unit of Oniris and Inra. During her PhD she guided one Master student, Luiza Wasiewska, from Wageningen University, and was involved in teaching activities of master and veterinary students in food safety and quality. During her last year of PhD she has been hired as a temporary associate professor in food safety, risk assessment and food processing (since January 2017).

During her Master and PhD, she specialized in quantitative risk assessment and modelling by attending several international trainings: Erasmus intensive program on "Risk assessment and modeling" (2 weeks in Malta), Workshop on "Advances in predictive modeling and quantitative microbiological risk assessment of foods" (2 weeks in Sao Paulo, Brazil), Erasmus + "Quantitative Tools for Sustainable Food and Energy in the food chain (Q-Safe)" (2 weeks in Malta), Research school RISQUALIM on risk assessment in food (1 week in France) as well as several risk assessment workshops.

UNIVERSITE BRETAGNE LOIRE

Oniris
Nantes-Atlantic National College of Veterinary Medicine, Food Science and Engineering
Health and food at the heart of life

Thèse de Doctorat

Géraldine BOUÉ

Public Health Risk-Benefit Assessment in Foods:
Methodological development with application to infant milk-based diet

Évaluation des Risques-Bénéfices de santé publique liés à l'alimentation :
Développement méthodologique et application à l'alimentation en lait des nourrissons

Abstract

The objective of the present PhD project was to develop a conceptual and methodological framework to assess quantitatively the overall impact of food on human health, including microbiological, chemical and nutritional dimensions.

This methodology was developed using a case study on infant milk-based diet (breast milk and infant formulas) taking into account the following selected factors: *Cronobacter sakazakii*, *Cryptosporidium*, arsenic, dioxin like polychlorinated biphenyls and docosahexaenoic acid. Five probabilistic mathematical models were developed to quantify risks / benefits associated with these factors. When possible, they were harmonised using a common public health indicator, the DALY. Results were obtained by second-order Monte Carlo simulation in order to quantify separately the uncertainty and the variability.

Probabilistic techniques enabled to take into account on the one hand the biology related to variability (heterogeneity between individuals of the same population) and on the other hand the uncertainty linked to the lack of knowledge and data. In addition, separation of variability and uncertainty strengthened the evaluation by enabling a more accurate interpretation of results and by providing more comprehensive information for policy makers.

The method used in this PhD thesis can be considered as a robust basis for other case studies and can be used to continue methodological development in risk-benefit assessment. This approach is also part of a broader area: the multi-criteria decision analysis of agronomic and food systems.

Key Words:
Risk-Benefit Assessment; food safety and nutrition; probabilistic techniques; infant milk.

Résumé

L'objectif de cette thèse était de développer un cadre conceptuel et méthodologique permettant d'évaluer quantitativement l'impact global de l'alimentation sur la santé des consommateurs, en prenant en compte les dimensions microbiologiques, chimiques et nutritionnelles.

Cette méthodologie a été développée à l'aide d'un cas d'étude portant sur l'alimentation des nourrissons (lait maternel et formules infantiles), incluant les facteurs suivants : *Cronobacter sakazakii, Cryptosporidium*, arsenic, polychlorobiphényles de type dioxine et acide docosahexaénoïque. Cinq modèles mathématiques probabilistes ont été développés pour quantifier les risques / bénéfices associés à chaque facteur. Ils ont été ensuite harmonisés, quand cela a été possible, à l'aide d'un indicateur commun de santé publique, le DALY. Les résultats ont été obtenus par simulation de Monte Carlo de second ordre afin de quantifier séparément l'incertitude et la variabilité.

Les techniques probabilistes ont permis de prendre en compte d'une part la variabilité inhérente à la biologie (hétérogénéité entre individus d'une même population) et d'autre part l'incertitude liée au manque de connaissances et de données. De plus, la séparation de la variabilité et de l'incertitude a consolidé l'évaluation, permettant une interprétation plus cohérente des résultats et donc fournissant des informations plus complètes aux décisionnaires.

La méthode mise en œuvre dans ce travail de thèse pourra servir de base pour d'autres cas d'études et pourra aussi être utilisée pour continuer le développement méthodologique de l'évaluation risque-bénéfice. Cette démarche s'inscrit dans une approche plus générale d'analyse multi-critères des systèmes agronomiques et alimentaires.

Mots clés :
Évaluation Risque-Bénéfice ; sécurité des aliments et nutrition ; techniques probabilistes ; lait infantile.