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Impact de la cuisson et de la digestion sur les micropolluants à risque des produits carnés

Christelle Planche

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N° D.U. 2749 ANNEE : 2016

ECOLE DOCTORALE SCIENCES DE LA VIE, SANTE, AGRONOMIE, ENVIRONNEMENT
N° d'ordre : 702

Thèse :

Présentée à l'Université Blaise Pascal
Pour l'obtention du grade de

DOCTEUR D'UNIVERSITE

(Spécialité : Nutrition et Sciences des aliments)

Soutenue le 18 novembre 2016

CHRISTELLE PLANCHE

IMPACT DE LA CUISSON ET DE LA DIGESTION SUR LES MICROPOLLUANTS A RISQUE DES PRODUITS CARNES

Président :	Pr M. ALRIC	UNIVERSITE D'AUVERGNE, Clermont-Ferrand
Rapporteurs :	Pr V. CAMEL	AGROPARISTECH, Paris
	Dr D. DUPONT	INRA, Rennes
Examinateur :	Dr O. BERDEAUX	INRA, Dijon
Directeurs de thèse :	Dr L. DEBRAUWER	INRA, Toulouse
	Dr E. ENGEL	INRA, Clermont-Ferrand

Laboratoires d'accueil :

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Résumé / Abstract

Résumé :

Au cours de leur élevage, les animaux sont exposés à divers contaminants chimiques, qui peuvent s'accumuler au niveau des tissus animaux et être retrouvés *in fine* dans les produits carnés consommés par l'Homme, représentant alors un risque pour la santé des consommateurs. L'évaluation de ce risque repose classiquement sur la mesure de teneur en contaminants de la viande fraîche alors que plusieurs travaux suggèrent que seule une fraction de ces contaminants est effectivement assimilée par le consommateur du fait des transformations technologiques comme la cuisson et physiologiques comme la digestion. L'objectif de cette thèse est d'étudier ces effets modulateurs de la cuisson et de la digestion sur les contaminants chimiques de la viande et leur bioaccessibilité.

En s'appuyant sur le développement d'une méthode multirésidus GC×GC-TOF/MS pour le suivi de 206 polluants environnementaux et sur des collaborations avec des Laboratoires Nationaux de Référence, le premier objectif de ce travail était d'étudier l'impact de la cuisson sur un large spectre de contaminants chimiques d'une viande intentionnellement contaminée. Les résultats montrent que la cuisson n'a pas d'impact significatif sur la teneur en PCDD/F et en métaux lourds de la viande alors que des pertes significatives (d'autant plus importantes que l'intensité de cuisson est élevée) en PCB, antibiotiques et pesticides ont pu être détectées. Ces pertes ont pour origine soit une libération dans le jus de cuisson des contaminants thermorésistants, soit une dégradation sous l'effet de la cuisson des contaminants thermosensibles, comme cela a été observé pour un antibiotique, le sulfaméthoxazole, pour lequel un schéma de dégradation thermique est proposé.

Le second objectif de ce travail était d'évaluer la bioaccessibilité des contaminants chimiques de la viande en s'appuyant sur un protocole standardisé de digestion *in vitro* statique. Dans le cas des PCB, les résultats obtenus indiquent que la bioaccessibilité dans la viande est relativement faible (26%). La teneur initiale de la viande en matière grasse et les variations physiologiques liées à l'âge du consommateur influencent significativement ces valeurs de bioaccessibilité alors que la cuisson de la viande a moins d'influence sur la bioaccessibilité.

A terme, les résultats obtenus durant ce travail devraient conduire à proposer de nouvelles procédures d'évaluation des risques liés aux contaminants chimiques de la viande, prenant en compte les modulations induites par la cuisson et la digestion.

Mots clés : Contaminants chimiques, Cuisson, Bioaccessibilité, Viande, Evaluation du risque, Sécurité sanitaire des aliments.

Abstract:

Livestock animals are exposed to various chemical contaminants during breeding. These contaminants are rapidly transferred from the environment to animal edible tissues, thus representing a public health risk. This risk is classically assessed based on the level of contaminants in raw meat. However, due to technological processes such as cooking or physiological transformations such as digestion, only a fraction of meat contaminants can be absorbed by the body. The purpose of this PhD thesis is to investigate the modulating effect of cooking and digestion on chemical contaminants in meat and on their bioaccessibility.

Thanks to a GC×GC-TOF/MS multiresidue method developed in this study for the analysis of 206 environmental pollutants and to collaborations with French National Reference Laboratories, the first aim of this work was to assess the effects of pan cooking on a broad range of chemical contaminants in spiked meat. Cooking did not impact the level of PCDD/Fs and heavy metals in meat whereas significant losses (more important as cooking conditions were more intense) of PCBs, antibiotics and pesticides were observed. These losses may originate from juice expelling of heat-resistant compounds or from degradation by breakdown of thermolabile compounds such as the sulfamethoxazole antibiotic.

The second aim was to assess the bioaccessibility of chemical contaminants in meat based on a standardized *in vitro* static digestion. For PCBs, results showed that their bioaccessibility was low (26%). Both the meat fat content and the age of consumer significantly affect this bioaccessibility value. In contrast, meat cooking was shown to have less influence on PCB bioaccessibility. The ultimate goal of the project will be to improve chemical risk assessment procedures taking into account the changes induced by cooking and digestion on micropollutants in meat.

Keywords: Chemical contaminants, Pan cooking, Bioaccessibility, Meat, Risk assessment, Food safety.

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Liste des travaux

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Articles soumis à des revues à comité de lecture

1. *Planche C.*, Ratel J., Mercier F., Blinet P., Debrauwer L., & Engel E. (2015). Assessment of comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry based methods for investigating 206 dioxin-like micropollutants in animal-derived food matrices. *Journal of Chromatography A, 1392*, 74-81.

2. Engel E., Ratel J., Bouhlel J., *Planche C.*, & Meurillon M. (2015). Novel approaches to improving the chemical safety of the meat chain towards toxicants. *Meat science, 109*, 75-85.

3. *Planche C.*, Ratel J., Blinet P., Mercier F., Angénieux M., Chafey C., Zinck J., Marchond N., Chevolleau S., Dervilly-Pinel G., Guérin T., Debrauwer L., Engel E., Effects of pan cooking on micropollutants in meat, *Soumis à Food Chemistry*

4. *Planche C.*, Chevolleau S., Noguer-Meireles M.-H., Jouanin I., Canlet C., Mompelat S., Ratel J., Verdon E., Engel E., Debrauwer L., Effects of pan cooking on sulfonamides and tetracyclines in meat, *En préparation pour soumission à Food Chemistry*

5. *Planche C.*, Ratel J., Mercier F., Zhang C., Angénieux M., Blinet P., Dervilly-Pinel G., Debrauwer L., Engel E., Bioaccessibilility of polychrorinated biphenyls in meat: influence of fat content, cooking level and consumer age, *En préparation pour soumission à Environment International*

Communications à des congrès Européens et Internationaux

6. *Planche C.*, Chevolleau S., Noguer-Meireles M.H., Ratel J., Engel E., Debrauwer L., Thermal degradation of sulfamethoxazole during food cooking, 21st International Mass Spectrometry Conference, 2016, Toronto, Canada. (Communication par affiche)

7. *Planche C.*, Ratel J., Mercier F., Zhang C., Angenieux M., Debrauwer L., Engel E., Assessment of digestive bioaccessibility of polychlorinated biphenyls in animal-derived food, 4th European Doctoral College on Environment and Health, 2016, Rennes, France (*Lauréate d'une bourse de participation*) (Communication orale)

8. *Planche C.*, Ratel J., Mercier F., Zhang C., Debrauwer L., Engel E., Application of GC×GC-TOF/MS to the assessment of the bioaccessibility of PCBs in meat after *in vitro* digestion, 13th GC×GC Symposium, 2016, Riva del Garda, Italie (*Lauréate d'une bourse de participation*) (Communication par affiche)

9. *Planche C.*, Ratel J., Mercier F., Blinet P., Zhang C., Debrauwer L., Engel E., A four-step method for the assessment of bioaccessibility of food contaminants by GC×GC-TOF/MS, 13th GC×GC Symposium, 2016, Riva del Garda, Italie (Communication par affiche)

10. *Planche C.*, Ratel J., Mompelat S., Chafey C., Verdon E., Guérin T., Debrauwer L., Engel E., Impact of cooking on meat contaminants, 7th International Symposium on Recent Advances in Food Analysis, 2015, Prague, Czech Republic (Communication orale)

11. Zhang C., *Planche C.*, Mercier F., Blinet P., Debrauwer L., Ratel J., Engel E., Assessment of the bioaccessibility of PCBs in meat after digestion, 7th International Symposium on Recent Advances in Food Analysis, 2015, Prague, Czech Republic (Communication par affiche)

12. Engel E., Ratel J., Bouhlel J., *Planche C.*, Meurillon M., Novel approaches to improving the chemical safety of the meat chain towards toxicants, 61st International Congress of Meat Science & Technology, 2015, Clermont Ferrand, France (Communication orale)

13. *Planche C.*, Ratel J., Mercier F., Blinet P., Debrauwer L., Engel E., Development of a GC×GC-TOF/MS based method to study the fate of 206 dioxin-like micropollutants in meat during cooking, 61st International Congress of Meat Science & Technology, 2015, Clermont Ferrand, France (Communication par affiche)

14. Engel E., *Planche C.*, Mercier F., Debrauwer L., Ratel J., Development of a GC×GC-TOF/MS-based method to investigate the fate of 206 dioxin-related micro-pollutants during food cooking, 11th GC×GC Symposium, 2014, Riva del Garda, Italy (Communication orale)

15. *Planche C.*, Ratel J., Fournier A., Blinet P., Marchand P., Le Bizec B., Jondreville C., Engel E., Back-tracing an emerging environmental toxicant (HBCD) in animal-derived food chain based on foodomics, 11th GC×GC Symposium, 2014, Riva del Garda, Italy (Communication par affiche)

16. Engel E., Ratel J., *Planche C.*, Giri A., Thomas C., Berdague J-L., Meurillon M., Analytical approaches for the determination of food quality markers: a focus on chemical safety and aroma of animal-derived products, 24th Conference of Residue Chemists, 2013, Melbourne, Australia (Communication orale)

17. *Planche C.*, Ratel J., Blinet P., Engel E., Development of a GC×GC-TOF/MS method to study the bioaccessibility of PCBs, PCDDs and PCDFs in meat products, 6th International Symposium on Recent Advances in Food Analysis, 2013, Prague, Czech Republic (Communication par affiche)

Communications à des congrès Nationaux

18. *Planche C.*, Chevolleau S., Noguer-Meireles M.-H., Ratel J., Engel E., Debrauwer L., Identification des produits de dégradation du Sulfaméthoxazole formés pendant la cuisson de viandes, 33èmes Journées Françaises de Spectrométrie de Masse, 2016 (Communication par affiche)

19. *Planche C.*, Ratel J., Mercier F., Blinet F., Debrauwer L., Engel E., Développement d'une méthode de GC×GC-TOF/MS pour le suivi de 206 micropolluants environnementaux au cours de la cuisson des produits carnés, 11ème congrès francophone de l'AFSEP sur les sciences séparatives et les couplages, 2015 (*Lauréate d'une bourse de participation*) (Communication orale)

20. Engel E., *Planche C.*, Meurillon M., Peyret P., Devenir des contaminants toxiques des aliments dans l'environnement digestif, Carrefours de l'innovation agronomique, 2014 (Communication orale)

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Liste des abréviations

Ah : Aryl hydrocarbon

ANSES : Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail

ASE : Extraction accélérée par solvant

DL : Dioxin-like

EAT : Etude de l'alimentation totale

GC×GC-TOF/MS : Chromatographie en phase gazeuse bidimensionelle couplée à la spectrométrie de masse à temps de vol

GPC : Chromatographie par perméation de gel

HAP : Hydrocarbures aromatiques polycycliques

HBCD : Hexabromocyclododécane

IARC : International Agency for Research on Cancer

ICP-MS : inductively coupled plasma mass spectrometry

LABERCA : Laboratoire d'étude des résidus et contaminants dans les aliments

LMR : Limite maximale de résidu

MRM : Multiresidue method

NDL : Non Dioxin-like

OMS : Organisation mondiale de la santé

PBDE : Polybromodiphenyl ethers

PCA : Principal component analysis

PCB : Polychlorobiphényles

PCDD : polychlorodibenzodioxines

PCDF : polychlorodibenzofuranes

RFB : Retardateur de flamme bromés

RMN : Résonnance magnétique nucléaire

$\mathbf{R_s}$: Resolution factor

SOMEAT : Safety of Organic MEAT

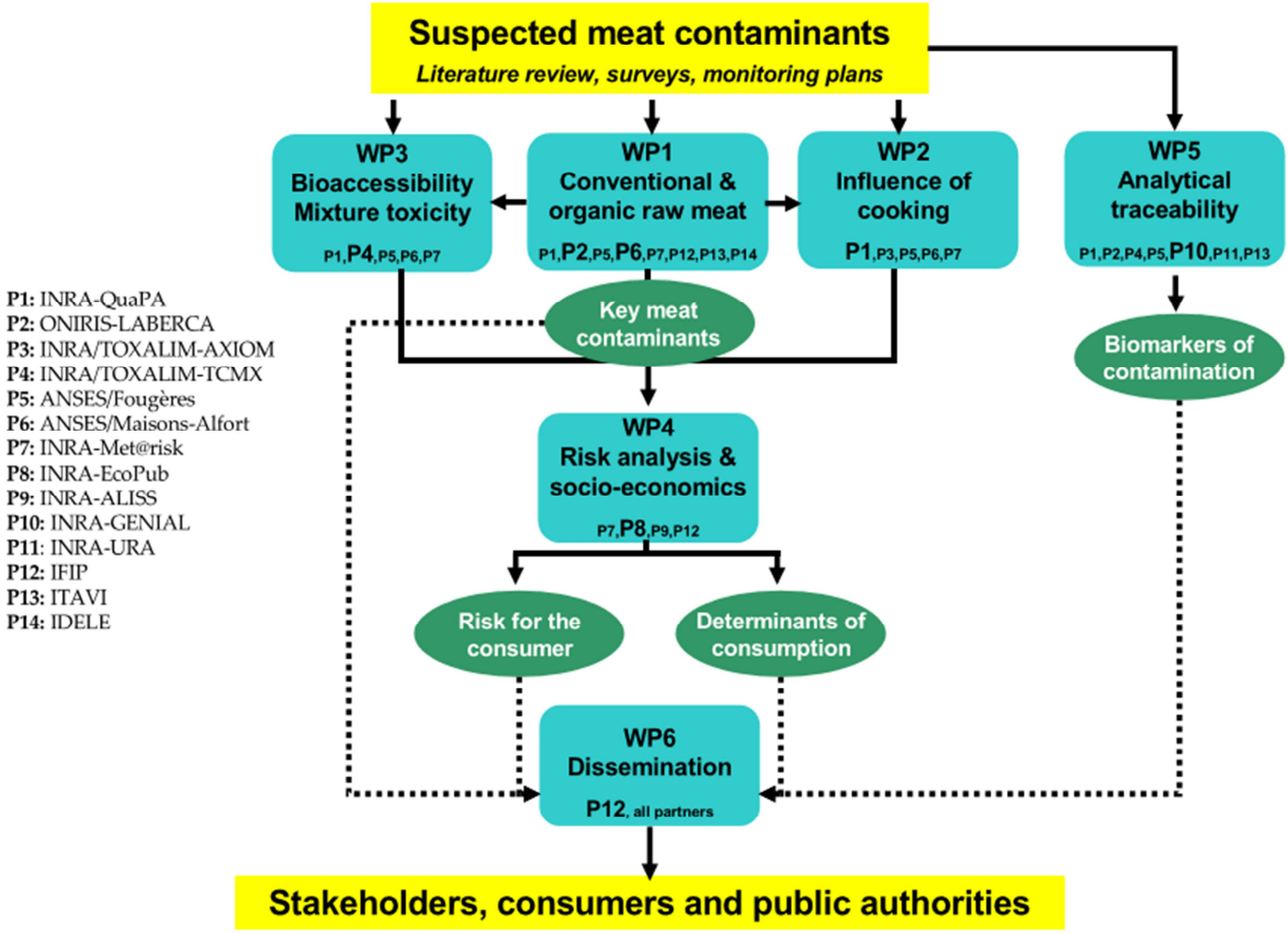
TEF : Facteur d'équivalence toxique

TIM-1 : TNO gastro-intestinal model 1

$\mathbf{t_R}$: retention time

$\mathbf{w_b}$: average peak width at the base

Introduction



<u>Figure 1 :</u> Organisation du projet ANR SOMEAT

La présence de contaminants chimiques dans l'alimentation et leur impact sur la santé de l'Homme représentent une préoccupation sociétale majeure. Même si les teneurs détectées sont la plupart du temps inférieures aux limites maximales résiduelles fixées par les autorités, les plans de surveillance nationaux montrent que cette présence est avérée dans la quasi-totalité des denrées alimentaires que nous consommons. Une augmentation de l'incidence de certaines pathologies (cancers, diabète, obésité, maladies métaboliques…) est observée dans les pays développés et des études épidémiologiques mettent en évidence des corrélations entre exposition aux contaminants chimiques et survenue de pathologies. Par ailleurs, de nombreuses études scientifiques ont montré les effets néfastes de ces contaminants sur la santé. Face à ces observations et suite aux différents scandales alimentaires survenus ces dernières années, les consommateurs ont pris conscience du lien entre alimentation et santé. Afin de pouvoir disposer de produits sains et sûrs, de nombreux consommateurs se sont tournés vers les aliments issus de la filière « bio ». Ainsi, 95% des consommateurs français citent le bénéfice santé comme premier motif d'achat des aliments issus de cette filière. Cependant, aucune étude scientifique n'a démontré clairement le bien-fondé de cette allégation. Face à ce constat, le projet SOMEAT (Safety of Organic MEAT, http://www.so-meat.fr/) dans lequel s'inscrit ce travail de thèse, a pour ambition de fournir des données scientifiques objectives pour évaluer les bénéfices et risques éventuels des systèmes de production de viandes conventionnelles ou « bio » au regard de leurs teneurs en contaminants. L'ambition plus large de ce projet est de pouvoir évaluer précisément le risque lié à la présence des contaminants chimiques dans la viande sur la santé des consommateurs. Cependant, deux aspects majeurs limitent actuellement cette évaluation du risque chimique. D'une part, le risque sur la santé humaine est classiquement estimé à partir de produits crus et non à partir de produits prêts à consommer, c'est à dire après cuisson pour la viande. D'autre part, l'évaluation du risque ne prend actuellement pas en compte la bioaccessibilité des contaminants au cours de la digestion alors que la toxicité dépend de la quantité de contaminants mobilisés de la matrice alimentaire dans le tractus gastrointestinal puisque seuls ces contaminants sont alors disponibles pour l'absorption. Afin de pouvoir *in fine* améliorer les modèles d'évaluation des risques chimiques existant, ces travaux de thèse ont consisté à caractériser et expliquer l'impact de la cuisson et de la digestion sur les contaminants chimiques susceptibles d'être retrouvés dans la viande (WP2 et WP3 du projet SOMEAT, Figure 1).

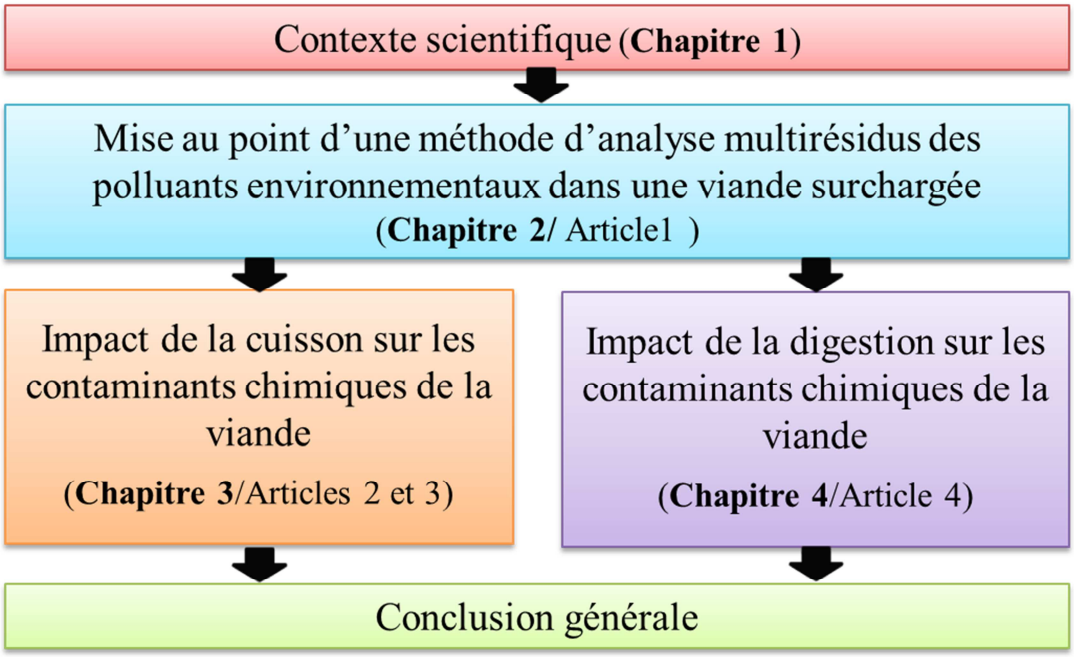


Figure 2 : Démarche adoptée dans cette étude

Le premier chapitre de ce manuscrit a pour vocation de présenter le contexte scientifique de ce travail (Figure 2). Après avoir rappelé les principales familles de contaminants chimiques susceptibles d'être retrouvées dans la viande, un état des connaissances actuelles sur leur devenir au cours de la cuisson et de la digestion est dressé. Les chapitres suivants présentent les résultats expérimentaux de cette thèse sous forme de publications introduites dans le manuscrit et complétées par des commentaires. Le Chapitre 2 a pour objectif de lever les deux principaux verrous méthodologiques de cette étude. Premièrement, il était nécessaire de pouvoir disposer d'une viande surchargée en contaminants de manière homogène, qui puisse être utilisée tout au long de cette étude. Une méthode reproductible de contamination intentionnelle à des concentrations connues a donc été développée. Deuxièmement, en ciblant les polluants environnementaux pertinents à étudier en raison de leur fréquence de détection dans la viande, il était nécessaire de pouvoir disposer d'une méthode d'analyse « multirésidus » permettant le suivi simultané d'un large spectre de ces contaminants. Une méthode analytique permettant finalement un suivi simultané de 206 micropolluants environnementaux dans la viande a donc été mise au point. En s'appuyant sur ces développements ainsi que sur des collaborations avec des Laboratoires Nationaux de Référence (Anses Maisons-Alfort, Anses Fougères, ONIRIS-LABERCA), le Chapitre 3 concerne l'étude du devenir des contaminants chimiques au cours de la cuisson de la viande. Divers contaminants aux propriétés physico-chimiques variées ont alors été ciblés : polychlorobiphenyles (PCB), polychlorodibenzodioxines et polychlorodibenzofuranes (PCDD/F), antibiotiques, métaux lourds et pesticides. Cette étude a ainsi permis d'examiner les devenirs des contaminants et de distinguer des composés thermorésistants et des composés thermosensibles subissant une dégradation au cours de la cuisson. Afin d'affiner les approches d'évaluation du risque, une étude spécifique a été menée sur un antibiotique thermosensible pour pouvoir identifier, après cuisson, ses produits de dégradation, composés ultimes auxquels le consommateur est réellement exposé. Suite aux travaux entrepris dans le chapitre 3 sur l'effet modulateur de la cuisson, le chapitre 4 concerne l'étude du devenir des contaminants chimiques au cours de la digestion de la viande en s'appuyant sur l'utilisation d'un protocole standardisé de digestion statique *in vitro,* développé afin de mimer les conditions physiologiques humaines. La bioaccessibilité des contaminants chimiques a alors été déterminée, la fraction bioaccessible correspondant à la quantité maximale de contaminants disponibles pour l'absorption intestinale. L'impact de la teneur en lipides de la viande, des conditions de cuisson et des variations physiologiques liées à l'âge du consommateur sur la bioaccessibilité des contaminants a été évalué. Les résultats de certaines

expérimentations de cuisson ou de digestion réalisées sur des échantillons contaminés par des doses très faibles ont été obtenus en collaboration avec le LABERCA. Enfin, le dernier chapitre « conclusion générale » présente les principales conclusions et perspectives de cette thèse.

Chapitre I : Contexte Scientifique

Chapitre I : Contexte Scientifique

Cette synthèse bibliographique a pour ambition de dresser un état des lieux des connaissances actuelles sur l'impact de la cuisson et de la digestion sur les micropolluants à risque susceptibles de contaminer les produits carnés. Volontairement concise, celle-ci est focalisée sur les aspects et considérations en relation directe avec les objectifs que nous nous sommes fixés et sur le travail expérimental réalisé pour les atteindre.

Comme nous l'avons mentionné dans l'introduction générale, nos recherches doivent cibler les contaminants les plus pertinents à étudier dans la viande de façon à produire des résultats en prise avec la réalité des contaminations faisant l'objet des préoccupations les plus importantes. Dans une première partie, les différentes familles de contaminants chimiques susceptibles d'être retrouvées dans les produits carnés seront donc présentées. Des données concernant leur toxicité ainsi que la contribution de la viande à l'exposition du consommateur à ces contaminants seront également discutées.

Il s'agit ensuite d'identifier les verrous à lever pour l'étude de l'impact de la cuisson sur les contaminants chimiques des aliments afin d'obtenir des conclusions pertinentes utilisables en analyse de risque. Pour cela, une analyse critique des données de la littérature qui traitent des conséquences des traitements thermiques sur les contaminants chimiques des aliments a été réalisée.

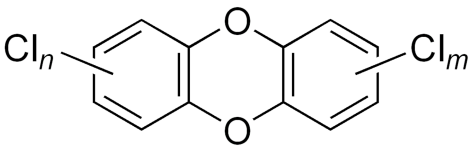
Enfin, concernant l'étude de l'impact de la digestion sur les contaminants chimiques de la viande, cette étude de la littérature a tout d'abord visé à déterminer le modèle de digestion *in vitro* le plus pertinent à utiliser dans ce travail de thèse. Pour cela, un bref rappel des différents phénomènes se produisant au cours de la digestion est effectué, permettant de discuter la pertinence des différents modèles de digestion *in vitro* retrouvés dans la littérature. Dans un second temps, afin de déterminer les facteurs modulateurs de la bioaccessibilité intéressants à étudier dans notre travail, les données de la littérature qui traitent de la bioaccessibilité des contaminants chimiques des aliments sont également discutées.

1/ Les contaminants chimiques de la viande

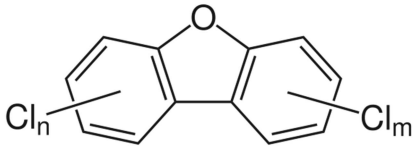
Les animaux d'élevage sont potentiellement exposés à des sources diverses de contamination (environnement, pâturage, alimentation…). Ils peuvent également être soumis à des traitements médicamenteux utilisés à titre prophylactique ou encore en tant que promoteurs de croissance. De ce fait, différents types de contaminants chimiques peuvent être détectés dans la viande. Ces contaminants peuvent provenir comme indiqué précédemment d'une exposition des animaux à des substances chimiques au cours de leur élevage, mais également d'une contamination de la viande au cours de son stockage ou encore d'une néoformation au cours de la cuisson de la viande (Püssa, 2013 ; Engel *et al.*, 2015). Nous nous intéresserons ici uniquement aux contaminants auxquels les animaux peuvent être exposés au cours de leur élevage et qui peuvent potentiellement se retrouver dans la viande consommée par l'Homme.

1.1/ Les métaux lourds

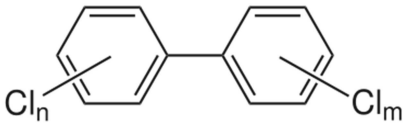
La famille des métaux lourds regroupe différentes substances parmi lesquelles les plus toxiques sont l'arsenic (As), le mercure (Hg), le plomb (Pb) et le cadmium (Cd), dont le risque actuel pour les consommateurs ne peut pas être écarté d'après l'Etude de l'Alimentation Totale française n°2 (EAT2) publiée par l'agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail en 2011 (ANSES, EAT2, Tome1). Ces substances, bien que naturellement présentes dans l'environnement, peuvent également provenir d'apports anthropiques (activités industrielles, minières et agricoles) (Marques *et al.*, 2011). Une exposition aux métaux lourds peut entraîner chez l'Homme des lésions cutanées, des développements de cancers, des retards de développement, une atteinte du système nerveux central, des maladies cardiovasculaires et rénales ou encore une perturbation du métabolisme du glucose entraînant un risque de diabète (ANSES, EAT2, Tome1). Les Valeurs Toxicologiques de Référence pour ces substances sont de 0,3 à 8 µg/kg pc/j pour l'arsenic, 0,5 à 1,5 µg/kg pc/jour pour le plomb, 0,36 µg/kg pc/jour pour le cadmium et 0,23 µg/kg pc/jour pour le mercure tandis que l'exposition moyenne de la population française adulte à ces substances a pu être estimée respectivement à 0,28 µg/kg pc/jour, 0,20 µg/kg pc/jour, 0,16 µg/kg pc/jour et 0,18 µg/kg pc/jour. Toujours selon l'EAT2, la consommation de viande contribue chez les adultes à 3% de l'exposition à l'arsenic, 4% de



PCDD



PCDF



PCB

<u>Figure 3</u>. Structure chimiques des polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) et polychlorobiphényles (PCB)

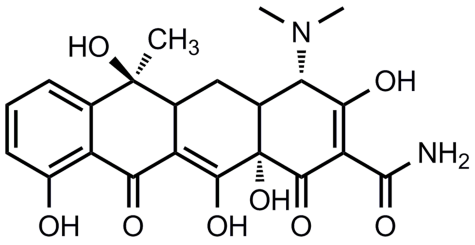
	Isomère ou groupe homologue (numéro IUPAC pour les isomères de PCB)	TEF (OMS 1998)	TEF (OMS 2005)
PCDD	2,3,7,8-tétraCDD	1	1
	1,2,3,7,8-pentaCDD	1	1
	1,2,3,4,7,8-hexaCDD	0,1	0,1
	1,2,3,6,7,8-hexaCDD	0,1	0,1
	1,2,3,7,8,9-hexaCDD	0,1	0,1
	1,2,3,4,6,7,8-heptaCDD	0,01	0,01
	OCDD	0,0001	**0,0003**
PCDF	2,3,7,8-TCDF	0,1	0,1
	1,2,3,7,8-pentaCDF	0,05	**0,03**
	2,3,4,7,8-pentaCDF	0,5	**0,3**
	1,2,3,4,7,8-hexaCDF	0,1	0,1
	1,2,3,6,7,8-hexaCDF	0,1	0,1
	1,2,3,7,8,9-hexaCDF	0,1	0,1
	2,3,4,6,7,8-hexaCDF	0,1	0,1
	1,2,3,4,6,7,8-heptaCDF	0,01	0,01
	1,2,3,4,7,8,9-heptaCDF	0,01	0,01
	OCDF	0,0001	**0,0003**
PCB non ortho	3,3',4,4'-TCB (77)	0,0001	0,0001
	3,3',4,4',5-TCB (81)	0,0001	**0,0003**
	3,3',4,4',5-PeCB (126)	0,1	0,1
	3,3',4,4',5,5'-HxCB (169)	0,01	**0,03**
PCB mono-ortho	2,3,3',4,4'-PeCB (105)	0,0001	**0,00003**
	2,3,4,4',5-PeCB (114)	0,0005	**0,00003**
	2,3',4,4',5-PeCB (118)	0,0001	**0,00003**
	2',3,4,4',5-PeCB (123)	0,0001	**0,00003**
	2,3,3',4,4',5-HxCB (156)	0,0005	**0,00003**
	2,3,3',4,4',5'-HxCB (157)	0,0005	**0,00003**
	2,3',4,4',5,5'-HxCB (167)	0,00001	**0,00003**
	2,3,3',4,4',5,5'-HpCB (189)	0,0001	**0,00003**

<u>Tableau 1 :</u> Facteurs d'équivalence toxique définis par l'OMS pour les PCB et PCDD/F

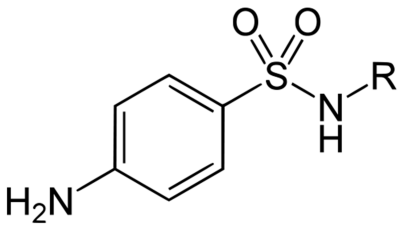
l'exposition au plomb, moins de 1% de l'exposition au cadmium et 3% de l'exposition au mercure.

1.2/ Les micropolluants environnementaux

La famille des micropolluants environnementaux regroupe différents contaminants dont les polychlorinated dibenzo-p-dioxins (PCDD), les polychlorinated dibenzofurans (PCDF) et les polychlorobiphényles (PCB) qui sont des composés aromatiques comptant respectivement 75, 135 et 209 congénères se distinguant par le nombre et la position des atomes de chlore sur les cycles aromatiques (Figure 3). Ces contaminants sont très stables chimiquement, insolubles dans l'eau mais très solubles dans les lipides et peu biodégradables (Jones *et al.*, 1999). Alors que les dioxines et furanes (PCDD/F) sont formés lors de combustions incomplètes ou de réactions chimiques, les PCB sont des mélanges industriels fabriqués et utilisés jusqu'en 1987 pour leurs propriétés isolantes et leur stabilité. D'un point de vue toxicologique, seuls 17 congénères de PCDD/F (7 congénères de PCDD et 10 congénères de PCDF) ont été considérés toxiques. 12 congénères de PCB se comportent comme les PCDD/F en se liant au récepteur cellulaire Ah (Aryl hydrocarbon), et sont dits « dioxin-like » (PCB-DL), par opposition aux autres PCB « non dioxin-like » (PCB-NDL). Ces 12 PCB-DL sont classiquement traités avec les PCDD/F pour l'évaluation des risques (ANSES, EAT2, Tome1). Le risque lié à l'exposition aux PCDD/F et PCB-DL est évalué à l'aide d'une grandeur appelée facteur d'équivalence toxique (TEF ou toxicological equivalent factor), qui représente en fait un coefficient de pondération permettant d'exprimer la toxicité de l'ensemble des congénères de même activité toxicologique dans une même unité, les équivalents toxiques (TEQ) (Tableau 1). Avec un TEF de 1, le 2,3,7,8–TCDD (congénère majeur de l'accident de Seveso de juillet 1976) est le seul des 17 congénères de PCDD/F à être classé cancérigène (groupe 1) pour l'homme par l'IARC (International Agency for Research on Cancer) (Püssa *et al.*, 2013). Une exposition aux PCB et PCDD/F peut également entraîner chez l'Homme des effets thyroïdiens, hépatotoxiques ou encore un dysfonctionnement du système immunitaire et de la fonction de reproduction. Une dose journalière tolérable a été fixée à 2,33 pg TEQ OMS /kg pc/jour pour les PCDD/F+PCB-DL tandis que l'exposition moyenne journalière de la population adulte française est estimée à 0,47 pg TEQ OMS /kg pc/jour. L'alimentation constitue la principale voie de contamination de la population générale aux PCB et PCDD/F (plus de 90 % de l'exposition). La viande représente l'un des vecteurs alimentaires importants de ces composés, en contribuant à 13,1%



Tétracyclines



Sulfamides

<u>Figure 4.</u> Structure chimiques des familles d'antibiotiques tétracyclines et sulfamides

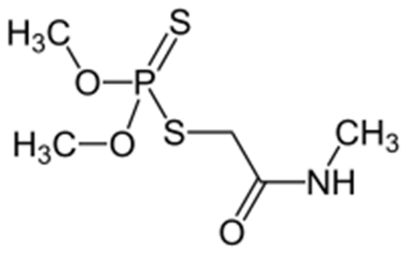
de l'exposition aux PCDD, 10,9% de l'exposition aux PCB-DL et 11,3% de l'exposition aux PCB-NDL (ANSES, EAT2, Tome1). Il est important de noter que, parmi les 209 congénères de PCB, six sont plus fréquemment retrouvés dans les matrices alimentaires (PCB-28, 52, 101, 138, 153 et 180), représentant jusqu'à 50 % de l'ensemble des congénères présents. Ces six congénères de PCB ajoutés aux 12 PCB-DL constituent les 18 PCB classiquement recherchés dans la viande (Sirot *et al.*, 2012).

Les retardateurs de flamme bromés (RFB) sont également des micropolluants environnementaux. Ces substances sont incorporées dans les matières plastiques d'appareils électriques et de circuits électroniques, mais également dans des mousses et des matériaux de capitonnage, les intérieurs de voitures et d'avions ainsi que dans certains textiles en vue de leur conférer des propriétés ignifuges. Une exposition aux RFB peut entraîner des dysfonctionnements des fonctions hépatiques, hormonales, reproductives, nerveuses et immunologiques. Les polybromodiphenyl ethers (PBDE) font partie de la famille des RFB mais l'EAT2 a conclu que leur risque pour la santé des consommateurs peut être écarté (ANSES, EAT2, Tome1). A l'inverse, il n'est à l'heure actuelle pas possible de conclure quant au risque lié à l'hexabromocyclododécane (HBCD), un autre RFB pour lequel les données toxicologiques restent parcellaires et pour lequel il n'existe actuellement pas de valeur toxicologique de référence pertinente. L'exposition moyenne journalière à l'HBCD s'élève chez les adultes à 0,211 ng/kg pc/j et la viande contribue à 24,7% de cette exposition.

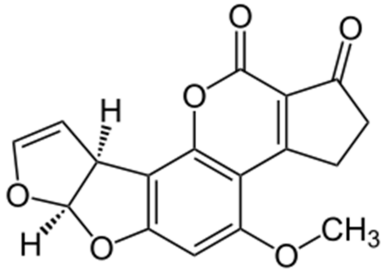
Les hydrocarbures aromatiques polycycliques (HAP) font également partie de la famille des micropolluants environnementaux mais le risque lié à la présence de ces contaminants dans les aliments sur la santé des consommateurs est actuellement écarté par les autorités (ANSES, EAT2, Tome1).

1.3/ Les antibiotiques

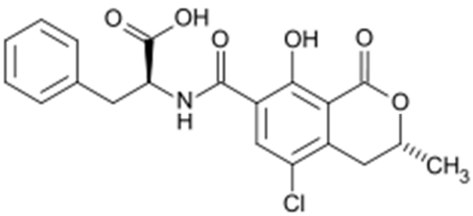
Les antibiotiques peuvent être administrés aux animaux à des fins thérapeutiques ou prophylactiques. Ils peuvent se retrouver dans les produits carnés consommés par l'Homme en cas de non-respect des doses d'administration ou des délais d'attente (Reig *et al.*, 2008). Les sulfamides et les tétracyclines (Figure 4) sont les deux familles d'antibiotiques les plus fréquemment détectées dans les derniers plans de surveillance et plans de contrôle réalisés en France en 2010, 2012 et 2014 (http://agriculture.gouv.fr/). Une exposition à ces deux familles



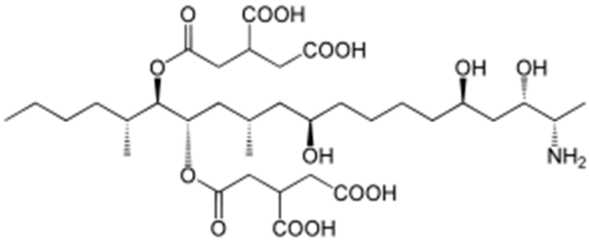
<u>Figure 5.</u> Structure chimiques du diméthoate



Aflatoxines



Ochratoxines



Fumonisines

<u>Figure 6.</u> Structure chimiques des mycotoxines aflatoxines, ochratoxines et fumonisines

d'antibiotiques peut entraîner chez l'Homme des réactions allergiques et le développement de résistance aux antibiotiques (Kishida, 2007). C'est pourquoi des Limites Maximales de Résidu (LMR) ont été fixées à 100µg/kg de viande pour ces deux familles de contaminants. Les antibiotiques n'ont pas fait partie des contaminants ciblés dans le cadre de l'EAT2.

1.4/ Les pesticides

Les pesticides comprennent en particulier les substances phytosanitaires utilisées pour des raisons agronomiques, et regroupent de nombreux composés de structure et de propriétés physico-chimiques différentes. Les substances les plus fréquemment retrouvées dans les aliments sont le pyrimiphos-méthyl, le chlorpyriphos-méthyl, le chlorpyriphos-éthyl, l'iprodione, le carbendazime et l'imazalil (ANSES, EAT2, Tome2). Une exposition aux pesticides peut entraîner un risque de cancer, des dysfonctionnements du système nerveux et du système de reproduction ou encore une perturbation endocrinienne (Püssa *et al.*, 2013), même si les modes d'action et les effets sont variables d'une famille de pesticides à l'autre. L'EAT2 a mis en évidence qu'il était impossible de conclure quant au risque sur la santé des consommateurs pour certains pesticides (dithiocarbamates, éthoprophos, carbofuran, diazinon, méthamidophos, disulfoton, dieldrine, endrine et heptachlore). Par ailleurs, un pesticide de la famille des organophosphorés, le diméthoate (Figure 5), présente des dépassements de la valeur toxicologique de référence chez certaines catégories de la population. Pour ce pesticide, une dose journalière admissible de 0,001 mg/kg pc/j a été établie tandis que l'exposition moyenne de la population adulte est estimée entre 0,02 µg/kg pc/j et 1,24 µg/ kg pc/j. Bien que peu fréquemment détectés, des résidus de pesticides peuvent se retrouver dans la viande si, au cours de leur élevage, les animaux consomment une alimentation elle-même contaminée en pesticides. La viande n'est cependant pas un contributeur significatif de l'exposition à ce contaminant.

1.5/ Les mycotoxines

Les mycotoxines sont des métabolites secondaires produits par les souches toxinogènes de moisissures (Aspergillus, Penicillium, Fusarium, Byssochlamys…) pouvant se développer en particulier sur les céréales et le fourrage dans des conditions de stockage favorables (température, humidité). A ce jour, plus de 250 mycotoxines ont été identifiées, parmi lesquelles les aflatoxines, les ochratoxines ou encore les fumonisines (Figure 6). La

Food Matrix	Contaminant	Cooking mode	Cooking losses	Référence
Environmental micropollutants				
Fish products	PCBs	Frying (10-15min)	12%	Rawn *et al.*, 2013
Fish	PCBs	Deep-frying (1min)	47%	Moya *et al.*, 1998
		Pan-frying (2min)	No significant loss	
		Broiling (2min)	No significant loss	
Beef	PCDD/Fs	Stewing (15min)	No significant loss	Rose *et al.*, 2001
Hamburger patties	PCDD/Fs	Pan-frying (210°C, 20min)	40-50%	Petroske *et al.*, 1998
Beef	PCDD/F and PCB-DL	Boiling (100°C, 5min)	31% (PCDD), 54% (PCDF), 29% (dl-PCB)	Hori *et al.*, 2005
		Broiling (approximately 5min)	41% (PCDD), 49% (PCDF), 31% (dl-PCB)	
Ground beef	PCDD/Fs and coplanar PCBs	Broiling (approximately 30min)	47% (PCDDs), 46% (PCDFs), 52% (coplnar PCBs)	Schecter *et al.*, 1998
Salmon	PCBs and PBDES	Pan-frying (180°C, 5min)	36% (PCBs), 42% (PBDEs)	Bayen *et al.*, 2005
		Microwaving (500W, 5min)	23% (PCBs), 25% (PBDEs)	
		Boiling (100°C, 5min)	28% (PCBs), 32% (PBDEs)	
		Baking (180°C, 30min)	28% (PCBs), 44% (PBDEs)	
Antibiotics				
Chicken thigh muscle	Sulfonamide	Boiling (100°C, 12min)	45-61%	Furusawa *et al.*, 2002
		Roasting (170°C, 12min)	38-40% except for sulfadiazine (no significant loss)	
		Microwaving (500W, 1min)	35-41%	
Chicken thigh muscle	Tetracyclines	Microwaving (20min)	46-99%	Abou-Raya *et al.*, 2013
		Boiling (100°C, 40min)	44-85%	
		Roasting (180°C, 80min)	42-95%	
Pesticides				
Ovine meat	Lindane	Grilling (85°C at core)	33%	Conchello *et al.*, 1993
		Roasting (100°C at core)	No significant loss	
		Pressure cooking (130°C, 30min)	No significant loss	
Salmon	DDT	Pan-frying (180°C, 5min)	31%	Bayen *et al.*, 2005
		Microwaving (500W, 5min)	21%	
		Boiling (100°C, 5min)	25%	
		Baking (180°C, 30min)	19%	

Tableau 2 : Pertes en contaminants chimiques observées dans la littérature lors de la cuisson de produits animaux

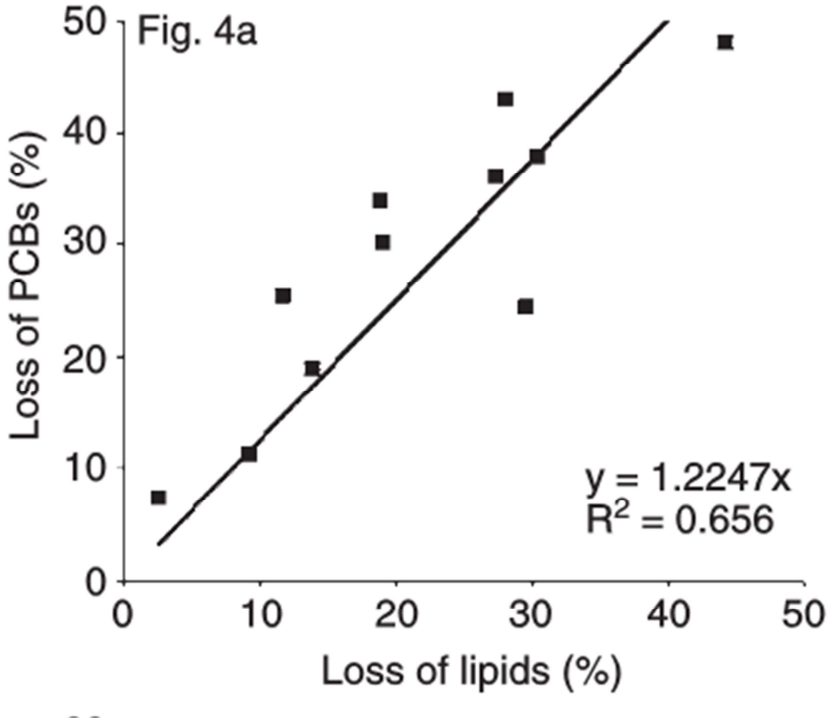
toxicité des mycotoxines chez l'homme et l'animal est variable selon la nature de la toxine et selon l'espèce. Des atteintes rénales, des anomalies du système nerveux ou du système immunitaire et des développements de cancers ont notamment été décrits dans la littérature suite à une exposition aux mycotoxines (ANSES, EAT2, Tome1 ; Püssa *et al.*, 2013). L'exposition moyenne de la population adulte s'élève à 0,89 ng/kg pc/j. Cette exposition peut être liée directement à la consommation d'aliments contaminés (céréales, fruits….) ou indirectement à la consommation de produits carnés suite à une contamination de l'alimentation animale (Marques *et al.*, 2011). D'après les résultats de l'EAT2, la seule mycotoxine dont le risque ne peut pas être écarté pour les consommateurs et pour laquelle la viande est un contributeur de son exposition est l'ochratoxine B. L'exposition moyenne de la population adulte est de 1,579 ng/ kg pc/j mais il n'existe pas de valeur toxicologique de référence. La consommation de viande contribue à 5% de l'exposition à cette toxine chez les adultes.

2/ Devenir des contaminants au cours de la cuisson des aliments

Au cours de la cuisson de la viande, des pertes en contaminants chimiques peuvent être induites par une libération de ces composés dans le jus de cuisson, une thermodégradation des composés thermosensibles ou encore par une évaporation des contaminants volatils et de faibles poids moléculaires (Abou-Arab, 1999; Bayen *et al*., 2005; Sallam *et al*., 2008; Rawn *et al.*, 2013; Muresan *et al.*, 2015). La cuisson est donc un phénomène à prendre en considération dans la perspective d'une évaluation du risque chimique des aliments parce que d'une part, seule une fraction des contaminants initialement présents dans la viande crue se retrouvera *in fine* dans la viande cuite et d'autre part, certains produits de dégradation des composés thermosensibles peuvent eux aussi être toxiques.

2.1/ Origine de l'hétérogénéité des données de la littérature

Le Tableau 2 recense les principales données de la littérature traitant des pertes en contaminants chimiques lors de la cuisson des aliments, en ciblant plus particulièrement les produits animaux (viandes et poissons). Ce tableau permet d'observer que les résultats obtenus sont très variables d'une étude à l'autre. Rose *et al*. (2001) n'ont par exemple observé aucune perte en PCDD/F au cours de la cuisson de viande de bœuf alors que Hori *et al.* (2005) ont décrit des pertes allant jusqu'à 41% pour les PCDD et 54% pour les PCDF. La variabilité



<u>Figure 7 :</u> Pertes en PCB observées par Bayen *et al.*, (2005) lors de la cuisson d'échantillons de saumon en fonction des pertes en lipides de ces échantillons.

des modes de cuisson étudiés dans la littérature pourrait en partie expliquer ces différences. Ainsi, dans une même étude, Moya *et al.* (1998) ont observé que les pertes moyennes en PCB dans des échantillons de poisson pouvaient varier entre 0 et 47% en fonction du mode de cuisson.

Au-delà du mode de cuisson en lui-même, les couples temps/température de cuisson utilisés varient également significativement entre les études. Il est cependant important de noter que les conditions de cuisson ne sont pas toujours précisées dans la littérature, limitant alors la comparaison des études entre elles. Ce manque d'information empêche également l'utilisation de ces données pour les analyses de risque. En effet, si l'on prend l'exemple de la viande, au moins deux critères doivent être pris en compte lors de l'évaluation du risque : le mode de cuisson, qui doit se rapprocher de celui qui est le plus répandu chez les consommateurs (la cuisson à la poêle pour les consommateurs français), mais également la température à cœur de la viande, qui doit se rapprocher des recommandations de l'OMS (70°C). Or, le manque d'informations concernant les températures à cœur obtenues dans les différentes études ne permet pas d'identifier celles qui se rapprochent le plus des recommandations de l'OMS. La comparaison des résultats obtenus dans les études de la littérature est aussi limitée par le fait que certains auteurs ne se sont pas intéressés aux pertes en contaminants au cours de la cuisson, comme cela est recensé dans le tableau 2, mais aux concentrations en contaminants dans les aliments avant et après cuisson (Perelló *et al.*, 2008 ; Perelló *et al.*, 2009 ; Perelló *et al.*, 2010 ; Nachman *et al*., 2013).

2.2/ Devenir à la cuisson des composés thermostables

Les principaux contaminants thermostables des produits animaux sont des micropolluants environnementaux tels que les PCB, les PCDD/F ou les métaux lourds. Etant donné que ces composés ne peuvent pas s'évaporer aux températures de cuisson des aliments, les pertes observées sont attribuables uniquement à leur expulsion dans le jus de cuisson. La montée en température de la matrice va induire des phénomènes de dénaturation des protéines et de thermocontraction du collagène induisant une libération de jus, mélange principalement composé d'eau et de lipides (Kondjoyan *et al.*, 2013).

La libération des contaminants dans le jus de cuisson a été tout particulièrement étudiée par Bayen *et al.* (2005) lors de l'étude du devenir des PCB au cours de la cuisson de poisson. Dans cette étude, quatre modes de cuisson différents ont été étudiés : cuisson à la poêle (5min à 180°C), cuisson aux micro-ondes (5min à 500W), cuisson par ébullition (5min

à 100°C) et cuisson au four (30min à 180°C). Selon ces auteurs, les PCB étant des composés particulièrement lipophiles, leur perte au cours de la cuisson est positivement corrélée aux pertes en lipides dans le jus de cuisson (Figure 7). Par conséquent, comme le précisent Bayen *et al.*, la concentration en PCB de la viande, si celle-ci est rapportée à la quantité de matière grasse de la matrice, n'est pas significativement différente entre la viande crue et la viande cuite. Plusieurs travaux montrent que les pertes en PCB sont positivement corrélées avec la teneur en matière grasse de l'échantillon étudié : plus la teneur de la viande en matière grasse est élevée, plus la quantité de lipides expulsés dans le jus est importante, et plus les pertes en PCB sont importantes (Bayen *et al.*, 2005 ; Oroszvári *et al*., 2006). En revanche, Bayen *et al.* (2005) n'ont pas observé de corrélation entre les pertes en PCB et la température à cœur de la viande cuite contrairement à ce que l'on pourrait supposer au regard des résultats obtenus par Aaslyng *et al.* (2003) indiquant que plus la température à cœur des produits animaux augmente et plus la perte en lipides est importante.

2.3/ Devenir à la cuisson des composés thermosensibles

Un autre phénomène pouvant expliquer les pertes en contaminants au cours de la cuisson des produits animaux est la dégradation de ces composés sous l'effet de la chaleur. Différentes études ont décrit ce phénomène pour des composés thermosensibles tels que des antibiotiques, des pesticides et certains polluants environnementaux.

2.3.1/ Antibiotiques

Concernant les antibiotiques, les deux principales familles qui seraient susceptibles de subir des phénomènes de dégradation au cours de la cuisson sont les sulfamides et les tétracyclines.

Parmi les molécules de la famille des tétracyclines, la doxycycline serait la substance la plus thermorésistante alors que l'oxytetracycline serait la plus sensible aux traitements thermiques (Hassani *et al*., 2008, Abou-Raya *et al.*, 2013). Concernant la tétracycline et la 4-epi-tétracycline, Gratacós-Cubarsí *et al.* (2007) ont observé qu'une cuisson par ébullition (14min à 100°C) ou aux micro-ondes (6min à 440W) de viande contaminée par ces deux substances pouvait entraîner leur dégradation en anhydrotétracycline (ATC) et 4-épi-anhydrotetracycline (4eATC). Ces produits de dégradation pourraient être impliqués dans le développement du syndrome de Fanconi caractérisé par un trouble de la fonction rénale

(Kühne *et al.*, 2001). Cette observation renforce la justification de la prise en compte de l'effet cuisson dans les évaluations toxicologiques.

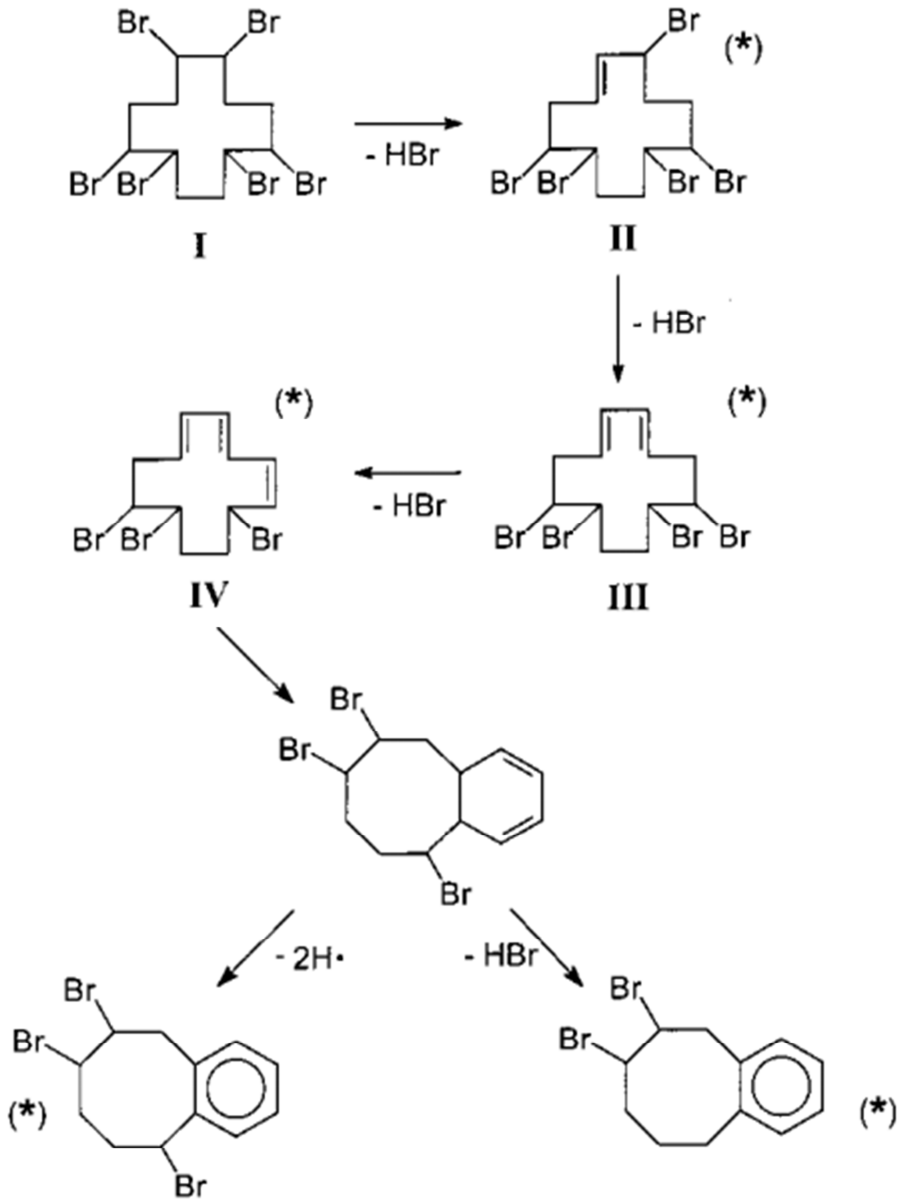
Concernant les sulfamides, bien qu'à notre connaissance aucune étude ne se soit intéressée à leur dégradation au cours de la cuisson des aliments, les pertes entre 45% et 61% après 12min à 100°C observées par Furusawa *et al.* (2002) (Tableau 2) laissent supposer que ce phénomène ne doit pas être exclu pour cette famille d'antibiotiques.

2.3.2/ Pesticides

Une isomérisation du lindane lors de la cuisson de la viande a été décrite dans la littérature. Le lindane (gamma-hexachlorocyclohexane) pourrait en effet être converti en ses isomères alpha (Jan *et al.*, 1982) ou béta (Conchello *et al.*, 1993) sous l'effet de traitements thermiques, l'isomère béta étant le plus toxique pour l'Homme. La cuisson pourrait également induire la formation de produits de dégradation du lindane lors de la cuisson de la viande de bœuf (2h à 115°C) (Jan *et al.*, 1982). Le dichlorobenzene et le trichlorobenzene ont été identifiés comme les produits de dégradation prédominants du lindane. D'un point de vue toxicologique, ces composés ont une toxicité équivalente au lindane. De plus faibles quantités de tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene, gamma-pentachlorocyclohexene et hexachlorocyclohexene ont également été identifiés comme produits de dégradation du lindane. D'après les auteurs de cette étude, une viande riche en lipides limiterait la dégradation du lindane.

Sous l'effet de traitements thermiques, le DDT pourrait se transformer en DDD et DDE (Sibanda *et al.*, 2011 ; Abou-Arab *et al.*, 1997 ; Skibniewska *et al.*, 2003 ; Bayarri *et al.*, 1994). La conversion du DDT en DDE a notamment été observée lors de cuisson de poulet (2h30 à 93°C) (Morgan *et al*., 1972). Ce phénomène est important à prendre en considération en raison de la toxicité du DDE. En effet, des études ont montré que le DDE est neurotoxique et qu'une exposition à ce contaminant peut entraîner une perturbation du système de reproduction chez l'Homme mais également peut augmenter le risque de développement du cancer du sein chez la femme (Snedeker *et al.*, 2001 ; Quan *et al*., 2014 ; Wnuk *et al*., 2016).

Les pesticides organophosphorés, très sensibles aux traitements thermiques, tels que le famphur (Coulibaly *et al.*, 1993), le fenthion (Coulibaly *et al.*, 1994) ou encore le parathion (Coulibaly *et al.*, 1993) pourraient également subir une dégradation sous l'effet de la cuisson.



<u>Figure 8 :</u> Réactions de thermodégradation de l'hexaBromoCycloDodécane (HBCD) pouvant se produire sous l'effet de traitements thermiques (Barontini *et al.*, 2001).

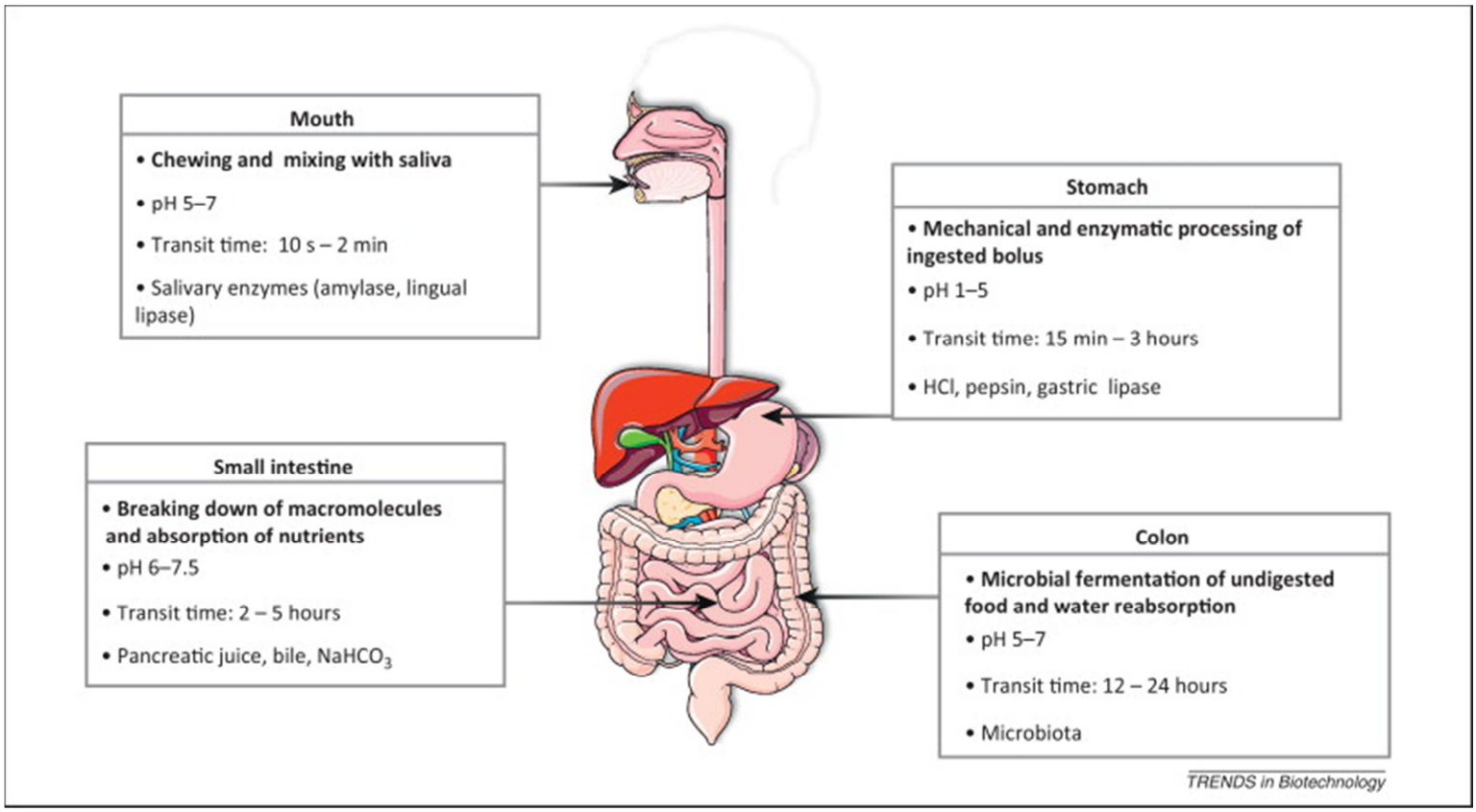
2.3.3/ Polluants environnementaux

Les polluants environnementaux, et notamment l'HexaBromoCycloDodecane (HBCD) et les PolyBromoDiphenylEthers (PBDE), pourraient également subir des phénomènes de dégradation au cours de la cuisson.

Bien qu'aucune étude n'ait validé cette hypothèse dans des matrices alimentaires, l'HBCD subirait des phénomènes d'isomérisation voire de thermodégradation lors de cuissons très intenses. En effet, au-delà de 160°C, le gamma-HBCD (isomère prédominant dans les formules commerciales) subit des phénomènes d'isomérisation principalement en alpha-HBCD (Barontini *et al.*, 2001). Au-delà de 230°C, l'HBCD peut se décomposer notamment en tribromocyclododecatriene. Des réactions de débromation secondaires conduisent alors à la formation de composés tétra- et penta-bromés (Figure 8). Compte tenu des températures élevées auxquelles ont été observées ces dégradations, ces phénomènes ne pourraient cependant se produire qu'à des conditions de cuissons très intenses, de type cuisson au grill ou au barbecue.

Pour les PBDE, une étude réalisée sur le BDE 209 a permis d'observer que ce composé pouvait subir des phénomènes de débromation au cours de la cuisson (Bendig *et al.*, 2012). En effet, la cuisson (15min à 200°C) de poisson contaminé en BDE 209 a entraîné la formation de composés nona- et octa-bromés.

La libération des contaminants chimiques thermorésistants dans le jus de cuisson et la thermodégradation des contaminants thermosensibles sont donc les deux principales explications des pertes en contaminants chimiques observées au cours de la cuisson des produits animaux. Le phénomène de thermodégradation n'a cependant que peu été étudié dans la littérature. Ceci laisse supposer que certains contaminants présentant des pertes significatives au cours de la cuisson, tels que les sulfamides, pourraient subir une thermodégradation, bien que ce phénomène n'ait pas encore été décrit dans la littérature pour ces molécules. Pour finir, bien que l'évaporation des contaminants de faible poids moléculaire, tels que certains pesticides, ait été observée lors de la cuisson de produits végétaux (Abou-Arab, 1999), à notre connaissance, ce phénomène n'a pas été décrit lors de la cuisson de produits animaux.



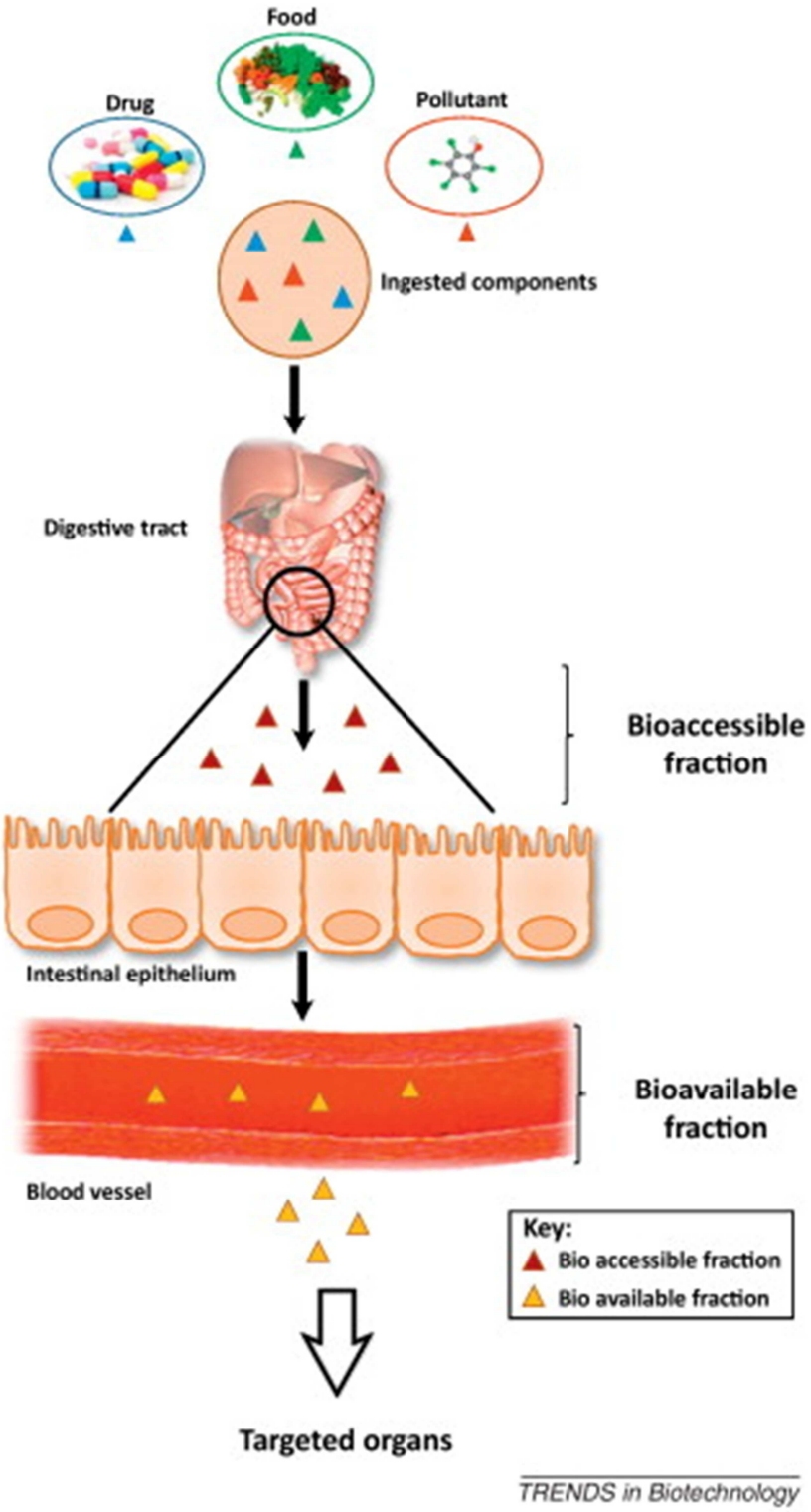
<u>Figure 9</u> : Principaux organes impliqués dans la digestion *(Guerra et al., 2012)***.**

3/ Devenir des contaminants chimiques au cours de la digestion des aliments

3.1/ Le processus de digestion

La digestion est un procédé séquentiel complexe faisant intervenir des transformations mécaniques et enzymatiques et lors duquel les aliments ingérés sont dégradés en nutriments qui seront utilisés pour les besoins de l'organisme (Figure 9). La digestion débute au niveau de la bouche où la mastication combinée à l'action de la salive et de ses enzymes permettent une première dégradation des aliments et la formation du bol alimentaire (Dean & Ma, 2007). Après un passage par l'œsophage, la digestion se poursuit dans l'estomac où le bol alimentaire entre en contact avec la solution gastrique qui contient en particulier de l'acide chlorhydrique (HCl) permettant une chute progressive du pH gastrique (d'approximativement 6,5 à 1,5), qui va permettre l'hydrolyse protéique (Guerra *et al.*, 2012). La dégradation protéique est amplifiée par la solution gastrique, composée d'enzymes comme la pepsine, alors que la dégradation mécanique des particules alimentaires par les ondes péristaltiques intervient au niveau distal de l'estomac et permet la formation du chyme (Guerra *et al.*, 2012). La digestion du chyme acide résultant des dégradations gastriques se poursuit au niveau des différentes parties de l'intestin grêle : le duodénum qui reçoit les sécrétions digestives du foie et du pancréas, le jéjunum puis l'iléon (Dean & Ma, 2007). Le bicarbonate de sodium ($NaHCO_3$) présent au niveau du duodénum permet tout d'abord de neutraliser l'acidité du chyme afin de rétablir un pH adapté aux activités des enzymes actives au niveau intestinal. Les enzymes pancréatiques (lipases, amylases, protéases) et les enzymes digestives produites par la paroi interne de l'intestin grêle agissent ensuite ensemble pour permettre la dégradation des constituants alimentaires. La bile produite au niveau du foie joue un rôle spécifique dans la digestion des lipides *via* leur émulsification en fines gouttelettes permettant le début de l'activité de la lipase pancréatique. Les résidus non absorbés au niveau de l'intestin grêle se retrouvent au niveau du colon où a lieu l'absorption d'eau et d'électrolytes, la fermentation des polysaccharides et des protéines par le microbiote, la réabsorption des sels biliaires et la formation, le stockage et l'élimination des fèces (Guerra *et al.*, 2012).

Lors de la digestion d'aliments contaminés, seule une fraction des contaminants pourra être libérée de la matrice alimentaire avant de pouvoir être absorbée par l'organisme et se retrouver au niveau de la circulation sanguine où elle pourra exercer des effets toxiques (Yu *et al.*, 2008 ; Yu *et al.*, 2011). Cette fraction est appelée la fraction bioaccessible. La bioaccessibilité d'un contaminant est alors calculée en déterminant le rapport entre la quantité



<u>Figure 10</u> : Représentation de la fraction bioaccessible qui correspond à la fraction de contaminants libérée de la matrice dans le tractus digestif au cours de la digestion *(Guerra et al., 2012).*

de contaminants présents dans cette fraction bioaccessible et la quantité totale de contaminants initialement présents dans la matrice alimentaire (Figure 10). Xing *et al.* (2008) ont par exemple montré que seuls 3% des PCB contenus dans des échantillons de poisson sont bioaccessibles. Ce faible pourcentage indique donc que, pour évaluer de manière fiable l'exposition humaine aux contaminants alimentaires, il est nécessaire de connaître cette fraction bioaccessible mobilisée de la matrice, qui correspond à la fraction maximale de contaminants effectivement disponibles pour l'absorption intestinale (Yu *et al.*, 2009).

3.2/ Les modèles *in vitro* d'étude de la bioaccessibilité digestive

L'étude *in vivo* du procédé complexe de la digestion est techniquement difficile, coûteux et limité par des contraintes éthiques lorsque l'étude porte sur des substances potentiellement dangereuses telles que des contaminants alimentaires. Afin d'étudier le devenir et la bioaccessibilité des contaminants au niveau du système digestif humain, il est donc nécessaire de développer des approches *in vitro* qui reproduisent les conditions physiologiques de la digestion (Guerra *et al.*, 2012).

Parmi les digesteurs *in vitro* développés pour évaluer la bioaccessibilité de polluants alimentaires, les modèles statiques représentatifs des conditions stomacales et intestinales avec un nombre limité de paramètres simulés sont les plus répandus et se sont révélés pertinents (Guerra *et al.*, 2012). La simulation de la phase gastrique de la digestion (pH=1-4 ; 37°C) s'appuie notamment sur l'utilisation d'une quantité déterminée de pepsine (Dean *et al.*, 2007). Cette étape peut être suivie par la modélisation d'une phase intestinale (pH=4-7,5 ; 37°C) avec l'utilisation d'enzymes pancréatiques et de sels biliaires.

Cependant, ces systèmes statiques ne reproduisent pas les processus dynamiques de la digestion tels que la vidange gastrique, les gradients de pH ou les flux de sécrétion. C'est pourquoi des modèles dynamiques ont été développés afin de se rapprocher des conditions réelles de la digestion chez l'Homme. Parmi eux, les digesteurs dynamiques à un seul compartiment permettent de modéliser la phase gastrique de la digestion avec notamment une acidification progressive du contenu gastrique *via* l'addition d'acide chlorhydrique. Les modèles dynamiques à deux compartiments miment quant à eux les conditions retrouvées au niveau de l'estomac et de l'intestin grêle proximal tandis que les modèles dynamiques multi-compartiments comme les systèmes de type TIM-1 (TNO gastro-Intestinal Model 1) représentent les conditions stomacales puis les conditions retrouvées au niveau du duodénum, du jéjunum et de l'iléon (Guerra *et al.*, 2012). Ces systèmes intègrent donc les variations de

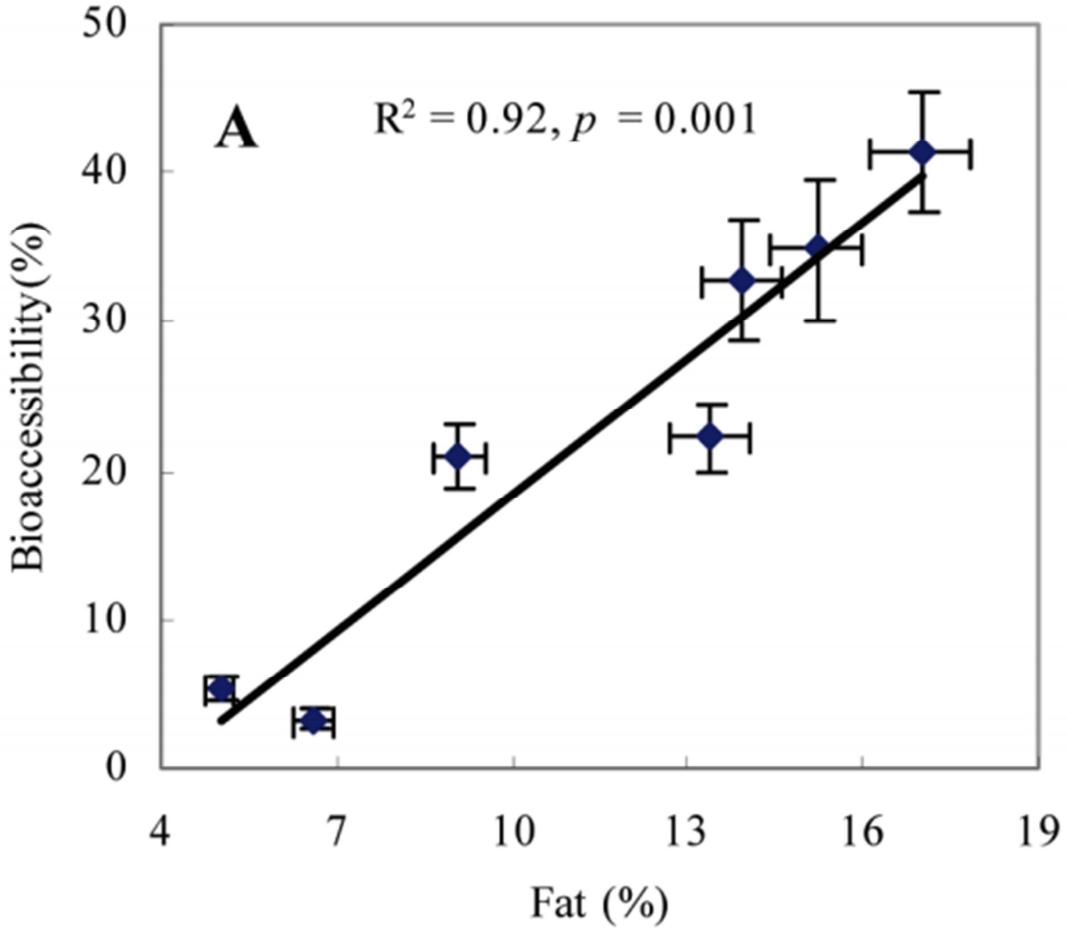
vitesse de vidange gastrique, de température, les cinétiques de pH ou encore les temps de transit retrouvés lors de la digestion, paramètres qui peuvent grandement influencer les valeurs de bioaccessibilité obtenues (Guerra *et al.*, 2012; Torres-Escribano *et al.*, 2011). Tous ces modèles, bien que pertinents, ne peuvent pas néanmoins rendre compte des mécanismes de rétrocontrôle, des contrôles hormonaux et nerveux, de l'intervention du système immunitaire ou encore de la complexité du microbiote et des mouvements péristaltiques retrouvés chez l'Homme lors de la digestion (Guerra *et al.*, 2012). Leur validation par des données *in vivo* reste donc nécessaire.

3.3/ Les facteurs de variation de la bioaccessibilité

Il existe une grande variabilité des données de bioaccessibilité disponibles dans la littérature. Plusieurs facteurs de variation pourraient expliquer ces différences : citons notamment la nature du contaminant étudié et ses propriétés physicochimiques, la variation de composition et de structure des matrices étudiées, les procédés de transformation des aliments avant ingestion (notamment la cuisson) ou encore les variations concernant les protocoles de digestion *in vitro* qui diffèrent entre les différentes études.

3.3.1/ Nature du contaminant

Les valeurs de bioaccessibilité dépendent tout d'abord de la famille de contaminants étudiés et, au sein d'une même famille, de la molécule ciblée. Lors d'une étude sur les métaux lourds contenus dans des fruits de mer, Amiard *et al.* (2008) ont par exemple observé que la bioaccessibilité moyenne de ces contaminants varie de 33% pour le plomb à 54% pour le cadmium. Xing *et al.* (2008) ont, quant à eux, remarqué que la bioaccessibilité des PCB contenus dans des échantillons de poissons ou de légumes variait selon le congénère étudié, les congénères les moins chlorés étant les plus bioaccessibles. Ces différences pourraient s'expliquer par les propriétés physico-chimiques des contaminants et notamment par leur caractère lipophile. Wang *et al.* (2010) ont ainsi suggéré que les HAP les plus lipophiles étaient les moins bioaccessibles. Cette hypothèse pourrait confirmer les résultats de Xing *et al.* (2008) précédemment discutés pour les PCB : les PCB les moins chlorés étant les moins lipophiles.



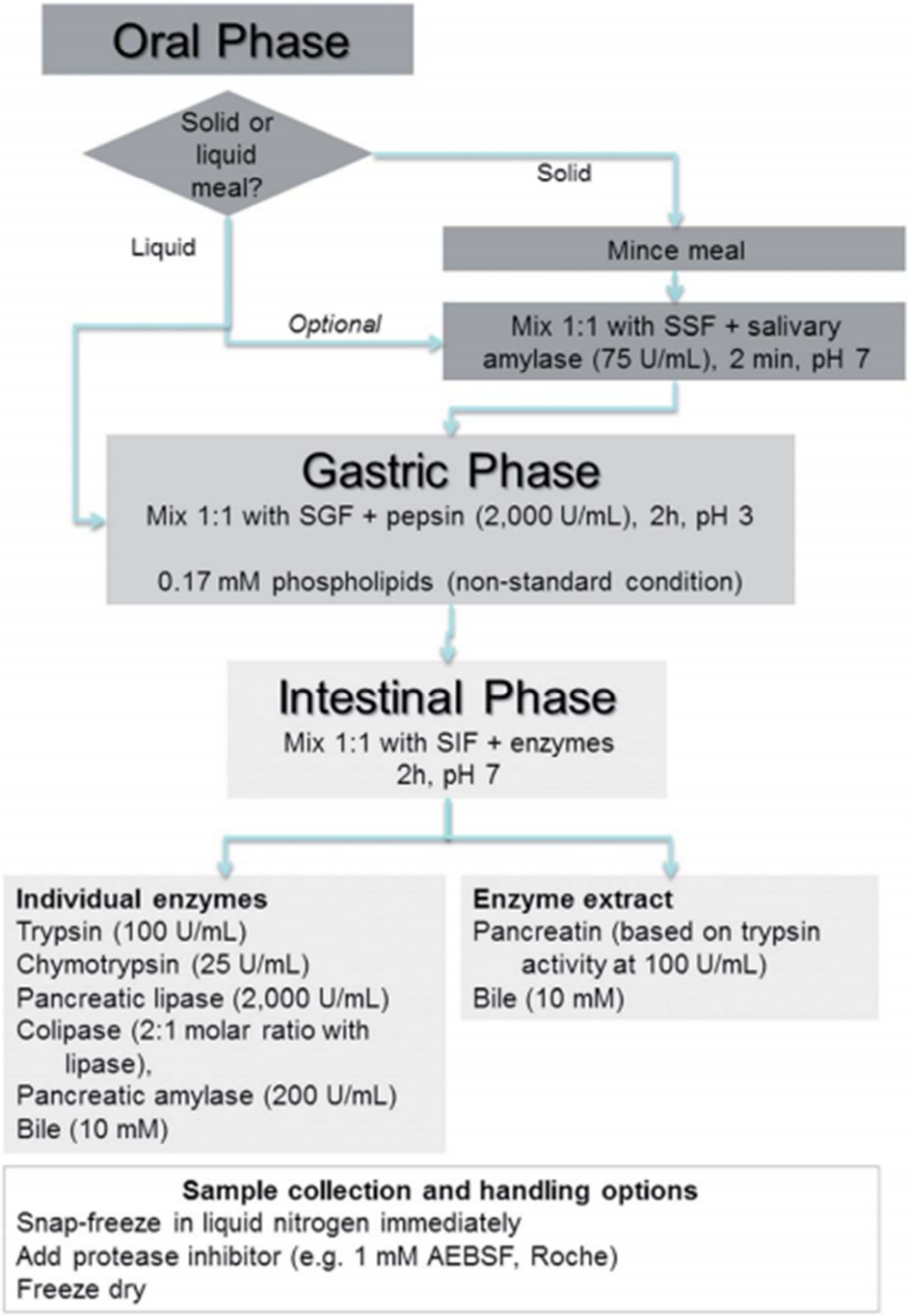
<u>Figure 11 :</u> Corrélation observée par Yu *et al.* (2011) entre la bioaccessibilité des PBDE et le taux de lipides des produits animaux étudiés.

3.3.2/ Nature de la matrice alimentaire

Au-delà de la nature du contaminant chimique, la composition et la structure de la matrice étudiée semblent impacter la bioaccessibilité. Des variations de la bioaccessibilité en fonction de la teneur en matière grasse des matrices étudiées ont notamment été mises en évidence. Xing *et al.* (2008) ont par exemple évalué à 3% la bioaccessibilité moyenne des PCB dans des échantillons de poisson contenant 3 à 15% de matière grasse alors que dans des échantillons d'aliments beaucoup moins gras tels que des épinards et des salades (0.1% de matière grasse en moyenne), la bioaccessibilité était significativement plus élevée (25%). En revanche, Yu *et al.* ont mis en évidence que la teneur en lipides correspondait au facteur prédominant affectant la bioaccessibilité des contaminants au-delà d'un seuil de 1.8% du poids frais (Yu *et al.*, 2011). A partir de ce seuil, ils ont observé une corrélation entre la bioaccessibilité des PBDE et le taux de lipides des produits animaux analysés (Figure 11). En ce qui concerne la structure de la matrice, Kulp *et al.* (2003) ont notamment observé qu'un broyage fin de la matrice alimentaire étudiée avant la réalisation des digestions *in vitro* pouvait augmenter les valeurs de bioaccessibilité des amines hétérocycliques (Kulp *et al.*, 2003).

3.3.3/ Influence des procédés de transformation : la cuisson

Les traitements préalables subis par les aliments avant leur consommation et leur digestion ont également une influence sur les valeurs de bioaccessibilité. La cuisson aurait par exemple un effet non négligeable, avec des variations observées selon les méthodes employées, l'intensité de la cuisson et la cinétique des traitements thermiques réalisés (Marques *et al.*, 2011; Maulvault *et al.*, 2011; Page *et al.*, 2012). Shen *et al.* (2016) ont par exemple observé que la bioaccessibilité des PCB de la viande était significativement plus faible ($p<0.05$) après cuisson par ébullition (5min à 100°C) qu'après friture dans de l'huile de cuisson (5min à 200-300°C). Différentes études réalisées sur des éléments métalliques traces (Cu, Cd, Zn…) ont également montré que la cuisson de poissons et produits de la mer entraînait généralement une diminution de la bioaccessibilité de ces aliments, quel que soit le mode de cuisson utilisé (cuisson à la poêle, au grill, par ébullition ou à la vapeur) (Amiard *et al.*, 2008 ; Metian *et al.*, 2009 ; He *et al.*, 2010). Une explication potentielle de la diminution de la bioaccessibilité lors d'une cuisson intense concernerait la dénaturation des protéines qui se produit lors de la cuisson, rendant la matrice plus compacte et limitant alors l'accès et donc



<u>Figure 12 :</u> Protocole international standardisé de digestion *in vitro* proposé par Minekus *et al.* (2014) afin de mimer les conditions physiologiques de digestion.

l'activité des enzymes digestives (Kulp *et al.*, 2003 ; He *et al.*, 2010). Ainsi, l'activité de la pepsine peut par exemple être réduite au cours de la digestion de viande cuite par rapport à de la viande crue comme cela a été démontré par Santé-Lhoutellier *et al.* (2008).

3.3.4/ Différences inter-laboratoires : les protocoles de digestion

Un autre facteur d'explication de la dispersion des valeurs retrouvées dans la littérature réside dans les différences entre les protocoles de digestion utilisés, que ce soit en termes de composition des solutions gastriques et intestinales, de pH ou de durée de digestion, alors que ces critères influencent significativement la bioaccessibilité (Maulvault *et al.*, 2011). La grande variabilité entre les protocoles en ce qui concerne les concentrations en enzymes et les activités enzymatiques est un premier facteur d'explication de la dispersion des valeurs de bioaccessibilité de la littérature (Minekus *et al.*, 2014). De plus, l'utilisation d'extraits biliaires d'espèces animales différentes et donc de composition et d'activité différentes peuvent influencer les mesures de bioaccessibilité. Ainsi, Oomen *et al.* (2004) ont par exemple observé que l'utilisation dans leur protocole *in vitro* de digestion de bile provenant de volaille entraînait des valeurs de bioaccessibilité 3 à 5,5 fois plus élevées que lors de l'utilisation de bile d'origine porcine ou bovine. Par ailleurs, le temps d'incubation de la matrice alimentaire avec les solutions gastro-intestinales, est lui aussi variable selon les protocoles et induit des différences de bioaccessibilité mesurée : une libération temps-dépendante des polluants alimentaires dans la solution intestinale a par exemple été observée lors de l'analyse d'échantillons de poisson (Yu *et al.*, 2009). Deux phases de libération successives ont été mises en évidence au cours du processus de digestion : une phase plus rapide durant les 2 premières heures où la bioaccessibilité augmente rapidement, puis une phase plus lente d'augmentation avant d'atteindre une valeur d'équilibre au bout de 6 heures (Yu *et al.*, 2009). Les valeurs de pH utilisées lors d'une digestion artificielle influencent également fortement le résultat final. Un faible pH au niveau de l'estomac induit par exemple des valeurs de bioaccessibilité plus élevées (Oomen *et al.*, 2002).

Afin de pouvoir *in fine* comparer objectivement les résultats obtenus entre les différents laboratoires, un réseau de scientifiques travaillant dans le domaine de la digestion a récemment proposé un protocole international standardisé de digestion *in vitro* permettant de mimer au mieux les conditions physiologiques de digestion (Minekus *et al.*, 2014). Ce protocole statique comprend les trois étapes classiques de digestion : orale, gastrique et intestinale (Figure 12). Ce protocole a été développé pour simuler une digestion modèle chez

l'adulte mais il est important de garder à l'esprit que des variations des conditions de digestion, notamment en termes de concentrations en enzymes digestives et en sels biliaires, existent physiologiquement chez l'Homme en fonction de l'âge (Levi *et al*., 2014 ; Dupont *et al*., 2010 ; Poquet *et al*., 2016 ; Rémond *et al*., 2015).

Pour conclure, l'état des lieux des données disponibles dans la littérature concernant la bioaccessibilité des contaminants montre que la variabilité des protocoles de digestion utilisés empêche de comparer objectivement les données entre elles et de tirer des conclusions générales. L'utilisation d'un protocole « consensus » standardisé semble donc un prérequis indispensable à la poursuite des travaux dans ce domaine. L'analyse des données de la littérature a par ailleurs permis de mettre en évidence les principaux facteurs de variation pertinents à étudier dans les études de la bioaccessibilité. Il s'agit de la composition de la matrice étudiée, de l'intensité de cuisson de cette matrice ou encore des concentrations et activités des enzymes digestives et des sels biliaires qui peuvent varier physiologiquement en fonction de l'âge du consommateur.

4/ Conclusion

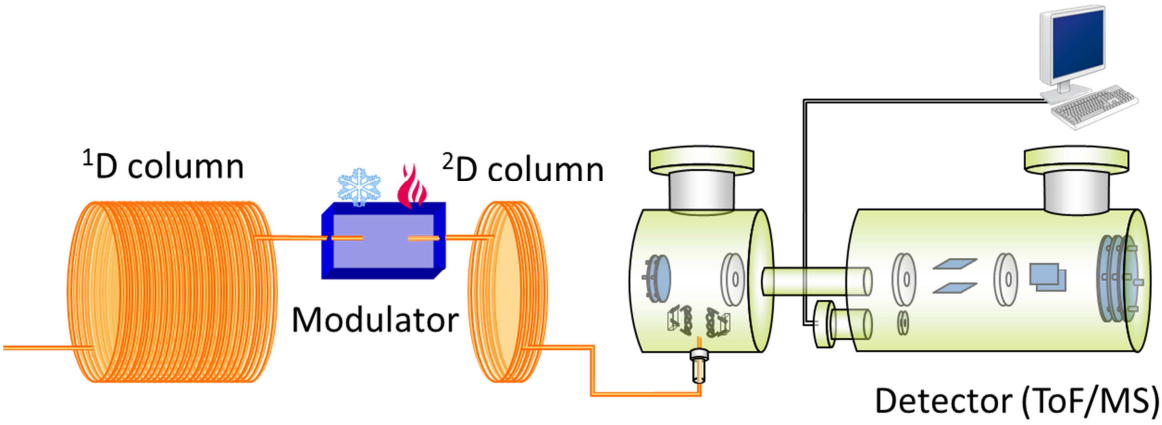
Cette synthèse bibliographique montre que la cuisson et la digestion sont des phénomènes qui peuvent moduler significativement l'exposition des consommateurs aux contaminants chimiques des aliments. Cependant, la variabilité importante des données disponibles dans la littérature ne permet actuellement pas de prendre en considération ces phénomènes dans les évaluations du risque.

La dispersion des données de la littérature s'explique en partie par la variabilité de la composition des matrices et des niveaux de contamination étudiés. Afin de pallier ce verrou, un des prérequis de ce travail de thèse est de pouvoir disposer tout au long de l'étude d'une **matrice de référence intentionnellement contaminée** de manière homogène avec une même dose de micropolluants. Cette synthèse souligne également que les études s'intéressant au devenir des contaminants au cours de la cuisson ou de la digestion ne ciblent le plus souvent qu'un faible nombre de contaminants alors qu'il serait intéressant de pouvoir élargir le spectre des micropolluants étudiés en analysant des matrices intentionnellement multi-contaminées avec des **méthodes multi-résidus**. Ces **développements méthodologiques** constituent l'objectif des recherches décrites dans le **chapitre 2** de ce manuscrit.

Ensuite, les travaux de la littérature qui concernent l'impact de la cuisson sur la teneur de la viande en micropolluants s'appuient sur des modes et des intensités de cuisson souvent peu décrits, peu contrôlés et parfois peu réalistes alors qu'il serait nécessaire d'obtenir des données fiables, reproductibles et réalistes en se focalisant sur le principal mode de cuisson domestique utilisé en France, à savoir la **cuisson à la poêle**. L'incidence de ce mode de cuisson sur les trois principales familles de contaminants de la viande, les **micropolluants organiques** environnementaux, les **métaux lourds** et les **antibiotiques** est le sujet des recherches présentées dans le **chapitre 3**.

Enfin, en ce qui concerne les protocoles de digestions *in vitro* utilisés pour déterminer la bioaccessibilité des contaminants, ceux-ci sont également très variables, limitant alors la comparaison des données entre elles et ainsi la portée des conclusions. L'utilisation du protocole standardisé développé par Minekus *et al.* (2014) pourrait cependant apporter une réponse pertinente à cette problématique. Dans ces conditions de **digestion standardisée**, l'étude des principaux facteurs de variation de la **bioaccessibilité** que sont la teneur en **lipides** de la matrice, l'intensité de **cuisson** et les variations physiologiques liées à **l'âge du consommateur** permettrait également d'apporter des informations complémentaires à celles de la littérature. Ces travaux constituent le cœur des recherches décrites dans le **chapitre 4**.

Chapitre II : Mise au point d'une méthode d'analyse multirésidus des polluants environnementaux dans une viande surchargée



<u>Figure 13 :</u> Schéma d'un système de chromatographie en phase gazeuse bidimensionnelle systématique couplé à un spectromètre de masse à temps de vol (GC×GC-TOF/MS).

Chapitre II : Mise au point d'une méthode d'analyse multirésidus des polluants environnementaux dans une viande surchargée

La première partie de ce travail de recherche a consisté à mettre en place une méthode analytique « multirésidus » pour suivre dans une viande intentionnellement contaminée deux familles de contaminants pertinentes en raison de leur fréquence de détection dans les produits carnés : les PCB et les PCDD/F.

209 congénères de PCB et 17 congénères toxiques de PCDD/F peuvent être retrouvés dans l'environnement. Les méthodes multirésidus, qui permettent le suivi simultané d'un large spectre de molécules, sont donc particulièrement intéressantes pour l'analyse de ces deux familles de contaminants (Tang *et al.*, 2013). L'utilisation de la chromatographie en phase gazeuse bidimensionnelle (GC×GC) est alors une technique de choix. Comme l'illustre la Figure 13, cette technique s'appuie sur la combinaison en série de deux colonnes de chromatographie en phase gazeuse, une première colonne de dimensions « classiques » et une deuxième colonne beaucoup plus courte dimensionnée pour des séparations dite « rapides ». Ces deux colonnes ont une sélectivité différente et sont séparées par une interface appelée modulateur dont le fonctionnement nécessite l'utilisation de liquide cryogénique, le plus souvent de l'azote liquide. Cet outil permet la séparation des analytes sur deux dimensions indépendantes et orthogonales avec un pouvoir de séparation élevé (Focant *et al.*, 2004a). Le spectromètre de masse à temps de vol (TOF/MS) est un détecteur de choix lors de l'analyse d'un mélange complexe de composés puisqu'il est doté d'une fréquence d'acquisition élevée requise notamment par la chromatographie rapide qui est réalisée à chaque modulation sur la seconde dimension (Focant *et al.*, 2004a). La combinaison GC×GC-TOF/MS s'avère donc particulièrement indiquée pour l'analyse de mélanges de contaminants avec des structures chimiques très proches (Focant *et al.*, 2004a, Engel *et al.*, 2013).

Plusieurs auteurs se sont déjà intéressés à l'analyse simultanée par GC×GC-TOF/MS de tous les congénères de PCB ou des congénères toxiques de PCDD/F (Megson *et al.*, 2013 ; Focant *et al.*, 2004a ; Focant *et al.*, 2004b ; Focant *et al.*, 2005 ; Hoh *et al.*, 2007 ; Zapadlo *et al.*, 2011). Cependant, aucune étude ne s'est encore intéressée à l'analyse simultanée des 209 congénères de PCB et des 17 congénères toxiques de PCDD/F. C'est donc un des objectifs de cette première partie. Le temps d'analyse maximum a ici été fixé à 75min afin de pouvoir utiliser, par la suite, cette méthode en routine. Une fois la méthode analytique développée en

solvant pur, cette dernière a été éprouvée en matrice complexe en prenant comme modèle de la viande intentionnellement contaminée en PCB et PCDD/F.

De la viande hachée a été choisie comme modèle dans cette étude. La viande de bœuf à 15% de matière grasse étant la viande hachée la plus consommée en France, cette matrice a été choisie comme référence. 40kg de steak haché issus d'un même lot nous ont alors été fournis par un fabricant de steaks hachés surgelés afin de pouvoir travailler sur le même produit tout au long de ce travail de thèse et ainsi limiter la variabilité entre échantillons. La viande hachée présente l'avantage de pouvoir être homogénéisée facilement après avoir été intentionnellement contaminée.

De nombreuses études de la littérature s'appuient sur l'utilisation de matrices alimentaires intentionnellement contaminées. En effet, à l'inverse des aliments naturellement contaminés, l'utilisation de ces matrices permet de limiter la variabilité des échantillons en termes de structure ou encore de composition en eau et en lipides qui sont des facteurs confondants pour les études que nous envisageons de mener sur la cuisson ou la digestion. La surcharge intentionnelle en micropolluants permet également de contrôler le niveau de contamination des aliments et d'éviter d'être confronté à des problèmes de sensibilité des méthodes analytiques ou à des biais éventuels liés à des différences de rendement de récupération à l'extraction en fonction de la concentration en contaminants. Bien que de nombreuses études de la littérature reposent sur l'utilisation de matrices intentionnellement contaminées, très peu de données sont disponibles concernant les méthodes employées pour réaliser la surcharge en contaminants. Afin d'obtenir une viande intentionnellement contaminée de manière homogène et reproductible, plusieurs techniques de contamination de la viande ont donc été testées et comparées. Ces techniques couplent toutes une étape de surcharge dans la viande des PCB et PCDD/F préalablement dilués dans du solvant et une étape d'homogénéisation de la viande.

L'extraction des PCB et PCDD/F de la viande est une étape lourde et délicate à mettre en œuvre au cours de laquelle des composés problématiques pour une analyse ultérieure par GC, tels que les lipides, peuvent être co-extraits avec les composés d'intérêt. Au cours des dix dernières années, le développement de l'extraction accélérée par solvant (ASE) qui permet de réaliser des extractions au solvant, sous pression et à une température élevée, a permis d'améliorer la vitesse et les rendements d'extraction (Sun *et al.*, 2012). Cette technique a donc été utilisée et optimisée dans cette étude. Cependant, en raison de la teneur en matière grasse élevée de la matrice ciblée, une délipidation des extraits a été nécessaire. Deux étapes de délipidation ont donc été mises en place *via* l'ajout d'alumine dans la cellule d'extraction

ASE, selon Bjorklund *et al.* (2000), permettant ainsi de limiter la co-extraction des lipides avec les PCB et PCDD/F et *via* une chromatographie par perméation de gel (GPC) post-extraction selon Saito *et al.* (2004).

Article 1

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Assessment of comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry based methods for investigating 206 dioxin-like micropollutants in animal-derived food matrices☆

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ABSTRACT

This paper evaluates different multiresidue methods based on comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC-TOF/MS) to analyze dioxin-related micropollutants in complex food matrices. In a first step, the column sets Rtx-PCB/BPX-50 and Rtx-Dioxin2/BPX-50 were compared in terms of peak shape (width and symmetry) and resolution for the separation of polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) in solvent. A satisfactory separation of 206 dioxin-related micropollutants including the 17 toxic PCDD/Fs was achieved in 75 min with the column set Rtx-Dioxin2/BPX-50. In a second time, the GC × GC-TOF/MS method was spread to the analysis of dioxin-related micropollutants in complex food matrices. An extraction procedure including accelerated solvent extraction (ASE), centrifugal evaporation and gel permeation chromatography (GPC) was optimized. Starting with meat as a model matrix, a micropollutant spiking method was then set up by comparing seven methods in terms of recoveries and reproducibility. The method combining immersion of the meat in a large volume of solvent containing micropollutants followed by homogenization by blender induced recoveries in the acceptable range of 70–130% and satisfactory standard deviations (≤10%) for most of the compounds studied. Limits of detection of the GC × GC-TOF/MS method ranged between 50 and 100 pg/g of spiked fresh meat for PCBs and between 65 and 227 pg/g for PCDD/Fs. Potential applications of this method are discussed.
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1. Introduction

The principal source of human exposure to persistent organic pollutants (POPs) is food, and most particularly, the consumption of animal products [1,2]. With the safety of health in mind, it is therefore important to accurately assess the risk the presence of these substances in food has on human health. This risk is currently assessed by determining the micropollutant content in raw food, yet knowing solely the content in the raw material is not enough to assess the amount actually assimilated by consumers. In actual fact, the transformations undergone by the food whether they be technological such as cooking [3,4] or physiological such as digestion [5,6] should also be taken into consideration. To assess the impact of these transformations on the contaminants contained in food, multiresidue methods (MRM) are particularly valuable as they allow for simultaneous monitoring of a large number of molecules in a single analysis. Of the toxic contaminants potentially present in food and remaining a current concern [7], polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are interesting candidates to monitor as these families of molecules have a wide variety of congeners whose structure is not affected by the different stages of the analytical protocol due to their thermoresistance. PCBs, used on a massive scale as insulating materials in industry up until the 1980s and PCDD/Fs, whose release is related to human and industrial activities and particularly combustion phenomena, are also persistent in the environment [8,9]. In addition, these molecules are carcinogenic, mutagenic and reprotoxic, mainly *via* their action

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on the aryl hydrocarbon receptor (AhR) [10,11]. Thus, the World Health Organization has defined toxic equivalency factors (WHO-TEF) to assess the toxicity of dioxin-like PCBs and PCDD/Fs [9]. The European Regulations laying down methods of sampling and analysis for the EU official control of levels of PCDD/Fs and PCBs in food and feed have been recently amended by EU Regulations No. 589/2014 and 709/2014.

To analyze PCBs and PCDD/Fs, several studies have shown the relevance of using two-dimensional gas chromatography (GC × GC) coupled with time-of-flight mass spectrometry (TOF-MS) [12,13]. This method is particularly useful on complex matrices because the high resolution provided by GC × GC enables to reduce any overlap between target analytes and matrix interferences, and to get lower LODs than with GC-TOF/MS [14,15]. Additionally, even if sensitivity and linearity range of GC × GC-TOF/MS are lower than that of GC-HRMS [13] which is used by reference laboratories, it could be a relevant alternative to study the impact of technological or physiological transformation on the level of contaminants in food. Although, to our knowledge, no study to date has explored the simultaneous analysis of all the congener profiles of PCBs and PCDD/Fs, several authors have worked on optimising the separation of 209 PCB congeners or 17 toxic dioxin congeners in solvent. For the PCBs, 188, 192 and 196 of the 209 congeners were respectively able to be separated with the Rtx-PCB/Rxi-17, HT8/BPX-50, and SPB-Octyl/SLB-IL59 columns sets [16–18]. For the dioxins, the 17 toxic congeners were able to be separated by GC × GC-TOF/MS coupled with an Rtx-Dioxin2/Rtx-PCB column set [19]. The Rtx-Dioxin2 column was also used coupled to a Rtx-500 column to analyze 17 toxic PCDD/F congeners and 4 dioxin-like PCB congeners [12]. In view of this research, the Rtx-PCB, HT8, SPB-Octyl and Rtx-Dioxin2 columns in first dimension seem to be the most promising to simultaneously analyze the existing 209 PCB congeners and the 17 toxic dioxin congeners by GC × GC-TOF/MS. For coupling with these different columns, the use of a BPX-50 type column in second dimension appears to be the preferred and classic choice [17,20].

To be able to study the health risk associated to the presence of PCBs and PCDD/Fs in food, the analytical methods assessed in pure solvent should then be validated on real and complex matrices the contaminants of which should be extracted with good recovery rates [21]. To do this, homogeneous and reproducible multicontaminated products are required [22]. Several studies have explored spiking with contaminants in environmental matrices such as soils [23], even if the spiking methods used were highly varied and the descriptions often incomplete. The literature relating to spiking food matrices with contaminants is virtually nil. Efficient methods for food spiking must therefore be found.

In order to ultimately improve the assessment of the risk related to chemical contaminants of food, this study is aimed at proposing an analysis method to monitor toxic dioxin-like PCB and PCDD/F congeners in complex food matrices such as meat products with a high water, protein and fat content. A first study will be conducted to assess the relevance of GC × GC-TOF/MS to simultaneously monitor the PCBs and PCDD/Fs. In order to obtain the best possible chromatographic separation of these contaminants, the performance of different column combinations will initially be compared in pure solvent on the most toxic compounds [24] and most frequently found in meat [25] based on peak shape [14] and peak resolution [18]. When the pure solvent analytical method is set up, it will then be proven in complex matrices taking high fat content ground meat as a model. To do this an extraction, concentration and clean-up method will be proposed working on a matrix destructured by freeze-drying. Different spiking scenarios will then be compared to a structured matrix based on the standard deviations and recovery rates obtained on the different contaminants studied. The performance of the analytical method will then be assessed in terms of linearity (R^2) and sensitivity (LOD) on a spiked matrix.

2. Materials and methods

2.1. Chemicals and standards

Hexane, dichloromethane and toluene were organic trace analysis grade solvents (Sigma-Aldrich, Saint-Quentin Fallavier, France). Activated aluminum oxide (acidic, Brockmann I) and diatomaceous earth used for the preparation of ASE cells were obtained from Sigma-Aldrich. Micropollutant reference standards were obtained from AccuStandard Europe (Niederbipp, Switzerland) and LGC Standards (Molsheim, France) for the 209 PCBs and the 17 PCDD/Fs, respectively. The 209 PCBs were divided into nine stock solutions concentrated to 10 ng/μL. For the PCDD/Fs, the concentration of the stock solution was different according to the congeners: tetra's 2.5 ng/μL, penta's, hexa's and hepta's 6.25 ng/μL and octa's 12.5 ng/μL. Internal standards were used for the accurate quantification of target compounds: 3′-F-PCB-28 (Chiron, Trondheim, Norway), 3′-F-PCB-81 (Chiron), 13C-labelled PCB-101 (Wellington laboratories, Guelph, ON, Canada) and 13C-labelled PCB-194 (Wellington).

2.2. Sample spiking

Ground beef samples (15% fat) were purchased from a French supplier. Matrix blanks consisting in spiking meat with pure dichloromethane without micropollutant were made on five different unspiked meat samples to determine the level of PCBs or PCDD/Fs in fresh meat. Seven methods for ground beef spiking were compared with a concentration of 2 ng/g of fresh meat chosen to give concentration in ready-to-run samples within the range of linearity of GC × GC-TOF/MS for these compounds. These methods combined micropollutant addition to 120 g of ground meat and matrix homogenization. Two approaches for micropollutant addition were tested: (i) addition of a micro-volume of dichloromethane (1 mL) containing micropollutants to ground beef, (ii) immersion of ground beef in a large volume of dichloromethane (20 mL) containing micropollutants followed by evaporation under hood. Dichloromethane (DCM) was used as solvent in the spiking method because of the high solubility of PCBs and PCDD/Fs in DCM and because of the high volatility of this solvent. After micropollutant addition, four homogenization methods were tested: 2 min with a stand mixer, 2 min with a blender, 2 min with an Ultra-turrax and 3 min with a liquid nitrogen grinder.

2.3. Extraction, clean-up and concentration

Extraction and clean-up were performed according to Saito *et al.* with slight modifications [26]. Briefly, all samples were extracted by Accelerated Solvent Extraction (ASE) using a Dionex ASE 350 extractor (Sunnyvale, CA, USA). 34 mL stainless-steel extraction cells were filled from bottom to top with 15 g of acidic alumina and 5 g of ground beef dispersed with diatomaceous earth according to Bjorklund *et al.* [27]. Paper filters were placed at the bottom of the cell and between the layers. ASE extraction included heating (5 min), static time (5 min) and purging (90 s) with two extractions per sample. Hexane was used as extraction solvent at a temperature of 100 °C and pressure of 1500 psi. After filtration through a 0.2 μm filter (Phenomenex, Torrance, CA), the extract (approximately 40 mL) was evaporated (Rocket, Genevac Ltd.) using toluene as keeper in order to minimize loss of analytes during the evaporation step, then 4 mL of dichloromethane were added. Gel Permeation Chromatography (GPC) (Gilson, Middleton,

WI, USA) purification was carried out on a S-X3 Bio-Beads column using dichloromethane as eluting solvent at a flow rate of 5 mL/min. The fraction obtained was evaporated to dryness (Rocket, Genevac Ltd.) and redissolved in 100 μL of hexane prior to analysis. All samples were spiked with internal standards at 100 ng/mL at different steps (ASE, evaporation and GC × GC analysis).

2.4. GC × GC-TOF/MS analysis

Samples were analyzed on a time-of-flight mass spectrometer (Pegasus 4D, Leco, St Joseph, MI) coupled to a two-dimensional gas chromatograph (6890, Agilent Technologies) equipped with a dual stage jet cryogenic modulator (licensed from Zoex). Four 1D columns were tested in the study: SPB-Octyl (60 m × 0.25 mm × 0.25 μm) (Sigma-Aldrich), HT8 (50 m × 0.25 mm × 0.25 μm) (SGE, Austin, TX, USA), Rtx-PCB (60 m × 0.18 mm × 0.18 μm) (Restek, Bellefonte, PA, USA) and Rtx-Dioxin2 (60 m × 0.25 mm × 0.25 μm) (Restek). The 1D column was connected by a deactivated ultimate union (Agilent Technologies, Santa Clara, CA) to a BPX-50 (2 m × 0.1 mm × 0.1 μm) (SGE) 2D column. A splitless injection of 1 μL of sample extract was performed through a CTC CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) with an inlet temperature set at 280 °C. A split/splitless inert liner (Restek, sky® 4.0 mm ID liner) was used. Ultra-pure grade helium (purity 99.9995%) was used as carrier gas with a constant flow rate of 1.5 mL min^{-1}. Purge time was set to 60 s with a flow rate of 50 mL min^{-1}. The primary oven temperature was initially set at 90 °C for 1 min, then increased to 200 °C at 20 °C min^{-1}, then to 308 °C at 2 °C min^{-1} and at 5 °C min^{-1} to 330 °C for 10 min. The secondary oven temperature was set at 5 °C higher than the primary oven temperature. The modulator temperature was set at 15 °C higher than the primary oven temperature and the modulation period was 5 s with 1.20 and 1.30 s for the hot and cold pulses, respectively. The transfer line temperature was set at 280 °C. The mass spectrometer was operated with an ionization energy of 70 eV, a detector voltage of 1800 V and a data acquisition rate of 100 spectra s^{-1}. The run time for each sample was 75 min. Analytical blank samples of pure solvent were run to check the absence of targeted analytes. GC × GC data were processed using the LECO ChromaTOF software (version 4.50.8.0).

2.5. Peak shape, resolution factor, and limit of detection

The peak shape of targeted compounds was studied through the peak width at half height and the tailing factor. The mass fragments used for identification and quantification of PCBs and PCDD/Fs are given in supporting information (Table SI). Peak width at half height is defined as the width of a peak (in time units) at half peak height. Tailing factor (T) provides a peak symmetry metric defined by the formula $T = w_{0.05}/2f$, where $w_{0.05}$ is the peak width defined at 5% of peak height and f is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline [14]. Resolution factor (R_s) was calculated according to Zapadlo *et al.* [18]. Briefly, $R_s = \Delta t_R/w_b$, where t_R is the retention time and w_b is the mean peak width at the base. Neighboring peaks were considered as resolved for a resolution factor $R_{s,1D} \geq 0.6$ in the 1D or $R_{s,2D} \geq 0.4$ in the 2D. Calibration curves were generated for 19 PCBs and 7 PCDD/Fs from seven concentration levels: 10, 25, 50, 100, 250, 500 and 1000 ng mL^{-1} prepared both in solvent and in matrix. Each concentration level was injected in triplicate. Calibration curves were constructed by plotting the analyte/internal standard peak area ratio against the concentration. The linearity of the calibration curves was assessed for each compound by calculating the coefficients of determination (R^2). The limit of detection (LOD), using the definition $3s/m$ (where s is the standard deviation of the intercept, and m is the slope of the linear calibration curve), was determined from the calibration curves for each individual compound studied [14]. The "lm" (linear model) function of R (release 2.14.0) [28] was used on the calibration curve data for the determination of R^2 as well as s and m requested for LOD calculation.

3. Results and discussion

3.1. Comparison of column sets in pure solvent

Preliminary analyses with the SPB-Octyl and HT8 columns revealed for the first, significant bleeding as well as a maximum operating temperature (280 °C) limited for the study of the PCDD/Fs and for the second, a low separation capacity (data not shown). Rtx-PCB and Rtx-Dioxin2 were therefore used as a first dimension with a run time determined to get the best separation of dioxin-like micropollutants without wrap-around.

3.1.1. Peak shape

In order to compare the two column sets selected for this study, Rtx-Dioxin2/BPX-50 and Rtx-PCB/BPX-50, the width at half height and the tailing factor of the peaks obtained on analysis of the 209 PCBs and 17 toxic PCDD/Fs in pure solution were determined [14].

Table 1 shows the widths at half height obtained for the 17 PCDD/Fs and 18 PCBs considered as the most relevant due to the frequency of their presence in meat (12 dioxin-like and 6 PCB congeners which represent up to 50% of the PCBs found in food matrices) [25]. Mean width at half height of the peaks obtained on analysis of the 209 PCBs is 0.119 ± 0.021 s for the column set Rtx-PCB/BPX-50 and 0.087 ± 0.014 s with the Rtx-Dioxin2/BPX-50 set. Width at half height of the peaks obtained for the 17 PCDD/Fs is greater than that obtained for the PCBs as it is 0.126 ± 0.005 and 0.099 ± 0.008 s, respectively, for the column sets Rtx-PCB/BPX-50 and Rtx-Dioxin2/BPX-50. The results show that the width at half height of the peaks obtained with the column set Rtx-Dioxin2/BPX-50 is less than that obtained with the column set Rtx-PCB/BPX-50 both for the 209 PCBs and the 17 PCDD/Fs. Despite optimizations of hot/cold pulses, modulation period and temperature offset (data not shown), peaks are wider than 0.06 s of peak width at half height obtained for PCBs and PCDD/Fs by Focant *et al.* [13].

Two or three modulations per peak could be observed for the 17 PCDD/Fs and 18 PCBs most relevant in meat showing no significant tailing in the first dimension. The tailing factors obtained for these compounds on the second dimension are shown in Table 1. The mean tailing factor of the peaks of the 209 PCBs is 1.08 ± 0.18 for the column set Rtx-PCB/BPX-50 and 1.21 ± 0.23 with the column set Rtx-Dioxin2/BPX-50. Concerning the 17 PCDD/Fs, the mean tailing factor obtained is 1.11 ± 0.17 and 1.32 ± 0.15, respectively, for the column sets Rtx-PCB/BPX-50 and Rtx-Dioxin2/BPX-50. The tailing factor obtained is therefore higher with the column set Rtx-Dioxin2/BPX-50 regardless of the family of compounds.

The column set Rtx-Dioxin2/BPX-50 allows for finer peaks to be obtained but with a greater tailing factor than the Rtx-PCB/BPX-50 set. It is therefore difficult to choose the column set on this criterion.

3.1.2. Peak resolution

In order to objectively compare the separation capacity of the two column sets studied, resolution factors were calculated as per Zapadlo *et al.* [18]. Out of the existing 209 PCB congeners, 189 were thus resolved with the column set Rtx-Dioxin2/BPX-50 compared to 194 with the column set Rtx-PCB/BPX-50. In both cases, PCBs 126 and 169 with the highest WHO-TEFs are not coeluted. These results are similar to those obtained by Focant *et al.* [17] and Megson *et al.* [16] who managed to respectively separate 192 PCBs with the column set HT8/BPX-50 and 188 PCBs with the Rtx-PCB/Rxi-17 set. However, the analysis time was 146 and 153 min, respectively,

Table 1
Comparison of the peak shape obtained by GC × GC-TOF/MS for 18 most relevant PCBs and 17 PCDD/Fs with Rtx-Dioxin2/BPX-50 and Rtx-PCB/BPX-50, respectively.

Compound	Congener	Rtx-Dioxin2/BPX50		Rtx-PCB/BPX50	
		W_h [1]	T [2]	W_h	T
2,4,4′-TCB	BZ-28	0.077	1.214	0.100	1.000
2,2′,5,5′-TCB	BZ-52	0.125	0.947	0.121	1.100
2,2′,4,5,5′-PCB	BZ-101	0.085	1.127	0.116	1.031
3,4,4′,5-TCB	BZ-81	0.092	1.250	0.120	0.833
3,3′,4,4′-TCB	BZ-77	0.088	1.417	0.124	0.752
2′,3,4,4′,5-PeCB	BZ-123	0.097	1.143	0.145	0.562
2,3′,4,4′,5-PeCB	BZ-118	0.089	1.106	0.116	1.027
2,3,4,4′,5-PeCB	BZ-114	0.117	1.261	0.128	0.875
2,2′,4,4′,5,5′-HxCB	BZ-153	0.088	1.563	0.128	0.875
2,3,3′,4,4′-PeCB	BZ-105	0.090	1.308	0.128	0.794
2,2′,3,4,4′,5′-HxCB	BZ-138	0.148	0.994	0.130	1.416
3,3′,4,4′,5-PeCB	BZ-126	0.083	1.278	0.059	0.969
2,3′,4,4′,5,5′-HxCB	BZ-167	0.040	1.071	0.107	1.071
2,3,3′,4,4′,5-HxCB	BZ-156	0.085	1.042	0.110	0.978
2,3,3′,4,4′,5′-HxCB	BZ-157	0.092	1.091	0.110	1.161
2,2′,3,4,4′,5,5′-HpCB	BZ-180	0.091	1.500	0.135	1.079
3,3′,4,4′,5,5′-HxCB	BZ-169	0.082	0.891	0.109	0.762
2,3,3′,4,4′,5,5′-HpCB	BZ-189	0.092	0.979	0.122	1.133
2,3,7,8-TCDF		0.092	1.409	0.118	0.974
2,3,7,8-TCDD		0.081	1.318	0.119	1.067
1,2,3,7,8-PeCDF		0.095	1.219	0.125	1.068
2,3,4,7,8-PeCDF		0.100	1.577	0.124	1.068
1,2,3,7,8-PeCDD		0.094	1.269	0.127	0.972
1,2,3,4,7,8-HxCDF		0.100	1.367	0.127	0.947
1,2,3,6,7,8-HxCDF		0.096	1.417	0.131	0.865
2,3,4,6,7,8-HxCDF		0.102	1.567	0.121	1.079
1,2,3,4,7,8-HxCDD		0.106	1.375	0.129	1.263
1,2,3,6,7,8-HxCDD		0.098	1.115	0.131	1.000
1,2,3,7,8,9-HxCDD		0.100	1.071	0.125	1.357
1,2,3,7,8,9-HxCDF		0.107	1.500	0.134	1.294
1,2,3,4,6,7,8-HpCDF		0.091	1.292	0.128	1.025
1,2,3,4,6,7,8-HpCDD		0.091	1.286	0.129	1.361
1,2,3,4,7,8,9-HpCDF		0.097	1.333	0.121	1.071
OCDD		0.109	1.200	0.121	1.452
OCDF		0.118	1.139	0.126	1.056

[1] Peak width at half height in 2D seconds, determined from the main peak modulation.
[2] Tailing factor defined by the formula $T = w_{0.05}/2f$, where $w_{0.05}$ is the peak width defined at 5% of peak height and f is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline [14].

in these two studies compared to only 75 min in our study. Even if the proposed analytical method led to wider peaks than former studies, it enables a separation comparable to literature data while reducing the analysis time significantly. With regard to the 18 most relevant PCBs for our meat model matrix, the two column sets have an equivalent separation capacity with 15 PCBs resolved out of the 18. Of these 18 PCBs, the coelution between PCBs 101 and 90 found with the two column sets were already reported by Megson *et al.*, who used a column set Rtx-PCB/Rxi-17 and by Zapadlo *et al.* with a column set SPB-Octyl/SLB-IL59 [16,18]. In addition, the coelutions found between PCBs 138 and 163 with the column set Rtx-Dioxin2/BPX-50 and between PCBs 123 and 107 with the Rtx-PCB/BPX-50 set were already described in other studies [17].

Out of the 17 toxic PCDD/Fs, the column set Rtx-Dioxin2/BPX-50 allows for all the compounds to be separated. In fact, although the calculation of the resolution factors shows coelution between 2,3,4,7,8-PeCDF and PCB 203, these two compounds were considered as resolved due to the presence of specific ions in their respective mass spectra allowing for individual monitoring. These results are consistent with those obtained by Focant *et al.* [12] and Hoh *et al.* [19] who succeeded in correctly separating the 17 toxic PCDD/F congeners *via* GC × GC–MS with the column set Rtx-Dioxin2 in the first dimension. With the column set Rtx-PCB/BPX-50, 2 congeners out of the 17 PCDD/Fs are coeluted, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD. With a TEF = 1, 1,2,3,7,8-PeCDD is however very relevant to analyze as it is part of the most toxic PCDD/Fs and the most monitored in food. Fig. 1 shows the coelution of this compound obtained with the Rtx-PCB/BPX-50 set compared to the Rtx-Dioxin2/BPX-50 set allowing for it to be monitored. It is interesting to also point out that, out of the toxic PCDD/Fs, TCDD which has already been found coeluting with PCB 126 [12], is resolved here with the two column sets tested.

As the two column sets are the same to monitor the 18 most relevant PCBs in meat, the column set Rtx-Dioxin2/BPX-50 which allows for the 17 toxic PCDD/Fs to be separated was therefore chosen for the rest of this study. Fig. 2 shows the contour plot obtained with this column set which therefore allows for 189 PCBs and 17 PCDD/Fs to be separated, i.e. 206 dioxin-like micropollutants (supporting information, Table SI). Further studies might enable to compare BPX-50 separation capacity with other 2D columns complementary from the Rtx-Dioxin2 in order to optimize the use of the 2D space for PCBs while maintaining the separation obtained for dioxins.

3.2. Assessment of an extraction, concentration and clean-up protocol

In order to be able to use the GC × GC-TOF/MS method to monitor toxic PCBs and PCDD/Fs in a high-fat complex matrix such as ground beef, a protocol of extraction of contaminants by ASE, defatting and concentration of the extract obtained was proposed according to Saito *et al.* [26] with slight modifications concerning the solvent used for extraction, the type of in-cell fat retainer, the clean-up of the resulting extract and evaporation time. Firstly, PCBs and PCDD/Fs were quantified in matrix blanks.

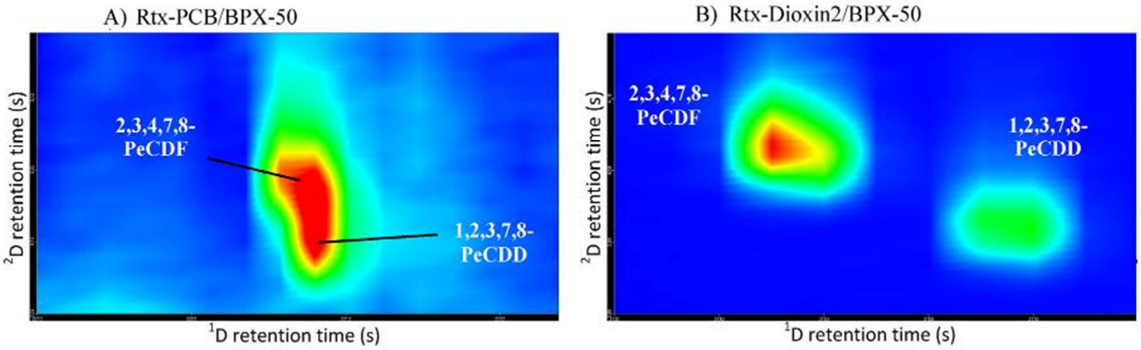

Fig. 1. Separation of 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF by GC × GC-TOF/MS with Rtx-PCB/BPX-50 (A) and Rtx- Dioxin2/BPX-50 (B).

Then, considering the high fat matrix used in this study, different clean-up procedures (cold extraction with acetonitrile, in-cell clean-up, GPC, ASE followed by GPC) were tested. Only the combination of in-cell clean-up with GPC enabled to concentrate the final extract and thus to get satisfactory reliability and sensitivity (data not shown). To assess the relevance of the protocol in concrete terms, a matrix contaminated at a known concentration was required. In order to initially overcome recovery and homogeneity problems posed by impregnation of the structured meat matrix, the extraction/concentration/clean-up protocol was from a spiked freeze-dried matrix according to Reid *et al.* [29]. The contaminant recovery rates after the ASE, GPC and concentration steps were measured on 5 PCBs spread over the chromatogram obtained after analysis of 5 extracts by GC × GC-TOF/MS. These recovery rates were within the 70–130% range generally considered as acceptable [21], with 92 ± 13% for BZ-1, 110 ± 7% for BZ-19, 118 ± 10% for BZ-172, 130 ± 10% for BZ-206 and 130 ± 8% for BZ-209. These results are of the same proportions as those obtained by Wang *et al.* who also explored the recoveries measured after extraction by ASE then defatting by GPC on samples of soil freeze–dried then spiked with PCBs [30]. The recoveries obtained therefore vary from 87% to 160% but only PCBs BZ-153 and BZ-180, not quantified in our study, had very high recoveries, outside the 70–130% range. In our study, the standard deviations were also measured for the same previously stated 5 PCBs. The values obtained are between 7.2% and 13.1%, only 2-chlorobiphenyl (BZ-1) has a standard deviation greater than 10%. This may be explained by its less repeatable extraction due to being the most water soluble congener of the 209 PCBs [31].

3.3. Comparison of different spiking methods and performance of GC × GC-TOF/MS with the column set Rtx-Dioxin2/BPX-50

In order to be able to monitor the future of PCBs and PCDD/Fs during the technological and physiological processes the food matrices are subjected to, a homogeneous and reproducible multi-contaminated, fixed concentration meat matrix is required. Based on protocols found in the literature for spiking contaminants in a real matrix *via* the addition of an organic solvent [23,32], seven different spiking methods were tested and compared. Each method includes a step where the matrix is impregnated with a contaminant solution (injection of a small volume or immersion) and a homogenizing step (ultra-turrax, blender or mixer). Although the use of an organic solvent to spike can alter the properties of the matrix, it was required due to the low water solubility of the POPs studied [33]. For the seven different spiking methods, solvent choice, volume of solvent used, evaporation time, homogenization method and homogenization time were optimised in order to have the best recoveries and standard deviations for targeted analytes. Table 2 shows the recovery rates and standard deviations of the areas corrected for 5 PCBs spread over the GC × GC-TOF/MS chromatogram with the seven spiking methods tested. Only the methods coupling the injection of a small volume of contaminant solution diluted in a solvent and homogenizing by blender or mixer don't allow for recoveries within the traditionally accepted limit of 70–130% to be obtained [21]. Among the other methods, the one coupling the injection of a small volume of solvent and mixing by ultra-turrax is the one inducing the least efficient homogenizing of the spike with standard deviations between 57% and

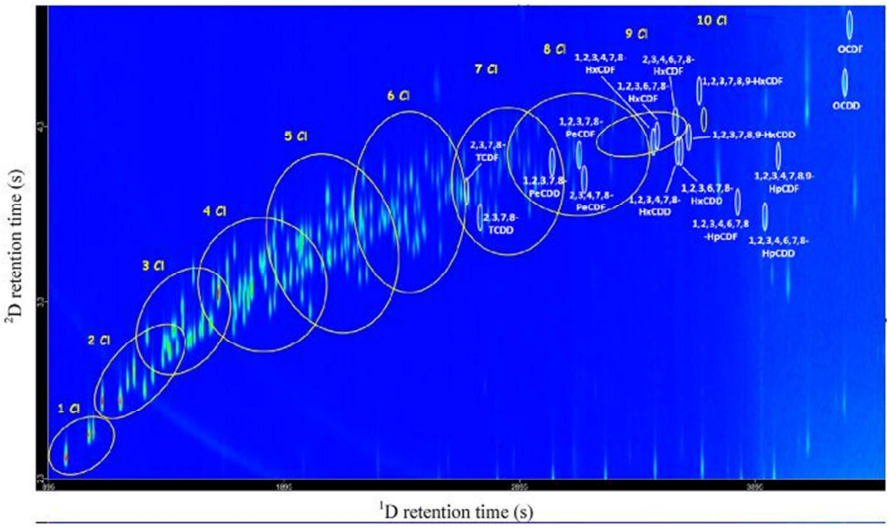

Fig. 2. GC × GC-TOF/MS contour plot of the 209 PCBs and 17 PCDD/Fs with the Rtx-Dioxin2/BP-X50 column set.

Table 2
Comparison of 7 spiking methods on the basis of the recovery rates and the standard deviations of the extraction of 5 PCBs in meat. These methods differed in their ways to add the micropollutants into the meat matrix and to homogenize the spiked matrix after micropollutant addition.

PCB	Immersion								Micro-volume addition					
	Blender		LN_2 grinder		Ultra-turrax		None		Blender		Stand mixer		Ultra-turrax	
	RR[1]	SD[2]	RR	SD	RR	SD	RR	SD	RR	SD	RR	SD	RR	SD
BZ-1	83.6	8.5	87.9	11.7	93.6	7.1	97.0	21.2	37.7	51.0	21.3	12.5	93.1	59.3
BZ-19	90.0	7.3	93.2	10.2	91.9	4.1	104.4	18.1	35.2	48.4	19.5	10.7	94.1	58.8
BZ-172	117.2	7.6	119.1	12.3	109.3	7.3	121.5	9.1	41.4	49.9	26.4	19.5	95.4	59.4
BZ-206	110.2	6.8	107.4	8.2	98.4	5.8	112.7	9.8	39.7	44.5	28.3	15.1	91.0	59.2
BZ-209	101	3.6	104.0	8.8	96.6	5.1	104.3	9.3	39.0	48.3	24.0	12.2	88.3	57.0

[1] Recovery rates (%).
[2] Standard deviations (%).

60%. The methods coupling an immersion step with homogenizing by blender or ultra-turrax are alternatively those allowing for the lowest standard deviations to be obtained, therefore the best homogeneity of the spike, which is essential for the statistical validity of future findings which could be conducted on this matrix [22]. In order to further assess the reliability of these two spiking methods, their reproducibility was then tested. Table 3 shows the standard deviations and recovery rates which were then obtained after extraction of the contaminants and analysis *via* GC × GC-TOF/MS for 20 different PCBs, among which the 15 most relevant PCBs in meat and not coeluted with the column set chosen in this study. For the majority of the PCBs, the recovery rates are greater when homogenizing with a blender is performed. Only BZ-28 has a recovery rate outside the 70–130% limits with this spiking method. Regarding the standard deviations obtained, they remain lower than 10% for the majority of the PCBs quantified when homogenizing with a blender is performed. Only PBZ-77 and BZ-114 have greater standard deviations with this spiking method. In addition to these two compounds, seven other PCBs and particularly the 4 PCBs quantified as the heaviest have standard deviations greater than 10% with the method using the ultra-turrax. These results may be compared to those of Suedel *et al.* who implemented, in samples of sediments, a protocol coupling the injection, in 1 g of matrix, of a few microlitres of fluoranthene dissolved in a very small volume of acetone, an evaporation step of the solvent for two minutes then a manual mixing step for 60 s [34]. A standard deviation of 10.3% of the fluoranthene was then reported which corresponds to the mean standard deviation obtained in our study with homogenizing by ultra-turrax. Reid *et al.* compared eleven different spiking protocols to contaminate soil samples with phenanthrene and benzo[*a*]pyrene [29]. In the eleven protocols, the standard deviations recorded could go up to 43% which is much greater than those obtained in our study. Alternatively, the best homogeneity (2.4% of standard deviation) was obtained on dehydrated samples. Among the protocols tested on non-dehydrated samples as is the case in our study, the lowest standard deviation (4.1%) was obtained using a small volume of ethanol (1 μL for 1 g of sample) to perform the spike solution before manual mixing coupled to mixing of the spiked matrix with a blender. This standard deviation is less than the lowest mean standard deviation obtained in our study on a structured matrix which was therefore 7.8% with homogenizing by blender. It is, however, to be pointed out that these results were obtained with polycyclic aromatic hydrocarbons which can explain for the differences found with our results relating to the PCBs. For the rest of this study, the method coupling a step of immersing the meat into a solvent solution containing the contaminants and a homogenizing step with a blender is the one retained after the recoveries obtained with the PCBs were validated on some dioxins (99.9 ± 12% for 2,3,7,8-TeCDD, 84.1 ± 9% for 2,3,7,8-TeCDF, 73.6 ± 11% for 2,3,4,7,8-PeCDF, 88.8 ± 12% for 1,2,3,7,8-PeCDD). In order to validate the method for official control procedures, these recovery results will have to be confirmed at a second spiking level.

Table 3
Comparison of the recovery rates and reproducibility for the two best spiking methods selected for 20 PCBs ($n = 5$ replicates).

	Immersion + blender		Immersion + ultra-turrax	
	RR[1]	SD[2]	RR	SD
BZ-1	87.0	4.7	81.2	9.7
BZ-19	92.0	8.6	82.3	8.7
BZ-28*	133.4	6.1	120.0	7.6
BZ-52*	112.2	9.4	101.9	8.2
BZ-77*	116.2	20.8	124.6	20.5
BZ-81*	102.5	8.0	92.0	10.7
BZ-105*	114.6	3.1	106.4	3.2
BZ-114*	110.7	10.1	111.8	10.8
BZ-118*	112.3	8.5	101.3	13.0
BZ-123*	105.1	5.8	100.4	7.8
BZ-126*	92.4	7.1	82.6	4.6
BZ-153*	123.4	9.1	109.7	21.6
BZ-156*	107.9	5.0	112.6	10.3
BZ-157*	91.3	8.6	91.4	5.5
BZ-167*	106.5	3.8	103.3	8.5
BZ-169*	96.1	6.1	87.2	6.1
BZ-172*	119.2	7.8	116.1	11.1
BZ-189*	94.3	6.1	93.0	15.7
BZ-206	120.7	9.7	116.3	10.4
BZ-209	111.4	8.3	104.9	12.0

[1] Recovery rates (%).
[2] Standard deviation (%).
*Non-coeluted PCBs forming part of the 18 most relevant in meat.

Table 4
Performance of GC × GC-TOF/MS used with a Rtx-Dioxin2/BPX50 column set for quantification of 19 PCBs and 7 PCDD/Fs.

Compound	$^{1}t_R$ [a] (s)	$^{2}t_R$ [b] (s)	Linearity (pg/g)	Correlation coefficient R^2	LOD[c] (pg/g)	LOQ[d] (pg/g)
BZ-1	970	2.42	50–5000	0.995	66	218
BZ-3	1085	2.57	50–5000	0.991	98	327
BZ-8	1260	2.89	50–5000	0.992	62	205
BZ-19	1315	3.10	50–5000	0.991	66	220
BZ-18	1385	3.08	50–5000	0.995	64	214
BZ-15	1440	3.04	50–5000	0.988	77	256
BZ-54	1510	3.45	50–5000	0.986	83	277
BZ-28	1580	3.17	50–5000	0.991	86	288
BZ-33	1615	3.34	50–5000	0.994	55	182
BZ-22	1655	3.39	50–5000	0.996	50	155
BZ-52	1705	3.35	50–5000	0.980	100	333
BZ-49	1725	3.34	50–5000	0.990	70	234
BZ-74	1970	3.61	50–5000	0.986	85	283
BZ-87	2220	3.79	50–5000	0.993	60	201
BZ-151	2300	3.73	50–5000	0.990	70	232
BZ-77	2320	3.76	50–5000	0.990	71	238
BZ-149	2350	3.85	50–5000	0.981	99	329
BZ-123	2390	3.72	50–5000	0.996	50	142
BZ-118	2415	3.70	50–5000	0.993	58	194
2,3,7,8-TCDF	2670	3.90	50–2500	0.989	75	251
2,3,7,8-TCDD	2725	3.77	50–2500	0.977	118	393
1,2,3,7,8-PeCDF	3030	4.08	50–5000	0.985	120	399
2,3,4,7,8-PeCDF	3145	4.11	50–5000	0.997	65	217
1,2,3,4,7,8-HxCDF	3460	4.20	50–2500	0.992	65	218
1,2,3,6,7,8-HxCDF	3475	4.21	50–2500	0.986	84	279
1,2,3,6,7,8-HxCDD	3575	4.16	50–5000	0.974	227	757

[a] Retention time in first dimension(s).
[b] Retention time in second dimension(s).
[c] Limit of detection. LOD is defined by the formula $3s/m$ (s is the standard deviation of the intercept, and m is the slope of the linear calibration curve).
[d] Limit of quantification.

In order to determine method performance, the limits of detection (LOD) and quantification (LOQ) were measured in spiked meat for 7 PCDD/F congeners and 19 PCB congeners spread over the whole chromatogram (Table 4). The linearity of the calibration lines allowing for these LODs and LOQs to be estimated was assessed by calculating coefficients of determination. All these values are greater than 0.980 except for one of the dioxin congeners (0.974). As shown in Table 4, the range of linearity spans from 50 to 5000 pg/g for the majority of the constituents. The LODs obtained vary from 50 to 100 pg/g for the PCBs and 65 to 227 pg/g for the PCDD/Fs. Excluding sample preparation that includes a concentration by a factor of about 100, our results may be compared to those obtained by Van der Lee *et al.* for several PCBs contained in different food matrices and analyzed on a GC × GC-TOF/MS with a column set RTX-CL pesticides/BPX-50 [35]. Of the seven PCBs considered, five therefore had LODs less than those obtained in our study (from 1.25 to 2.5 ng/g) whereas two PCBs had LODs comparable to our results (10 ng/g). The results obtained for the PCBs are therefore consistent with those obtained by Van der Lee *et al.* which enables our method to be validated. With LOQs at pg level [36], TOF/MS has a lower sensitivity for PCB and PCDD/F analysis [13] than HRMS or MS/MS whose LOQs range between 30 and 80 fg for tetra- to hexa-chlorinated compounds and between 80 and 320 fg for hepta- to octa-chlorinated compounds [37]. However, GC × GC and adapted sample preparation procedures may help to partially compensate the lower sensitivity of TOF/MS detection in case of trace contaminants in complex matrices [14,15].

4. Conclusion

The GC × GC-TOF/MS method assessed in this study allows for simultaneous analysis of 206 dioxin-like micropolluants thanks to the use of a column set Rtx-Dioxin2/BPX-50. To be able to study these compounds present in trace form in a complex and high fat matrix such as ground beef, this method was coupled to accelerated solvent extraction, GPC clean-up and concentration protocols allowing for good recovery rates to be obtained. This work has also allowed for a sample preparation optimization to obtain a matrix of ground beef homogeneously spiked with these contaminants. Before reference laboratories use it, more extensive investigations will be necessary to validate reproducibility and robustness of the spiking method proposed by the present study. The aim is now to use these methodological breakthroughs to monitor these contaminants during technological or physiological transformations which the food may be subjected to. These data could be incorporated into the analyses of risks relating to the presence of these contaminants in food.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2015.02.054.

References

[1] H.W. Vallack, D.J. Bakker, I. Brandt, E. Brostrom-Lunden, A. Brouwer, K.R. Bull, C. Gough, R. Guardans, I. Holoubek, B. Jansson, R. Koch, J. Kuylenstierna, A. Lecloux, D. Mackay, P. McCutcheon, P. Mocarelli, R.D.F. Taalman, Controlling persistent organic pollutants—what next? Environ. Toxicol. Pharmacol. 6 (1998) 143–175.
[2] B.E. Fisher, Most unwanted, Environ. Health Perspect. 107 (1999) A18–A23.
[3] J.L. Domingo, Influence of cooking processes on the concentrations of toxic metals and various organic environmental pollutants in food: a review of the published literature, Crit. Rev. Food Sci. Nutr. 51 (2011) 29–37.

[4] G. Perelló, R. Martí-Cid, V. Castell, J.M. Llobet, J.L. Domingo, Influence of various cooking processes on the concentrations of PCDD/PCDFs, PCBs and PCDEs in foods, Food Control 21 (2010) 178–185.
[5] G.H. Xing, Y. Yang, J.K.Y. Chan, S. Tao, M.H. Wong, Bioaccessibility of polychlorinated biphenyls in different foods using an in vitro digestion method, Environ. Pollut. 156 (2008) 1218–1226.
[6] A. Marques, H.M. Lourenco, M.L. Nunes, C. Roseiro, C. Santos, A. Barranco, S. Rainieri, T. Langerholc, A. Cencic, New tools to assess toxicity, bioaccessibility and uptake of chemical contaminants in meat and seafood, Food Res. Int. 44 (2011) 510–522.
[7] R. Malisch, A. Kotz, Dioxins and PCBs in feed and food—review from European perspective, Sci. Total Environ. 491–492 (2014) 2–10.
[8] R. Weber, C. Gaus, M. Tysklind, P. Johnston, M. Forter, H. Hollert, E. Heinisch, I. Holoubek, M. Lloyd-Smith, S. Masunaga, P. Moccarelli, D. Santillo, N. Seike, R. Symons, J.P. Machado Torres, M. Verta, G. Varbelow, J. Vijgen, A. Watson, P. Costner, J. Woelz, P. Wycisk, M. Zennegg, Dioxin-and POP-contaminated sites—contemporary and future relevance and challenges, Environ. Sci. Pollut. Res. 15 (2008) 363–393.
[9] K. Srogi, Levels and congener distributions of PCDDs, PCDFs and dioxin-like PCBs in environmental and human samples: a review, Environ. Chem. Lett. 6 (2008) 1–28.
[10] T.M. Crisp, E.D. Clegg, R.L. Cooper, W.P. Wood, D.G. Anderson, K.P. Baetcke, J.L. Hoffmann, M.S. Morrow, D.J. Rodier, J.E. Schaeffer, L.W. Touart, M.G. Zeeman, Y.M. Patel, Environmental endocrine disruption: an effects assessment and analysis, Environ. Health Perspect. 106 (1998) 11–56.
[11] J.P. Whitlock, Induction of cytochrome P4501A1, Annu. Rev. Pharmacol. Toxicol. 39 (1999) 103–125.
[12] J.F. Focant, E.J. Reiner, K. MacPherson, T. Kolic, A. Sjodin, D.G. Patterson, S.L. Reese, F.L. Dorman, J. Cochran, Measurement of PCDDs, PCDFs, and non-ortho-PCBs by comprehensive two-dimensional gas chromatography-isotope dilution time-of-flight mass spectrometry (GC × GC-IDTOFMS), Talanta 63 (2004) 1231–1240.
[13] J.F. Focant, G. Eppe, M.L. Scippo, A.C. Massart, C. Pirard, G. Maghuin-Rogister, E. De Pauw, Comprehensive two-dimensional gas chromatography with isotope dilution time-of-flight mass spectrometry for the measurement of dioxins and polychlorinated biphenyls in foodstuffs: comparison with other methods, J. Chromatogr. A 1086 (2005) 45–60.
[14] E. Engel, J. Ratel, P. Blinet, S.T. Chin, G. Rose, P.J. Marriott, Benchmarking of candidate detectors for multiresidue analysis of pesticides by comprehensive two-dimensional gas chromatography, J. Chromatogr. A 1311 (2013) 140–148.
[15] P.Q. Tranchida, F.A. Franchina, P. Dugo, L. Mondello, Comprehensive two-dimensional gas chromatography-mass spectrometry: Recent evolution and current trends, Mass Spectrom. Rev. XX (2014) 1–11.
[16] D. Megson, R. Kalin, P.J. Worsfold, C. Gauchotte-Lindsay, D.G. Patterson, M.C. Lohan, S. Comber, T.A. Brown, G. O'Sullivan, Fingerprinting polychlorinated biphenyls in environmental samples using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry, J. Chromatogr. A 1318 (2013) 276–283.
[17] J.F. Focant, A. Sjodin, D.G. Patterson, Improved separation of the 209 polychlorinated biphenyl congeners using comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry, J. Chromatogr. A 1040 (2004) 227–238.
[18] M. Zapadlo, J. Krupcik, T. Kovalczuk, P. Majek, I. Spanik, D.W. Armstrong, P. Sandra, Enhanced comprehensive two-dimensional gas chromatographic resolution of polychlorinated biphenyls on a non-polar polysiloxane and an ionic liquid column series, J. Chromatogr. A 1218 (2011) 746–751.
[19] E. Hoh, K. Mastovska, S.J. Lehotay, Optimization of separation and detection conditions for comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans, J. Chromatogr. A 1145 (2007) 210–221.
[20] M. Herrero, E. Ibanez, A. Cifuentes, J. Bernal, Multidimensional chromatography in food analysis, J. Chromatogr. A 1216 (2009) 7110–7129.
[21] EPA Method 8000C, Determinative Chromatographic Separations, March 2003.
[22] G.L. Northcott, K.C. Jones, Spiking hydrophobic organic compounds into soil and sediment: a review and critique of adopted procedures, Environ. Toxicol. Chem. 19 (2000) 2418–2430.
[23] G.L. Northcott, K.C. Jones, Developing a standard spiking procedure for the introduction of hydrophobic organic compounds into field-wet soil, Environ. Toxicol. Chem. 19 (2000) 2409–2417.
[24] M. Van den Berg, L. Birnbaum, A.T.C. Bosveld, B. Brunstrom, P. Cook, M. Feeley, J.P. Giesy, A. Hanberg, R. Hasegawa, S.W. Kennedy, T. Kubiak, J.C. Larsen, F.X.R. van Leeuwen, A.K.D. Liem, C. Nolt, R.E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillitt, M. Tysklind, M. Younes, F. Waern, T. Zacharewski, Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife, Environ. Health Perspect. 106 (1998) 775–792.
[25] V. Sirot, A. Tard, A. Venisseau, A. Brosseaud, P. Marchand, B. Le Bizec, J.C. Leblanc, Dietary exposure to polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls of the French population: results of the second French Total Diet Study, Chemosphere 88 (2012) 492–500.
[26] K. Saito, A. Sjodin, C.D. Sandau, M.D. Davis, H. Nakazawa, Y. Matsuki, D.G. Patterson, Development of a accelerated solvent extraction and gel permeation chromatography analytical method for measuring persistent organohalogen compounds in adipose and organ tissue analysis, Chemosphere 57 (2004) 373–381.
[27] E. Bjorklund, T. Nilsson, S. Bowadt, Pressurised liquid extraction of persistent organic pollutants in environmental analysis, Trac—Trends Anal. Chem. 19 (2000) 434–445.
[28] R.C. Team, 2012 (http://www.R-project.org).
[29] B.J. Reid, G.L. Northcott, K.C. Jones, K.T. Semple, Evaluation of spiking procedures for the introduction of poorly water soluble contaminants into soil, Environ. Sci. Technol. 32 (1998) 3224–3227.
[30] P. Wang, Q. Zhang, Y. Wang, T. Wang, X. Li, L. Ding, G. Jiang, Evaluation of Soxhlet extraction, accelerated solvent extraction and microwave-assisted extraction for the determination of polychlorinated biphenyls and polybrominated diphenyl ethers in soil and fish samples, Anal. Chim. Acta 663 (2010) 43–48.
[31] Y.B. Wang, C.S. Hong, Effect of hydrogen peroxide, periodate and persulfate on photocatalysis of 2-chlorobiphenyl in aqueous TiO_2 suspensions, Water Res. 33 (1999) 2031–2036.
[32] U.C. Brinch, F. Ekelund, C.S. Jacobsen, Method for spiking soil samples with organic compounds, Appl. Environ. Microb. 68 (2002) 1808–1816.
[33] M. Howsam, R.A. Verweij, N.M. Van Straalen, A solvent-free method for spiking terrestrial algae (Desmococcus spp.) with pyrene for use in bioassays, Chemosphere 53 (2003) 253–262.
[34] B.C. Suedel, J.H. Rodgers, P.A. Clifford, Bioavailability of fluoranthene in freshwater sediment toxicity tests, Environ. Toxicol. Chem. 12 (1993) 155–165.
[35] M.K. Van der Lee, G. Van der Weg, W.A. Traag, H.G. Mol, Qualitative screening and quantitative determination of pesticides and contaminants in animal feed using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry, J. Chromatogr. A 1186 (2008) 325–339.
[36] J.-F. Focant, C. Pirard, G. Eppe, E. De Pauw, Recent advances in mass spectrometric measurement of dioxins, J. Chromatogr. A 1067 (2005) 265–275.
[37] A.M. Ingelido, G. Brambilla, A. Abballe, A. Di Domenico, A.R. Fulgenzi, N. Iacovella, A.L. Iamiceli, S. Valentini, E. De Felip, PCDD, PCDF, AND. DL-PCB analysis in food: performance evaluation of the high-resolution gas chromatography/low-resolution tandem mass spectrometry technique using consensus-based samples, Rapid Commun. Mass Spectrom. 26 (2012) 236–242.

Supplementary Data

Table 1. Elution order of PCB and PCDD/F congeners using the Rtx-Dioxin2/BPX-50 column set. Coeluting congeners determined from the resolution factor proposed by Zapadlo *et al*. [18] are enclosed in a box.

Compound	Congener	ID mass (m/z)	$^{1}t_R$ (s)	$^{2}t_R$ (s)
2-MoCB	BZ-1	188*	970	2.42
3-MoCB	BZ-2	188	1065	2.55
4-MoCB	BZ-3	188*	1085	2.57
2,6-DiCB	BZ-10	222	1120	2.75
2,2'-DiCB	BZ-4	222	1125	2.75
2,4-DiCB	BZ-7	222	1200	2.74
2,5-DiCB	BZ-9	222	1205	2.74
2,3'-DiCB	BZ-6	222	1230	2.85
2,3-DiCB	BZ-5	222	1255	2.92
2,4'-DiCB	BZ-8	222*	1260	2.89
3,5-DiCB	BZ-14	222	1305	2.80
2,2',6-TrCB	BZ-19	186*	1315	3.10
2,4,6-TrCB	BZ-30	256	1340	2.88
3,3'-DiCB	BZ-11	222	1380	2.98
2,2',5-TrCB	BZ-18	256*	1385	3.08
2,2',4-TrCB	BZ-17	256	1400	3.06
3,4-DiCB	BZ-12	222	1405	3.02
3,4'-DiCB	BZ-13	222	1410	3.01
2,3',6-TrCB	BZ-27	256	1425	3.14
2,3,6-TrCB	BZ-24	256	1435	3.15
4,4'-DiCB	BZ-15	222*	1440	3.04
2,2',3-TrCB	BZ-16	256	1460	3.27
2,4',6-TrCB	BZ-32	256	1465	3.17
2',3,5-TrCB	BZ-34	256	1490	3.09
2,3,5-TrCB	BZ-23	256	1500	3.08
2,2',6,6'-TeCB	BZ-54	292*	1510	3.45
2,4,5-TrCB	BZ-29	256	1515	3.10
2,3',5-TrCB	BZ-26	256	1540	3.14
2,3',4-TrCB	BZ-25	256	1550	3.16
2,2',4,6-TeCB	BZ-50	292	1555	3.25
2,4,4'-TrCB	BZ-28	256*	1580	3.17
2,4',5-TrCB	BZ-31	256	1585	3.19
2,2',5,6'-TeCB	BZ-53	292	1595	3.38
2,3,3'-TrCB	BZ-20	256	1610	3.34
2,3,4-TrCB	BZ-21	256	1615	3.34
2,3',4'-TrCB	BZ-33	256*	1615	3.34
2,2',4,6'-TeCB	BZ-51	292	1620	3.39
2,3,4'-TrCB	BZ-22	256*	1655	3.39

2,2',3,6-TeCB	BZ-45	292	1655	3.51
3,3',5-TrCB	BZ-36	256	1680	3.16
2,3',5',6-TeCB	BZ-73	292	1680	3.31
2,2',3,6'-TeCB	BZ-46	292	1685	3.59
2,3',4,6-TeCB	BZ-69	292	1690	3.25
2,2',3,5-TeCB	BZ-43	292	1700	3.35
2,2',5,5'-TeCB	BZ-52	292*	1705	3.35
3,4',5-TrCB	BZ-39	256	1725	3.22
2,2',4,5'-TeCB	BZ-49	292*	1725	3.34
2,2',4,5-TeCB	BZ-48	292	1730	3.41
2,2',4,4'-TeCB	BZ-47	292	1740	3.32
2,4,4',6-TeCB	BZ-75	292	1745	3.32
2,3,5,6-TeCB	BZ-65	292	1750	3.39
2,2',4,6,6'-PeCB	BZ-104	326	1755	3.51
3,4,5-TrCB	BZ-38	256	1760	3.35
2,3,4,6-TeCB	BZ-62	292	1765	3.39
2,2',3,5'-TeCB	BZ-44	292	1795	3.54
2,3,3',6-TeCB	BZ-59	292	1805	3.49
2,2',3,4'-TeCB	BZ-42	292	1810	3.54
3,3',4-TrCB	BZ-35	256	1815	3.42
2,3',5,5'-TeCB	BZ-72	292	1835	3.28
2,3',4',6-TeCB	BZ-71	292	1835	3.57
2,2',3,4-TeCB	BZ-41	292	1850	3.65
2,3',4,5'-TeCB	BZ-68	292	1855	3.31
2,2',3,6,6'-PeCB	BZ-96	326	1855	3.79
2,2',4,5',6-PeCB	BZ-103	326	1860	3.42
3,4,4'-TrCB	BZ-37	256	1860	3.46
2,3,4',6-TeCB	BZ-64	292	1860	3.58
2,2',3,3'-TeCB	BZ-40	292	1885	3.76
2,2',4,4',6-PeCB	BZ-100	326	1890	3.44
2,3,3',5-TeCB	BZ-57	292	1900	3.40
2,2',3,5,6'-PeCB	BZ-94	326	1910	3.61
2,3',4,5-TeCB	BZ-67	292	1920	3.43
2,3,3',5'-TeCB	BZ-58	292	1925	3.49
2,2',4,5,6'-PeCB	BZ-102	326	1950	3.46
2,3,4',5-TeCB	BZ-63	292	1950	3.64
2,3,4,5-TeCB	BZ-61	292	1960	3.57
2,2',3',4,6-PeCB	BZ-98	326	1965	3.63
2,3',4,5',6-PeCB	BZ-121	326	1970	3.34
2,4,4',5-TeCB	BZ-74	292*	1970	3.61
2,2',3,5,6-PeCB	BZ-93	326	1970	3.67
2',3,4,5-TeCB	BZ-76	292	1975	3.47
2,2',3,5',6-PeCB	BZ-95	326	1975	3.66
2,3',4',5-TeCB	BZ-70	292	1985	3.53
2,2',3,4,6-PeCB	BZ-88	326	1990	3.68
3,3',5,5'-TeCB	BZ-80	292	2005	3.25

2,3',4,4'-TeCB	BZ-66	292	2005	3.55
2,2',3,4',6-PeCB	BZ-91	326	2005	3.70
2,2',4,4',6,6'-HxCB	BZ-155	360	2010	3.51
2,3,3',4-TeCB	BZ-55	292	2045	3.68
2,2',3,5,5'-PeCB	BZ-92	326	2055	3.53
2,3,3',4'-TeCB	BZ-56	292	2080	3.77
2,2',3,3',6-PeCB	BZ-84	326	2080	3.91
2,2',3,4,6'-PeCB	BZ-89	326	2080	3.91
2,2',3,4',5-PeCB	BZ-90	326	2085	3.56
2,2',4,5,5'-PeCB	BZ-101	326*	2085	3.56
2,3,3',5',6-PeCB	BZ-113	326	2090	3.55
2,3,4,4'-TeCB	BZ-60	292	2095	3.72
2,2',4,4',5-PeCB	BZ-99	326	2110	3.57
2,2',3,4',6,6'-HxCB	BZ-150	360	2120	3.75
2,3',4,4',6-PeCB	BZ-119	326	2140	3.59
2,2',3,5,6,6'-HxCB	BZ-152	360	2150	3.88
2,3,3',5,6-PeCB	BZ-112	326	2155	3.63
2,2',3,3',5-PeCB	BZ-83	326	2155	3.75
3,3',4,5'-TeCB	BZ-79	292	2160	3.51
2,3,3',4,6-PeCB	BZ-109	326	2165	3.65
2,3,3',5,5'-PeCB	BZ-111	326	2170	3.79
2,2',3,4,5-PeCB	BZ-86	326	2180	3.81
2,2',3',4,5-PeCB	BZ-97	326	2185	3.78
2,2',3,4,6,6'-HxCB	BZ-145	360	2185	3.91
2,2',3,4',5,6'-HxCB	BZ-148	360	2190	3.59
3,3',4,5-TeCB	BZ-78	292	2205	3.63
2',3,4,5,6'-PeCB	BZ-125	326	2215	3.44
2,3,4,5,6-PeCB	BZ-116	326	2215	3.72
2,3,4',5,6-PeCB	BZ-117	326	2215	3.72
2,2',3,4,5'-PeCB	BZ-87	326*	2220	3.79
2,3,4,4',6-PeCB	BZ-115	326	2230	3.73
2,2',3,3',6,6'-HxCB	BZ-136	360	2230	4.00
2,2',4,4',5,6'-HxCB	BZ-154	360	2235	3.62
2,2',3,4,4'-PeCB	BZ-85	326	2240	3.84
2,3',4,5,5'-PeCB	BZ-120	326	2245	3.47
3,4,4',5-TeCB	BZ-81	292*	2265	3.69
2,3,3',4',6-PeCB	BZ-110	326	2265	3.83
2,2',3,5,5',6-HxCB	BZ-151	360*	2300	3.73
2,2',3,3',5,6'-HxCB	BZ-135	360	2310	3.81
3,3',4,4'-TeCB	BZ-77	292*	2320	3.76
2,2',3,3',4-PeCB	BZ-82	326	2320	4.03
2,2',3,4,5',6-HxCB	BZ-144	360	2325	3.76
2,2',3,4',5,6-HxCB	BZ-147	360	2340	3.79
2,2',3,4',5',6-HxCB	BZ-149	360*	2350	3.85
2,2',3,4,4',6-HxCB	BZ-139	360	2360	3.68
2',3,4,5,5'-PeCB	BZ-124	326	2360	3.81

2,2',3,4,4',6'-HxCB	BZ-140	360	2375	3.86
2,3,3',4,5'-PeCB	BZ-108	326	2380	3.72
2,2',3,4,5,6'-HxCB	BZ-143	360	2380	4.01
2,3,3',4',5-PeCB	BZ-107	326	2385	3.72
2',3,4,4',5-PeCB	BZ-123	326*	2390	3.72
2,3,3',4,5-PeCB	BZ-106	326	2410	3.78
2,2',3,4',5,6,6'-HpCB	BZ-188	392	2410	3.81
2,2',3,3',5,6-HxCB	BZ-134	360	2410	3.96
2,3',4,4',5-PeCB	BZ-118	326*	2415	3.70
2,2',3,3',5,5'-HxCB	BZ-133	360	2420	3.64
2,2',3,3',4,6-HxCB	BZ-131	360	2430	3.99
2,2',3,4,5,6-HxCB	BZ-142	360	2435	4.00
2,3,3',5,5',6-HxCB	BZ-165	360	2445	3.61
2,2',3,4,4',6,6'-HpCB	BZ-184	392	2450	3.84
2,2',3,4',5,5'-HxCB	BZ-146	360	2455	3.69
2,3,3',4',5'-PeCB	BZ-122	326	2460	3.95
2,3,3',4,5',6-HxCB	BZ-161	360	2465	3.64
2,3,4,4',5-PeCB	BZ-114	326*	2465	3.87
2,3',4,4',5',6-HxCB	BZ-168	360	2485	3.72
2,2',4,4',5,5'-HxCB	BZ-153	360*	2490	3.70
2,2',3,3',4,6'-HxCB	BZ-132	360	2500	4.11
2,2',3,3',5,6,6'-HpCB	BZ-179	392	2525	4.03
2,3,3',4,4'-PeCB	BZ-105	326*	2545	4.01
3,3',4,5,5'-PeCB	BZ-127	326	2555	3.61
2,2',3,4,5,5'-HxCB	BZ-141	360	2555	3.86
2,2',3,3',4,6,6'-HpCB	BZ-176	392	2565	4.06
2,2',3,4,4',5-HxCB	BZ-137	360	2580	3.91
2,2',3,3',4,5'-HxCB	BZ-130	360	2595	3.97
2,2',3,4,5,6,6'-HpCB	BZ-186	392	2600	4.20
2,3,3',4',5',6-HxCB	BZ-164	360	2610	3.96
2,2',3,3',5,5',6-HpCB	BZ-178	392	2630	3.82
2,2',3,4,4',5'-HxCB	BZ-138	360*	2630	3.93
2,3,3',4',5,6-HxCB	BZ-163	360	2630	3.93
2,3,3',4,4',6-HxCB	BZ-158	360	2645	3.93
2,3,3',4,5,6-HxCB	BZ-160	360	2650	3.93
2,2',3,3',4,5-HxCB	BZ-129	360	2655	4.14
2,2',3,3',4,5',6-HpCB	BZ-175	392	2665	3.88
2,2',3,4,4',5,6'-HpCB	BZ-182	392	2665	3.88
2,3,7,8-TCDF		306*	2670	3.90
2,2',3,4',5,5',6-HpCB	BZ-187	392	2680	3.90
2,2',3,4,4',5',6-HpCB	BZ-183	392	2710	3.90
2,3,4,4',5,6-HxCB	BZ-166	360	2715	4.02
2,3,7,8-TCDD		322*	2725	3.77
3,3',4,4',5-PeCB	BZ-126	326*	2730	3.88
2,3,3',4,5,5'-HxCB	BZ-159	360	2735	3.74
2,3,3',4',5,5'-HxCB	BZ-162	360	2760	3.80

2,2',3,4,5,5',6-HpCB	BZ-185	392	2770	4.00
2,2',3,3',4,4'-HxCB	BZ-128	360	2770	4.26
2,2',3,3',4,5,6'-HpCB	BZ-174	392	2790	4.15
2,3',4,4',5,5'-HxCB	BZ-167	360*	2795	3.83
2,2',3,3',5,5',6,6'-OcCB	BZ-202	426	2805	4.02
2,2',3,4,4',5,6-HpCB	BZ-181	392	2815	4.08
2,2',3,3',4,5',6'-HpCB	BZ-177	392	2830	4.14
2,2',3,3',4,5',6,6'-OcCB	BZ-201	426	2850	4.07
2,2',3,3',4,4',6-HpCB	BZ-171	392	2855	4.21
2,2',3,4,4',5,6,6'-OcCB	BZ-204	426	2860	4.09
2,2',3,3',4,5,6-HpCB	BZ-173	392	2885	4.29
2,2',3,3',4,4',6,6'-OcCB	BZ-197	426	2890	4.12
2,3,3',4,4',5-HxCB	BZ-156	360*	2915	4.02
2,2',3,3',4,5,5'-HpCB	BZ-172	392*	2920	3.95
2,3,3',4,4',5'-HxCB	BZ-157	360*	2930	4.12
2,3,3',4,5,5',6-HpCB	BZ-192	392	2935	3.83
2,2',3,4,4',5,5'-HpCB	BZ-180	392*	2960	3.97
2,3,3',4',5,5',6-HpCB	BZ-193	392	2960	3.97
2,2',3,3',4,5,6,6'-OcCB	BZ-200	426	2975	4.33
2,3,3',4,4',5',6-HpCB	BZ-191	392	2985	4.00
1,2,3,7,8-PeCDF		340*	3030	4.08
2,2',3,3',4,5,5',6-OcCB	BZ-198	426	3100	4.06
2,2',3,3',4',5,5',6-OcCB	BZ-199	426	3105	4.12
2,2',3,3',4,4',5-HpCB	BZ-170	392	3105	4.27
3,3',4,4',5,5'-HxCB	BZ-169	360*	3125	3.94
2,3,3',4,4',5,6-HpCB	BZ-190	392	3125	4.17
2,2',3,3',4,4',5',6-OcCB	BZ-196	426	3140	4.15
2,2',3,4,4',5,5',6-OcCB	BZ-203	426	3145	4.11
2,3,4,7,8-PeCDF		340*	3145	4.11
1,2,3,7,8-PeCDD		356*	3170	4.00
2,2',3,3',4,5,5',6,6'-NoCB	BZ-208	460	3245	4.26
2,3,3',4,4',5,5'-HpCB	BZ-189	392*	3280	4.07
2,2',3,3',4,4',5,6,6'-NoCB	BZ-207	460	3290	4.33
2,2',3,3',4,4',5,6-OcCB	BZ-195	426	3300	4.41
2,2',3,3',4,4',5,5'-OcCB	BZ-194	426	3415	4.20
2,3,3',4,4',5,5',6-OcCB	BZ-205	426	3450	4.18
1,2,3,4,7,8-HxCDF		374*	3460	4.20
1,2,3,6,7,8-HxCDF		374*	3475	4.21
2,3,4,6,7,8-HxCDF		374*	3555	4.30
1,2,3,4,7,8-HxCDD		390*	3565	4.14
2,2',3,3',4,4',5,5',6-NoCB	BZ-206	460*	3565	4.35
1,2,3,6,7,8-HxCDD		390*	3575	4.16
1,2,3,7,8,9-HxCDD		390*	3615	4.22
1,2,3,7,8,9-HxCDF		374*	3655	4.48
2,2',3,3',4,4',5,5',6,6'-DeCB	BZ-209	498*	3670	4.39
1,2,3,4,6,7,8-HpCDF		408*	3820	3.84

1,2,3,4,6,7,8-HpCDD	424*	3935	3.75
1,2,3,4,7,8,9-HpCDF	408*	3990	4.12
OCDD	460*	4275	4.53
OCDF	444*	4295	4.85

*Mass fragment also used for quantification in the study according to [13] for PCDD/Fs and [16] for PCBs

Complément de discussion sur la mise au point d'une méthode d'analyse multirésidus des polluants environnementaux dans une viande surchargée :

Les développements méthodologiques mis en œuvre dans cette première partie ont tout d'abord permis la mise au point d'une méthode d'extraction et d'analyse multirésidus des PCB et PCDD/F. Cette méthode permet le suivi simultané d'un mélange de 189 PCB et des 17 PCDD/F toxiques en 75min. Cette méthode pourra donc être utilisée dans la suite de l'étude pour déterminer l'impact des transformations technologiques et physiologiques sur les PCB et PCDD/F de la viande. Les données de la littérature et l'expérience du laboratoire (Engel *et al.*, 2013 ; Giri *et al.*, 2015) permettent cependant de penser que cette méthode pourrait être élargie au suivi d'autres contaminants chimiques dont les pesticides dans la suite de cette étude.

La méthode de surcharge de la viande en contaminants mise au point dans ce chapitre permet de travailler sur une matrice contaminée de façon similaire tout au long de cette étude, permettant ainsi une comparaison objective de tous les résultats obtenus. Moyennant des adaptations (modification du solvant de surcharge, adaptation de la durée d'évaporation du solvant), ce protocole de contamination intentionnelle de la viande développé pour le cas des PCB et PCDD/F peut également être utilisé pour d'autres familles de contaminants (pesticides, antibiotiques, métaux lourds), après vérification des taux de récupération et de l'homogénéité de la matrice. Cependant, il n'est pas impossible que l'utilisation de solvant et l'homogénéisation au blender induisent des modifications de structure de la viande étudiée et que l'imprégnation des contaminants dans la matrice diffère par rapport à une viande « naturellement » contaminée. Il est donc pertinent de s'interroger sur l'impact que ces phénomènes pourraient avoir sur le devenir des contaminants au cours de la cuisson et de la digestion. Pour élucider cette question, des échantillons naturellement contaminés prélevés en abattoir dans le cadre du projet SOMEAT seront analysés en parallèle des échantillons surchargés dans la suite de cette étude afin de comparer les résultats obtenus.

Chapitre III : Impact de la cuisson sur les contaminants chimiques de la viande

Chapitre III : Impact de la cuisson sur les contaminants chimiques de la viande

Comme nous l'avons indiqué dans le chapitre 1, plusieurs auteurs se sont intéressés à l'étude de l'impact de la cuisson sur le devenir des contaminants chimiques des aliments. Cependant, les résultats obtenus dans ces études sont très différents et ne permettent pas de tirer de conclusion générale. Beaucoup d'auteurs ont en effet travaillé sur des échantillons naturellement contaminés avec une variabilité inter-échantillons importante, en termes par exemple de concentration initiale en contaminants ou de teneur en eau et en lipides. Ces facteurs influencent le devenir des contaminants à la cuisson, et doivent donc être impérativement pris en compte et contrôlés lors des études. Pour pallier ce biais potentiel, des échantillons de viande intentionnellement contaminés selon la méthode développée dans le chapitre précédent ont été utilisés dans cette étude.

L'étude des données de la littérature a permis d'observer une grande variabilité en termes de modes et d'intensités de cuisson avec souvent peu d'informations concernant les temps et températures de cuisson utilisés. Dans cette étude, une cuisson à la poêle a été ciblée, ce mode de cuisson étant le plus répandu en France pour la cuisson de steaks hachés. A partir de la viande surchargée en contaminants, des steaks hachés ont été calibrés, préformés dans un moule puis systématiquement pesés avant cuisson afin d'être le plus reproductible possible. Une plaque à induction contrôlée en température et des poêles en inox permettant une diffusion plus homogène de la chaleur ont été utilisées afin de limiter la variabilité de la montée en température dans la viande. Un couple temps/température a été déterminé grâce à l'utilisation de thermocouples afin d'obtenir une température à cœur de la viande de 70°C en accord avec les recommandations faites par l'OMS. Cependant, les habitudes de cuisson étant très variables entre les consommateurs, l'influence de l'intensité de cuisson sur le devenir des contaminants a été étudiée en utilisant deux autres conditions de cuisson : une cuisson saignante (50°C à cœur) et une cuisson intense (85°C à cœur).

Afin de pouvoir suivre le devenir des contaminants dans la viande cuite, la méthode d'extraction et d'analyse multirésidus décrite dans le chapitre 2 a été étendue à l'analyse de 16 pesticides après que leurs taux de récupération aient été déterminés. L'analyse des pesticides a pu être réalisée car la combinaison des colonnes chromatographiques utilisée pour l'analyse des PCB et PCDD/F est également fréquemment utilisée pour l'analyse multirésidus des pesticides en GC×GC. Par ailleurs, les taux de récupération des PCB et PCDD/F déterminés

dans le chapitre 2 dans la viande crue ont été déterminés dans ce chapitre sur de la viande cuite. De plus, afin d'élargir le spectre de contaminants étudiés, des collaborations ont été établies avec des Laboratoires Nationaux de Référence disposant de matériel de pointe et de méthodes validées pour l'analyse des contaminants. Une partie des analyses a ainsi été réalisée en collaboration avec d'une part l'Anses Fougères pour l'analyse des antibiotiques, et d'autre part l'Anses Maisons-Alfort pour l'analyse des métaux lourds. En revanche, les résultats préliminaires obtenus dans le cadre du projet SOMEAT ayant indiqué que l'étude des mycotoxines s'avère peu pertinente dans la viande, ces molécules n'ont pas été ciblées dans cette étude.

Le large spectre de contaminants ciblés a ainsi permis de distinguer les contaminants thermorésistants des contaminants thermosensibles et donc susceptibles de subir une dégradation dans certaines conditions de cuisson. Une étude spécifique a été menée sur l'un des composés qui s'est révélé fortement thermosensible. Il s'agit d'un antibiotique, le sulfaméthoxazole, pour lequel nous avons cherché à identifier, après cuisson, les produits de dégradation, composés ultimes auxquels le consommateur est réellement exposé. La détection spécifique des produits de dégradation et leur quantification absolue ont été facilitées grâce à l'utilisation de l'antibiotique modèle sous sa forme radiomarquée au ^{14}C, et leur identification a été réalisée grâce à des analyses par LC-HRMS et par RMN. La difficulté d'obtention des contaminants radiomarqués au ^{14}C n'a cependant pas permis d'étudier la thermodégradation d'autres contaminants thermosensibles tels que l'hexabromocyclododecane ou certains pesticides.

Article 2

Effects of pan cooking on micropollutants in meat

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Abstract

This work presents the effects of pan cooking on polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs), pesticides and heavy metals in meat in a risk assessment perspective. Three different realistic cooking intensities were studied. A GC×GC-TOF/MS method was set up for the multiresidue analysis of 189 PCBs, 17 PCDD/Fs and 16 pesticides whereas Cd, As, Pb and Hg were assayed by ICP-MS. In terms of quantity, average PCB losses after cooking were 18 ± 5% for rare, 30 ± 3% for medium, and 48 ± 2% for well-done meat. In contrast, average PCDD/F losses were not significant. For pesticides, no loss occurred for aldrin, lindane, DDE or DDD whereas losses exceeding 80% were found for dieldrin, sulfotep or phorate. Losses close to the margin of error of the method were observed for heavy metals. These results are discussed in light of the physico-chemical properties of the micropollutants as well as of water and fat losses into cooking juice.

Keywords

Meat; Pan cooking; Polychlorinated biphenyls (PCBs); Polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs); Heavy metals; Pesticides; Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOF/MS); Chemical risk assessment

1. Introduction

Food represents one of the main sources of human exposure to harmful pollutants, of which meat in particular can form a non-negligible source (Engel *et al*., 2015). For example, about 90–98% of the average exposure of humans to PCBs and PCDD/Fs results from dietary intake, with food of animal origin being the predominant source (Malisch *et al.*, 2014). Most micropollutants, whether environmental or phytosanitary, can accumulate in animal tissues during stock raising and remain in meat end-products for human consumption (Takaki *et al*., 2015). Among the toxic contaminants liable to being found in foods, and in particular meat products, polychlorinated biphenyls (PCBs), polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs), pesticides and heavy metals are important to monitor because these chemicals include many toxic congeners that are especially persistent in the environment (Darko *et al*., 2007 ; Zheng *et al*., 2013 ; Malisch *et al*., 2014). PCBs were massively used in industry as insulators until the 1980s. PCDD/Fs are released in the environment by industrial and other human activities. Pesticides used as crop-control agents

persist in cereals and other plants consumed by animals, and levels of heavy metals naturally present in the environment can be increased by industrial activities or pollution.

Although these micropollutants are generally found in trace amounts in animal tissues, the human health risk they imply cannot be ignored. It is therefore important to assess precisely the risk incurred by consumers. To this end, the technological processes the food undergoes before consumption, in particular cooking (Hori *et al*., 2005) have to be considered. Indeed, levels of contaminants can be affected by cooking, since contaminants can be released in cooking juices, evaporate, or be broken down by heat (Abou-Arab, 1999; Bayen *et al*., 2005; Rawn *et al.*, 2013; Muresan *et al.*, 2015). A wide examination of literature data regarding the effects of cooking on food micropollutants show major discrepancies between laboratories (Stachiw *et al.*, 1988; Zabik *et al.*, 1995; Schecter *et al.*, 1998; Petroske *et al.*, 1998; Rose *et al*., 2001). For instance, Hori *et al*. (2005) reported an approximately 40% cooking-induced decrease of PCBs and PCDD/Fs in beef (250g chunks) whereas Perelló *et al.* (2010) found that cooking enhanced PCB concentration in veal steak, loin of pork, breast and thigh chicken or even steak and rib of lamb. At least two factors may explain these inconsistencies. First, cooking conditions could vary a lot between studies but the extent of these variations is difficult to assess because cooking protocols are generally poorly documented, with no or very few information regarding temperature monitoring and control. Second, most of these studies deal with naturally contaminated food matrices. Thus, these matrices differ significantly not only in micropollutant concentration but also in water and fat content, that may explain the variability of the cooking effects observed on micropollutants. Due to these discrepancies, no clear conclusion can be drawn regarding the effect of cooking on micropollutants. Thus, these former studies are not sufficient to feed risk assessment models which require robust and reproducible data. To address this challenging issue, a realistic, standardized and reproducible cooking method must be set up. It is also necessary to use a homogeneous matrix intentionally and uniformly contaminated at a known and high enough concentration to be detected, even after cooking (Northcott *et al.*, 2000). Finally, since it is expected that micropollutants have different behaviors during cooking depending on their physicochemical properties, there is a need to widen the range of micropollutants investigated.

To enable a high-throughput assessment of the effects of cooking on a wide variety of harmful contaminants in food, multiresidue methods (MRMs) are particularly suitable (Tang *et al.*, 2013). Several studies demonstrated the relevance of comprehensive two-dimensional gas chromatography (GC×GC) in tandem with time-of-flight mass spectrometry (TOF-MS) to

assay PCBs, PCDD/Fs (Focant *et al*., 2004a; Focant *et al*., 2005) and pesticides (Engel *et al*, 2013). Some authors have focused on the combined assay of PCBs and PCDD/Fs (Planche *et al*., 2015) or PCBs and pesticides (Van der Lee *et al*., 2008), but to our knowledge, no GC×GC-TOF/MS method had yet been developed for the simultaneous assay of PCBs, toxic PCDD/Fs, and organochlorine and organophosphorus pesticides. For heavy metals, a method was validated by the French National Reference Laboratory (Chevallier *et al*., 2015) to quantify 31 essential and non-essential trace elements in food by inductively coupled plasma mass spectrometry (ICP-MS).

With the ultimate aim of making a better assessment of risks due to chemical contamination of food, we have studied the effects of cooking on the levels of PCBs, PCDD/Fs, pesticides and heavy metals in meat. For this purpose the first part of this paper was focused on the set up of a GC×GC-TOF/MS multiresidue method for the simultaneous assay of PCBs, PCDD/Fs and pesticides in cooked ground beef. In a second part, this method, together with the ICP-MS method, were used to assess the effects of different realistic cooking intensities on micropollutant levels in contaminant-spiked meat. The validity of these results obtained with spiked meat will be discussed in the light of contaminant levels measured after cooking in naturally contaminated meat samples.

2. Materials and Methods

2.1. Chemicals and standards

Hexane, dichloromethane, acetone, methanol and toluene were organic trace analysis grade solvents (Sigma-Aldrich, Saint-Quentin-Fallavier, France). Activated aluminum oxide (acidic, Brockmann I) and diatomaceous earth used for the preparation of Accelerated Solvent Extraction (ASE) cells were obtained from Sigma-Aldrich. Micropollutant reference standards were obtained from AccuStandard Europe (Niederbipp, Switzerland) for the 209 PCBs, the organochlorine and organophosphorus pesticides, and from LGC Standards (Molsheim, France) for the 17 PCDD/Fs. The 209 PCBs were divided into nine 10 ng μL^{-1} stock solutions. For PCDD/Fs, the concentration of the stock solution was different according to the congeners: tetra- 2.5 ng μL^{-1}, penta-, hexa- and hepta- 6.25 ng μL^{-1} and octa- 12.5 ng μL^{-1}. Organochlorine and organophosphorus pesticide solution concentrations were 2000 ng μL^{-1}. Internal standards were used for the accurate quantification of target compounds: 3’-F-PCB-28 (Chiron, Trondheim, Norway), 3’-F-PCB-81 (Chiron), 13C-labeled PCB-111 (Wellington laboratories, Guelph, ON, Canada), 13C-labeled PCB-194 (Wellington) and

fenchlorphos (Sigma-Aldrich). For heavy metal analysis, standard solutions containing arsenic, cadmium, mercury and lead and internal standard solutions were purchased from Analytika (Prague, Czech Republic) or from Ultra Scientific (North Kingstown, USA). Suprapur HNO3 (67% v/v) and Rectapur HNO3 (54% v/v) was purchased from VWR (Fontenay-sous-Bois, France).

2.2. Meat samples

Two types of meat samples were used, corresponding to both intentionally and naturally contaminated meat. Intentionally contaminated meat was prepared with ground beef samples from a same blend of muscles (11% fat) purchased from a French supplier. 125 g weight aliquots were stored at −80 °C before use. Matrix blanks of these samples were made before spiking. Naturally contaminated meat samples were obtained in the frame of the French project SOMEAT (Contract No. ANR-12-ALID-0004. Safety of Organic Meat. Available at www.so-meat.fr) and were ground before use.

2.3. Spiking and cooking

2.3.1. Sample spiking

Ground beef was spiked according to Planche *et al.* (2015) combining micropollutant addition to ground meat with matrix homogenization. Briefly, ground beef (120 g) was immersed in dichloromethane (20 mL) containing the micropollutants (PCBs, PCDD/Fs, pesticides and heavy metals) followed by evaporation under a hood and homogenization for 2 min in a blender. For PCBs, PCDD/Fs and pesticides, a spiking concentration of 8 ng g^{-1} of fresh meat was chosen to give concentrations in ready-to-run samples within the range of linearity of our GC×GC-TOF/MS method. For heavy metals, meat was spiked at a concentration of 0.1 ng g^{-1} fresh meat corresponding to the authorized limit for lead in meat according to EU Directive 466/2001.

2.3.2. Cooking method

To study the fate of micropollutants during cooking, circular small ground beef patties weighing 26 g (2.5 cm thickness) were shaped to resemble commercial ground beef patties. Ground beef patties were cooked in a stainless steel frying pan (17 cm diameter) on a

controlled-temperature induction hob (Bosch Electroménager, Saint-Ouen, France). A sheet of 11 µm thick aluminum foil was laid on the bottom of the frying pan to recover juice released during meat cooking. Three different cooking conditions were used to simulate rare (core 50 °C), medium (core 70 °C, according to WHO recommendations for ground meats) and well-done (core 85 °C) meat (*n* = 3 for each cooking condition). These cooking conditions corresponded to: 7 min heating (patty turned over once) at 160 °C at the bottom of the pan, 14 min heating (turned over three times) at 200 °C at the bottom of the pan and 14 min heating (turned over three times) at 250 °C at the bottom of the pan, respectively. Temperatures at the core of the meat and at the bottom of the pan were monitored by thermocouples (RS Components, Beauvais, France).

2.3.3. Determination of fat content

The determination of fat content was realized according to Blanchet-Letrouvé *et al.* (2014) with slight modifications. Raw and cooked meat were freeze-dried. 1 g of the powder obtained was then extracted by accelerated solvent extraction (ASE) using a Dionex ASE 350 extractor (Sunnyvale, CA, USA) fitted with 22 mL stainless-steel extraction cells. A toluene-acetone (70:30) mixture was used as extraction solvent at a temperature of 120 °C and a pressure of 1500 psi with three extraction cycles per sample. The extract obtained was then evaporated under a hood and weighed to determine fat content.

2.4. Sample extraction

2.4.1. Extraction of PCBs, PCDD/Fs and pesticides

Before extraction, the cooked ground beef patties were powdered with a liquid nitrogen grinder. Extraction, clean-up and concentration of the extract were then carried out according to Planche *et al.* (2015) with slight modifications. Briefly, 5 g of meat powder were extracted by accelerated solvent extraction (ASE) using a Dionex ASE 350 extractor (Sunnyvale, CA, USA). 34 mL stainless-steel extraction cells were used, with 12 g of acidic alumina placed at the bottom of the cells. Paper filters were placed at the bottom and top of the alumina layer. The cells were then filled with 5 g of ground beef dispersed in diatomaceous earth. Hexane was used as extraction solvent at a temperature of 100 °C and pressure of 1500 psi. ASE extraction included heating (5 min), static time (5 min) and purging (90 s) with two extraction cycles per sample. After filtration through a glass fiber prefilter

and a 0.2 µm nylon filter (Phenomenex, Torrance, CA), the extract (ca. 40 mL) was evaporated (Rocket, Genevac Ltd.) using toluene as a keeper to minimize analyte losses during the evaporation step, and 4 mL of dichloromethane were then added. To clean up extracts, gel permeation chromatography (GPC) (Gilson, Middleton, WI, USA) was carried out on an S-X3 Bio-Beads column (Bio-Rad, Philadelphia, USA) using dichloromethane as eluting solvent at a flow rate of 5 mL min^{-1}. The fraction obtained was evaporated to dryness (Rocket, Genevac Ltd.), and 100 µL of hexane were then added prior to analysis. All the samples were spiked with internal standards (100 ng mL^{-1}) at different steps.

2.4.2. Sample digestion for heavy metals analysis

Samples were digested using a Multiwave 3000 microwave digestion system (Anton-Paar, Courtaboeuf, France), equipped with a rotor for eight 80 mL quartz vessels (operating pressure, 80 bar). The sample digestion procedure was performed according to the EN 13805 standard and had previously been optimized (Noël *et al.*, 2003). Before use, the quartz vessels were decontaminated with 6 mL of 50% HNO_3 (54%, v/v) in the microwave digestion system, then rinsed with ultrapure water and dried in a 40°C oven. From 0.3 to 0.5 g (dry food) and from 0.5 to 2.0 g (fresh diet) of dietary samples were weighed precisely in the quartz digestion vessels and wet-oxidized with 3 mL ultrapure water and 3 mL Suprapur HNO_3 (67%) in the microwave digestion system. One randomly selected vessel was filled with reagents only and taken through the entire procedure as a blank. After cooling to room temperature, sample solutions were transferred into 50 mL polyethylene flasks. Then, 100 µL of the internal standard solution (1 mg L^{-1} internal standards and 10 mg L^{-1}Au) were added to a final concentration of 2 µg L^{-1} internal standards and 20 µg L^{-1} Au; the digested samples were filled with ultrapure water to the final volume before analysis by ICP-MS.

2.5. Micropollutant analysis

2.5.1. Multiresidue analysis of PCBs, PCDD/Fs and pesticides

Samples were analyzed on a time-of-flight mass spectrometer (Pegasus 4D, Leco, St Joseph, MI) coupled to a two-dimensional gas chromatograph (6890, Agilent Technologies) equipped with a dual stage jet cryogenic modulator (licensed from Zoex) according to Planche *et al.* (2015). A Rtx-Dioxin2 1D column (60 m × 0.25 mm × 0.25 µm) (Restek, Bellefonte, PA, USA) was connected to a BPX-50 2D column (2 m × 0.1 mm × 0.1 µm) (SGE, Austin,

TX, USA) with a deactivated ultimate union (Agilent Technologies, Santa Clara, CA). A splitless injection of 1 µL of sample extract was performed through a CTC CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) with an inlet temperature set at 280 °C. A split/splitless inert liner (Restek, sky® 4.0 mm ID liner) was used. Ultra-pure grade helium (purity 99.9995%) was used as carrier gas with a constant flow rate of 1.5 mL min^{-1}. Purge time was set to 60 s with a flow rate of 50 mL min^{-1}. The primary oven temperature was initially set at 90 °C for 1 min, then ramped to 200 °C at 20 °C min^{-1}, then to 308 °C at 2 °C min^{-1} and lastly to 330°C at 5 °C min^{-1} for 10 min. The secondary oven temperature was set 5 °C higher than the primary oven temperature. The modulator temperature was set 15 °C higher than the primary oven temperature, and the modulation period was 5 s with 1.20 and 1.30 s for the hot and cold pulses, respectively. The transfer line temperature was set at 280 °C. The mass spectrometer was operated with an electron ionization source (ionization energy: 70 eV), a detector voltage of 1800 V and a data acquisition rate of 100 spectra s^{-1}. The run time for each sample was 75 min. Analytical blank samples of pure solvent were run to check for absence of targeted analytes. GC×GC data were processed using LECO ChromaTOF software (version 4.50.8.0).

In order not to be restricted by the sensitivity of the GC×GC-TOF/MS method, naturally contaminated samples, in which the concentration of contaminants is unknown, were analyzed according to Berge *et al.* (2011) by a French National Reference Laboratory (LABERCA, Nantes, France).

2.5.2. Analysis of heavy metals

ICP-MS measurements were performed with a 7700 Series x (Agilent Technologies, Courtaboeuf, France) equipped with a third generation Octopole Reaction System (ORS^3) using He gas. The sample solutions were pumped by a peristaltic pump from tubes arranged on a CETAC ASX-500 Series auto-sampler (CETAC Technologies, Omaha, NE, USA). The torch position, ion lenses, gas output, resolution axis (10% of peak height, m ± 0.05 a.m.u) and background (<20 shots) were optimized daily with the tuning solution (1 mg L^{-1}) to carry out a short-term stability test of the instrument, to maximize ion signals and to minimize interference effects due to high oxide levels (CeO^+/ Ce^+ < 1.2%) and doubly charged ions (Ce^{2+}/Ce^+ < 2%). Linearity response in the pulsed and analogue modes (P/A factor determination) was verified daily using PA tuning solutions. Further details of instrumental

settings, optimization and validation process for the simultaneous determination of 31 elements are given in Chevallier *et al.* (2015).

2.6. Data processing

Micropollutant losses induced by cooking were determined according to Rawn *et al.* (2013):

$$\text{Loss} = 1 - \frac{([\text{Micropollutant cooked meat}] \times \text{mass cooked meat})}{([\text{Micropollutant raw meat}] \times \text{mass raw meat})}$$

where [Micropollutant raw meat] and [Micropollutant cooked meat] are the micropollutant concentrations in raw or cooked meat (ng g^{-1} of meat) and mass raw meat and mass cooked meat are the mass of meat (g) before and after cooking.

Data were processed using Statistica software version 10 (StatSoft, Maisons-Alfort, France). Principal component analysis (PCA) was performed on the level of micropollutants in meat before and after cooking to visualize the structure of the data. To determine whether the cooking process had an effect on the level of micropollutants in meat, a one-way analysis of variance (ANOVA; $p < 0.05$) was performed on data from GC×GC-TOF/MS and ICP-MS analyses. A Newman-Keuls mean comparison test was then performed on the resulting dataset to determine which cooking intensity distinguished between the three ($p<0.05$).

3. Results and Discussion

3.1. Multiresidue assay of PCBs, PCDD/Fs and pesticides in cooked meat

To study the effects of cooking on levels of PCBs, PCDD/Fs and pesticides in meat, we first designed a multiresidue method based on GC×GC-TOF/MS for the simultaneous monitoring of these three families of contaminants in cooked meat. Thus, we adapted the method recently developed by Planche *et al.* for the simultaneous monitoring of PCBs and PCDD/Fs to extend its scope to the assay of pesticides (Planche *et al.*, 2015).

A set of 16 pesticides (12 organochlorine and 4 organophosphorus) likely to be found in meat were targeted in this work. The separative capacity of the column set used (Rtx-Dioxin2/BPX50) was determined by calculating resolution factors (R_s) according to Zapadlo *et al.* with $R_s = \Delta t_R/w_b$, where t_R is the retention time and w_b the average peak width at the base (Zapadlo *et al.*, 2011). Two successive peaks were considered resolved when $R_{s,1D} \geq 0.6$ in 1D or $R_{s,2D} \geq 0.4$ in 2D. The average resolution factors for pesticides were 2.44 in the first

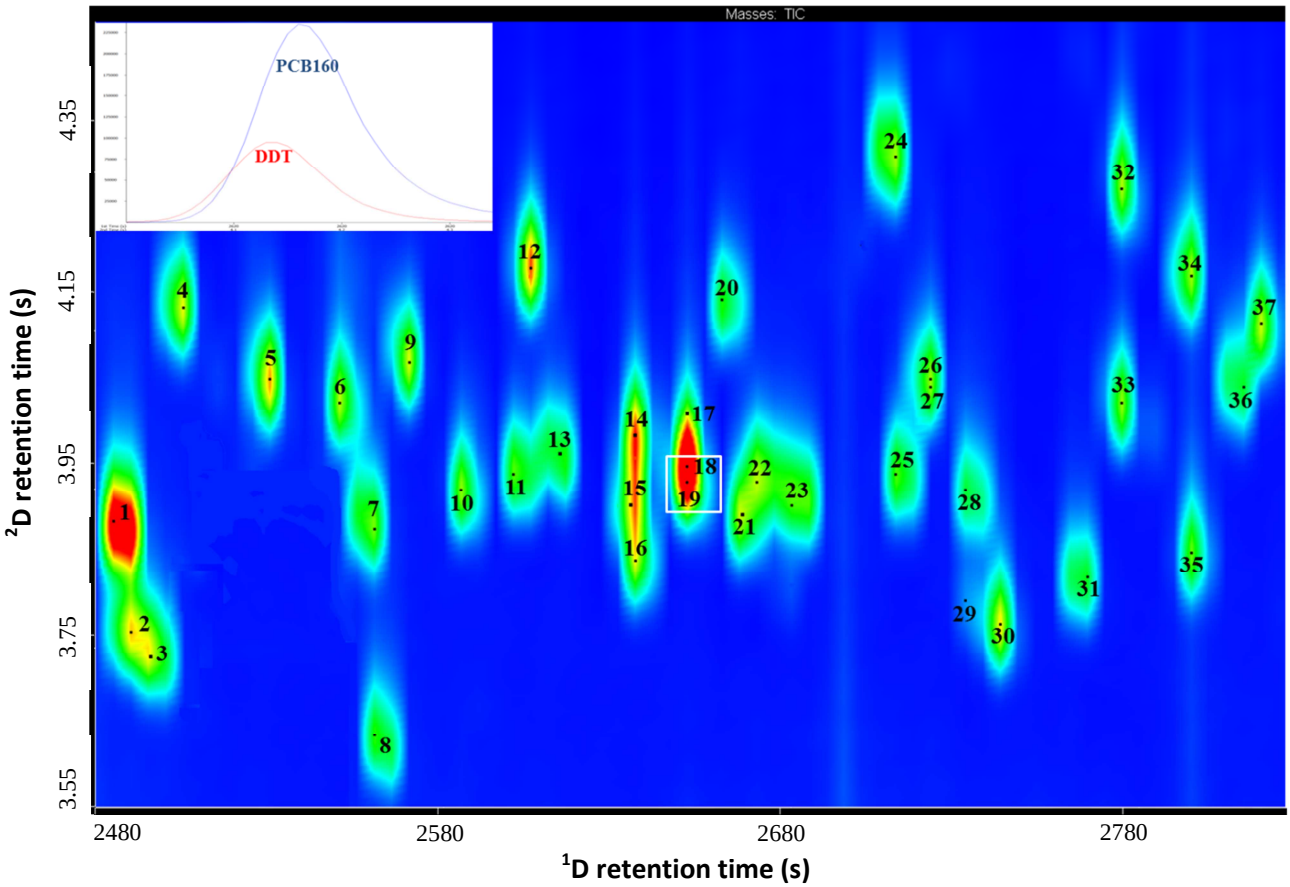

Fig. 1. GC×GC-TOF/MS contour plot of a set of PCBs, PCDD/Fs and pesticides in medium-cooked meat. The zoom shows how DDT (No. 19) and PCB160 (No. 18) can be resolved by means of their specific ions.

1-DDD; 2-PCB168; 3-PCB153; 4-PCB132; 5-PCB179; 6-PCB105; 7-PCB141; 8-PCB127; 9-PCB176; 10-PCB137; 11-PCB130; 12-PCB186; 13-PCB164; 14-PCB163; 15-PCB138; 16-PCB178; 17-PCB158; 18-PCB160; 19-DDT; 20-PCB129; 21-PCB175; 22-PCB182; 23-2,3,7,8-TCDF; 24-Methoxychlor; 25-PCB187; 26-PCB166; 27-PCB183; 28-PCB126; 29-2,3,7,8-TCDD; 30-PCB159; 31-PCB162; 32-PCB128; 33-PCB185; 34-PCB174; 35-PCB167; 36-PCB202; 37-PCB181.

Table 1. Recovery rates obtained for a set of PCDD/Fs, PCBs and pesticides, both organochlorine (OC) and organophosphorus (OP), after spiking, extraction and analysis of medium cooked meat (*n* = 3). *: significantly lower at $p < 0.05$ than recovery rates obtained in raw meat by Planche *et al.* (2015).

Family	Compound	Recovery rate (%)	RSD (%)
PCDD/F	2,3,7,8-TCDD	86	8.3
PCDD/F	2,3,7,8-TCDF	94	11
PCDD/F	1,2,3,7,8-PeCDD	74	6.3
PCDD/F	2,3,4,7,8-PeCDF	72	5.4
PCDD/F	1,2,3,4,7,8-HxCDD	67	12
PCDD/F	1,2,3,4,7,8-HxCDF	69	12
PCDD/F	1,2,3,6,7,8-HxCDF	62	14
PCB (1)	2	91	6.7
PCB (8)	2,4'	102*	5.9
PCB (19)	2,2',6	91	6.8
PCB (28)	2,4,4'	103*	6.4
PCB (52)	2,2’,5,5’	94*	8.1
PCB (77)	3,3',4,4'	107*	4.1
PCB (81)	3,4,4',5	83*	6.5
PCB (105)	2,3,3',4,4'	72*	7.3
PCB (114)	2,3,4,4’,5	71*	6.5
PCB (118)	2,3',4,4',5	70*	6.4
PCB (123)	2',3,4,4',5	80*	3.2
PCB (126)	3,3',4,4',5	71*	3.2
PCB (153)	2,2',4,4',5,5'	80*	0.6
PCB (156)	2,3,3',4,4',5	59*	9.0
PCB (157)	2,3,3’,4,4’,5’	106	4.0
PCB (167)	2,3’,4,4’,5,5’	65*	7.5
PCB (169)	3,3',4,4',5,5'	113	5.0
PCB (172)	2,2',3,3',4,5,5'	107*	4.0
PCB (189)	2,3,3’,4,4’,5,5’	94	2.3
PCB (206)	2,2',3,3',4,4',5,5',6	83*	3.0
PCB (209)	2,2',3,3',4,4',5,5',6,6'	95*	21
Pesticide (OC)	aldrin	107	6.8
Pesticide (OC)	dieldrin	117	9.7
Pesticide (OC)	4,4’-DDT	136	10
Pesticide (OC)	4,4’-DDD	90	7.8
Pesticide (OC)	4,4’-DDE	92	3.3
Pesticide (OC)	lindane	88	9.0
Pesticide (OC)	alpha BHC	94	5.4
Pesticide (OC)	béta BHC	87	5.9

Pesticide (OC)	delta BHC	77	9.2
Pesticide (OC)	heptachlor	138	4.1
Pesticide (OC)	heptachlor epoxide	76	5.7
Pesticide (OC)	methoxychlor	96	11
Pesticide (OP)	sulfotep	101	7.6
Pesticide (OP)	O,O,O-triethylphosphorothioate	68	7.3
Pesticide (OP)	phorate	112	7.3
Pesticide (OP)	disulfoton	72	7.4

dimension and 0.45 in the second one. These values are of the same order of magnitude as those obtained by Planche *et al.* (2015) for PCBs and PCDD/Fs (on average, $R_{s,1D}$ = 2.05 and $R_{s,2D}$ = 0.51). The GC×GC-TOF/MS method optimized for the analysis of PCBs and PCDD/Fs was thus also satisfactory for the analysis of pesticides. Figure 1 presents an expanded region of the contour plot obtained from the analysis of an extract of cooked meat spiked beforehand with PCBs, PCDD/Fs and pesticides, showing the separation of PCBs 105, 141 and 127 (Nos. 6, 7 and 8 in Figure 1), and of PCB 126 (No. 28) and 2,3,7,8-TCDD (No. 29) using the second chromatographic dimension of the GC×GC-TOF/MS. In addition, compounds that were not chromatographically resolved could be individually monitored provided that they displayed specific ions in their respective mass spectra: this was the case for DDT (No. 19) and PCB 160 (No. 18). The GC×GC-TOF/MS method used thus allowed the simultaneous assay of 222 micropollutants (189 PCBs, 17 PCDD/Fs and 16 pesticides), in 75 min (Table S1. Supplementary material). These results further support GC×GC-TOF/MS as an effective method for multiresidue analysis.

In order to further use this method for PCB, PCDD/F and pesticide quantification after cooking, recovery rates of these micropollutants were determined after spiking, extraction and analysis of cooked meat using the protocol developed by Planche *et al.* on raw meat (Planche *et al.*, 2015). Table 1 shows the recovery rates obtained from medium cooked meat for a set of 21 PCBs (including 15 of the 18 PCBs considered as the most relevant due to their occurrence in meat), the 16 pesticides listed in Table S1. (Supplementary material), and a set of 7 PCDD/Fs including 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, the most toxic dioxins with WHO-TEF = 1 (Sirot *et al.*, 2012). If the expanded uncertainty is taken as twice the RSD, we can note that many of the component recoveries shown for 2 degrees of freedom are not significantly different from 100%. Indeed, most of the recovery rates lay between 70% and 130%, a classically acceptable interval (EPA Method 8000C, 2003), with RSD ≤ 10%. Hexachlorodibenzo-dioxins/furans, PCBs 156, 167 and 209, heptachlor, DDT and O,O,O-triethylphosphorothioate had recovery rates and/or RSD values outside the classical acceptable limits. For these compounds, the results subsequently obtained in this study must therefore be interpreted with caution. Note that for 16 of the 21 PCBs monitored, cooking lowered recovery rates relative to the values obtained in raw meat by Planche *et al.* (2015). This decrease was especially marked for PCBs 105 and 156, with respective recovery rates of 72% and 59% after extraction and analysis of cooked meat, against 115% and 108% from raw meat. Sporring *et al.* (2004) also showed for an ASE extraction method that PCB recovery rates varied widely according to the matrix, for example averaging 113% (RSD = 1.8%) when

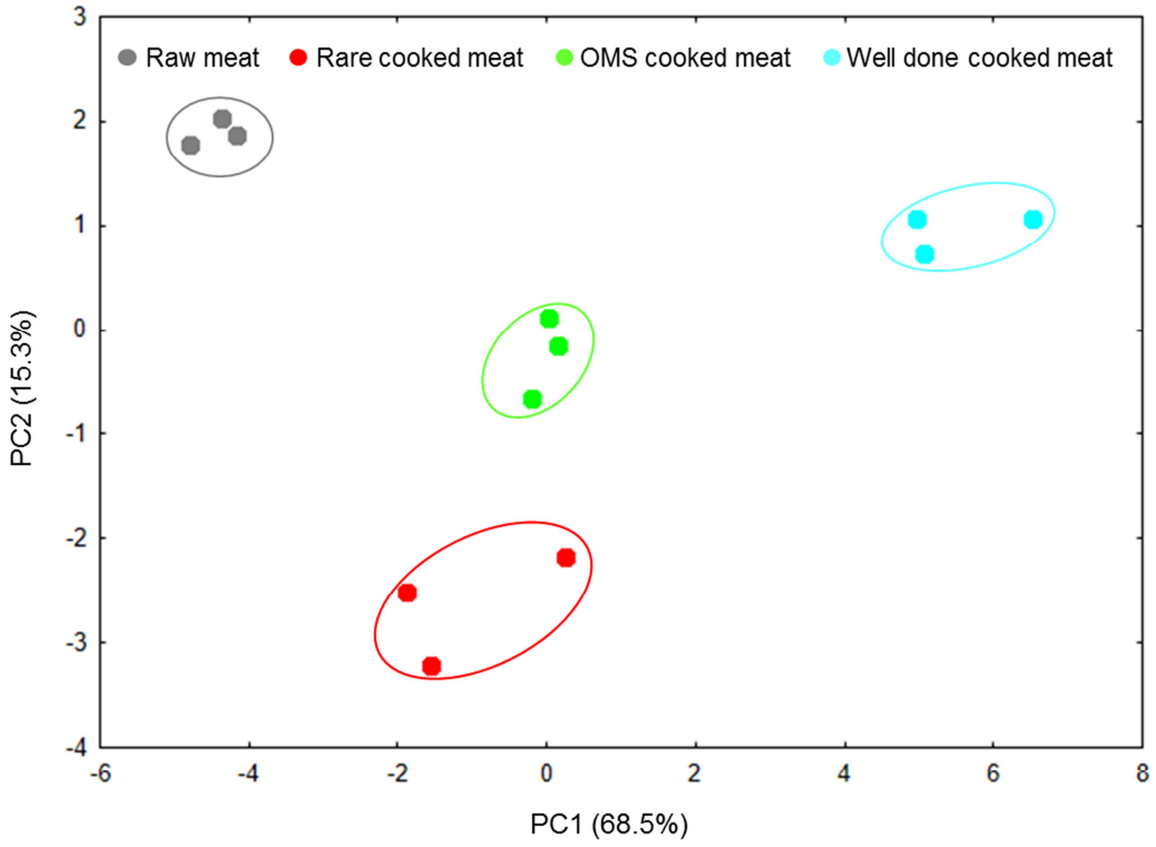

Fig. 2. Discrimination of concentration (ng g^{-1} of fat) of PCBs in meat according to intensity of cooking of samples.

Table 2. Concentrations (n = 3) of PCBs and PCDD/Fs in spiked raw meat and in spiked meat cooked at different intensities (rare, medium and well-done). All results are corrected for individual recovery rates. [a,b,c,d]: The letters represent the groups given by the Newman-Keuls mean comparison test when compared for each compound, based on the concentration obtained for the different cooking conditions. Two groups with distinct letters can be considered as significantly different ($p < 0.05$).

		Raw meat		**Rare-cooked meat**		**Medium-cooked meat**		**Well-done meat**	
Lipid content		11%		13%		15%		16%	
Cooking mass loss				23%		34%		36%	
Compound	Toxic equivalency factor (TEF)	Concentration (ng g^{-1} of fat)	RSD (%)	Concentration (ng g^{-1} of fat)	RSD (%)	Concentration (ng g^{-1} of fat)	RSD (%)	Concentration (ng g^{-1} of fat)	RSD (%)
PCB 1		64^{b}	4.6	49^{a}	8.2	51^{a}	12	47^{a}	11
PCB 8		77^{a}	5.6	75^{a}	14	79^{a}	13	60^{a}	23
PCB 19		68^{a}	8.6	55^{a}	11	58^{a}	32	53^{a}	15
PCB 28		99^{d}	0.60	81^{c}	1.3	73^{b}	2.9	63^{a}	7.5
PCB 52		78^{bc}	2.2	92^{c}	8.2	73^{b}	11	56^{a}	20
PCB 77	0.0001	84^{d}	5.3	49^{c}	5.1	44^{b}	3.1	38^{a}	5.3
PCB 81	0.0003	76^{b}	6.4	70^{b}	13	66^{ab}	3.1	55^{a}	12
PCB 105	0.00003	87^{c}	0.62	68^{b}	11	69^{b}	3.5	47^{a}	7.7
PCB 114	0.00003	76^{b}	1.6	79^{b}	11	68^{b}	8.5	48^{a}	6.6
PCB 118	0.00003	78^{b}	3.1	71^{b}	11	70^{b}	2.2	49^{a}	8.0
PCB 123	0.00003	81^{c}	4.1	68^{b}	11	71^{b}	2.7	30^{a}	6.1
PCB 126	0.1	72^{c}	2.3	62^{b}	11	67^{bc}	4.5	54^{a}	2.8
PCB 153		97^{c}	3.9	47^{b}	8.6	50^{b}	15	32^{a}	27
PCB 156	0.00003	80^{b}	2.7	76^{b}	4.5	80^{b}	4.8	38^{a}	7.3
PCB 157		67^{b}	4.6	105^{c}	7.8	73^{b}	2.1	55^{a}	3.0

PCB 167	0.00003	77[b]	0.90	102[c]	7.8	71[b]	0.43	51[a]	4.4
PCB 169	0.03	74[c]	1.3	80[d]	4.1	36[b]	9.5	26[a]	15
PCB 172		88[c]	7.6	56[b]	11	61[b]	1.1	40[a]	8.9
PCB 189	0.00003	72[c]	3.7	60[b]	6.5	72[c]	1.4	32[a]	8.7
PCB 206		89[c]	9.9	50[b]	8.9	41[b]	4.4	15[a]	8.3
PCB 209		82[d]	8.1	49[c]	10	39[b]	5.3	13[a]	3.3
2,3,7,8-TCDD	1	74[a]	12	70[a]	17	71[a]	15	67[a]	16
2,3,7,8-TCDF	0.1	63[a]	9.4	72[a]	15	77[a]	1.8	68[a]	16
1,2,3,7,8-PeCDD	1	66[a]	12	77[a]	5.0	65[a]	30	49[a]	6.4
2,3,4,7,8-PeCDF	0.3	55[a]	11	66[a]	25	74[a]	8.3	58[a]	3.7
1,2,3,4,7,8-HxCDD	0.1	62[a]	18	90[a]	15	97[a]	7.2	74[a]	35
1,2,3,4,7,8-HxCDF	0.1	51[a]	9.6	85[b]	11	81[b]	1.4	64[a]	16
1,2,3,6,7,8-HxCDF	0.1	45[a]	7.9	72[b]	10	75[b]	18	49[a]	14

extracting from a vegetable feedstuff, against 46% (RSD = 2.2%) when extracting from pork fat. According to Ishiwatari *et al.* (2013), the different matrix structures could explain these differences. In our study conditions, the protein denaturation that occurs during cooking causes a marked change in the structure of the meat matrix that may account for the decreased recovery rates obtained from cooked relative to raw meat.

The recovery rates of the micropollutants monitored were determined in the same way from rare-cooked and well-done meat. Contrary to what one might expect, the results were of the same order of magnitude as those obtained after medium cooking (data not shown). Indeed, although high cooking intensity raised the lipid content of the meat (13%, 15% and 16% of lipids respectively in rare, medium and well-done cooked meat) and lowered its water content (61%, 52% and 50% of water respectively in rare, medium and well-done cooked meat), the recovery rates obtained were not significantly affected.

3.2. Effects of cooking on contaminant loads in meat

3.2.1. PCBs and PCDD/Fs

As shown in the first map of the PCA (Figure 2), clear differences in meat PCB concentrations can be observed according to the cooking intensity. A similar separation was obtained starting with the quantities of PCBs in raw or cooked meat (Figure S1. Supplementary material). The examination of the effect of the different cooking intensities on each PCB (Table 2 and Table S2. Supplementary material) confirms these observations. The average total PCB concentration decreases ($p < 0.05$) from 79 ng g^{-1} of fat in raw meat (n=3) to 69, 63 and 43 ng g^{-1} of fat after rare (n=3), medium (n=3), and intense (n=3) cooking. This corresponds to a loss of respectively 18 %, 30 % and 48 % of total PCBs (ng) in the meat. Higher cooking intensity thus causes significantly greater PCB losses ($p < 0.05$), which induce a decrease in PCB concentration in meat. Although both the matrix studied and the cooking intensities were different, these results are in line with those of Bayen *et al*. (2005) for pan-fried fish (5 min at 180 °C) containing 8.3–18.3% lipids, in which average losses of PCBs were 36 ± 11% (Bayen *et al*., 2005). Loss observed for intense cooking is also consistent with the average decrease of 44% in PCDD/F and dioxin-like PCB concentration reported by Hori *et al.* (2005) after a 10 minute broiling of ground beef (15.7 ± 4.6% fat). In contrast, Rawn *et al.* (2013) reported narrow variations in PCB concentrations when cooking fish, these variations being partly attributed to the uncertainty in the quantification method used. Considering the different PCB congeners (Table S2. Supplementary material), cooking

effect varied with the congener assayed, with for example non-significant losses of PCBs 8, 19 and 52, but losses greater than 50% of PCBs 77, 153, 169, 206 and 209 after medium cooking. Note that the losses observed for PCBs 126 and 169, which had the highest WHO-TEF values of all the PCB congeners studied (0.1 and 0.03), were respectively 16% and 57%, showing the importance of taking into account the mitigating effects of cooking when assessing risks ascribed to this family of contaminants. These losses cannot be explained by a volatilization of PCBs during cooking because PCBs are resistant to temperatures up to 300°C whereas, even in the most intense cooking condition, the temperature at the bottom of the pan is 250°C and only 85°C at the core of the meat. In our study, the greatest losses (>55% for medium cooking and >80% for intense cooking) were observed for PCBs 206 and 209, the most highly chlorinated PCB congeners (9 and 10 chlorine atoms, respectively). Bayen *et al*. also found greater losses of more highly chlorinated PCBs after cooking: they explained this effect by the expulsion of these particularly lipophilic compounds into cooking juices along with lipids contained in the food matrix (Bayen *et al*., 2005). This explanation also fits our results, where PCBs 206 and 209 were the most lipophilic owing to their high chlorination level (highest log K_{ow} values of 7.9 and 8.3, respectively). Also, Aaslyng *et al*. (2003) showed that the higher the core temperature in the meat during cooking, the greater were the lipid losses in the cooking juice. Extraction of PCBs in the expelled lipids thus explains why losses caused by intense cooking (core 85 °C) were greater than those observed with gentle cooking (core 50 °C). The matrix studied, and in particular its initial lipid content and the method of cooking chosen thus seem to strongly influence variations in PCB loads observed during cooking.

For total PCDD/Fs in meat, the average losses were not significant, irrespective of the cooking intensity (Table S2. Supplementary material). These results are consistent with those obtained by Rose *et al*., who also found no significant losses of PCDD/Fs after beef cooking, whatever the method used (Rose *et al*., 2001). Table 2 shows that meat concentration in 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF (in ng g^{-1} of fat) increase after rare or medium cooking whereas no significant increase could be observed after intense cooking. However, these results must be interpreted with caution for these two compounds, their recovery rates being respectively 63.8% and 63.9% after intense cooking, with RSD $\geq$ 10%. For the other targeted PCDD/Fs, no significant difference in concentration during cooking could be observed, whatever the cooking intensity. These results contrast with those obtained by Hori *et al*. (2005), who showed an average decrease in concentration (pg TEQ g^{-1}) of 54% for PCDD/Fs when ground beef (15.7 ± 4.6% fat) was broiled (approximately 10 min) (Hori *et*

Table 3. Concentrations (*n* = 3) of organochlorine (OC) and organophosphorus (OP) pesticides in spiked raw meat and in spiked meat cooked at different intensities (rare, medium and well-done). All results are corrected for individual recovery rates. [a,b,c,d]: The letters represent the groups given by the Newman-Keuls mean comparison test when compared for each compound, based on the concentration obtained for the different cooking conditions. Two groups with distinct letters can be considered as significantly different ($p < 0.05$).

Compound		Raw meat		Rare-cooked meat		Medium-cooked meat		Well-done meat	
		Concentration (ng g^{-1} of meat)	RSD (%)	Concentration (ng g^{-1} of meat)	RSD (%)	Concentration (ng g^{-1} of meat)	RSD (%)	Concentration (ng g^{-1} of meat)	RSD (%)
Aldrin	OC	7.8^{a}	3.9	10^{b}	9.6	13^{b}	12	12^{b}	12
Dieldrin	OC	8.4^{c}	8.5	1.3^{a}	5.9	2.4^{b}	4.7	0.87^{a}	16
4,4’-DDT	OC	8.5^{c}	5.9	6.5^{b}	9.9	3.4^{a}	13	4.3^{a}	4.6
4,4’-DDD	OC	7.5^{a}	12	9.4^{b}	12	11^{b}	3.3	12^{c}	8.4
4,4’-DDE	OC	6.9^{ab}	14	8.1^{b}	3.2	9.9^{c}	6.3	6.4^{a}	10
Lindane (Gamma BHC)	OC	7.4^{a}	6.6	12^{b}	9.8	10^{b}	4.3	10^{b}	12
Alpha BHC	OC	6.9^{a}	7.3	7.0^{a}	10	8.5^{a}	7.0	8.1^{a}	12
Béta BHC	OC	6.5^{a}	5.4	9.8^{c}	4.5	10^{c}	4.6	8.3^{b}	11
Delta BHC	OC	5.9^{b}	4.9	3.6^{a}	15	3.2^{a}	4.6	4.2^{a}	20
Heptachlor	OC	8.2^{c}	1.8	4.5^{ab}	7.9	5.0^{b}	5.1	4.1^{a}	10
Heptachlor epoxide	OC	6.5^{a}	6.1	5.8^{a}	11	5.1^{a}	18	6.8^{a}	13
Methoxychlor	OC	7.3^{a}	4.5	10^{b}	6.0	10^{b}	4.7	7.5^{a}	12
Sulfotep	OP	7.5^{b}	6.8	1.2^{a}	17	1.5^{a}	29	1.2^{a}	6.9
O,O,O-triethylphosphorothioate	OP	6.7^{b}	12	1.6^{a}	17	1.9^{a}	10	1.5^{a}	11
Phorate	OP	8.4^{b}	6.4	2.1^{a}	15	2.3^{a}	20	1.7^{a}	28
Disulfoton	OP	6.0^{a}	4.6	7.8^{a}	26	8.8^{a}	11	8.0^{a}	14

al., 2005). As already reported by Perelló *et al*. (2010), we thus observed different behavior during cooking between key PCBs and PCDD/Fs, even though both families of compounds are lipophilic.

In order to determine whether a difference may exist in the fate of contaminants during cooking, between spiked contaminants and contaminants bioaccumulated in muscles during animal breeding, beef (n = 3), pork (n = 3) and chicken (n = 3) samples naturally contaminated with PCBs and PCDD/Fs were studied. Table S3. (Supplementary material) presents concentrations of PCBs and PCDD/Fs in these raw or medium-cooked samples. Except one beef sample (n°3), an increase was observed in the concentration of PCBs and PCDD/Fs in meat after cooking. In terms of quantity, no significant losses were observed for these compounds during cooking (data not shown), which is consistent with the results observed for PCDD/Fs in spiked meat but differs from those obtained for PCBs in spiked meat (30% loss). These differences could be partly explained by the fact that meat fat content may vary significantly between samples and these variations could affect the fate of micropollutants during cooking (Watkins *et al.*, 2010). Moreover, it is also important to note that the lipid content of naturally contaminated samples (from 1.0% to 5.3% fat) is much lower than in spiked samples (11% fat). As PCBs are expelled in the cooking juice with fat, it can be hypothesized that for lean meat, there are, according to Oroszvári *et al*. (2006), small lipid losses in the cooking juice which thus induce no significant loss of PCBs during cooking. To confirm this hypothesis, additional analyses were carried out on chicken meat samples (mean fat level: 1.5%) intentionally spiked with PCBs before cooking. Consistent with the results observed with naturally contaminated samples, no significant losses of PCBs were observed during cooking for these low-fat spiked samples (data not shown). Thus, differences that were observed between 11% fat spiked samples and naturally contaminated samples seems to be due to the different lipid level between these samples and not to the spiking protocol.

3.2.2. Pesticides

Table 3 gives the concentrations in ng g^{-1} of meat of the 16 pesticides (12 organochlorine and 4 organophosphorus) for the different cooking intensities, these concentration units being classically used in risk assessment according to Reg 396/2005/EC. Losses in the total load of pesticides in meat during cooking are reported in Table S2. (Supplementary material).

Different behaviors can be observed in Table 3. First, an increase in concentration during cooking could be observed for 6 pesticides (aldrin, DDE, DDD, lindane, methoxychlor, and beta-BHC). This increase may be explained by the mass loss of meat during cooking (22.6 ± 1.5%, 34.0 ± 1.5% and 35.6 ± 1.8% of mass loss after respectively rare, medium and well-done cooking) that exceed pesticide losses reported in Table S2. (Supplementary material), resulting in a concentration effect. Secondly, three pesticides (alpha-BHC, heptachlor epoxide and disulfoton) showed no significant variation in concentration during cooking. Indeed the losses reported in Table S2. (Supplementary material) were of the same order of magnitude than mass loss of meat during cooking. These losses can be explained by an elimination of these compounds with juice during cooking or by their volatilization, in particular for disulfoton which is more volatile than the others. Note that the high variability of the results obtained for disulfoton (RSD ≥ 10%) may also explain that its small concentration increase after cooking observed in Table 3 was not significant. Lastly, a decrease in concentration could be observed for 7 pesticides (DDT, heptachlor, dieldrin, sulfotep, phorate, O,O,O-triethylphosphorothioate and delta-BHC), with losses of contaminants, that can exceed 80% after medium cooking (Table S2. Supplementary material), greater than meat mass losses. These 7 pesticides included 3 organophosphorus pesticides (sulfotep, phorate and O,O,O-triethylphosphorothioate) which are known to be thermosensitive even at current cooking temperature (Abou-Arab, 1999). Their breakdown during cooking could thus explain their lowered concentration in cooked meat. For heptachlor, dieldrin and DDT, Muresan *et al.* (2015) reported lower losses than in our study after baking pork (1 h at 110 °C): loss of 44.1%, 46.3% and 51.1% for respectively heptachlor, dieldrin and DDT (loss of 61.6%, 81.9% and 74.7% in our study for medium cooking). In our study, the conversion of DDT into DDD by reductive dechlorination or into DDE by dehydrochlorination under the effect of heat can explain the marked losses of DDT during cooking (Bayarri *et al*., 1994; Muresan *et al*., 2015). There seems to be an equilibrium state for DDD and DDE between losses in cooking juice and formation from DDT, which could explain why their quantities in meat remained almost constant during cooking (except for DDE during intense cooking with losses ≥40%), as shown in Table S2 (Supplementary material). According to Muresan *et al*. (2015), the oxidation of heptachlor into heptachlor epoxide could also explain the marked losses and lowered concentration of heptachlor during cooking. This is consistent with the smaller losses of heptachlor epoxide during intense cooking than during medium cooking (Table S2. Supplementary material), possibly due to the formation of heptachlor epoxide by oxidation of heptachlor. In contrast, neither the physical

Table 4. Concentrations (*n* = 3) of heavy metals in spiked raw meat and in spiked medium-cooked meat. All results are corrected for individual recovery rates. *: significantly different at $p < 0.05$.

Compound	Raw meat		Medium-cooked meat	
	Concentration (mg kg^{-1} of meat)	RSD (%)	Concentration (mg kg^{-1} of meat)	RSD (%)
Arsenic	0.13	5.6	0.22*	3.7
Cadmium	0.12	4.9	0.15*	4.8
Mercury	0.11	6.0	0.15*	6.7
Lead	0.12	3.6	0.15*	3.8

and chemical properties of dieldrin and delta-BHC, nor their high temperature stability data available in the literature can explain the decrease in concentration of these two pesticides during cooking.

3.2.3. Heavy metals

Table 4 presents concentrations in mg kg^{-1} of meat (classical expression of heavy metal concentration in food for risk assessment according to Reg 466/2001/EC) of the four heavy metals studied (arsenic, cadmium, mercury and lead). A significant increase ($p < 0.05$) was observed in the concentration of heavy metals in the meat during cooking for all 4 metals monitored. This increase was of the same order of magnitude for cadmium, mercury and lead, with an average concentration for each metal before cooking of 0.116 mg kg^{-1} of meat (RSD = 1.83%) and an average concentration after cooking of 0.151 mg kg^{-1} of meat (RSD = 0.66%). This increase may be explained by the mass loss of meat during cooking that exceed heavy metal losses reported in Table S2. (Supplementary material), resulting in a concentration effect. A more marked concentration effect was observed for arsenic (from 0.133 mg kg^{-1} of meat before cooking (RSD = 5.60%) to 0.215 mg kg^{-1} of meat after cooking (RSD = 3.67%)) because its quantity in meat stays constant during cooking while meat mass decreases. This increase in the arsenic concentration in meat during cooking was already reported in earlier studies (Devesa *et al.*, 2001; Ersoy *et al.*, 2006; Perelló *et al.*, 2008). Note that losses of heavy metals observed during medium cooking (Table S2. Supplementary material) are close to the margin of error of the method used to assay the heavy metals, making difficult their critical reading (Chevallier *et al.*, 2015). Thus, given the weak or nil effect of cooking on the heavy metal levels in meat, we did not collect data for rare or intense cooking in addition to medium cooking.

In order to assess the impact of spiking on the fate of heavy metals during cooking, beef (n = 9), pork (n = 7) and chicken (n = 6) samples naturally contaminated with arsenic were studied. Table S4. (Supplementary material) presents concentrations (in mg kg^{-1} of meat) of arsenic in these raw or medium-cooked samples. Except one beef sample (n°6) and one pork sample (n°15), an increase was observed in the concentration of arsenic in meat after cooking, whereas its quantity in meat stays constant (data not shown). These results are consistent with those observed with spiked meat. Thus, spiking does not seem to affect the fate of heavy metals during cooking.

4. Conclusion

The GC×GC-TOF/MS multiresidue method set up enabled to study the effects of cooking on a broad range of PCBs, PCDD/Fs and pesticides in spiked ground beef meat. The fate of some heavy metals in meat was assessed by ICP-MS analysis. Results show that cooking could induce a significant decrease in levels of PCBs and pesticides in meat, this decrease being more marked as cooking conditions were more intense. By contrast, cooking only slightly affected levels of PCDD/Fs and heavy metals in meat. Our results indicate that meat lipid levels, hydrophobicity of the contaminants studied and their sensitivity to heat represent the major factors explaining the variations observed. Results obtained on spiked meat proved to be relevant for future modeling / prediction of micropollutant fate in naturally contaminated meat during cooking. The ultimate goal of the project will be to improve chemical risk assessment procedures taking into account the changes induced by cooking on micropollutants in meat.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

Aaslyng, M. D., Bejerholm, C., Ertbjerg, P., Bertram, H. C., & Andersen, H. J. (2003). Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food quality and preference, 14(4),* 277-288.

Abou-Arab, A. A. K. (1999). Behavior of pesticides in tomatoes during commercial and home preparation. *Food chemistry, 65(4),* 509-514.

AFNOR-NF EN 13805 (2014). Foodstuffs - Determination of trace elements - Pressure digestion, Saint Denis, France.

Bayarri, S., Conchello, P., Arino, A., Lazaro, R., & Herrera, A. (1994). DDT, DDT metabolites, and other organochlorines as affected by thermal processing in three commercial cuts of lamb. *Bulletin of environmental contamination and toxicology, 52(4)*, 554-559.

Bayen, S., Barlow, P., Lee, H. K., & Obbard, J. P. (2005). Effect of cooking on the loss of persistent organic pollutants from salmon. *Journal of Toxicology and Environmental Health, Part A, 68(4)*, 253-265.

Berge, P., Ratel, J., Fournier, A., Jondreville, C., Feidt, C., Roudaut, B., Le Bizec, B. & Engel, E. (2011). Use of volatile compound metabolic signatures in poultry liver to back-trace dietary exposure to rapidly metabolized xenobiotics. *Environmental science & technology, 45(15)*, 6584-6591.

Blanchet-Letrouvé, I., Zalouk-Vergnoux, A., Vénisseau, A., Couderc, M., Le Bizec, B., Elie, P., Herrenknecht, C., Mouneyrac, C., & Poirier, L. (2014). Dioxin-like, non-dioxin like PCB and PCDD/F contamination in European eel (Anguilla anguilla) from the Loire estuarine continuum: spatial and biological variabilities. *Science of the Total Environment, 472*, 562-571.

Chevallier, E., Chekri, R., Zinck, J., Guérin, T., & Noël, L. (2015). Simultaneous determination of 31 elements in foodstuffs by ICP-MS after closed-vessel microwave digestion: Method validation based on the accuracy profile. *Journal of Food Composition and Analysis, 41*, 35-41.

Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs.

Commission Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

Darko, G., & Acquaah, S. O. (2007). Levels of organochlorine pesticides residues in meat. *International Journal of Environmental Science & Technology*, *4*(4), 521-524.

Dasgupta, S., Banerjee, K., Patil, S. H., Ghaste, M., Dhumal, K. N., & Adsule, P. G. (2010). Optimization of two-dimensional gas chromatography time-of-flight mass spectrometry for separation and estimation of the residues of 160 pesticides and 25 persistent organic pollutants in grape and wine. *Journal of Chromatography A, 1217(24)*, 3881-3889.

Devesa, V., Macho, M. L., Jalón, M., Urieta, I., Muñoz, O., Súñer, M. A., López, F., Dinoraz Vélez, D., & Montoro, R. (2001). Arsenic in cooked seafood products: study on the effect of cooking on total and inorganic arsenic contents. *Journal of Agricultural and Food Chemistry, 49(8)*, 4132-4140.

Engel, E., Ratel, J., Blinet, P., Chin, S. T., Rose, G., & Marriott, P. J. (2013). Benchmarking of candidate detectors for multiresidue analysis of pesticides by comprehensive two-dimensional gas chromatography. *Journal of Chromatography A, 1311*, 140-148.

Engel, E., Ratel, J., Bouhlel, J., Planche, C., & Meurillon, M. (2015). Novel approaches to improving the chemical safety of the meat chain towards toxicants. *Meat Science, 109*, 75-85.

Environmental Protection Agency (EPA) (2003). EPA Method 8000C, Determinative Chromatographic Separations, Revision 3, Washington, DC, USA.

Ersoy, B., Yanar, Y., Küçükgülmez, A., & Çelik, M. (2006). Effects of four cooking methods on the heavy metal concentrations of sea bass fillets (Dicentrarchus labrax Linne, 1785). *Food Chemistry, 99(4)*, 748-751.

Focant, J. F., Reiner, E. J., MacPherson, K., Kolic, T., Sjödin, A., Patterson, D. G., Reesec, S. L., Dormand, F. L., & Cochran, J. (2004a). Measurement of PCDDs, PCDFs, and non-ortho-PCBs by comprehensive two-dimensional gas chromatography-isotope dilution time-of-flight mass spectrometry (GC× GC-IDTOFMS). *Talanta*, *63*(5), 1231-1240.

Focant, J. F., Sjödin, A., Turner, W. E., & Patterson, D. G. (2004b). Measurement of selected polybrominated diphenyl ethers, polybrominated and polychlorinated biphenyls, and organochlorine pesticides in human serum and milk using comprehensive two-dimensional gas chromatography isotope dilution time-of-flight mass spectrometry. *Analytical chemistry, 76(21)*, 6313-6320.

Focant, J. F., Eppe, G., Scippo, M. L., Massart, A. C., Pirard, C., Maghuin-Rogister, G., & De Pauw, E. (2005). Comprehensive two-dimensional gas chromatography with isotope dilution time-of-flight mass spectrometry for the measurement of dioxins and polychlorinated biphenyls in foodstuffs: Comparison with other methods. *Journal of Chromatography A*, *1086*(1), 45-60.

Hori, T., Nakagawa, R., Tobiishi, K., Iida, T., Tsutsumi, T., Sasaki, K., & Toyoda, M. (2005). Effects of cooking on concentrations of polychlorinated dibenzo-p-dioxins and related compounds in fish and meat. *Journal of agricultural and food chemistry*, *53*(22), 8820-8828.

Ishiwatari, N., Fukuoka, M., & Sakai, N. (2013). Effect of protein denaturation degree on texture and water state of cooked meat. *Journal of Food engineering, 117(3)*, 361-369.

Malisch, R., & Kotz, A. (2014). Dioxins and PCBs in feed and food—review from European perspective. *Science of the Total Environment*, *491*, 2-10.

Muresan, C., Covaci, A., Socaci, S., Suharoschi, R., Tofana, M., Muste, S., & Pop, A. (2015). Influence of Meat Processing on the Content of Organochlorine Pesticides. *Journal of Food Processing & Technology, 6(11)*, 1.

Noël, L., Leblanc, J.C., Guérin, T. (2003). Determination of several elements in duplicate meals from catering establishment using closed vessels microwave digestion with inductively coupled plasma mass spectrometry detection: estimation of daily dietary intake. *Food Additives and Contaminants, 20*, 44–56.

Northcott, G. L., & Jones, K. C. (2000). Spiking hydrophobic organic compounds into soil and sediment: a review and critique of adopted procedures. *Environmental toxicology and chemistry, 19(10),* 2418-2430.

Oroszvári, B. K., Rocha, C. S., Sjöholm, I., & Tornberg, E. (2006). Permeability and mass transfer as a function of the cooking temperature during the frying of beefburgers. *Journal of food engineering, 74(1),* 1-12.

Perelló, G., Martí-Cid, R., Llobet, J. M., & Domingo, J. L. (2008). Effects of various cooking processes on the concentrations of arsenic, cadmium, mercury, and lead in foods. *Journal of Agricultural and Food Chemistry, 56(2*3), 11262-11269.

Perelló, G., Martí-Cid, R., Castell, V., Llobet, J. M., & Domingo, J. L. (2010). Influence of various cooking processes on the concentrations of PCDD/PCDFs, PCBs and PCDEs in foods. *Food Control, 21*(2), 178-185.

Petroske, E., Zaylskie, R. G., & Feil, V. J. (1998). Reduction in polychlorinated dibenzodioxin and dibenzofuran residues in hamburger meat during cooking. *Journal of Agricultural and Food Chemistry, 46(8),* 3280-3284.

Planche, C., Ratel, J., Mercier, F., Blinet, P., Debrauwer, L., & Engel, E. (2015). Assessment of comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry based methods for investigating 206 dioxin-like micropollutants in animal-derived food matrices. *Journal of Chromatography A, 1392,* 74-81.

Rawn, D. F., Breakell, K., Verigin, V., Tittlemier, S. A., Del Gobbo, L., Diamond, M., Vanderlinden, L., & Sit, D. (2013). Impacts of cooking technique on polychlorinated biphenyl and polychlorinated dioxins/furan concentrations in fish and fish products with intake estimates. *Journal of agricultural and food chemistry, 61(4),* 989-997.

Rose, M., Thorpe, S., Kelly, M., Harrison, N., & Startin, J. (2001). Changes in concentration of five PCDD/F congeners after cooking beef from treated cattle. *Chemosphere, 43(4),* 861-868.

Schecter, A., Dellarco, M., Päpke, O., & Olson, J. (1998). A comparison of dioxins, dibenzofurans and coplanar PCBs in uncooked and broiled ground beef, catfish and bacon. *Chemosphere, 37(9)*, 1723-1730.

Sirot, V., Tard, A., Venisseau, A., Brosseaud, A., Marchand, P., Le Bizec, B., & Leblanc, J. C. (2012). Dietary exposure to polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls of the French population: results of the second French Total Diet Study. *Chemosphere*, *88*(4), 492-500.

Sporring, S., & Björklund, E. (2004). Selective pressurized liquid extraction of polychlorinated biphenyls from fat-containing food and feed samples: Influence of cell dimensions, solvent type, temperature and flush volume. *Journal of Chromatography A, 1040(2)*, 155-161.

Stachiw, N. C., Zabik, M. E., Booren, A. M., & Jabik, M. J. (1988). Tetrachlorodibenzo-p-dioxin residue reduction through cooking/processing of restructured carp fillets. *Journal of agricultural and food chemistry, 36(4)*, 848-852.

Takaki, K., Wade, A. J., & Collins, C. D. (2015). Modelling the bioaccumulation of persistent organic pollutants in agricultural food chains for regulatory exposure assessment. *Environmental Science and Pollution Research*, 1-9.

Tang, H. P. O. (2013). Recent development in analysis of persistent organic pollutants under the Stockholm Convention. *TrAC Trends in Analytical Chemistry, 45*, 48-66.

Van der Lee, M. K., Van der Weg, G., Traag, W. A., & Mol, H. G. (2008). Qualitative screening and quantitative determination of pesticides and contaminants in animal feed using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. *Journal of chromatography A*, *1186*(1), 325-339.

Watkins, P. J., Rose, G., Salvatore, L., Allen, D., Tucman, D., Warner, R. D., Dunshea, F. R., & Pethick, D. W. (2010). Age and nutrition influence the concentrations of three branched chain fatty acids in sheep fat from Australian abattoirs. *Meat science, 86(3)*, 594-599.

Zabik, M. E., & Zabik, M. J. (1995). Tetrachlorodibenzo-p-dioxin residue reduction by cooking/processing of fish fillets harvested from the Great Lakes. *Bulletin of environmental contamination and toxicology, 55(2)*, 264-269.

Zapadlo, M., Krupčík, J., Kovalczuk, T., Májek, P., Špánik, I., Armstrong, D. W., & Sandra, P. (2011). Enhanced comprehensive two-dimensional gas chromatographic resolution of polychlorinated biphenyls on a non-polar polysiloxane and an ionic liquid column series. *Journal of Chromatography A, 1218(5)*, 746-751.

Zheng, J., Chen, K. H., Yan, X., Chen, S. J., Hu, G. C., Peng, X. W., Yuan, J. G., Mai, B. X., & Yang, Z. Y. (2013). Heavy metals in food, house dust, and water from an e-waste recycling area in South China and the potential risk to human health. *Ecotoxicology and environmental safety, 96*, 205-212.

Supplementary Material

Table S1. Elution order of PCBs, PCDD/Fs and pesticides in cooked meat using the Rtx-Dioxin2/BPX-50 column set. Coeluting congeners determined from the resolution factor proposed by Zapadlo *et al*. (2011) are listed in a box.

Compound	Congener	ID mass (m/z)	$^{1}t_R$ (s)	$^{2}t_R$ (s)
O,O,O-triethylphosphorothioate		198	640	1.75
2-MoCB	1	188	970	2.42
3-MoCB	2	188	1065	2.55
4-MoCB	3	188	1085	2.57
sulfotep		322	1115	2.68
2,6-DiCB	10	222	1120	2.75
2,2'-DiCB	4	222	1125	2.75
phorate		260	1185	2.77
2,4-DiCB	7	222	1200	2.74
2,5-DiCB	9	222	1205	2.74
2,3'-DiCB	6	222	1230	2.85
2,3-DiCB	5	222	1255	2.92
α-BHC		219	1260	2.88
2,4'-DiCB	8	222	1260	2.89
3,5-DiCB	14	222	1305	2.80
2,2',6-TrCB	19	256	1315	3.10
2,4,6-TrCB	30	256	1340	2.88
3,3'-DiCB	11	222	1380	2.98
disulfoton		88	1380	3.00
β-BHC		219	1380	3.13
2,2',5-TrCB	18	256	1385	3.08
2,2',4-TrCB	17	256	1400	3.06
3,4-DiCB	12	222	1405	3.02
3,4'-DiCB	13	222	1410	3.01
2,3',6-TrCB	27	256	1425	3.14
2,3,6-TrCB	24	256	1435	3.15
4,4'-DiCB	15	222	1440	3.04
lindane		181	1450	3.06
2,2',3-TrCB	16	256	1460	3.27
2,4',6-TrCB	32	256	1465	3.17
2',3,5-TrCB	34	256	1490	3.09
2,3,5-TrCB	23	256	1500	3.08
2,2',6,6'-TeCB	54	292	1510	3.45
2,4,5-TrCB	29	256	1515	3.10
2,3',5-TrCB	26	256	1540	3.14
2,3',4-TrCB	25	256	1550	3.16
δ-BHC		181	1550	3.32
2,2',4,6-TeCB	50	292	1555	3.25

2,4,4'-TrCB	28	256	1580	3.17
2,4',5-TrCB	31	256	1585	3.19
2,2',5,6'-TeCB	53	292	1595	3.38
2,3,3'-TrCB	20	256	1610	3.34
2,3,4-TrCB	21	256	1615	3.34
2,3',4'-TrCB	33	256	1615	3.34
heptachlor		272	1620	3.06
2,2',4,6'-TeCB	51	292	1620	3.39
2,3,4'-TrCB	22	256	1655	3.39
2,2',3,6-TeCB	45	292	1655	3.51
3,3',5-TrCB	36	256	1680	3.16
2,3',5',6-TeCB	73	292	1680	3.31
2,2',3,6'-TeCB	46	292	1685	3.59
2,3',4,6-TeCB	69	292	1690	3.25
2,2',3,5-TeCB	43	292	1700	3.35
2,2',5,5'-TeCB	52	292	1705	3.35
3,4',5-TrCB	39	256	1725	3.22
2,2',4,5'-TeCB	49	292	1725	3.34
2,2',4,5-TeCB	48	292	1730	3.41
2,2',4,4'-TeCB	47	292	1740	3.32
2,4,4',6-TeCB	75	292	1745	3.32
2,3,5,6-TeCB	65	292	1750	3.39
2,2',4,6,6'-PeCB	104	326	1755	3.51
3,4,5-TrCB	38	256	1760	3.35
aldrin		263	1765	3.22
2,3,4,6-TeCB	62	292	1765	3.39
2,2',3,5'-TeCB	44	292	1795	3.54
2,3,3',6-TeCB	59	292	1805	3.49
2,2',3,4'-TeCB	42	292	1810	3.54
3,3',4-TrCB	35	256	1815	3.42
2,3',5,5'-TeCB	72	292	1835	3.28
2,3',4',6-TeCB	71	292	1835	3.57
2,2',3,4-TeCB	41	292	1850	3.65
2,3',4,5'-TeCB	68	292	1855	3.31
2,2',3,6,6'-PeCB	96	326	1855	3.79
2,2',4,5',6-PeCB	103	326	1860	3.42
3,4,4'-TrCB	37	256	1860	3.46
2,3,4',6-TeCB	64	292	1860	3.58
2,2',3,3'-TeCB	40	292	1885	3.76
2,2',4,4',6-PeCB	100	326	1890	3.44
2,3,3',5-TeCB	57	292	1900	3.40
2,2',3,5,6'-PeCB	94	326	1910	3.61
2,3',4,5-TeCB	67	292	1920	3.43
2,3,3',5'-TeCB	58	292	1925	3.49
2,2',4,5,6'-PeCB	102	326	1950	3.46
2,3,4',5-TeCB	63	292	1950	3.64

heptachlor epoxide		353	1955	3.46
2,3,4,5-TeCB	61	292	1960	3.57
2,2',3',4,6-PeCB	98	326	1965	3.63
2,3',4,5',6-PeCB	121	326	1970	3.34
2,4,4',5-TeCB	74	292	1970	3.61
2,2',3,5,6-PeCB	93	326	1970	3.67
2',3,4,5-TeCB	76	292	1975	3.47
2,2',3,5',6-PeCB	95	326	1975	3.66
2,3',4',5-TeCB	70	292	1985	3.53
2,2',3,4,6-PeCB	88	326	1990	3.68
3,3',5,5'-TeCB	80	292	2005	3.25
2,3',4,4'-TeCB	66	292	2005	3.55
2,2',3,4',6-PeCB	91	326	2005	3.70
2,2',4,4',6,6'-HxCB	155	360	2010	3.51
2,3,3',4-TeCB	55	292	2045	3.68
2,2',3,5,5'-PeCB	92	326	2055	3.53
2,3,3',4'-TeCB	56	292	2080	3.77
2,2',3,3',6-PeCB	84	326	2080	3.91
2,2',3,4,6'-PeCB	89	326	2080	3.91
2,2',3,4',5-PeCB	90	326	2085	3.56
2,2',4,5,5'-PeCB	101	326	2085	3.56
2,3,3',5',6-PeCB	113	326	2090	3.55
2,3,4,4'-TeCB	60	292	2095	3.72
2,2',4,4',5-PeCB	99	326	2110	3.57
2,2',3,4',6,6'-HxCB	150	360	2120	3.75
2,3',4,4',6-PeCB	119	326	2140	3.59
2,2',3,5,6,6'-HxCB	152	360	2150	3.88
2,3,3',5,6-PeCB	112	326	2155	3.63
2,2',3,3',5-PeCB	83	326	2155	3.75
3,3',4,5'-TeCB	79	292	2160	3.51
2,3,3',4,6-PeCB	109	326	2165	3.65
2,3,3',5,5'-PeCB	111	326	2170	3.79
2,2',3,4,5-PeCB	86	326	2180	3.81
2,2',3',4,5-PeCB	97	326	2185	3.78
2,2',3,4,6,6'-HxCB	145	360	2185	3.91
2,2',3,4',5,6'-HxCB	148	360	2190	3.59
4,4’-DDE		246	2205	3.59
3,3',4,5-TeCB	78	292	2205	3.63
2',3,4,5,6'-PeCB	125	326	2215	3.44
2,3,4,5,6-PeCB	116	326	2215	3.72
2,3,4',5,6-PeCB	117	326	2215	3.72
2,2',3,4,5'-PeCB	87	326	2220	3.79
2,3,4,4',6-PeCB	115	326	2230	3.73
2,2',3,3',6,6'-HxCB	136	360	2230	4.00
2,2',4,4',5,6'-HxCB	154	360	2235	3.62
2,2',3,4,4'-PeCB	85	326	2240	3.84

2,3',4,5,5'-PeCB	120	326	2245	3.47
3,4,4',5-TeCB	81	292	2265	3.69
dieldrin		263	2265	3.73
2,3,3',4',6-PeCB	110	326	2265	3.83
2,2',3,5,5',6-HxCB	151	360	2300	3.73
2,2',3,3',5,6'-HxCB	135	360	2310	3.81
3,3',4,4'-TeCB	77	292	2320	3.76
2,2',3,3',4-PeCB	82	326	2320	4.03
2,2',3,4,5',6-HxCB	144	360	2325	3.76
2,2',3,4',5,6-HxCB	147	360	2340	3.79
2,2',3,4',5',6-HxCB	149	360	2350	3.85
2,2',3,4,4',6-HxCB	139	360	2360	3.68
2',3,4,5,5'-PeCB	124	326	2360	3.81
2,2',3,4,4',6'-HxCB	140	360	2375	3.86
2,3,3',4,5'-PeCB	108	326	2380	3.72
2,2',3,4,5,6'-HxCB	143	360	2380	4.01
2,3,3',4',5-PeCB	107	326	2385	3.72
2',3,4,4',5-PeCB	123	326	2390	3.72
2,3,3',4,5-PeCB	106	326	2410	3.78
2,2',3,4',5,6,6'-HpCB	188	392	2410	3.81
2,2',3,3',5,6-HxCB	134	360	2410	3.96
2,3',4,4',5-PeCB	118	326	2415	3.70
2,2',3,3',5,5'-HxCB	133	360	2420	3.64
2,2',3,3',4,6-HxCB	131	360	2430	3.99
2,2',3,4,5,6-HxCB	142	360	2435	4.00
2,3,3',5,5',6-HxCB	165	360	2445	3.61
2,2',3,4,4',6,6'-HpCB	184	392	2450	3.84
2,2',3,4',5,5'-HxCB	146	360	2455	3.69
2,3,3',4',5'-PeCB	122	326	2460	3.95
2,3,3',4,5',6-HxCB	161	360	2465	3.64
2,3,4,4',5-PeCB	114	326	2465	3.87
4,4’-DDD		165	2485	3.85
2,3',4,4',5',6-HxCB	168	360	2485	3.72
2,2',4,4',5,5'-HxCB	153	360	2490	3.70
2,2',3,3',4,6'-HxCB	132	360	2500	4.11
2,2',3,3',5,6,6'-HpCB	179	392	2525	4.03
2,3,3',4,4'-PeCB	105	326	2545	4.01
3,3',4,5,5'-PeCB	127	326	2555	3.61
2,2',3,4,5,5'-HxCB	141	360	2555	3.86
2,2',3,3',4,6,6'-HpCB	176	392	2565	4.06
2,2',3,4,4',5-HxCB	137	360	2580	3.91
2,2',3,3',4,5'-HxCB	130	360	2595	3.97
2,2',3,4,5,6,6'-HpCB	186	392	2600	4.20
2,3,3',4',5',6-HxCB	164	360	2610	3.96
2,2',3,3',5,5',6-HpCB	178	392	2630	3.82
2,2',3,4,4',5'-HxCB	138	360	2630	3.93

2,3,3',4',5,6-HxCB	163	360	2630	3.93
2,3,3',4,4',6-HxCB	158	360	2645	3.97
2,3,3',4,5,6-HxCB	160	360	2650	3.93
4,4’-DDT		235	2650	3.90
2,2',3,3',4,5-HxCB	129	360	2655	4.14
2,2',3,3',4,5',6-HpCB	175	392	2665	3.88
2,2',3,4,4',5,6'-HpCB	182	392	2665	3.88
2,3,7,8-TCDF		306	2670	3.90
methoxychlor		227	2680	4.30
2,2',3,4',5,5',6-HpCB	187	392	2680	3.90
2,2',3,4,4',5',6-HpCB	183	392	2710	3.90
2,3,4,4',5,6-HxCB	166	360	2715	4.02
2,3,7,8-TCDD		322	2725	3.77
3,3',4,4',5-PeCB	126	326	2730	3.88
2,3,3',4,5,5'-HxCB	159	360	2735	3.74
2,3,3',4',5,5'-HxCB	162	360	2760	3.80
2,2',3,4,5,5',6-HpCB	185	392	2770	4.00
2,2',3,3',4,4'-HxCB	128	360	2770	4.26
2,2',3,3',4,5,6'-HpCB	174	392	2790	4.15
2,3',4,4',5,5'-HxCB	167	360	2795	3.83
2,2',3,3',5,5',6,6'-OcCB	202	426	2805	4.02
2,2',3,4,4',5,6-HpCB	181	392	2815	4.08
2,2',3,3',4,5',6'-HpCB	177	392	2830	4.14
2,2',3,3',4,5',6,6'-OcCB	201	426	2850	4.07
2,2',3,3',4,4',6-HpCB	171	392	2855	4.21
2,2',3,4,4',5,6,6'-OcCB	204	426	2860	4.09
2,2',3,3',4,5,6-HpCB	173	392	2885	4.29
2,2',3,3',4,4',6,6'-OcCB	197	426	2890	4.12
2,3,3',4,4',5-HxCB	156	360	2915	4.02
2,2',3,3',4,5,5'-HpCB	172	392	2920	3.95
2,3,3',4,4',5'-HxCB	157	360	2930	4.12
2,3,3',4,5,5',6-HpCB	192	392	2935	3.83
2,2',3,4,4',5,5'-HpCB	180	392	2960	3.97
2,3,3',4',5,5',6-HpCB	193	392	2960	3.97
2,2',3,3',4,5,6,6'-OcCB	200	426	2975	4.33
2,3,3',4,4',5',6-HpCB	191	392	2985	4.00
1,2,3,7,8-PeCDF		340	3030	4.08
2,2',3,3',4,5,5',6-OcCB	198	426	3100	4.06
2,2',3,3',4',5,5',6-OcCB	199	426	3105	4.12
2,2',3,3',4,4',5-HpCB	170	392	3105	4.27
3,3',4,4',5,5'-HxCB	169	360	3125	3.94
2,3,3',4,4',5,6-HpCB	190	392	3125	4.17
2,2',3,3',4,4',5',6-OcCB	196	426	3140	4.15
2,2',3,4,4',5,5',6-OcCB	203	426	3145	4.11
2,3,4,7,8-PeCDF		340	3145	4.11
1,2,3,7,8-PeCDD		356	3170	4.00

2,2',3,3',4,5,5',6,6'-NoCB	208	460	3245	4.26
2,3,3',4,4',5,5'-HpCB	189	392	3280	4.07
2,2',3,3',4,4',5,6,6'-NoCB	207	460	3290	4.33
2,2',3,3',4,4',5,6-OcCB	195	426	3300	4.41
2,2',3,3',4,4',5,5'-OcCB	194	426	3415	4.20
2,3,3',4,4',5,5',6-OcCB	205	426	3450	4.18
1,2,3,4,7,8-HxCDF		374	3460	4.20
1,2,3,6,7,8-HxCDF		374	3475	4.21
2,3,4,6,7,8-HxCDF		374	3555	4.30
1,2,3,4,7,8-HxCDD		390	3565	4.14
2,2',3,3',4,4',5,5',6-NoCB	206	460	3565	4.35
1,2,3,6,7,8-HxCDD		390	3575	4.16
1,2,3,7,8,9-HxCDD		390	3615	4.22
1,2,3,7,8,9-HxCDF		374	3655	4.48
2,2',3,3',4,4',5,5',6,6'-DeCB	209	498	3670	4.39
1,2,3,4,6,7,8-HpCDF		408	3820	3.84
1,2,3,4,6,7,8-HpCDD		424	3935	3.75
1,2,3,4,7,8,9-HpCDF		408	3990	4.12
OCDD		460	4275	4.53
OCDF		444	4295	4.85

Table S2. Losses (*n*=3) of PCBs, PCDD/Fs, pesticides and heavy metals in spiked meat cooked at different intensities (rare, medium and well-done) relative to the total load of POPs in the raw meat. All results are corrected for individual recovery rates. [a,b,c]: The letters represent the groups given by the Newman-Keuls mean comparison test when compared for each compound, based on the loss obtained for the different cooking conditions. Two groups with distinct letters can be considered as significantly different ($p < 0.05$). N.D.: non detectable.

Compound	Rare-cooked meat	Medium-cooked meat	Well-done meat
	Loss (%)	Loss (%)	Loss (%)
PCB 1	28.4 ± 5.8 [a]	29.2 ± 9.8 [a]	30.0 ± 6.7 [a]
PCB 8	10.5 ± 9.1 [a]	10.0 ± 13.8 [a]	25.1 ± 15.8 [a]
PCB 19	23.4 ± 8.3 [a]	24.4 ± 25.0 [a]	24.6 ± 9.7 [a]
PCB 28	22.8 ± 2.2 [a]	34.1 ± 0.7 [b]	38.6 ± 3.4 [b]
PCB 52	N.D. [a]	16.2 ± 11.8 [a.b]	31.4 ± 12.5 [b]
PCB 77	44.2 ± 2.4 [a]	53.5 ± 1.9 [b]	56.9 ± 3.1 [b]
PCB 81	11.7 ± 12.0 [a]	22.5 ± 3.2 [a]	30.5 ± 7.0 [a]
PCB 105	25.8 ± 9.1 [a]	29.4 ± 1.6 [a]	47.8 ± 3.2 [b]
PCB 114	5.1 ± 8.9 [a]	20.3 ± 5.9 [b]	39.2 ± 3.0 [c]
PCB 118	13.7 ± 10.5 [a]	20.8 ± 1.2 [a]	40.0 ± 4.3 [b]
PCB 123	19.4 ± 10.2 [a]	21.3 ± 4.7 [a]	64.2 ± 2.8 [b]
PCB 126	18.3 ± 10.1 [a]	16.4 ± 2.4 [a]	27.0 ± 1.2 [a]
PCB 153	54.2 ± 4.3 [a]	54.1 ± 7.7 [a]	67.9 ± 8.2 [a]
PCB 156	9.3 ± 2.6 [a]	10.5 ± 1.4 [a]	53.7 ± 3.5 [b]
PCB 157	N.D. [a]	2.4 ± 1.6 [a]	20.7 ± 1.4 [b]
PCB 167	None [a]	17.3 ± 3.3 [b]	36.2 ± 1.6 [c]
PCB 169	None [a]	56.8 ± 2.6 [b]	66.4 ± 5.6 [c]
PCB 172	39.4 ± 6.9 [a]	37.7 ± 2.7 [a]	56.0 ± 3.4 [b]
PCB 189	20.6 ± 5.5 [b]	11.7 ± 2.0 [a]	57.1 ± 4.7 [c]
PCB 206	47.0 ± 4.4 [a]	58.6 ± 2.7 [b]	83.5 ± 1.7 [c]
PCB 209	43.9 ± 5.2 [a]	57.4 ± 3.1 [b]	84.4 ± 0.8 [c]
2,3,7,8-TCDD	12.7 ± 12.1 [a]	15.1 ± 9.9 [a]	12.2 ± 12.6 [a]
2,3,7,8-TCDF	2.5 ± 4.3 [a]	N.D. [a]	3.4 ± 3.3 [a]
1,2,3,7,8-PeCDD	N.D. [a]	13.5 ± 23.4 [a]	28.5 ± 6.2 [a]
2,3,4,7,8-PeCDF	6.6 ± 11.4 [a]	N.D. [a]	1.0 ±0.9 [a]
1,2,3,4,7,8-HxCDD	N.D. [a]	N.D. [a]	6.0 ± 10.4 [a]
1,2,3,4,7,8-HxCDF	N.D. [a]	N.D. [a]	N.D. [a]
1,2,3,6,7,8-HxCDF	N.D. [a]	N.D. [a]	1.2 ± 2.0 [a]
Aldrin	2.9 ± 2.8 [a]	2.2 ± 2.3 [a]	3.3 ± 5.7 [a]
Dieldrin	88.0 ± 0.6 [a]	81.9 ± 1.1 [b]	93.4 ± 1.0 [c]
4,4’-DDT	41.6 ± 6.2 [a]	74.7 ± 2.3 [b]	67.9 ± 2.0 [b]
4,4’-DDD	3.0 ± 11.0 [a]	10.4 ± 4.9 [a]	0.4 ± 0.7 [a]
4,4’-DDE	9.5 ± 4.2 [a]	8.6 ± 7.6 [a]	40.2 ± 4.8 [b]

Lindane	N.D. [a]	14.9 ± 6.3 [a]	13.0 ± 8.6 [a]
Alpha BHC	22.2 ± 8.4 [a]	22.7 ± 8.1 [a]	25.2 ± 8.2 [a]
Béta BHC	N.D. [a]	1.5 ± 2.2 [a]	18.5 ± 10.3 [b]
Delta BHC	52.6 ± 7.7 [a]	65.8 ± 2.7 [a]	53.9 ± 8.5 [a]
Heptachlor	57.7 ± 2.8 [a]	61.6 ± 3.0 [a.b]	67.6 ± 3.8 [b]
Heptachlor epoxide	31.4 ± 8.6 [a]	51.0 ± 7.4 [b]	33.8 ± 7.3 [a]
Methoxychlor	N.D. [a]	11.6 ± 0.9 [b]	33.7 ± 9.5 [c]
Sulfotep	88.0 ± 2.2 [a]	87.3 ± 3.3 [a]	89.6 ± 0.7 [a]
O,O,O-triethylphosphorothioate	81.5 ± 3.1 [a]	81.9 ± 1.7 [a]	85.7 ± 1.4 [a]
Phorate	81.0 ± 3.1 [a]	82.7 ± 2.8 [a]	87.5 ± 3.3 [a]
Disulfoton	10.1 ± 8.9 [a]	7.0 ± 8.0 [a]	14.6 ± 10.5 [a]
Arsenic	/	N.D.	/
Cadmium	/	16.5 ± 3.2	/
Mercury	/	14.4 ± 4.3	/
Lead	/	18.1 ± 1.1	/

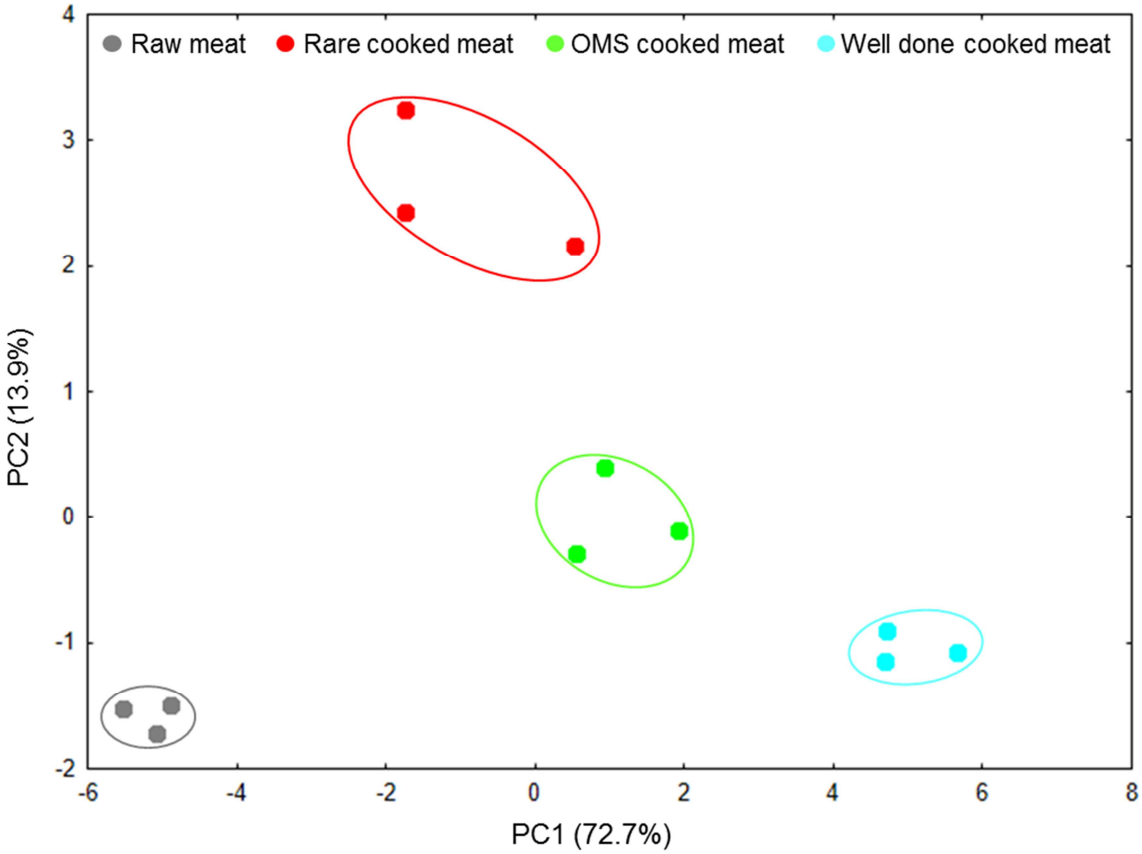

Fig. S1. Discrimination of quantity (ng) of PCBs in meat according to intensity of cooking of samples.

Table S3. Concentrations of PCBs and PCDD/Fs in naturally contaminated meat samples raw or medium-cooked. Beef (n = 3), pork (n = 3) and chicken (n = 3) samples were studied.

	PCBs		PCDD/Fs	
	Concentration in raw meat (ng g^{-1} of fat)	Concentration in medium-cooked meat (ng g^{-1} of fat)	Concentration in raw meat (pg g^{-1} of fat)	Concentration in medium-cooked meat (pg g^{-1} of fat)
Beef				
Sample 1	8.74 ± 1.94	10.3 ± 2.53	3.08 ± 0.54	7.97 ± 1.39
Sample 2	10.3 ± 2.29	11.1 ± 2.74	3.17 ± 0.55	6.77 ± 1.18
Sample 3	48.6 ± 10.9	46.0 ± 11.6	1.99 ± 0.35	5.29 ± 0.92
Pork				
Sample 4	2.69 ± 0.67	7.59 ± 1.69	1.43 ± 0.25	6.18 ± 1.08
Sample 5	3.65 ± 0.92	6.43 ± 1.43	1.91 ± 0.33	5.12 ± 0.89
Sample 6	6.31 ± 1.51	13.5 ± 3.00	2.18 ± 0.38	4.58 ± 0.80
Chicken				
Sample 7	2.68 ± 0.60	5.96 ± 1.48	5.39 ± 0.94	20.3 ± 3.53
Sample 8	0.58 ± 0.14	0.71 ± 0.17	4.50 ± 0.78	12.0 ± 2.09
Sample 9	1.72 ± 0.37	3.71 ± 0.92	3.93 ± 0.68	7.20 ± 1.25

Table S4. Concentrations of arsenic in naturally contaminated meat samples raw or medium-cooked. Beef (*n* = 9), pork (*n* = 7) and chicken (*n* = 6) samples were studied.

	Concentration in raw meat (mg kg^{-1} of meat)	Concentration in medium-cooked meat (mg kg^{-1} of meat)
Beef		
Sample 1	0.014 ± 0.003	0.021 ± 0.004
Sample 2	0.004 ± 0.001	0.013 ± 0.002
Sample 3	0.009 ± 0.002	0.016 ± 0.003
Sample 4	0.007 ± 0.002	0.009 ± 0.002
Sample 5	0.022 ± 0.005	0.034 ± 0.006
Sample 6	0.023 ± 0.006	0.014 ± 0.002
Sample 7	0.022 ± 0.005	0.040 ± 0.007
Sample 8	0.004 ± 0.001	0.035 ± 0.006
Sample 9	0.007 ± 0.002	0.013 ± 0.002
Pork		
Sample 10	0.003 ± 0.001	0.008 ± 0.001
Sample 11	0.005 ± 0.001	0.010 ± 0.003
Sample 12	0.007 ± 0.002	0.009 ± 0.002
Sample 13	0.011 ± 0.003	0.014 ± 0.002
Sample 14	0.011 ± 0.003	0.012 ± 0.002
Sample 15	0.023 ± 0.006	0.010 ± 0.002
Sample 16	0.005 ± 0.001	0.011 ± 0.002
Chicken		
Sample 17	0.012 ± 0.003	0.019 ± 0.003
Sample 18	0.013 ± 0.003	0.022 ± 0.004
Sample 19	0.008 ± 0.002	0.015 ± 0.003
Sample 20	0.007 ± 0.002	0.012 ± 0.002
Sample 21	0.010 ± 0.002	0.016 ± 0.003
Sample 22	0.013 ± 0.003	0.020 ± 0.003

Article 3

Effects of pan cooking on sulfonamides and tetracyclines in meat

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Abstract

Even if it is now admitted that food represents one of the main sources of human exposure to harmful pollutants, little is known about the fate of these toxicants during cooking. This work presents the effects of pan cooking on 15 sulfonamides and 6 tetracyclines in meat in a risk assessment perspective. The levels of these antibiotics in raw and cooked spiked meat were determined by HPLC-MS/MS analyses realized by a French National Reference Laboratory. Results show that cooking could induce a significant decrease in levels of 4-epi-chlortetracycline (43% loss) and sulfamethoxazole (55% loss). In contrast, no significant losses of tetracycline and doxycycline were observed. To determine the origin of losses observed, the thermal degradation of sulfamethazine and sulfamethoxazole was investigated by use of radiolabeling associated to both radiodetection and HRMS, allowing for both absolute quantification and structural identification of degradation compounds. No degradation products were observed for sulfamethazine. In contrast, six different degradation products of sulfamethoxazole were formed depending on the cooking conditions. Degradation products were identified using LC-HRMS and NMR. A degradation scheme of sulfamethoxazole during food cooking is proposed and discussed.

Highlights

• No significant loss of tetracycline and doxycycline was observed during meat cooking

• Cooking losses exceed 40% for 4-epi-chlortetracycline and 50% for sulfamethoxazole

• No degradation product of sulfamethazine was detected

• Six degradation products of sulfamethoxazole were formed depending upon cooking conditions

• A degradation scheme of sulfamethoxazole during food cooking was proposed

Keywords

Sulfonamides; Tetracyclines; Radiolabeling; Degradation products; Cooking

1. Introduction

Veterinary drugs are extensively used in food animal production for therapeutic and prophylactic purposes. Among these drugs, sulfonamides and tetracyclines are the most commonly used antibiotics. These compounds are broad spectrum antibiotics widely used for the prevention and treatment of animal farm to cure several infectious diseases or used as feed additives to promote animal growth (Sukul & Spiteller, 2006 ; Gaghrir & Drogui, 2013). However, residues of these compounds may remain in animal food products if these drugs are incorrectly used or if recommended drug withdrawal periods are not observed (Reig *et al.*, 2008). Although these antibiotics are generally found in trace amounts in animal tissues, the human health risk they imply cannot be ignored. Currently this risk is most often assessed from their levels in raw foods. However, such knowledge alone does not enable a precise assessment of the risk incurred by consumers, because levels of antibiotics can be affected by the technological processes the food undergoes before consumption, in particular cooking (Engel *et al.*, 2015).

A wide examination of literature data regarding the effects of cooking on sulfonamides and tetracyclines show major discrepancies between reported results (Fischer *et al.*, 1992 ; Ibrahim *et al.*, 1994 ; Rose *et al.*, 1995 ; Rose *et al.*, 1996 ; Xu *et al.*, 1996 ; Fedeniuk *et al.*, 1997 ; Lan *et al.*, 2001 ; Furusawa *et al.*, 2002 ; Ismail-Fitry *et al.*, 2008 ; Abou-Raya *et al.*, 2013). For instance, Ibrahim *et al.* (1994) reported an approximately 80% decrease of oxytetracycline in ground lamb after 20 min boiling whereas Abou-Raya *et al.* (2013) found that 20 min boiling induced 51.5% loss of oxytetracycline in thigh meat. For sulfadiazine, Furusawa *et al.* (2002) found no significant reduction of this antibiotic in chicken muscles after 12 min roasting whereas Ismail-Fitry *et al.* (2008) reported a 37.5% loss after deep-frying chicken meat-balls at 190°C for 9 min. At least two factors may explain these inconsistencies. First, cooking conditions could vary a lot between studies. The extent of these variations is difficult to assess because cooking protocols are generally poorly documented, with no or very few information regarding temperature monitoring and control. Second, most of these studies deal with naturally contaminated food matrices which differ significantly in micropollutant concentration. Due to these discrepancies, these former studies cannot be used to feed risk assessment models which require robust and reliable data. To address this challenging issue, a realistic, standardized and reproducible cooking method must be set up. It is also necessary to use a homogeneous matrix intentionally and uniformly contaminated at a known and high enough concentration to be detected, even after cooking. It is important to

note that little is known about the thermal degradation of sulfonamides and tetracyclines during cooking whereas the potential toxic effects of the breakdown compounds should be investigated and their formation in cooked meat should be taken into account when maximum residue limits are established (Gratacós-Cubarsí *et al.*, 2007).

With the ultimate aim of getting a better assessment of risks related to chemical contamination of food, we have studied the effects of cooking on sulfonamides and tetracyclines in meat. For this purpose the first part of this paper was focused on the impact of a realistic medium cooking on the level of 15 sulfonamides and 6 tetracyclines in meat samples. In a second part, the thermal degradation during meat cooking of two sulfonamides, namely sulfamethazine and sulfamethoxazole, has been studied using radio-labeled compounds and analysis by LC-HRMS and NMR. The impact of cooking level on the formation of degradation products will be discussed and a degradation scheme is proposed.

2. Materials and Methods

2.1. Chemicals and standards

Methanol, acetonitrile, and ethanol were HPLC grade (Sharlau, Barcelona, Spain). Hexane, formic acid and diatomaceous earth used for the preparation of ASE cells were from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Ultra-pure water was obtained using a Milli-Q system (Millipore, St Quentin en Yvelines, France). Flo-Scint II and Ultima Gold liquid scintillation cocktails were purchased from PerkinElmer Life and Analytical Sciences (Courtaboeuf, France). Sulfamethazine, sulfamethoxazole, sulfanilamide, sulfamethoxazole impurity F and 1-sulfanilylurea were purchased from Sigma Aldrich. N-acetyl sulfamethoxazole was purchased from Cayman Chemical (Ann Arbor, MI, USA). ^{14}C-Sulfamethazine (specific activity of 55 mCi/mmol) and ^{14}C-Sulfamethoxazole (specific activity of 77 mCi/mmol) were obtained from American Radiolabeled Chemicals Inc. (St Louis, MO, USA). Radiopurities were checked by radio-HPLC and chemical purities obtained for these standards were greater than 99%. Stock solutions were prepared in ethanol, and kept at -20°C.

All non-radiolabeled sulfonamides and tetracyclines standards (purity > 95%) were from Sigma-Aldrich (Saint-Quentin Fallavier, France), except for 4-Epi-tetracycline hydrochloride, Epi-oxytétracycline chlorhydrate which were from VWR International (Fontenay-sous-Bois, France). Sulfadiméthoxine-D6, sulfadiazine-13C6, sulfathiazole-13C6, sulfadimidine-13C6 and sulfadoxine-D3and demeclocyclin hydrochloride used as internal

standards during LC-MS/MS analysis were purchased from Witega (Berlin, Germany), andSigma-Aldrich (Saint-Quentin Fallavier, France), respectively.

HPLC grade and LC-MS grade acetonitrile (ACN), HPLC grade methanol (MeOH), were purchased from Fisher Scientific (Illkirch, France). Trichloroacetic acid, ethylenediaminetetraacetic acidtrisodium salt trihydrate (EDTA), citric acid monohydrate were from Fisher Scientific (Illkirch, France). Glacial acetic acid, oxalic acid dehydrate were from VWR International (Fontenay-sous-Bois, France). Anhydrous Disodium hydrogen phosphate (Na_2HPO_4) was from Roth Sochiel (Lauterbourg, France).

Heptafluorobutyric acid 99.5 %, and pentafluoropropionic acid were from Sigma-Aldrich (Saint-Quentin Fallavier, France). Ultrapure water was obtained using a Milli-Q system (Millipore, Molsheim, France).

2.2. Meat samples

Two types of meat samples were used: intentionally and naturally contaminated samples. Intentionally contaminated meat was prepared with ground beef samples from a same blend of muscles (11% fat) purchased from a French supplier. 125 g weight aliquots were stored at −80 °C before use. Matrix blanks of these samples were made before spiking. Naturally contaminated meats were sampled in the frame of the French project SOMEAT (Contract No. ANR-12-ALID-0004. Safety of Organic Meat. Available at www.so-meat.fr) and were ground before use.

2.3.Spiking and cooking

2.3.1. Spiking

Ground beef samples from the same blend of muscles (11% fat) were purchased from a French supplier. 125 g weight aliquots were stored at −80 °C before use. Matrix blanks made by spiking meat with pure methanol without micropollutant were used to determine the level of sulfonamides and tetracyclines in fresh unspiked meat. Ground beef spiking combining micropollutants addition to ground meat with matrix homogenization was realized according to Planche *et al*. (2015) with slight modifications. Briefly, ground beef (120 g) was immersed in methanol (20 mL) containing the antibiotics followed by evaporation under a hood and homogenization 2 min with a blender. For non-radiolabeled sulfonamides and tetracyclines, a spiking concentration of 200 µg kg^{-1} of fresh meat was chosen whereas for

radiolabeled sulfamethazine and sulfamethoxazole, meat was spiked at a concentration of 0.019 µCi/g fresh meat. Small ground beef patties weighing 26 g (2.5 cm thickness) were then shaped to resemble commercial ground beef patties.

2.3.2. Cooking method

To study the fate of sulfonamides and tetracyclines during cooking, ground beef patties were cooked in a stainless steel frying pan (17 cm diameter) on a controlled-temperature induction hob (Bosch Electroménager, Saint-Ouen, France). A sheet of 11 µm thick aluminum foil was laid on the bottom of the frying pan to recover juice released during meat cooking. Three different cooking conditions were used to simulate rare (core 50°C), medium (core 70 °C, according to WHO recommendations for ground meats) or well-done (core 85°C) meat (n = 3 for each cooking condition). These cooking conditions corresponded to: 7 min heating (patty turned over once) at 160 °C at the bottom of the pan, 14 min heating (turned over three times) at 200 °C at the bottom of the pan and 14 min heating (turned over three times) at 250 °C at the bottom of the pan, respectively. Temperatures at the core of the meat and at the bottom of the pan were monitored by thermocouples (RS Components, Beauvais, France).

2.4. Sample extraction and analysis

2.4.1. Extraction of non-radiolabeled sulfonamides and tetracyclines

The two implemented methods for national monitoring and control of sulfonamides and tetracyclines in raw meat of muscle were previously validated according to Commission Decision 2002/657/EC and accredited by COFRAC under standard ISO17025.

For the analysis of sulfonamides, the extraction procedure consisted in the weighting of 2g of ground beef sample (raw or cooked) followed by the addition of 200 µL of internal standard (isotopically labeled sulfonamides standards) solution at 1 µg mL^{-1} and 200 µL of water into the samples. Then, samples were extracted with 8 mL of acetonitrile, rotary-stirring at 100 rpm during 10 minutes before centrifugation at 14 000 x g at 4°C during 5 minutes. 6 mL of resulting supernatant were transferred into a new sample tube and were evaporated to dryness at 50°C. The extracts were dissolved in 1 mL of ultrapure water and filtered through a 0.45 µm syringe filter before LC-MS/MS analysis.

The preparation of raw and cooked samples for the analysis of tetracylcines consisted in the weighting of 2 g of meat, following the addition of 200 µL of a demeclocyclin (internal standard) solution at 1 µg mL^{-1} and 600 µL of purified water. Then, 10 mL of Mac Ilvaine/EDTA solution were added to samples and rotary-stirring at 100 rpm for 10 minutes before centrifugation at 14 000 x g at 4°C during 10 minutes. Deproteinization of samples was then carried out by adding 1 mL of trichloroacetic acid at 1g mL^{-1}, vortexed during 10 seconds and stored at -20°C during 15 minutes. Samples were then centrifuged at 14 000 x g at 4°C during 5 minutes and the supernatants were sampled and loaded onto pre-conditioned Solid Phase Extraction cartridges (Bond-Elut). After the cleaning of cartridge with 1 mL of purified water and 5 minutes drying of cartridge, elution was performed with 2 x 0.6 mL of an oxalic solution in methanol at 0.01 mol L^{-1}, and 1.8 mL of purified water. Extracts were then filtered before through a 0.45 µm syringe filter before LC-MS/MS analysis.

2.4.2. Analysis of non-radiolabeled sulfonamides and tetracyclines

For the analysis of sulfonamides by HPLC-MS/MS, 10 µL of the sample extract was injected to a LC-20ADXR system (Shimadzu, Marne la Vallée, France) for the chromatographic separation step using a RP C18 Symmetry column (100 mm × 2.1 mm i.d., 3.5 µm particle size, Waters, Saint-Quentin en Yvelines, France) with a security guard column C18, 4.0 mm × 2.0 mm i.d. (Phenomenex, Le Pecq, France). Elution gradient was performed at a constant flow rate of 0.250 mL min^{-1} and binary mobile phase: ultra-pure water (A), and acetonitrile (B), each solvent containing 1 mmol L^{-1} heptafluorobutyric acid. The elution gradient started with 10 % of B, and linearly rose to 30 % during the fourth minutes. Then B was held at 30 % during one minute before a linear raise to 70 % over 2 minutes and being held at 70 % over the next within 3 minutes, a linear gradient to 10% over one minute (return to initial conditions), and held at 10 % over three minutes for a re-equilibration time.

Detection and identification of the sulfonamides of interest were performed on an API 5500 triple quadrupole mass detector (AB-Sciex, Les Ulis, France). The electrospray ionization source was set on the positive mode and the following parameters was set: source temperature set at 650°C, the curtain gas was set at 20 psi, the ion spray voltage set at 4000 V, the ion source gas 1 and 2 was set at 25 psi and 30 psi, respectively. The scan mode of MS/MS detection was set on scheduled Multiple Reaction Monitoring (MRM).

For the analysis of tetracyclines by HPLC-MS/MS, 25 µL of the sample extract was injected to a LC-20ADXR system (Shimadzu, Marne la Vallée, France) for the chromatographic separation step using a RP C18 Symmetry column (100 mm × 2.1 mm i.d., 3.5 µm particle size, Waters, Saint-Quentin en Yvelines, France) with a security guard column C18, 4.0 mm × 2.0 mm i.d. (Phenomenex, Le Pecq, France). Separation was performed at a flow rate of 300 µL/min during a run analysis of 20 minutes with a binary mobile phase as following: ultra-pure water + 0.1% heptafluoropropionic acid (A), and acetonitrile (B). The elution gradient started with 15 % of B, and linearly rose to 50 % during the seventh minutes. Then B was held at 50 % during two minutes before a linear gradient to return to initial conditions, and held at 15 % over ten minutes for a re-equilibration time.

Detection and identification of the tetracyclines of interest were performed on an API 5500 triple quadrupole mass detector (AB-Sciex, Les Ulis, France). The electrospray ionization source was set on the positive mode and the following parameters was set: source temperature set at 500°C, the curtain gas was set at 20 psi, the ion spray voltage set at 4000 V, the ion source gas 1 and 2 was set at 40 psi and 50 psi, respectively. The scan mode of MS/MS detection was set on scheduled Multiple Reaction Monitoring (MRM).

The presence of internal standards was checked in all samples. Identification was performed according to the Commission Decision 2002/657/EC.

Quantification of sulfonamides and tetracyclines in raw, cooked samples were made thanks to a calibration curve which was prepared from blank raw muscle samples spiked at different levels of concentration in µg kg^{-1}. Quality control samples of spiked blank samples of cooked muscle were added to the sequence of analysis in order to check the validity of the analysis of cooked samples.

2.4.3. Extraction of ^{14}C-sulafamethazine and ^{14}C-sulfamethoxazole

Before extraction, the cooked ground beef patties were powdered with a liquid nitrogen grinder. Then, 5 g of meat powder were extracted by accelerated solvent extraction (ASE) using a Dionex ASE 350 extractor (Sunnyvale, CA, USA). 34 mL stainless-steel extraction cells were used. The cells were filled with 5 g of ground beef dispersed in diatomaceous earth. Paper filters were placed at the bottom and top of the extraction cell. According to Hoff *et al.* (2015), prior to extraction, the cells were submitted to a clean-up

method in order to remove the lipids from the samples by using hexane as solvent. ASE conditions were as follows: temperature 60°C, 2 cycles of 5 min each one, 5 min static time, and pressure 1500 psi. Total flush volume of 80% and 300 s of purge time with nitrogen flow were applied. After that, the same ASE cells (with the samples) were submitted to an extraction method using acetonitrile as extraction solvent at a temperature of 90°C and a pressure of 1500 psi. Three cycles of 5 min each were carried out. A total flush volume of 80% and 60 s of purge with nitrogen flow were applied. The acetonitrile extract was then evaporated firstly in a Rotavapor apparatus and secondly under nitrogen flow until dryness. Extracts were redissolved in 500 µL of mobile phase mixture (95% water / 5% acetonitrile + 0.2% Formic Acid) and transferred to a HPLC vial.

Radioactivity losses were checked at each step of the protocol by direct counting on a liquid scintillation analyzer (Tri-Carb 2910 TR / PerkinElmer, France), using Ultima Gold (PerkinElmer) as scintillation cocktail.

2.4.4. Detection and identification of ^{14}C-sulafamethazine and ^{14}C-sulfamethoxazole degradation products

^{14}C-Sulfamethazine, ^{14}C-Sulfamethoxazole and their degradation products were analyzed on an Agilent 1200 (Agilent Technologies, Les Ulis, France) liquid chromatograph connected to a Flo-one/β A500 instrument (Radiomatic, La-Queue-Lez-Yvelines, France), with Flow-scint II as scintillation cocktail (Packard Instruments).The HPLC system consisted of a C18 Nucleodur column (250×4 mm, 5 µm; Macherey Nagel) maintained at a temperature of 35°C. Conditions were as follows: mobile phases consisted of A, water / acetonitrile 95:5 v/v with 0.2 % formic acid and B, acetonitrile. The flow rate was 1 mL min^{-1}. For Sulfamethazine, the following gradient was used: 0-2 min 100% A; 2-4 min linear gradient from 0 to 12 % B; 4-27 min linear gradient from 12 to 30 % B; 27-30 min linear gradient from 30 to 100 % B, held for 3 min. For Sulfamethoxazole, the gradient was : 0-2 min 100% A; 2-4 min linear gradient from 0% to 12% B; 4-27 min linear gradient from 12% to 15% B, then 27-30 min linear gradient from 15% to 100% B, held for 10 min. Degradation compounds were quantified by integrating the area under the peaks monitored by radioactivity detection.

For structural characterization of the degradation compounds, analyses were performed by HPLC coupled to a LTQ Orbitrap XL hybrid high resolution mass spectrometer

(Thermo Scientific, Les Ulis, France) fitted with an electrospray ionization source operating in the positive mode, using the HPLC conditions described for radio-HPLC profiling, and a 1/4 post-column splitting. Typical operating parameters used for ion production were as follows: spray voltage (4.5 kV), sheath gas (N2) flow rate (35 arbitrary units (au)), auxiliary gas (N2) flow rate (5 au), heated transfer capillary temperature (350 °C), heated transfer capillary voltage (20 V), tube lens voltage (75 V). High resolution mass spectra were acquired at a resolution power of 60000 from m/z 80 to 600. Identifications were performed by tandem MS experiments using the ion-trap mass analyzer of the LTQ Orbitrap spectrometer. For MS/MS experiments, resolution power was reduced to 7500, and the excitation parameters (isolation width, normalized collision energy, excitation time) were adjusted to obtain the maximum structural information on the compound of interest. All analyses were achieved under automatic gain control conditions using helium as damping as well as collision gas for MS/MS experiments. NMR experiments were carried out on a Bruker 600MHz instrument (5mm cryoprobe with 3mm tube shuttle) for compound 3.

2.5. Data processing

Antibiotic losses induced by cooking were determined according to Rawn *et al.* (2013):

$$\text{Loss} = 1 - \frac{([\text{Antibiotic cooked meat}] \times \text{mass cooked meat})}{([\text{Antibiotic raw meat}] \times \text{mass raw meat})}$$

where [Antibiotic raw meat] and [Antibiotic cooked meat] are the antibiotic concentrations in raw or cooked meat (µg kg^{-1} of meat) and mass raw meat and mass cooked meat are the mass of meat before and after cooking.

Data were processed using Statistica software version 10 (StatSoft, Maisons-Alfort, France). To determine whether the cooking process had an effect on the level of micropollutants in meat, a one-way analysis of variance (ANOVA; $p < 0.05$) was performed. A Newman-Keuls mean comparison test was then performed on the resulting dataset to determine which cooking variables distinguished between the three cooking conditions.

Table 1. Concentrations of sulfonamides and tetracyclines in raw (*n* = 3) and medium cooked meat (*n* = 3). *: significantly different at $p < 0.05$.

Compound	Raw meat		Medium-cooked meat	
	Concentration (μg kg^{-1} of meat)	RSD (%)	Concentration (μg kg^{-1} of meat)	RSD (%)
Sulfaguanidine	180.7	5.7	209.1	11.4
Sulfacetamide	147.7	6.9	211.0*	6.7
Sulfadiazine	163.9	6.8	233.8*	4.8
Sulfamethoxazole	155.1	5.0	127.1*	9.2
Sulfathiazole	154.0	20.0	206.3*	3.0
Sulfamerazine	191.9	20.0	241.9	4.0
Sulfamethizole	150.2	6.6	237.3*	2.9
Sulfamethazine	159.4	4.8	241.1*	4.2
Sulfamethoxypyridazine	163.8	3.7	239.1*	2.2
Sulfamonomethoxine	158.7	4.9	243.8*	8.8
Sulfaquinoxaline	146.7	8.4	228.9*	7.4
Sulfadoxine	173.6	0.7	240.7*	4.3
Sulfadimethoxine	171.5	4.3	252.6*	6.0
Sulfaclozine	155.0	6.7	239.3*	7.0
Sulfachloropyridazine	155.4	5.9	219.3*	8.5
Tetracycline	222.8	3.7	309.9*	7.4
Doxycycline	211.4	4.1	266.9*	9.5
Oxytetracycline	227.8	3.9	239.3	5.3
Chlortetracycline	220.5	2.7	205.2	8.6
4-epi-tetracycline	199.3	3.4	219.1	6.2
4-epi-chlortetracycline	188.7	4.8	158.6*	6.2

3. Results and Discussion

3.1. Impact of cooking on the level of sulfonamides and tetracyclines in meat

15 sulfonamides and 6 tetracyclines were focused in this study and their fate during cooking was assessed. Table 1 presents the concentrations of these antibiotics in µg kg^{-1} of meat (classical expression of antibiotics concentration in food for risk assessment) in raw or medium cooked (70°C at core) spiked ground beef. Complementarily, Table S1 (Supplementary material) gives the losses caused by cooking on the total quantity (ng) of micropollutants in meat, according to Rawn *et al*. (2013). These tables show that antibiotics display different behaviors according to their families and structures. First, an increase in concentration during cooking ($p<0.05$) was observed for 12 sulfonamides and 2 tetracyclines (Tetracycline and Doxycycline). This increase may be explained by the mass loss of meat during cooking (34.0 ± 1.5% after medium cooking) that exceeds losses reported for these antibiotics in Table S1 (Supplementary material), resulting in a concentration effect. Secondly, 2 sulfonamides (sulfaguanidine and sulfamerazine) and 3 tetracyclines (oxytetracycline, chlortetracycline and 4-epi-tetracycline) showed no significant variation in concentration during cooking. Indeed, for oxytetracycline, chlortetracycline and 4-epi-tetracycline, the losses reported in Table S1 (Supplementary material) were of the same order of magnitude than the mass loss of meat during medium cooking, suggesting that these hydrophilic compounds (log K_{ow} in the -1.3 - 0.44 range) were lost with water during cooking. For sulfaguanidine and sulfamerazine, the high variability of the results (RSD ≥ 10%) may also explain that the concentration increase during cooking, observed in Table 1 for these compounds, was not significant. Lastly, a decrease in concentration ($p<0.05$) could be observed for sulfamethoxazole and 4-epi-chlortetracycline with losses (respectively 55.0 ± 6.5% and 42.8 ± 2.1% after medium cooking) greater than the meat mass loss (Table S1, Supplementary material). Although the matrix studied and the cooking mode were different, these last results are in line with those of Ismail-Fitry *et al.* (2008) for deep-fried chicken meat-ballsfish, in which average losses of sulfamethoxazole were 40.7%, suggesting that this sulfonamide is the most sensitive to cooking process (Ismail-Fitry *et al.*, 2008). In contrast, Furusawa *et al.* (2002) reported narrow differences between sulfonamides when roasting chicken thigh muscles 12min at 170°C : 38% loss of sulfaquinoxaline (23% in our study), 39% of sulfamethoxazole (55% in our study) and 40% of sulfamonomethoxine (17% in our study). For tetracyclines, Abou Raya *et al.* (2013) found greater losses than in our study after roasting chicken thigh meat 40 min at 180°C: 61% loss of oxytetracycline (27% in our study),

58% loss of tetracycline (no loss in our study), 43% loss of chlortetracycline (34% in our study) and 15% loss of doxycycline (1% in our study). Although no supporting data is available, these differences can be tentatively explained by greater quantities of juice expelled under the cooking conditions used by Abou Raya *et al.*, thus carrying off more hydrophilic tetracyclines. Moreover, cooking conditions of these authors might be more favorable to potential thermal degradation of tetracyclines as it was already described by Gratacós-Cubarsí *et al.* (2007) for tetracycline and 4-epi-tetracycline when boiling or microwaving chicken and pig meat.

In order to determine whether a difference may exist in the fate of antibiotics during cooking between spiked contaminants and contaminants bioaccumulated in muscles during animal breeding, beef (n = 3) and pork (n = 5) samples naturally contaminated with some sulfonamides or tetracyclines were studied. Tables S2 and S3 (Supplementary material) gives concentrations of 3 sulfonamides (sulfadiazine, sulfamethazine and sulfadimethoxine) and 3 tetracyclines (tetracycline, oxytetracycline and 4-epi-tetracycline) in these raw or medium-cooked samples. An increase in concentration of sulfonamides and tetracyclines was observed in all studied samples. These results are consistent with those observed for sulfadiazine, sulfamethazine, sulfadimethoxine and for tetracycline in spiked meat with ~20% loss for sulfadiazine, sulfamethazine, sulfadimethoxine and no significant loss of tetracycline during cooking. However, for oxytetracycline and 4-epi-tetracycline, lower losses were observed during cooking with naturally contaminated samples compared to spiked meat: 20.6% average loss for oxytetracycline (27.2% with spiked meat) and 17.3% average loss for 4-epi-tetracycline (24.1% with spiked meat).

3.2. Thermal degradation of sulfamethazine and sulfamethoxazole during cooking

To determine whether losses of sulfonamides observed during meat cooking could be the consequence of a thermal degradation of these compounds, their fate during cooking was investigated by use of radiolabeling associated to both radiodetection and HRMS analysis, to get information on both absolute quantification and structural identification of degradation compounds. Two sulfonamides were studied as models: sulfamethazine considered as relevant due to its frequency of occurrence in meat (2010, 2012 and 2013 French food safety

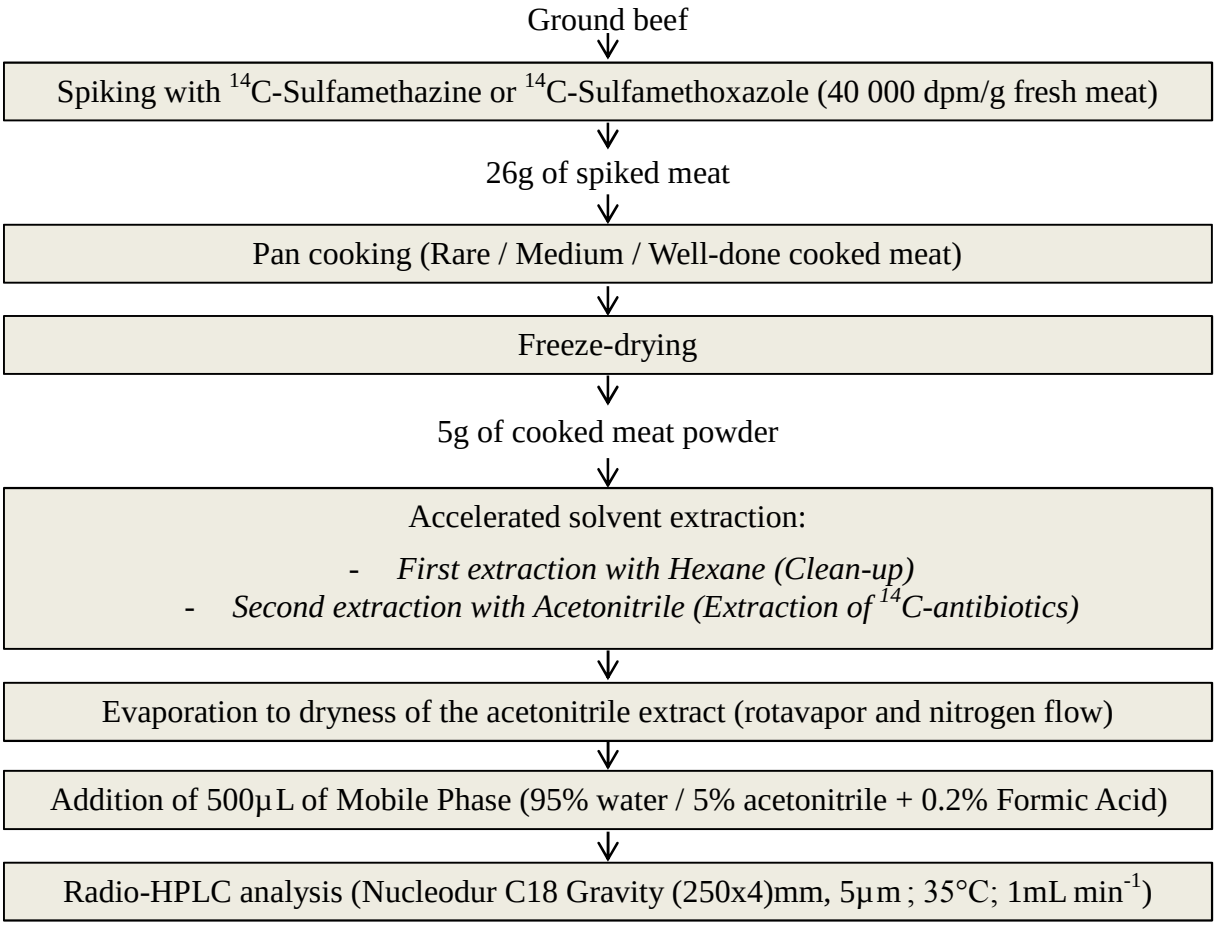

Fig 1: Experimental flowchart showing sample preparation, extraction, clean-up and analysis of ^{14}C-Sulfamethazine or ^{14}C-Sulfamethoxazole and their cooking degradation products.

Table 2. Losses of ^{14}C-Sulfamethazine and ^{14}C-Sulfamethoxazole during sample preparation and extraction, depending on the cooking level (*n*=3 for each level). Relative standard deviations (%) are presented in brackets.

	^{14}C-Sulfamethazine			^{14}C-Sulfamethoxazole		
	Rare meat	Medium-cooked meat	Well done meat	Rare meat	Medium-cooked meat	Well done meat
Spiking losses (%)	1.1 (11.9)	1.1 (11.9)	1.1 (11.9)	1.3 (24.7)	1.3 (24.7)	1.3 (24.7)
Pan cooking losses (%)	2.4 (4.0)	2.3 (4.2)	2.0 (17.3)	1.7 (16.7)	2.2 (22.3)	1.7 (24.9)
ASE extraction losses (%)	6.8 (2.4)	11.1 (1.5)	17.0 (1.2)	6.6 (4.4)	13.0 (3.5)	26.1 (4.2)
Evaporation losses (%)	25.5 (18.7)	23.5 (17.2)	16.0 (9.9)	12.8 (12.8)	25.3 (9.5)	16.3 (3.1)
Total losses (%)	**35.8 (17.0)**	**38.0 (17.5)**	**36.1 (9.2)**	**22.4 (14.6)**	**41.8 (7.3)**	**45.4 (5.3)**

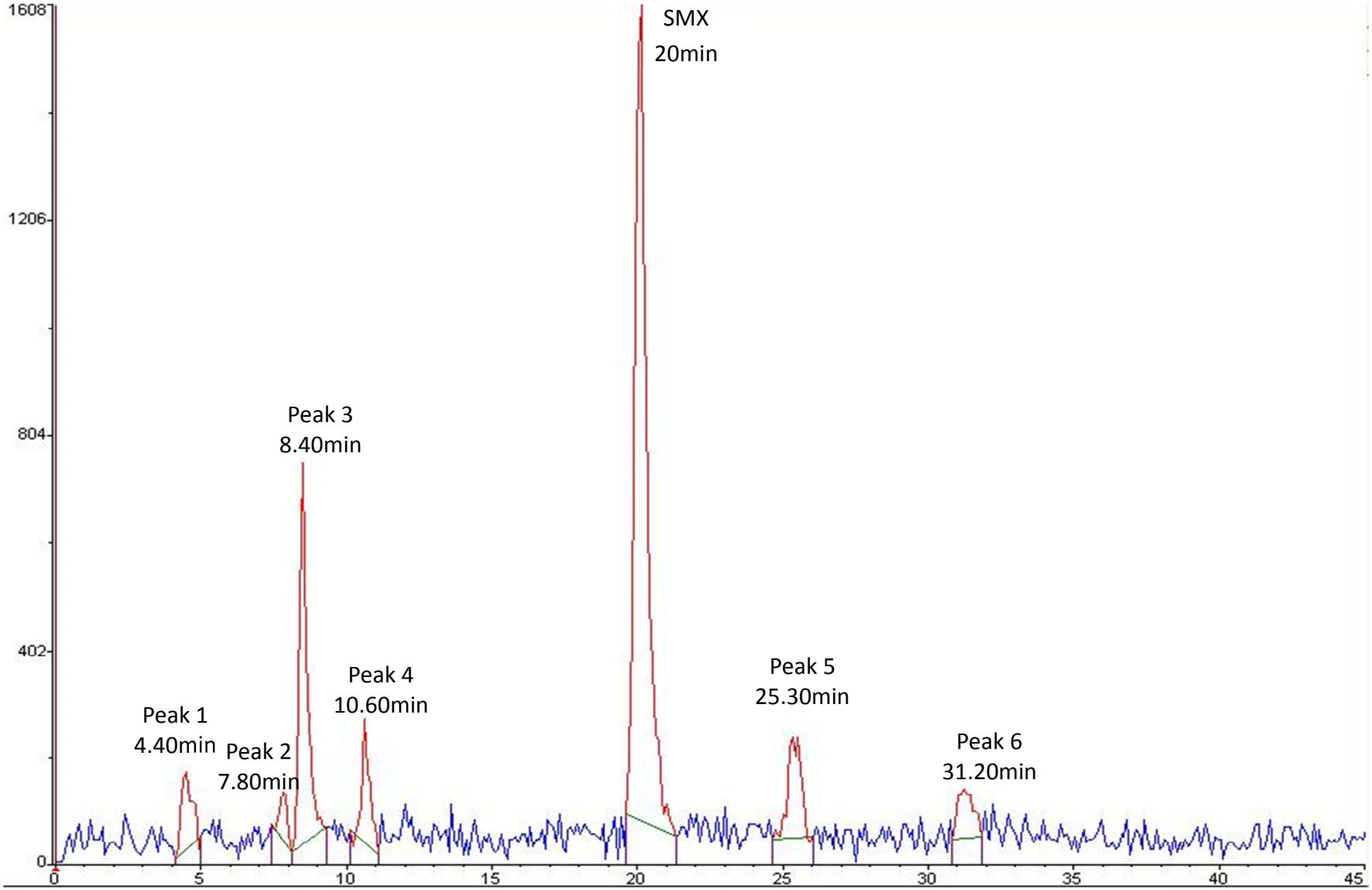

Fig. 2: Typical radio-HPLC chromatogram of an extract of meat spiked with ^{14}C-sulfamethoxazole (SMX) before cooking. Six sulfamethoxazole degradation products can be detected.

Table 3. Percentage of the radioactivity detected by Radio-HPLC represented by each peak according to cooking level. [a,b,c]: significantly different at $p < 0.05$.

	Raw meat	Rare meat	Medium-cooked meat	Well done meat
SMX	76.1 ± 5.8 [ab]	78.6 ± 1.7 [b]	72.0 ± 2.2 [a]	59.6 ± 3.9 [a]
Peak 1	None [a]	None [a]	3.0 ± 2.2 [b]	4.8 ± 1.9 [c]
Peak 2	None [a]	None [a]	None [a]	0.6 ± 1.1 [a]
Peak 3	1.9 ± 3.3 [a]	6.2 ± 2.1 [ab]	12.7 ± 4.0 [ab]	17.0 ± 10.3 [b]
Peak 4	None [a]	None [a]	1.2 ± 1.4 [ab]	2.7 ± 2.3 [b]
Peak 5	8.1 ± 4.4 [ab]	7.9 ± 3.3 [b]	6.1 ± 3.4 [a]	8.0 ± 3.1 [ab]
Peak 6	13.9 ± 4.6 [b]	7.3 ± 1.4 [ab]	5.1 ± 3.6 [a]	7.6 ± 6.3 [ab]

surveillance and control programmes), and sulfamethoxazole which was found to be the most cooking- sensitive sulfonamide according to Table S1 (Supplementary material).

3.2.1. Set-up of a sample preparation, extraction, concentration and clean-up protocol

To study the thermal degradation of ^{14}C-Sulfamethazine and ^{14}C-sulfamethoxazole in meat, we first designed a protocol for sample preparation, extraction, concentration and clean-up of the extracts, which is presented in Figure 1. Radioactivity enabled checking losses by direct counting at each step of the protocol . Table 2 reports losses observed according to the cooking level of meat (rare, medium or well done) (n=3 for each level). Total losses of ^{14}C-Sulfamethazine during the protocol were 36% (RSD: 17%), 38% (RSD: 18%) and 36% (RSD: 9.2%) for respectively rare, medium and well done meat. For, ^{14}C-sulfamethoxazole, total losses were respectively 22% (RSD: 15%), 42% (RSD: 7%) and 45% (RSD: 5%). Most losses were observed during the evaporation step. Moreover, we can note that losses observed during the extraction process increase with the intensity of meat cooking. These differences in extraction efficiency may be due to the protein denaturation that occurs during cooking, such that the tissues shrink and then become harder and more compact when cooking intensity increases (Tornberg, 2005), thus limiting the solvent extraction.

3.2.2. Detection and identification of thermal degradation products

^{14}C-Sulfamethazine, ^{14}C-Sulfamethoxazole and their potential degradation products separated by HPLC were monitored by online radioactivity detection. For ^{14}C-Sulfamethazine, irrespective of the cooking level, no degradation products were detected in meat extracts, suggesting that losses observed during medium cooking (Table S1. Supplementary material) were only due to an expelling into cooking juice. In contrast, for ^{14}C-Sulfamethoxazole, six degradation products were detected, as shown in Figure 2. Thus, for this antibiotic, the 55.0% losses observed during medium cooking (Table S1. Supplementary material) may be not only due to an expelling into cooking juice but also to thermal degradation processes. Although the cooking juice was collected, and subjected to sulfonamides extraction, the quantitative results were not sufficiently reproducible to be of use. Table 3 presents the percentage of the radioactivity detected in meat extracts by Radio-HPLC for each peak according to the cooking level. Peak 1 and peak 4 appeared after medium

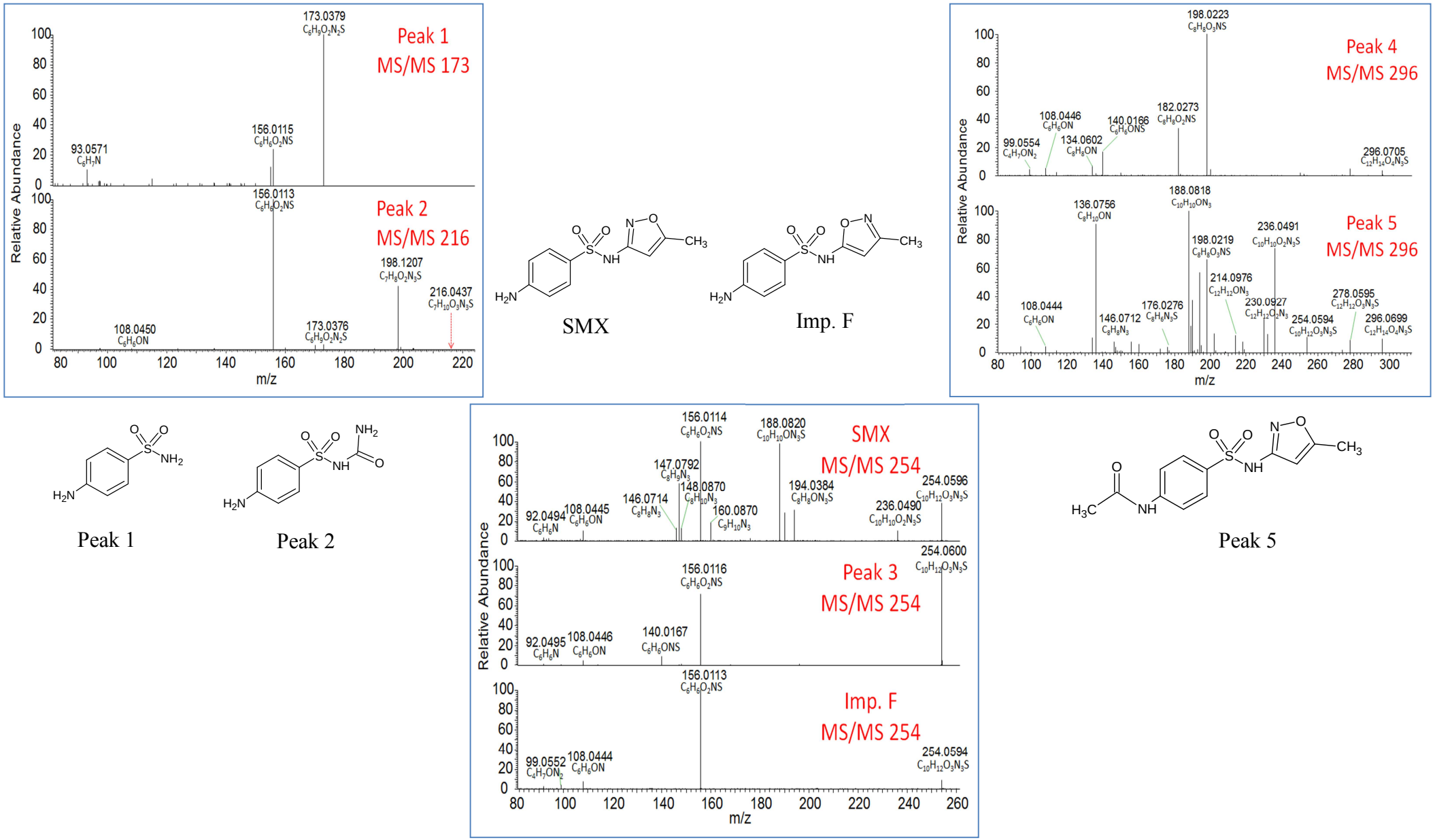

Fig. 3: Fragmentation mass spectra of SMX degradation compounds

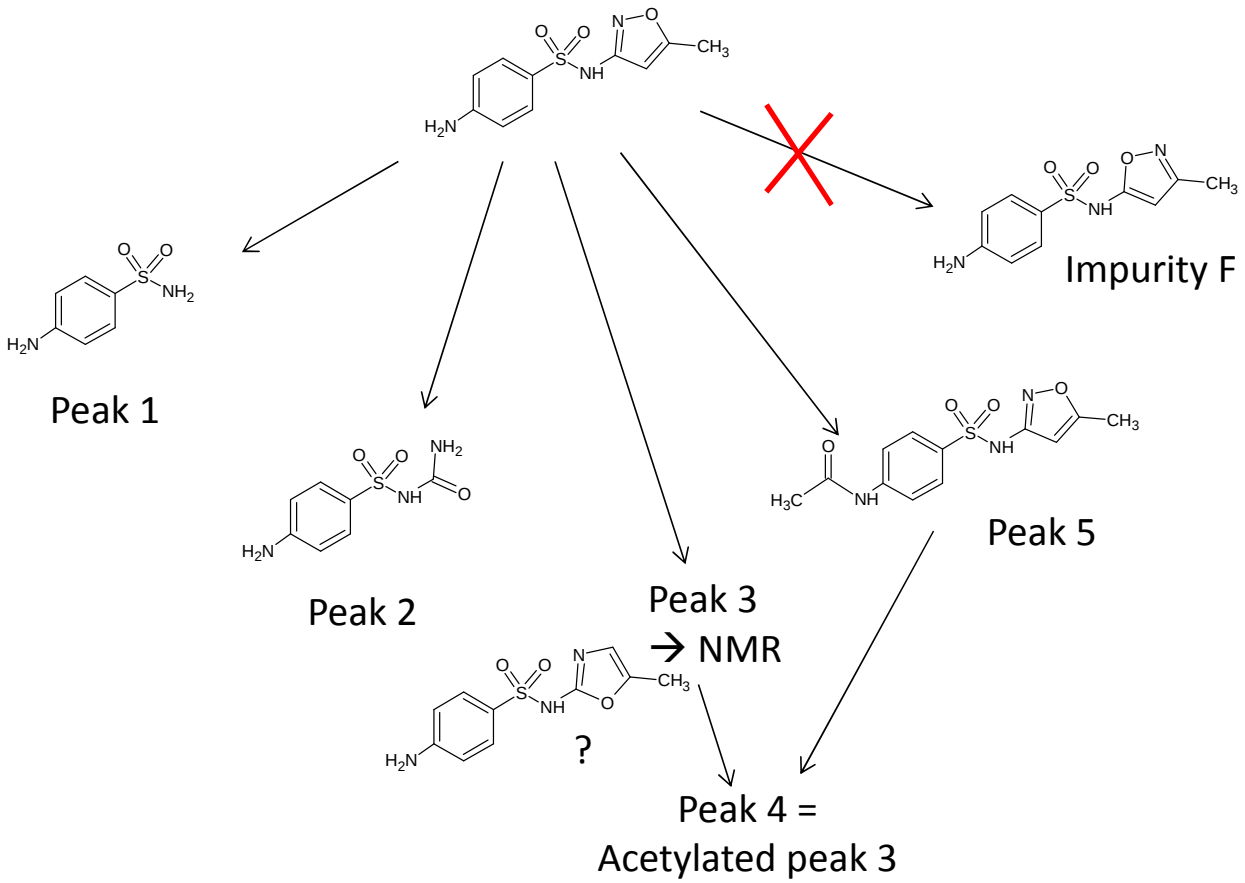

Fig. 4: Proposed degradation scheme of SMX during meat cooking

and intense cooking but not in rare meat, whereas Peak 2 appeared only after intense cooking. Peak 3 represents the major degradation compound. It can be found in trace amount in raw meat, suggesting that this compound can appear during meat storage, but its formation significantly increased ($p<0.05$) during cooking. Finally, peaks 5 and 6 are found at the same level in raw and in cooked meat, suggesting that these compounds appeared during meat storage but not during meat cooking.

Structural identification of the degradation compounds was then carried out by using MS and MS/MS experiments, and high-resolution exact mass measurements on both molecular species and fragment ions. Molecular ions were detected at m/z 173 for Peak 1, m/z 216 for peak 2, m/z 254 for peak 3 as well as for SMX, and m/z 296 for both peak 4 and peak 5. Peak 6 gave no response under our analytical conditions and was not investigated further. The MS/MS spectra of Sulfamethoxazole (SMX), peaks 1, 2 and 3 all displayed a common diagnostic fragment ion at m/z 156 corresponding to the sulfonyl aniline ion (Figure 3), indicating that for these three degradation compounds, this part of the molecule was not modified. Peak 4 and 5 displayed a characteristic fragment ion at m/z 198 (42 amu shift from the m/z 156 ion) corresponding to the N-acetyl sulfonyl aniline ion (Figure 3). For these two compounds, the amine function of the aniline moiety of SMX was thus acetylated. Based on these results, SMX was thus shown to undergo thermal degradation into polar compounds (peak 1, sulfanilamide and peak 2, 1-sulfanilylurea ; Rt and spectra identical to those of standard compounds) resulting from the opening / breakage of the parent SMX oxazole moiety. These oxazole modified compounds (peaks 1, 2) have already been identified as SMX photodegradation products (Zhou *et al.*, 1994, Trovo *et al.*, 2009a, 2009b), but they are described for the first time in cooked meat. The major degradation compound (peak 3) corresponded to an isomerization product from the initial 3-isoxazole form of SMX. By comparison of Rt and MS/MS spectra with that of a standard compound, the formation of the 3-methyl-5-isoxazolyl isomeric form of SMX ("impurity F", Figure 4) could be ruled out. However, mass spectra of peak 3 (Figure 3) were not informative enough to get the precise structure of this compound, and further investigations using NMR are currently under progress in order to check if it could correspond to the 5-methyl-2-oxazolyl isomer of SMX (Figure 4), another SMX photodegradation product (Zhou *et al.*, 1994, Trovo *et al.*, 2009a, 2009b). Peaks 4 and 5 were identified as the N-acetylated derivatives of SMX (N-acetyl sulfomethoxazole, comparison with the available corresponding standard compound) and (very likely) peak 3, respectively. The acetylated derivative of SMX is known as a metabolite of SMX (Vree *et al.*, 1994). However, to the best of our knowledge, peak 4 is described for

the first time in this work. Based on these results, a degradation scheme of sulfamethoxazole during meat cooking is proposed in Figure 4.

4. Conclusion

The fate of 15 sulfonamides and 6 tetracyclines during meat cooking meat was assessed. Results show that cooking could induce a significant decrease in levels of 4-epi-chlortetracycline (43% loss) and sulfamethoxazole (55% loss). In contrast, no significant losses of tetracycline and doxycycline were observed. To determine whether losses observed could be the consequence of a degradation of the antibiotics during cooking, the thermal degradation of sulfamethazine and sulfamethoxazole was investigated by use of radiolabeling associated to both radiodetection and HRMS. No degradation products were observed for sulfamethazine whereas, for sulfamethoxazole, six different degradation compounds were formed depending on the cooking conditions. Toxicological studies should now be undertaken in order to assess the potential toxicity of these degradation products, for a better risk assessment.

Acknowledgements

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References

Abou-Raya, S. H., Shalaby, A. R., Salama, N. A., Emam, W. H., & Mehaya, F. M. (2013). Effect of Ordinary Cooking Procedures on Tetracycline Residues in Chicken Meat. *Journal of Food & Drug Analysis, 21(1).*

Daghrir, R., & Drogui, P. (2013). Tetracycline antibiotics in the environment: a review. *Environmental chemistry letters, 11(3)*, 209-227.

Engel, E., Ratel, J., Bouhlel, J., Planche, C., & Meurillon, M. (2015). Novel approaches to improving the chemical safety of the meat chain towards toxicants. *Meat science, 109*, 75-85.

Fedeniuk, R. W., Shand, P. J., & McCurdy, A. R. (1997). Effect of thermal processing and additives on the kinetics of oxytetracycline degradation in pork muscle. *Journal of Agricultural and Food Chemistry, 45(6),* 2252-2257.

Fischer, L. J., Thulin, A. J., Zabik, M. E., Booren, A. M., Poppenga, R. H., & Chapman, K. J. (1992). Sulfamethazine and its metabolites in pork: Effects of cooking and gastrointestinal absorption of residues. *Journal of agricultural and food chemistry, 40(9),* 1677-1682.

Furusawa, N., & Hanabusa, R. (2002). Cooking effects on sulfonamide residues in chicken thigh muscle. *Food research international, 35(1),* 37-42.

Gratacós-Cubarsí, M., Fernandez-García, A., Picouet, P., Valero-Pamplona, A., García-Regueiro, J. A., & Castellari, M. (2007). Formation of tetracycline degradation products in chicken and pig meat under different thermal processing conditions. *Journal of agricultural and food chemistry, 55(11),* 4610-4616.

Hoff, R. B., Pizzolato, T. M., Peralba, M. D. C. R., Díaz-Cruz, M. S., & Barceló, D. (2015). Determination of sulfonamide antibiotics and metabolites in liver, muscle and kidney samples by pressurized liquid extraction or ultrasound-assisted extraction followed by liquid chromatography–quadrupole linear ion trap-tandem mass spectrometry (HPLC–QqLIT-MS/MS). *Talanta, 134,* 768-778.

Ibrahim, A., & Moats, W. A. (1994). Effect of cooking procedures on oxytetracycline residues in lamb muscle. *Journal of agricultural and food chemistry, 42(11),* 2561-2563.

Ismail-Fitry, M. R., Jinap, S., Jamilah, B., & Saleha, A. A. (2008). Effect of deep-frying at different temperature and time on sulfonamide residues in chicken meat-balls. *Journal of Food & Drug Analysis, 16(6).*

Lan, C. C., HWANG, B. S., & Tu, M. F. (2001). Effect of microwave and roast treatment on the degradation of sulfamethazine residue in tilapia meat. *Journal of Food and Drug Analysis, 9(2).*

Planche, C., Ratel, J., Mercier, F., Blinet, P., Debrauwer, L., & Engel, E. (2015). Assessment of comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry based methods for investigating 206 dioxin-like micropollutants in animal-derived food matrices. *Journal of Chromatography A, 1392*, 74-81.

Reig, M., & Toldrá, F. (2008). Veterinary drug residues in meat: Concerns and rapid methods for detection. *Meat science, 78(1)*, 60-67.

Rose, M. D., Farrington, W. H., & Shearer, G. (1995). The effect of cooking on veterinary drug residues in food: 3. sulphamethazine (sulphadimidine). *Food Additives & Contaminants, 12(6)*, 739-750.

Rose, M. D., Bygrave, J., Farrington, W. H., & Shearer, G. (1996). The effect of cooking on veterinary drug residues in food: 4. Oxytetracycline. *Food Additives & Contaminants, 13(3)*, 275-286.

Sukul, P., & Spiteller, M. (2006). Sulfonamides in the environment as veterinary drugs. *Reviews of environmental contamination and toxicology* (pp. 67-101). Springer New York.

Tornberg, E. (2005). Effects of heat on meat proteins–Implications on structure and quality of meat products. *Meat science, 70(3)*, 493-508.

Trovó, A. G., Nogueira, R. F., Agüera, A., Fernandez-Alba, A. R., Sirtori, C., & Malato, S. (2009a). Degradation of sulfamethoxazole in water by solar photo-Fenton. Chemical and toxicological evaluation. *Water research, 43(16)*, 3922-3931.

Trovó, A. G., Nogueira, R. F., Agüera, A., Sirtori, C., & Fernández-Alba, A. R. (2009b). Photodegradation of sulfamethoxazole in various aqueous media: persistence, toxicity and photoproducts assessment. *Chemosphere, 77(10)*, 1292-1298.

Vree, T. B., Van der Ven, A. J. A. M., Verwey-van Wissen, C. P. W. G. M., Kolmer, E. V. E. B., Swolfs, A. E. M., Van Galen, P. M., & Amatdjais-Groenen, H. (1994). Isolation, identification and determination of sulfamethoxazole and its known metabolites in human

plasma and urine by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications, 658(2),* 327-340.

Xu, D., Grizzle, J. M., Rogers, W. A., & Santerre, C. R. (1996). Effect of cooking on residues of ormetoprim and sulfadimethoxine in the muscle of channel catfish. *Food research international, 29(3),* 339-344.

Zhou, W., & Moore, D. E. (1994). Photochemical decomposition of sulfamethoxazole. *International journal of pharmaceutics, 110(1),* 55-63.

Supplementary Material

Table S1. Losses of sulfonamides and tetracyclines in medium cooked meat (*n* = 3) relative to the total load of antibiotics in the raw meat. *: significantly different at $p < 0.05$.

Compound	Loss (%)
Sulfaguanidine	22.9 ± 11.9 *
Sulfacetamide	26.6 ± 9.8 *
Sulfadiazine	21.4 ± 8.4 *
Sulfamethoxazole	55.0 ± 6.5 *
Sulfathiazole	18.1 ± 18.0
Sulfamerazine	18.9 ± 17.0
Sulfamethizole	18.7 ± 8.8 *
Sulfamethazine	18.5 ± 7.2 *
Sulfamethoxypyridazine	19.3 ± 5.4 *
Sulfamonomethoxine	16.8 ± 11.6
Sulfaquinoxaline	23.4 ± 10.0 *
Sulfadoxine	17.9 ± 4.2 *
Sulfadimethoxine	19.2 ± 7.5 *
Sulfaclozine	20.6 ± 10.6 *
Sulfachloropyridazine	21.5 ± 11.4 *
Tetracycline	None
Doxycycline	1.0 ± 5.0
Oxytetracycline	27.2 ± 0.5 *
Chlortetracycline	34.3 ± 2.6 *
4-epi-tetracycline	24.1 ± 3.0 *
4-epi-chlortetracycline	42.8 ± 2.1 *

Table S2. Concentrations in µg kg^{-1} of meat of sulfonamides in naturally contaminated samples raw or medium cooked.

	Sulfadiazine		Sulfamethazine		Sulfadimethoxine	
	Raw meat	Cooked meat	Raw meat	Cooked meat	Raw meat	Cooked meat
Beef						
Sample 1	/	/	/	/	/	/
Sample 2	/	/	/	/	/	/
Sample 3	/	/	/	/	/	/
Pork						
Sample 4	/	/	/	/	7.6 ± 0.3	11.0 ± 0.1
Sample 5	/	/	/	/	/	/
Sample 6	/	/	51.1 ± 1.6	69.6 ± 2.0	62.8 ± 1.4	77.9 ± 0.3
Sample 7	/	/	/	/	/	/
Sample 8	105.0 ± 1.3	133.5 ± 2.1	/	/	/	/

Table S3. Concentrations in µg kg^{-1} of meat of tetracyclines in naturally contaminated samples raw or medium cooked.

	Tetracycline		Oxytetracycline		4-epi-tetracycline	
	Raw meat	Cooked meat	Raw meat	Cooked meat	Raw meat	Cooked meat
Beef						
Sample 1	/	/	223.5 ± 10.6	310.6 ± 5.9	/	/
Sample 2	36.8 ± 9.6	64.7 ± 4.2	/	/	18.1 ± 5.8	34.9 ± 1.9
Sample 3	/	/	327.0 ± 12.7	387.5 ± 10.6	/	/
Pork						
Sample 4	/	/	/	/	/	/
Sample 5	129.5 ± 4.9	138.8 ± 0.4	/	/	69.3 ± 0.3	77.7 ± 1.1
Sample 6	/	/	/	/	/	/
Sample 7	/	/	418.8 ± 34.8	583.8 ± 18.7	/	/
Sample 8	/	/	/	/	/	/

Complément de discussion sur l'étude de l'impact de la cuisson sur les contaminants chimiques de la viande :

Ce troisième chapitre a permis de montrer que l'impact de la cuisson était très variable selon la nature des contaminants chimiques étudiés et selon l'intensité de cuisson. Alors que la cuisson n'a pas montré d'impact significatif sur la teneur en PCDD/F et en métaux lourds de la viande, des pertes significatives en PCB, antibiotiques et pesticides ont pu être détectées, ces pertes étant d'autant plus importantes que l'intensité de cuisson est élevée.

Le caractère thermorésistant des PCB permet de conclure que les pertes observées pour ce contaminant sont très probablement liées à une libération dans le jus de cuisson. Bien que des résidus de PCB aient été détectés dans le jus de cuisson, la difficulté à collecter le jus après cuisson et à extraire les contaminants de ce dernier n'a cependant pas permis d'obtenir des résultats quantitatifs précis en raison d'une forte variabilité.

Les pesticides et les antibiotiques étant, d'après la littérature, plus sensibles aux températures élevées que les PCB, des phénomènes de dégradation de ces contaminants sous l'effet de la chaleur pourraient expliquer les pertes observées lors de la cuisson. Cette hypothèse n'a pas pu être validée pour les pesticides concernés faute de disponibilité commerciale de ces composés sous forme radiomarquée au ^{14}C, stratégie que nous avons choisi d'utiliser dans ce travail. En revanche, les études menées ont permis de confirmer une thermodégradation de certains antibiotiques comme le sulfaméthoxazole. Dans le cas de cet antibiotique, l'ensemble des produits de dégradation a pu être caractérisé en mettant en œuvre des analyses en LC-MS et LC-MS/MS haute résolution. Cependant, pour l'un des composés de dégradation caractérisé en LC-HRMS comme un produit d'isomérisation du sulfaméthoxazole, des analyses RMN, actuellement en cours, ont dû être effectuées afin de pouvoir proposer une identification structurale non ambigüe, de façon à pouvoir *in fine* proposer un schéma de dégradation thermique de cet antibiotique. Lorsque l'ensemble des produits de dégradation du sulfaméthoxazole seront identifiés, des analyses toxicologiques supplémentaires devront être réalisées pour pouvoir comparer objectivement la toxicité des produits de dégradation du sulfamethoxazole par rapport à celle de la molécule mère.

Chapitre IV : Impact de la digestion sur la bioaccessibilité des contaminants chimiques de la viande

Chapitre IV : Impact de la digestion sur la bioaccessibilité des contaminants chimiques de la viande

Comme cela a été décrit dans le chapitre 1, une grande diversité de protocoles de digestion *in vitro* ont été utilisés dans la littérature, ce qui peut en partie expliquer la grande variabilité des résultats obtenus. C'est pourquoi, afin de pouvoir comparer des résultats provenant de différents laboratoires, un réseau de scientifiques européens travaillant dans le domaine de la digestion a récemment proposé un protocole international standardisé permettant de mimer au mieux les conditions physiologiques de digestion (Minekus *et al.*, 2014). Minekus *et al.* (2014) ont ainsi décrit un protocole consensus comprenant trois étapes : digestion orale, gastrique et intestinale. Ce protocole de digestion statique a donc été mis en place dans cette étude. L'utilisation d'un protocole statique a par ailleurs l'avantage, lors de l'étude de contaminants chimiques, de limiter les risques de contamination qui seraient plus difficiles à contrôler avec un système dynamique.

La méthode de contamination intentionnelle de la viande développée dans le chapitre 2 a été réutilisée dans cette partie. Les résultats préliminaires obtenus dans le cadre du projet SOMEAT avant la réalisation des mesures de bioaccessibilité ont permis de montrer que les PCB correspondaient aux contaminants les plus fréquemment détectés dans la viande. En raison de la lourdeur du protocole de digestion *in vitro* mis en place et du protocole d'extraction des contaminants en vue de l'analyse par GC×GC-TOF/MS, seuls les 18 PCB les plus pertinents à étudier dans la viande selon Sirot *et al.* (2012) ont ici été ciblés.

L'état des lieux de la littérature réalisé dans le chapitre 1 montre que plusieurs facteurs peuvent influencer significativement les valeurs de bioaccessibilité obtenues. Premièrement, la composition de la matrice étudiée, et notamment sa teneur en lipides, semble être un facteur de variation de la bioaccessibilité. Deuxièmement, l'étape de cuisson des aliments ne doit pas être négligée car elle semble également pouvoir influencer la libération des contaminants chimiques de la matrice. Enfin, les différences de protocoles utilisés en termes de teneurs en enzymes digestives ou en sels biliaires peuvent expliquer les différences observées entre les résultats de la littérature. Des variations physiologiques de ces concentrations existent chez l'Homme en fonction de l'âge et pourraient donc suggérer que la bioaccessibilité varie selon l'âge du consommateur. Ces trois facteurs ont donc été étudiés dans ce chapitre. L'impact de la teneur initiale de la viande en lipides a été déterminé en comparant les résultats obtenus avec une viande hachée à 5% ou 15% de matière grasse. Il est important de noter que la

mesure expérimentale de la teneur en matière grasse de la viande étiquetée à 15% de matière grasse a révélé que cette viande ne contenait finalement que 11% de matière grasse. L'impact de la cuisson a été étudié en réalisant les trois conditions de cuisson décrites dans le chapitre précédent (cuisson douce, medium ou intense). Enfin, l'impact des variations physiologiques liées à l'âge du consommateur a été étudié en faisant varier les concentrations en sels biliaires et en enzymes selon les modèles décrits par Dupont *et al.* (2010) pour l'enfant et par Levi *et al.* (2014) pour la personne âgée.

Article 4

Bioaccessibility of polychlorinated biphenyls in meat: Influence of fat content, cooking level and consumer age

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Abstract

In a risk assessment perspective, this work studies the bioaccessibility of polychlorinated biphenyls (PCBs) in meat according to fat level, cooking intensity and physiological differences related to the age of the consumers. A standardised *in vitro* static digestion protocol was set up and coupled with extraction, clean-up and GC×GC-TOF/MS multiresidue analysis to monitor the fate of the 18 most relevant PCBs in meat during digestion. Starting with intentionally contaminated meat, average PCB bioaccessibility was 26 ± 2% in 11% fat meat. PCB bioaccessibility varied inversely with the fat level of meat, increasing to 48 ± 2% in 5% fat meat. The age of the consumer was a further key factor since PCB bioaccessibility decreased to 8 ± 1% and to 17 ± 2% in *in vitro* digestion physiological conditions mimicking infants and elderly, respectively. By contrast, meat cooking was shown to have less influence on PCB bioaccessibility, although intense cooking significantly decreased it to 23 ± 3%. The validity of the bioaccessibility data obtained with spiked meat is discussed in the light of bioaccessibility measurements carried out in naturally contaminated meat samples.

Highlights

• A standardised protocol was set up to assess the bioaccessibility of PCBs in meat

• The mean PCB bioaccessibility was 26% in 11% fat meat

• PCB bioaccessibility increased when the fat level decreased in meat

• Intense cooking decreased PCB bioaccessibility compared with raw or moderately cooked meat

• PCB bioaccessibility was lower in infants and in elderlies than in adults

Keywords

Bioaccessibility; Polychlorinated biphenyls; Meat; *In vitro* digestion; Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-ToF/MS)

1. Introduction

Many micropollutants may be found in foods, and in particular in products of animal origin. Among these micropollutants are polychlorinated biphenyls (PCBs), persistent organic pollutants (POPs) used massively as insulators until the 1980s. PCBs accumulate in animal tissues during growth, and end up in meat products intended for human consumption (Takaki

et al., 2015). The consumption of foods of animal origin is considered the main source of human exposure to PCBs (Malisch & Kotz, 2014). Although these micropollutants generally occur only in trace amounts in foods, they still represent a health hazard for consumers. At present risk is generally assessed from the total concentration of micropollutants in fresh foods. However, the bioaccessible fraction of the micropollutants present in the food (i.e. the fraction liable to cross the intestinal barrier and induce toxic effects) can be considerably lower than the total amount (Collins *et al.*, 2015), because the physiological processes occurring during digestion modify the amounts of contaminants truly available for absorption in the systemic circulation. The bioaccessibility of micropollutants must therefore be taken into account to make an accurate assessment of their human impact (Guerra *et al.*, 2012).

To study the bioaccessibility of micropollutants such as PCBs, *in vitro* digestion models are largely used because they offer the advantage of not being restricted by ethical considerations, while also being faster, better controlled and less costly than *in vivo* methods (Guerra *et al.*, 2012). However, marked variations are observed among the different *in vitro* digestion models described in the literature (incubation time, pH, constituents and concentrations of digestive and intestinal solutions, etc.), yielding widely disparate results. For example, Xing *et al.* (2008) observed a mean bioaccessibility of PCBs contained in raw fish samples of 3%, whereas more recently Shen *et al.* (2016) reported a bioaccessibility of PCBs in fried fish samples of 77%. Owing to the differences in protocols between the two studies, the results obtained are very hard to compare and interpret, and so the moderating influence of cooking on the bioaccessibility values cannot be accurately assessed. To allow comparison of results from different laboratories, a network of scientists working in the field of digestion recently proposed a standardised international protocol to mimic the physiological conditions of digestion as closely as possible (Minekus *et al.*, 2014). Minekus *et al.* (2014) have specified a consensus protocol in three steps: oral, gastric and intestinal digestion. This protocol has been used to assess the bioaccessibility of micro- and macro-nutrients, but remained to be adapted to the study of food contaminants.

To assess the bioaccessibility of PCBs in food after digestion, appropriate analytical methods are required for the monitoring and quantification of contaminants. Multiresidue methods (MRMs) are of particular interest because they allow the simultaneous monitoring of many substances in a single run (Tang *et al.*, 2013). Several studies have shown the utility of two-dimensional gas phase chromatography (GC×GC) in tandem with time-of-flight mass spectrometry (TOF-MS) to analyse the different PCB congeners (Focant *et al.*, 2004; Planche *et al.*, 2015).

Various factors are liable to influence the bioaccessibility of contaminants in meat. The composition of the raw material, and in particular its fat content could be one of them (Xing *et al.*, 2008; Shen *et al.*, 2016). Food processing steps such as cooking could also cause variations in bioaccessibility values (Amiard *et al.*, 2008; Metian *et al.*, 2009; He *et al.*, 2010). Lastly, age-related physiological variations in the human digestion process exist, in particular in concentrations of digestive enzymes: the age of consumers could therefore also affect bioaccessibility values (Levi *et al.*, 2014; Dupont *et al.*, 2010).

The final aim of this study was to achieve a better assessment of the impact on health of chemical contaminants in food. To this end we set out to determine the bioaccessibility of PCBs in meat. We first developed a protocol to assess the bioaccessibility of chemical contaminants in food, the influence of fat level, cooking intensity and the age of consumers on the bioaccessibility of PCBs in meat. The validity of our results obtained with spiked meat are discussed in the light of measurements carried out on naturally contaminated samples.

2. Materials and Methods

2.1. Chemicals and standards

Hexane, dichloromethane, acetone and toluene were organic trace analysis grade solvents (Sigma-Aldrich, Saint-Quentin Fallavier, France). For *in vitro* digestion, constituents of simulated salivary fluid, simulated gastric fluid and simulated intestinal fluid (KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2(H_2O)_6$, $(NH_4)_2CO_3$), together with HCl, NaOH, $CaCl_2$, pepsin from porcine gastric mucosa (P6887), pancreatin from porcine pancreas (P7545) and porcine bile extract (B8631) were from Sigma-Aldrich. For PCB extraction, trichloroacetic acid, activated aluminium oxide (acidic, Brockmann I) and diatomaceous earth were from Sigma-Aldrich. PCB reference standards were from AccuStandard Europe (Niederbipp, Switzerland). Internal standards were used during *in vitro* digestion and PCB extraction for the accurate quantification of target compounds: 3’-F-PCB-28, 3-F-PCB-52, 3’-F-PCB-81, and 5’-F-PCB-156 (Chiron, Trondheim, Norway), 13C-labeled PCB-111 and 13C-labeled PCB-194 (Wellington laboratories, Guelph, ON, Canada).

2.2. Meat samples

Two types of meat samples were used: intentionally and naturally contaminated. Intentionally contaminated meat was prepared with ground beef samples from the same blend of muscles (<15% fat) purchased from a French supplier. The exact fat level in these samples was found to be 11%. Aliquots weighing 125 g were stored at −80 °C before use. Matrix blanks of these samples were made before spiking. Naturally contaminated samples were obtained under the French project SOMEAT (Contract No. ANR-12-ALID-0004. Safety of Organic Meat, available at www.so-meat.fr), and were ground before use.

2.3. Spiking and cooking

2.3.1. Sample spiking

Ground beef was spiked according to Planche *et al*. (2015), combining contaminant addition to ground meat with matrix homogenisation. Briefly, ground beef (120 g) was immersed in a large volume of dichloromethane (20 mL) containing PCBs, the mixture evaporated down under a hood, and the residue homogenised for 2 min in a blender. A spiking concentration of 20 ng g^{-1} of fresh meat was chosen to give concentrations in ready-to-run samples within the range of linearity of GC×GC-TOF/MS for these compounds.

2.3.2. Cooking method

To study the effect of cooking on the bioaccessibility of PCBs in meat, circular small ground beef patties weighing 26 g (2.5 cm thick) were then shaped to copy commercial ground beef patties. These ground beef patties were cooked in a stainless steel frying pan (diameter 17 cm) on a temperature-controlled induction hob (Bosch Electroménager, Saint-Ouen, France). Aluminium foil (11 µm thickness) was placed on the bottom of the frying pan to recover juices released during meat cooking. Three different cooking conditions were used to simulate rare (50 °C at the core), medium (70 °C at the core according to WHO recommendations for ground meats) and well-done (85 °C at the core) meat ($n = 3$ for each cooking condition). These cooking conditions were obtained by 7 min heating (turned once) at 160 °C at the bottom of the pan, 14 min heating (turned three times) at 200 °C at the bottom of the pan, and 14 min heating (turned three times) at 250 °C at the bottom of the pan, respectively. Temperatures at the core of the meat and at the bottom of the pan were measured with thermocouples (RS Components,

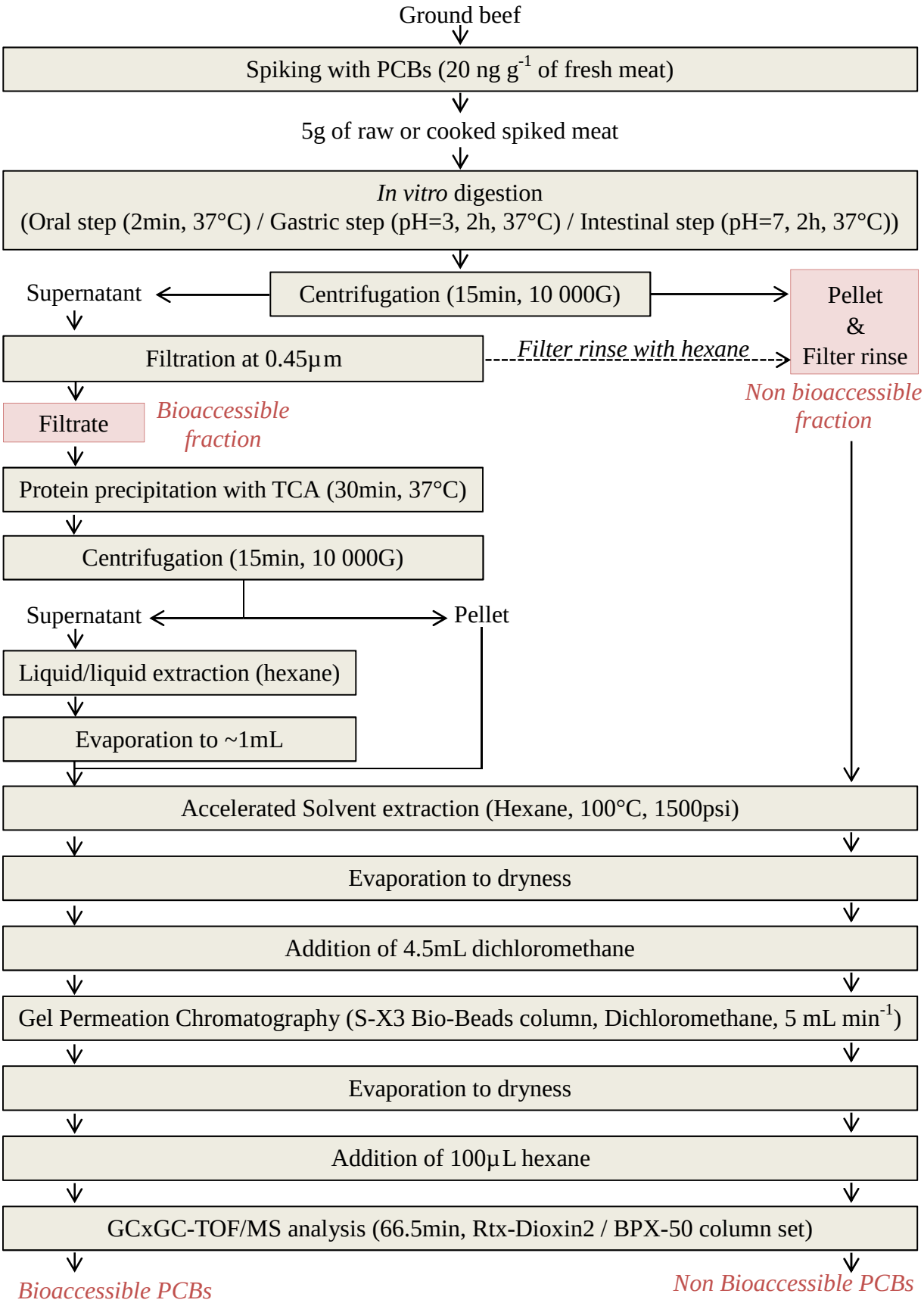

Fig 1: Experimental flowchart showing sample preparation, *in vitro* digestion and PCB extraction and analysis

Beauvais, France). Before *in vitro* digestion, the cooked meat was minced in a blender to simulate mastication.

2.3.3. Determination of fat content

The determination of fat content was realized according to Blanchet-Letrouvé *et al.* (2014) with slight modifications. Raw and cooked meat were first freeze-dried. Aliquots (1 g) of the resulting powder were then extracted by accelerated solvent extraction (ASE) using a Dionex ASE 350 extractor (Sunnyvale, CA, USA) with 22 mL stainless-steel extraction cells. Toluene-acetone (70:30) was used as extraction solvent at a temperature of 120 °C and pressure of 1500 psi with three extraction cycles per sample. The extracts obtained were then evaporated down under a hood and weighed to determine fat content.

2.4. *In vitro* digestion

In vitro adult digestions ($n = 3$ for each condition) were performed according to Minekus *et al*. (2014). Firstly, to simulate the oral phase, 5 g of raw or cooked meat was mixed with 3.5 mL of simulated salivary fluid (SSF), 25 µL of 0.3 M $CaCl_2$ and 1.475 mL of ultrapure water for 2 min at 150 rpm in a water bath at 37 °C. A gastric phase was then carried out by adding 7.5 mL of simulated gastric fluid (SGF), 1.6 mL of porcine pepsin solution (25 000 U mL^{-1}) and 5 µL of 0.3 M $CaCl_2$. The pH was adjusted to 3.0 with 1 M HCl, and the mixture was shaken in darkness for 2 h at 150 rpm in a water bath at 37 °C. To simulate the intestinal step, 11 mL of simulated intestinal fluid (SIF), 2.5 mL of 160 mM bile salts, 5 mL of pancreatin solution (800 U mL^{-1}) and 40 µL of 0.3 M $CaCl_2$ were added to gastric chyme. The pH was adjusted to 7.0 with 1 M NaOH, and the mixture was shaken in darkness for 2 h at 150 rpm in a water bath at 37 °C. The digesta obtained were centrifuged for 15 min at 10,000 × *g*. After filtration of the supernatant obtained through a glass fiber prefilter and a 0.45 µm nylon filter (Phenomenex, Torrance, CA), the filters were rinsed with hexane. The filtrate contained the bioaccessible fraction of PCBs, and the pellet and filter rinse contained the non-bioaccessible fraction of PCBs (Figure 1).

Following Dupont *et al.* (2010), *in vitro* infant digestion was simulated by reducing the pepsin concentration by a factor of 8, the bile salt concentration by a factor of 4 and the pancreatin concentration by a factor of 10 compared with *in vitro* adult digestion.

Following Levi & Lesmes (2014), the digestion of elderly persons (age 75 years) was simulated *in vitro* by reducing the pepsin concentration by a factor of 1.3, the bile salt concentration by a factor of 1.5 and the pancreatin concentration by a factor of 2.2 compared with *in vitro* adult digestion.

The meat digestion protocol was validated by measuring the quantity of peptides produced by proteolysis during the meat digestion. Absorbance of the digesta at 280 nm was then measured after the gastric step according to Gatellier *et al.* (2009) (UVIKON 923, double beam UV/VIS spectrophotometer). Before this measurement, trichloroacetic acid (TC) (50% by volume) was added to the gastric digesta TCA/digesta, v/v 1:2) to precipitate undigested proteins at 37 °C for 15 min (Gatellier *et al.*, 2009). The mixture was then spun at 4000 rpm at 4 °C for 15 minutes. The absorbance at 280 nm (n = 3) was then measured on the supernatant, and compared with that of the simulated gastric fluid (SGF), the SGF + raw meat mixture and the SGF + pepsin mixture at the same concentrations as those used in the gastric step of the digestion protocol. This verification of the quantity of peptides produced could not be carried out for the intestinal digestion step because the pancreatin and bile salts present in this step also absorbed at 280 nm.

2.5. Multiresidue analysis of PCBs

2.5.1. Extraction

The flowchart in Figure 1 shows the steps followed for PCB extraction. To extract bioaccessible PCBs, the filtrate obtained after *in vitro* digestion was first mixed with a trichloroacetic acid (TCA) solution (0.5 g TCA mL^{-1}) in a volume ratio of 2:1 (filtrate: TCA solution) for protein precipitation. The mixture was left for 30 min at 37 °C and then centrifuged for 15 min at 10,000 × *g*. A liquid-liquid extraction of the resulting supernatant was then performed with 70 mL of solvent and repeated three times. For this step, the PCB recovery rates after an extraction by hexane and dichloromethane (DCM) were compared, these two solvents having already been used for the extraction of organochlorine contaminants in bioaccessible fractions (Xing *et al.*, 2008; Wang *et al.*, 2011). For this comparison, a mixture of PCBs was spiked at 2.5 µg/mL in 40 mL of ultrapure water (Millipore, Bedford, MA, USA) and a liquid-liquid extraction was performed with 40 mL of hexane (repeated three times) or with 40 mL of DCM (repeated three times) (n = 3 for each solvent). The organic solvent recovered was then evaporated to dryness

(Rocket, Genevac Ltd.), and 100 µL of hexane was added for the analysis of PCBs by GC×GC-TOF/MS.

For the bioaccessible fraction, after the liquid-liquid extraction with the more efficient solvent, the organic phase containing PCBs was evaporated to ~1 mL. A 34 mL stainless-steel extraction cell was then prepared to extract bioaccessible PCBs by accelerated solvent extraction (ASE). Briefly, 10 g of acidic alumina was placed at the bottom of the cell according to Bjorklund *et al*. (2001). Filter papers were placed underneath and on the top of the alumina layer. The cell was then filled with the pellet obtained after TCA protein precipitation and centrifugation, dispersed in 5 g of diatomaceous earth. The evaporated (~1 mL) solvent phase obtained after liquid-liquid extraction was added at the top of the cell.

Same ASE extraction was performed to extract non-bioaccessible PCBs contained in the pellet obtained at the end of the *in vitro* digestion. The hexane filter rinse was added at the top of the cell.

ASE was then carried out according to Planche *et al.* (2015) using a Dionex ASE 350 extractor (Sunnyvale, CA, USA). Hexane was used as extraction solvent at a temperature of 100 °C and pressure of 1500 psi. ASE extraction included heating (5 min), static time (5 min) and purging (90 s) with two extraction cycles per sample. The extract (approximately 40 mL per extraction cell) was evaporated down (Rocket, Genevac Ltd.) using toluene as a keeper to minimise loss of analytes during the evaporation step; 4.5 mL of dichloromethane was then added. For cleaning extracts, gel permeation chromatography (GPC) (Gilson, Middleton, WI, USA) was carried out on an S-X3 Bio-Beads column (Bio-Rad, Philadelphia, USA) using dichloromethane as eluting solvent at a flow rate of 5 mL min^{-1}. The fraction obtained was evaporated to dryness (Rocket, Genevac Ltd.); 100 µL of hexane was then added before analysis. All the samples were spiked with internal standards at 100 ng mL^{-1} at the different steps.

2.5.2. GC×GC-TOF/MS

Samples were analysed with a time-of-flight mass spectrometer (Pegasus 4D, Leco, St Joseph, MI) coupled to a two-dimensional gas chromatograph (6890, Agilent Technologies) equipped with a dual stage jet cryogenic modulator (licensed from Zoex) according to Planche *et al.* with slight modifications (Planche *et al*., 2015). A Rtx-Dioxin2 1D column (60 m × 0.25 mm × 0.25 µm) (Restek, Bellefonte, PA, USA) was connected by a deactivated Ultimate

Union (Agilent Technologies, Santa Clara, CA) to a BPX-50 2D column (2 m × 0.1 mm × 0.1 µm) (SGE, Austin, TX, USA). A splitless injection of 1 µl of sample extract was performed through a CTC CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) with an inlet temperature set at 280 °C. A split/splitless inert liner (Restek, Sky® 4.0 mm ID liner) was used. Ultra-pure grade helium (purity 99.9995%) was used as carrier gas with a constant flow rate of 1.5 mL min^{-1}. Purge time was set to 60 s with a flow rate of 50 mL min^{-1}. The primary oven temperature was initially set at 90 °C for 1 min, ramped to 200 °C at 20 °C min^{-1}, and then to 300°C at 2°C min^{-1} for 10 min. The secondary oven temperature was set at 5 °C higher than the primary oven temperature. The modulator temperature was set at 15 °C higher than the primary oven temperature, and the modulation period was 5 s, with 1.20 and 1.30 s for the hot and cold pulses, respectively. The transfer line temperature was set at 280 °C. The mass spectrometer was operated with an electron ionisation source (ionisation energy of 70 eV), a detector voltage of 1800 V and a data acquisition rate of 100 spectra s^{-1}. The run time for each sample was 66.5 min. Analytical blank samples of pure solvent were run to check the absence of targeted analytes. GC×GC data were processed using the LECO ChromaTOF software (version 4.50.8.0).

The separative capacity of the column set used (Rtx-Dioxin2/BPX50) was determined by calculating resolution factors (R_s) according to Zapadlo *et al.* with $R_s = \Delta t_R/w_b$, where t_R is the retention time (Table S1. Supplementary material), and w_b the average peak width at the base (Zapadlo *et al.*, 2011). Two successive peaks were considered resolved when $R_{s,1D} \geq 0.6$ in 1D or $R_{s,2D} \geq 0.4$ in 2D.

In order not to be restricted by the sensitivity of the GC×GC-TOF/MS method, naturally contaminated samples, in which the concentration of contaminants is unknown, were analyzed according to Berge *et al.* (2011) by a French National Reference Laboratory (LABERCA, Nantes, France).

2.6. Data processing

Bioaccessibility of PCBs was calculated by:

$$\text{Bioaccessibility (\%)} = \frac{\text{Bioaccessible PCBs}}{\text{Bioaccessible PCBs + Non-bioaccessible PCBs}},$$

where Bioaccessible PCBs and Non-bioaccessible PCBs are the amounts of PCBs found after *in vitro* digestion in the bioaccessible and non-bioaccessible fractions, respectively. Data were processed using the Statistica Software version 12 (Dell Software, Paris, France). Principal component analyses

(PCA) were performed on the bioaccessibility data of PCBs in meat after *in vitro* digestion to visualise the structure of the data. To determine whether the cooking process, the fat content of meat or the physiological differences due to the age of consumers had any impact on the bioaccessibility of PCBs in meat, a one-way analysis of variance (ANOVA; $p < 0.05$) was performed on bioaccessibility data from GC×GC-TOF/MS. A Newman-Keuls mean comparison test was then performed on the resulting dataset.

3. Results and Discussion

3.1. Analysis of PCBs in meat digesta

To evaluate the bioaccessibility of the 18 most relevant PCBs in meat (Sirot *et al.*, 2012), the standard static *in vitro* digestion protocol described by Minekus *et al.* (2014) was implemented starting with 5 g of meat spiked with PCBs. Briefly, as shown in Figure 1, the digestion protocol comprised an oral step (2 min, 150 rpm, 37 °C), a gastric step (pH = 3, 2 h, 150 rpm, 37 °C) and an intestinal step (pH = 7, 2 h, 150 rpm, 37 °C). After these steps the digesta were centrifuged and the supernatant filtered, thereby separating the bioaccessible fraction (filtrate) from the non-bioaccessible fraction (pellet and filter rinse). The protocol was validated by verifying the increase in the amount of peptides produced by proteolysis during digestion. According to Gatellier *et al.* (2009), the mean absorbance of the digesta was 1.56 against nil for the SGF solution, 0.49 for the SGF + meat mixture and 0.48 for the SGF + pepsin mixture. The absorbance of the gastric digesta (1.56) was thus greater than the other absorbances measured ($p < 0.05$), confirming the increase due to proteolysis in the course of the gastric digestion of the meat. Allowing for dilution, the mean absorbance (1.18) was of the same order of magnitude as that found by Gatellier *et al.* (2009) in their gastric raw meat digesta (0.98), thus validating our *in vitro* digestion set-up. A protocol for the extraction and analysis of PCBs in the bioaccessible fraction (filtrate) and non-bioaccessible fraction (pellet and filter rinse) was then developed. As shown in Figure 1, the proteins contained in this fraction were first precipitated with trichloroacetic acid (TCA) to extract the PCBs contained in the bioaccessible fraction, consisting mainly of water. After centrifugation, a liquid/liquid extraction was carried out on the aqueous supernatant obtained with a comparison between hexane and dichloromethane (DCM) as the extraction solvent (Xing *et al.*, 2008; Wang *et al.*, 2011). The results showed a mean recovery rate of PCBs in the water of 98% when hexane was used for the liquid/liquid extraction, with values ranging from 56 ± 0.7% for PCB 28 to 112 ± 19% for PCB 156. With DCM, the mean recovery rate was 90%, with values ranging

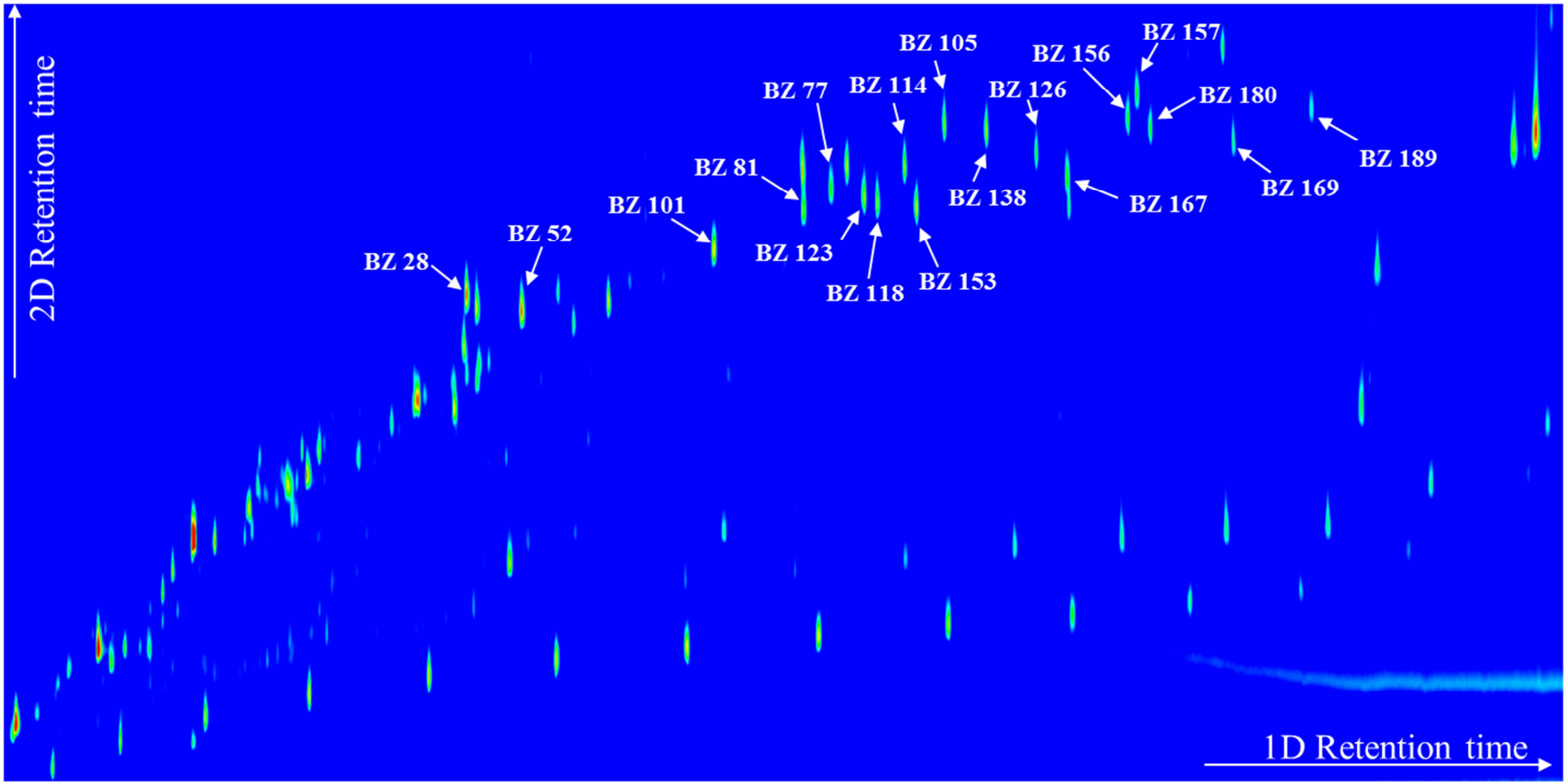

Fig 2: GC×GC-ToF/MS contour plot with the 18 PCB congeners more relevant to monitor in meat.

Table 1. Bioaccessibility (BA) (%) of PCBs in 5% fat meat (*n* = 3) and 11% fat meat (*n* = 3). [a,b]: significantly different at $p < 0.05$.

Compound	Congener	WHO-TEF	5% fat meat		11% fat meat	
			BA (%)	RSD (%)	BA (%)	RSD (%)
2,4,4'-Trichlorobiphenyl	28		49.5[b]	9.9	24.9[a]	1.4
2,2',5,5'-Tetrachlorobiphenyl	52		48.7[b]	12.4	25.9[a]	2.2
3,3',4,4'-Tetrachlorobiphenyl	77	0.0001	44.4[b]	9.8	20.6[a]	6.1
3,4,4',5-Tetrachlorobiphenyl	81	0.0003	45.9[b]	7.8	23.0[a]	4.3
2,2',4,5,5'-Pentachlorobiphenyl	101		48.1[b]	4.7	26.9[a]	3.5
2,3,3',4,4'-Pentachlorobiphenyl	105	0.00003	47.5[b]	12.5	26.4[a]	2.7
2,3,4,4',5-Pentachlorobiphenyl	114	0.00003	47.6[b]	8.6	25.2[a]	5.0
2,3',4,4',5-Pentachlorobiphenyl	118	0.00003	48.4[b]	9.1	24.9[a]	6.1
2',3,4,4',5-Pentachlorobiphenyl	123	0.00003	48.7[b]	8.7	25.8[a]	5.1
3,3',4,4',5-Pentachlorobiphenyl	126	0.1	44.7[b]	7.7	24.0[a]	2.7
2,2',3,4,4',5'-Hexachlorobiphenyl	138		48.4[b]	8.0	27.5[a]	6.7
2,2',4,4',5,5'-Hexachlorobiphenyl	153		50.1[b]	8.3	27.9[a]	6.1
2,3,3',4,4',5-Hexachlorobiphenyl	156	0.00003	46.8[b]	7.6	26.7[a]	6.2
2,3,3',4,4',5'-Hexachlorobiphenyl	157	0.00003	46.5[b]	6.0	23.8[a]	4.7
2,3',4,4',5,5'-Hexachlorobiphenyl	167	0.00003	48.2[b]	9.3	28.0[a]	2.3
3,3',4,4',5,5'-Hexachlorobiphenyl	169	0.03	44.2[b]	3.0	25.9[a]	3.0
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180		49.4[b]	4.8	30.5[a]	4.7
2,3,3',4,4',5,5'-Heptachlorobiphenyl	189	0.00003	47.3[b]	4.8	28.5[a]	3.1

from 71 ± 4.9% for PCB 101 to 118 ± 6.5% for PCB 180. Except for PCB 28, all the recovery rates lay in the classically accepted range 70–130% (data not shown), with RSD ≤ 10% for most of the compounds (EPA Method 8000C, 2003). Of the 18 PCBs targeted in this study, 14 showed higher recovery rates, and closer to 100%, with hexane than with DCM. Hexane was therefore subsequently used in this study for the liquid/liquid extraction of PCBs in the bioaccessible fraction.

After ASE extraction of PCBs and GPC defatting of the extracts obtained for bioaccessible and non bioaccessible fractions (Figure 1), an analysis by GC×GC-TOF/MS (Rtx-Dioxin2/BPX-50 column set) of PCBs was carried out in 66.5 min using the protocol developed by Planche *et al.* (2015). The separation of the 18 PCBs targeted in this study (Figure 2) was validated by calculating resolution factors defined by Zapadlo *et al.* (2011) (data not shown).

Lastly, to verify the soundness of our protocol for the study of bioaccessibility, the recovery rates for PCBs contained in the raw meat were determined after completion of the flowchart sequence (*in vitro* digestion, extraction and analysis by GC×GC-TOF/MS). The PCBs contained in the meat before digestion were thus quantified (n = 3) and compared with the sum of the PCBs found in the bioaccessible and non-bioaccessible fractions (n = 3) after digestion. The mean of the recovery rates obtained was 95 ± 16%, confirming the efficiency and robustness of the protocol.

3.2. Impact of meat fat content on bioaccessibility of PCBs

To determine the impact of fat level in meat on the bioaccessibility of PCBs, *in vitro* digestions were carried out, using the previously described protocol, on ground beef containing 11% (n = 3) or 5% fat (n = 3) pan-fried as recommended by WHO (70 °C at the core). It is important to note that there was no difference between 11% and 5% fat meat in terms of lipid composition (data not shown) which could influence the bioaccessibility (Zhang *et al.*, 2016 ; Zou *et al.*, 2016). Bioaccessibility results of the 18 most relevant PCBs in meat are reported in Table 1. For meat with 11% fat, the mean bioaccessibility of PCBs was 25.9 ± 2.3% against 47.5 ± 1.7% for meat with 5% fat. Note that the bioaccessibility of PCB 126, which has the highest toxic equivalent factor (WHO-TEF) (0.1) of all the 18 PCBs studied, was 24.0 ± 0.6% for meat with 11% fat and 44.7 ± 3.4% for meat with 5% fat, confirming that bioaccessibility is a key factor to be taken into account in risk analysis. These values are of the same order of magnitude as those recently obtained by Shen *et al.* (2016), who report a

mean bioaccessibility of PCBs in beef samples (4.8% fat) of 49.0 ± 3.3% after boiling (5 min at 100 °C). By contrast, they are at variance with those of Xing *et al*. (2008), who found a mean bioaccessibility of PCBs of 3% in fish samples (3–15% fat). It is however noteworthy that Xing *et al.* worked on raw matrices whereas we used cooked ones, and that there were wide disparities between the *in vitro* digestion protocols used, in particular in the incubation time for the samples with the digestive enzymes used in the different steps of the protocol. In the gastric digestion step, this time was 1 h in Xing *et al.* against 2 h in our study, but 6 h (against 2 h) in the intestinal digestion step. These differences in protocols thus rule out any objective comparison of the data obtained. However, it is interesting to note that these authors, like us, found a significant increase in the mean bioaccessibility of the PCBs ($p < 0.05$) when the food fat content is lower. Xing *et al.* measured thus a mean bioaccessibility of PCBs of 3% in fish samples containing 3–15% fat, whereas in samples of spinach and salads with much less fat (0.1% on average), bioaccessibility was significantly higher at 25%. In our study, we found the relation between cooked meat lipid levels and bioaccessibility of PCBs to be inversely proportional: when the percentage of fat was divided by 1.9, the bioaccessibility was multiplied by 1.8. As PCBs are lipophilic compounds (Log K_{ow} = 4.09–8.18 according to Hawker & Connell (1988)), they can be sequestered by lipids during digestion. Lipids are difficult to digest when fat content is high (Xing *et al.*, 2008), which can explain the lower solubilisation of PCBs in the bioaccessible fraction, and so a lower bioaccessibility as the meat fat content rises. The same explanation was given by Xia *et al.* (2017) who observed that the bioaccessibility of the hydrophobic β-carotene was significantly lower in a simulated high-fat diet than in a simulated low-fat diet: an appreciable fraction of the lipid phase was not fully digested and, therefore, a fraction of the β-carotene was not released into the intestinal fluids, remaining in the nondigested lipid phase (Xia *et al.*, 2017).

As shown in Figure S1. (Supplementary material), fat content is thus a key determinant in the bioaccessibility of PCBs. It is of interest that in several studies, (Bordajandi *et al.*, 2004; Carlson *et al.*, 2005), the higher the fat content of foods, the higher their PCB concentrations. The results obtained in our study thus suggest that PCBs in fat-rich foods are less bioaccessible, mitigating the hazard related to their presence in such foods.

Table 2. Bioaccessibility (BA) (%) of PCBs in raw meat (*n* = 3) and in meat cooked at different intensities (rare (*n* = 3), medium (*n* = 3) and well-done (*n* = 3)). [a,b,c]: significantly different at $p < 0.05$.

Compound	Congener	WHO-TEF	Raw meat		Rare cooked meat		Medium cooked meat		Well done cooked meat	
			BA (%)	RSD (%)	BA (%)	RSD (%)	BA (%)	RSD (%)	BA (%)	RSD (%)
2,4,4'-Trichlorobiphenyl	28		26.1[a]	13.5	24.5[a]	7.4	24.9[a]	1.4	22.6[a]	5.1
2,2',5,5'-Tetrachlorobiphenyl	52		27.4[a]	11.8	26.9[a]	8.9	25.9[a]	2.2	24.1[a]	2.3
3,3',4,4'-Tetrachlorobiphenyl	77	0.0001	24.1[b]	12.7	20.5[ab]	4.7	20.6[ab]	6.1	18.3[a]	6.7
3,4,4',5-Tetrachlorobiphenyl	81	0.0003	24.6[c]	8.1	21.4[b]	2.0	23.0[bc]	4.3	19.0[a]	5.1
2,2',4,5,5'-Pentachlorobiphenyl	101		28.9[a]	7.5	27.3[a]	6.8	26.9[a]	3.5	26.7[a]	4.2
2,3,3',4,4'-Pentachlorobiphenyl	105	0.00003	26.1[b]	7.1	24.2[b]	5.4	26.4[b]	2.7	20.5[a]	7.5
2,3,4,4',5-Pentachlorobiphenyl	114	0.00003	25.8[a]	10.1	24.2[a]	4.5	25.2[a]	5.0	22.6[a]	6.5
2,3',4,4',5-Pentachlorobiphenyl	118	0.00003	25.5[a]	9.3	24.3[a]	4.3	24.9[a]	6.1	21.5[a]	5.9
2',3,4,4',5-Pentachlorobiphenyl	123	0.00003	27.0[a]	8.5	26.4[a]	6.8	25.8[a]	5.1	25.8[a]	2.5
3,3',4,4',5-Pentachlorobiphenyl	126	0.1	25.2[b]	10.0	22.1[b]	4.3	24.0[b]	2.7	18.0[a]	8.1
2,2',3,4,4',5'-Hexachlorobiphenyl	138		27.4[a]	8.4	26.7[a]	7.5	27.5[a]	6.7	24.6[a]	5.0
2,2',4,4',5,5'-Hexachlorobiphenyl	153		28.2[a]	8.7	28.2[a]	6.6	27.9[a]	6.1	26.3[a]	7.2
2,3,3',4,4',5-Hexachlorobiphenyl	156	0.00003	27.7[b]	8.7	25.7[b]	1.0	26.7[b]	6.2	21.9[a]	6.2
2,3,3',4,4',5'-Hexachlorobiphenyl	157	0.00003	27.5[b]	10.9	25.4[ab]	2.4	23.8[ab]	4.7	21.9[a]	5.5
2,3',4,4',5,5'-Hexachlorobiphenyl	167	0.00003	28.0[b]	6.0	25.9[ab]	6.0	28.0[b]	2.3	23.3[a]	11.6
3,3',4,4',5,5'-Hexachlorobiphenyl	169	0.03	23.3[ab]	9.1	23.7[ab]	3.8	25.9[b]	3.0	21.2[a]	5.7
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180		29.0[ab]	11.1	27.9[ab]	4.3	30.5[b]	4.7	24.4[a]	7.2
2,3,3',4,4',5,5'-Heptachlorobiphenyl	189	0.00003	35.1[c]	13.9	27.9[b]	4.1	28.5[b]	3.1	21.7[a]	7.5

3.3. Impact of cooking intensity on bioaccessibility of PCBs

To determine the impact of cooking and its intensity on PCB bioaccessibility in meat, *in vitro* digestions were carried out on ground beef containing 11% fat, raw and cooked at three intensities: rare (50 °C at the core), medium, (70 °C at the core according to WHO recommendation), and well-done (85 °C at the core) ($n = 3$ for each condition). Results presented in Table 2 show that the mean bioaccessibility of the PCBs was 27.1 ± 2.6% for raw meat, 25.2 ± 2.3% for rare-cooked meat, 25.9 ± 2.3% for medium-cooked meat (WHO recommendations), and 22.5 ± 2.6% for well-done meat. No significant difference was observed between raw, rare- and WHO-cooked meat, but the bioaccessibility measured on well-done meat was significantly lower ($p < 0.05$). For PCB 126, which displays the highest toxic equivalent factor (WHO-TEF) of all the PCBs studied (0.1), the bioaccessibility decreased from 25.2% (RSD: 10.0%) for raw meat to 18.0% (RSD: 8.1%) after intense cooking. Although they did not evaluate the impact of cooking intensity on PCB bioaccessibility, Shen *et al.* (2016) showed that cooking conditions need to be taken into account in bioaccessibility studies: for example, the bioaccessibility of PCBs in meat was significantly lower ($p < 0.05$) after boiling (5 min at 100 °C) than after frying in cooking oil. Various studies conducted on trace elements (Cu, Cd, Zn, etc.) have also shown that the cooking of fish and other seafood generally lowers bioaccessibility, irrespective of the cooking method used: frying, grilling, boiling or steaming (Amiard *et al.*, 2008; Metian *et al.*, 2009; He *et al.*, 2010). This decrease in bioaccessibility may be explained by the modification in meat fat content induced by cooking. Whereas raw meat contains 11% fat, cooked meat contains respectively 13%, 15% and 16% fat after rare, WHO or intense cooking, respectively. As discussed above, the decrease in bioaccessibility could thus be at least partly linked to the increase in meat fat content. Another possible explanation for the reduced bioaccessibility after intense cooking is the protein denaturing that occurs during cooking, shrinking tissues and so making them harder and more compact, therefore restricting the activity of digestive enzymes (Kulp *et al.*, 2003; He *et al.*, 2010). The activity of pepsin can thus be reduced during the digestion of cooked meat compared with raw meat as demonstrated by Santé-Lhoutellier *et al.* (2008), who observed a decrease of 58% in the activity of pepsin on myofibrillary proteins after 45 min cooking at 100 °C. Cooking may also cause the formation of disulphide-bonded proteins, which make the proteins less digestible (He *et al.*, 2010). All these processes are especially important under extreme cooking

Table 3. Bioaccessibility (BA) (%) of PCBs in medium-cooked meat (11% of fat) after simulating the digestion of an infant (*n*=3), an adult (*n*=3) or an elderly person (*n*=3). [a,b,c]: significantly different at $p < 0.05$.

Compound	Congener	WHO-TEF	Infant		Adult		Elderly	
			BA (%)	RSD (%)	BA (%)	RSD (%)	BA (%)	RSD (%)
2.4.4'-Trichlorobiphenyl	28		7.0 [a]	14.1	24.9 [c]	1.4	15.0 [b]	5.0
2.2'.5.5'-Tetrachlorobiphenyl	52		6.4 [a]	9.6	25.9 [c]	2.2	15.5 [b]	5.0
3.3'.4.4'-Tetrachlorobiphenyl	77	0.0001	7.2 [a]	9.7	20.6 [c]	6.1	14.6 [b]	9.5
3.4.4'.5-Tetrachlorobiphenyl	81	0.0003	6.8 [a]	4.6	23.0 [c]	4.3	15.0 [b]	5.1
2.2'.4.5.5'-Pentachlorobiphenyl	101		8.0 [a]	10.2	26.9 [c]	3.5	17.9 [b]	4.6
2.3.3'.4.4'-Pentachlorobiphenyl	105	0.00003	7.7 [a]	8.3	26.4 [c]	2.7	16.8 [b]	5.8
2.3.4.4'.5-Pentachlorobiphenyl	114	0.00003	7.5 [a]	8.1	25.2 [c]	5.0	16.7 [b]	5.8
2.3'.4.4'.5-Pentachlorobiphenyl	118	0.00003	7.3 [a]	6.5	24.9 [c]	6.1	16.3 [b]	6.3
2'.3.4.4'.5-Pentachlorobiphenyl	123	0.00003	8.1 [a]	7.8	25.8 [c]	5.1	17.7 [b]	4.0
3.3'.4.4'.5-Pentachlorobiphenyl	126	0.1	7.2 [a]	12.9	24.0 [c]	2.7	15.3 [b]	5.5
2.2'.3.4.4'.5'-Hexachlorobiphenyl	138		8.6 [a]	8.7	27.5 [c]	6.7	18.4 [b]	4.2
2.2'.4.4'.5.5'-Hexachlorobiphenyl	153		8.8 [a]	9.5	27.9 [c]	6.1	18.6 [b]	4.8
2.3.3'.4.4'.5-Hexachlorobiphenyl	156	0.00003	7.4 [a]	9.7	26.7 [c]	6.2	18.1 [b]	3.9
2.3.3'.4.4'.5'-Hexachlorobiphenyl	157	0.00003	8.2 [a]	6.7	23.8 [c]	4.7	18.5 [b]	3.2
2.3'.4.4'.5.5'-Hexachlorobiphenyl	167	0.00003	8.4 [a]	6.6	28.0 [c]	2.3	18.1 [b]	5.8
3.3'.4.4'.5.5'-Hexachlorobiphenyl	169	0.03	6.2 [a]	14.3	25.9 [c]	3.0	17.0 [b]	5.3
2.2'.3.4.4'.5.5'-Heptachlorobiphenyl	180		9.1 [a]	7.8	30.5 [c]	4.7	19.4 [b]	3.7
2.3.3'.4.4'.5.5'-Heptachlorobiphenyl	189	0.00003	8.0 [a]	6.3	28.5 [c]	3.1	19.1 [b]	4.5

conditions (Dadorama, 1996), which explains why the significant decrease in bioaccessibility was observed only in the case of intense cooking.

3.4. Impact of consumer age on bioaccessibility of PCBs

To determine the impact of physiological conditions related to the age of the consumer on PCB bioaccessibility, *in vitro* digestions of meat (11% fat, WHO cooking) were carried out, simulating the digestion of an infant ($n = 3$) according to Dupont *et al*. (2010), and that of an elderly person ($n = 3$) following Levi & Lesmes (2014). These data could thus be compared with those previously obtained for adult digestion simulation (Minekus *et al.*, 2014). According to Table 3, the mean bioaccessibility of PCBs was 7.7 ± 0.8% in the infant and 17.1 ± 1.5% in the elderly person, against 25.9 ± 2.3% in the adult. As shown in Figure S1. (Supplementary material), bioaccessibility thus varies significantly ($p < 0.05$) according to the physiological conditions related to the age of the consumer. These variations are consistent with concentrations of pepsin, bile salts and pancreatin, which were 8, 4 and 10 times lower in the infant, and 1.3, 1.5 and 2.2 times lower in the elderly person compared to the adult data (Dupont *et al.*, 2010; Levi & Lesmes, 2014). The lower were the concentrations of these enzymes, the lower were the PCB bioaccessibility values. These results are consistent with with those obtained by Jadán-Piedra *et al.* (2016), who observed that the bioaccessibility of Hg in fish increased with the concentration of pepsin, which facilitated the cleavage of protein-contaminant bonds (Periago *et al.*, 2013). Likewise, Kulp *et al.* (2003) showed that when the concentration of pancreatin (containing lipases) increased, the bioaccessibility of the heterocyclic amines in the meat likewise increased. Our results also suggest that the concentration of bile salts is a key determinant of bioaccessibility: in the infant, the concentration of bile salts is 4 times lower than in the adult, and we observed that the bioaccessibility was divided by 3.4 relative to that of the adult. Likewise, in the elderly person, the concentration of bile salts was 1.5 times lower than in the adult, and bioaccessibility was also divided by 1.5 relative to the adult. These results are consistent with those reported by Yu *et al.* (2011b), who studied the bioaccessibility of PBDEs in dust. These authors observed that when the concentration of bile salts was multiplied by 2.2, the bioaccessibility of PBDEs rose 2.8-fold. During digestion, bile salts may facilitate the emulsification of fats containing lipophilic substances such as PBDEs or PCBs, thereby inducing higher bioaccessibilities when their concentration increases (Oomen *et al.*, 2000 ; Yu *et al.*, 2011b).

Table 4. Bioaccessibility (%) of PCBs in four medium-cooked naturally contaminated samples after simulating adult digestion.

	Sample 1 *2.9% of fat* *10.3 ng PCBs/g fat*	**Sample 2** *5.0% of fat* *48.6 ng PCBs/g fat*	**Sample 3** *5.3% of fat* *8.7 ng PCBs/g fat*	**Sample 4** *9.5% of fat* *5.0 ng PCBs/g fat*
PCB 28	61.6	63.9	76.8	70.8
PCB 52	56.4	51.4	65.8	48.7
PCB 77	44.9	46.5	48.8	35.8
PCB 81	46.2	45.1	49.3	38.5
PCB 101	61.2	59.8	65.9	45.7
PCB 105	40.9	39.8	39.7	23.9
PCB 114	44.7	38.3	54.5	37.1
PCB 118	45.0	34.8	47.9	22.3
PCB123	48.9	54.8	57.2	35.3
PCB 126	43.6	47.1	42.5	30.9
PCB 138	31.8	34.5	41.2	20.4
PCB 153	37.9	36.6	39.7	19.9
PCB 156	40.1	38.0	42.9	25.0
PCB 157	39.2	38.3	42.6	22.4
PCB 167	44.8	35.2	45.4	23.0
PCB 169	38.2	48.2	41.3	27.0
PCB 180	39.1	37.0	38.8	16.0
PCB 189	35.9	37.4	41.4	20.5

3.5. Comparison of results with naturally contaminated samples

This study was designed with samples intentionally spiked in order to obtain a matrix in unlimited amounts with PCBs at a known concentration. However, in order to determine whether a difference may exist in the bioaccessibility of PCBs, between spiked PCBs and PCBs bioaccumulated in muscles during animal breeding. For this purpose, *in vitro* digestions were carried out on samples of meat identified as contaminated ($n = 4$) by a French national reference laboratory (LABERCA, Nantes, France) in its monitoring programs. After mincing and blending, the four raw samples targeted were analysed, showing concentrations of PCBs in the range 5.0–48.6 ng/g of fat. As given by Table 4, mean bioaccessibility of the PCBs was 45.0 ± 8.2% for sample 1 (2.9% fat), 44.0 ± 9.0% for sample 2 (5.0% fat), 49.4 ± 10.9% for sample 3 (5.3% fat) and 31.9 ± 13.6% for sample 4 (9.5% fat). The mean values of bioaccessibility obtained for samples 2 and 3, which both had a fat content close to 5% were of the same order of magnitude as those obtained on spiked meat with 5% fat used in our study (47.5 ± 1.7%). In addition, there was no significant difference between the results obtained with meat containing 2.9% fat and meat with near 5% fat. There may thus be a threshold fat content below which the bioaccessibility of PCBs remains constant, as already described for PBDEs by Yu *et al.* (2009), who observed that the bioaccessibility of PBDEs was significantly correlated with the fat content of foods only when this exceeded 1.8% of their fresh weight. In the case of PCB bioaccessibility, this hypothesis would, however, need to be validated on a greater number of samples. The data obtained also tell us that for a higher fat content, as for sample 4 (9.5% fat), the values of bioaccessibility were significantly lower ($p < 0.05$) than for the other samples, in line with our results with spiked meat, thus confirming the inversely proportional relation between PCB bioaccessibility and fat content of meat. In addition, unlike the spiked samples, the naturally contaminated samples were found to present bioaccessibility values that varied according to the PCB congener: bioaccessibility was higher for the least chlorinated congeners and lower for the most chlorinated, with bioaccessibility values significantly higher ($p < 0.05$) for PCB 28 (trichlorobiphenyl) than for PCBs 180 and 189 (heptachlorobiphenyls). Xing *et al*. (2008) also observed that the bioaccessibility of PCBs in fish samples was generally higher for the least chlorinated congeners, and the same trend was observed by Kang *et al.* (2013) and by Wang *et al.* (2013) evaluating the bioaccessibility of PCBs in dust. The most chlorinated PCBs are those displaying the highest Log K_{ow} values, so that these congeners are more readily retained by the fat in the meat, and so harder to solubilise in the bioaccessible fraction. However, it is still

difficult to explain why this trend is observed in naturally contaminated samples, and not in spiked samples. It would therefore be of interest to extend this study and analyse a greater number of naturally contaminated samples.

4. Conclusion

The standardised *in vitro* digestion protocol coupled with extraction and analysis protocols implemented in this study enabled to assess the bioaccessibility of PCBs in meat. For ground beef meat with 11% fat, mean bioaccessibility was 26 ± 2%. Meat fat content is a key factor to be taken into account, results showing that bioaccessibility was inversely proportional to fat content. The data obtained on naturally contaminated samples confirmed these findings, and revealed a threshold fat content below which the bioaccessibility of PCBs remained constant. The physiological conditions related to the age of the consumer were also found to be an important factor, the bioaccessibility of PCBs being significantly lower in the infant and elderly person than in the adult. To compare the results obtained in this study with those from other laboratories, it would, however, be useful to use consensus digestion protocols for young and older consumers, in addition to adults. Lastly, although it had less impact on our results than the two preceding variables, cooking conditions cannot be ignored. While no significant difference was observed between raw, rare-cooked and medium-cooked (WHO) samples, intense cooking caused a decrease in PCB bioaccessibility. These findings show that the fat content of foods, the age of the consumer and to a lesser extent, cooking intensity are factors that cause bioaccessibility to vary. The next step will be to take these data into account to propose a new model for the assessment of risks due to chemical contaminants in food.

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References

Amiard, J. C., Amiard-Triquet, C., Charbonnier, L., Mesnil, A., Rainbow, P. S., & Wang, W. X. (2008). Bioaccessibility of essential and non-essential metals in commercial shellfish from Western Europe and Asia. *Food and Chemical Toxicology, 46(6)*, 2010-2022.

Berge, P., Ratel, J., Fournier, A., Jondreville, C., Feidt, C., Roudaut, B., Le Bizec, B., & Engel, E. (2011). Use of volatile compound metabolic signatures in poultry liver to back-trace dietary exposure to rapidly metabolized xenobiotics. *Environmental science & technology, 45(15)*, 6584-6591.

Blanchet-Letrouvé, I., Zalouk-Vergnoux, A., Vénisseau, A., Couderc, M., Le Bizec, B., Elie, P., Herrenknecht, C., Mouneyrac, C., & Poirier, L. (2014). Dioxin-like, non-dioxin like PCB and PCDD/F contamination in European eel (Anguilla anguilla) from the Loire estuarine continuum: spatial and biological variabilities. *Science of the Total Environment, 472*, 562-571.

Björklund, E., Müller, A., & von Holst, C. (2001). Comparison of fat retainers in accelerated solvent extraction for the selective extraction of PCBs from fat-containing samples. *Analytical chemistry, 73(16)*, 4050-4053.

Bordajandi, L. R., Gómez, G., Abad, E., Rivera, J., Fernández-Bastón, M. D. M., Blasco, J., & González, M. J. (2004). Survey of persistent organochlorine contaminants (PCBs, PCDD/Fs, and PAHs), heavy metals (Cu, Cd, Zn, Pb, and Hg), and arsenic in food samples from Huelva (Spain): levels and health implications. *Journal of agricultural and food chemistry, 52(4)*, 992-1001.

Carlson, D. L., & Hites, R. A. (2005). Polychlorinated biphenyls in salmon and salmon feed: global differences and bioaccumulation. *Environmental science & technology, 39(19)*, 7389-7395.

Collins, C. D., Craggs, M., Garcia-Alcega, S., Kademoglou, K., & Lowe, S. (2015). Towards a unified approach for the determination of the bioaccessibility of organic pollutants. *Environment international, 78*, 24-31.

Dadorama, S. (1996). Amino acids, peptides, and proteins. In O. R. Fennema (Ed.), F*ood chemistry* (3rd ed., pp. 321–429). New York, NY, USA: Marcel Dekker Inc.

Dean, J. R., & Ma, R. (2007). Approaches to assess the oral bioaccessibility of persistent organic pollutants: a critical review. *Chemosphere, 68(8)*, 1399-1407.

Dupont, D., Mandalari, G., Molle, D., Jardin, J., Léonil, J., Faulks, R. M., ... & Mackie, A. R. (2010). Comparative resistance of food proteins to adult and infant in vitro digestion models. *Molecular nutrition & food research, 54(6)*, 767-780.

Engel, E., Ratel, J., Bouhlel, J., Planche, C., & Meurillon, M. (2015). Novel approaches to improving the chemical safety of the meat chain towards toxicants. *Meat science, 109*, 75-85.

Environmental Protection Agency (EPA) (2003). EPA Method 8000C, Determinative Chromatographic Separations, Revision 3, Washington, DC, USA.

Focant, J. F., Sjödin, A., & Patterson, D. G. (2004). Improved separation of the 209 polychlorinated biphenyl congeners using comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry. *Journal of Chromatography A, 1040(2)*, 227-238.

Gatellier, P., & Santé-Lhoutellier, V. (2009). Digestion study of proteins from cooked meat using an enzymatic microreactor. *Meat science, 81(2)*, 405-409.

Guerra, A., Etienne-Mesmin, L., Livrelli, V., Denis, S., Blanquet-Diot, S., & Alric, M. (2012). Relevance and challenges in modeling human gastric and small intestinal digestion. *Trends in biotechnology, 30(11)*, 591-600.

Hawker, D. W., & Connell, D. W. (1988). Octanol-water partition coefficients of polychlorinated biphenyl congeners. *Environmental science & technology, 22(4)*, 382-387.

He, M., Ke, C. H., & Wang, W. X. (2010). Effects of cooking and subcellular distribution on the bioaccessibility of trace elements in two marine fish species. *Journal of agricultural and food chemistry, 58(6),* 3517-3523.

Jadán-Piedra, C., Clemente, M. J., Devesa, V., & Vélez, D. (2016). Influence of Physiological Gastrointestinal Parameters on the Bioaccessibility of Mercury and Selenium from Swordfish. *Journal of agricultural and food chemistry, 64(3),* 690-698.

Kang, Y., Yin, Y., Man, Y., Li, L., Zhang, Q., Zeng, L., & Wong, M. H. (2013). Bioaccessibility of polychlorinated biphenyls in workplace dust and its implication for risk assessment. *Chemosphere, 93(6),* 924-930.

Kulp, K. S., Fortson, S. L., Knize, M. G., & Felton, J. S. (2003). An in vitro model system to predict the bioaccessibility of heterocyclic amines from a cooked meat matrix. *Food and Chemical Toxicology, 41(12),* 1701-1710.

Levi, C. S., & Lesmes, U. (2014). Bi-compartmental elderly or adult dynamic digestion models applied to interrogate protein digestibility. Food & function, 5(10), 2402-2409.

Li, L., Xie, S., Cai, H., Bai, X., & Xue, Z. (2008). Quantitative structure–property relationships for octanol–water partition coefficients of polybrominated diphenyl ethers. *Chemosphere, 72(10),* 1602-1606.

Malisch, R., & Kotz, A. (2014). Dioxins and PCBs in feed and food—review from European perspective. *Science of the Total Environment, 491,* 2-10.

Metian, M., Charbonnier, L., Oberhänsli, F., Bustamante, P., Jeffree, R., Amiard, J. C., & Warnau, M. (2009). Assessment of metal, metalloid, and radionuclide bioaccessibility from mussels to human consumers, using centrifugation and simulated digestion methods coupled with radiotracer techniques. *Ecotoxicology and Environmental Safety, 72(5),* 1499-1502.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., ... & Dufour, C. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. *Food & function, 5(6)*, 1113-1124.

Oomen, A. G., Sips, A. J., Groten, J. P., Sijm, D. T., & Tolls, J. (2000). Mobilization of PCBs and lindane from soil during in vitro digestion and their distribution among bile salt micelles and proteins of human digestive fluid and the soil. *Environmental science & technology, 34(2)*, 297-303.

Periago, M. J., Bravo, S., García-Alonso, F. J., & Rincón, F. (2013). Detection of key factors affecting lycopene in vitro accessibility. *Journal of agricultural and food chemistry, 61(16)*, 3859-3867.

Planche, C., Ratel, J., Mercier, F., Blinet, P., Debrauwer, L., & Engel, E. (2015). Assessment of comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry based methods for investigating 206 dioxin-like micropollutants in animal-derived food matrices. *Journal of Chromatography A, 1392*, 74-81.

Santé-Lhoutellier, V., Astruc, T., Marinova, P., Greve, E., & Gatellier, P. (2008). Effect of meat cooking on physicochemical state and in vitro digestibility of myofibrillar proteins. *Journal of Agricultural and Food Chemistry, 56(4)*, 1488-1494.

Shen, H., Starr, J., Han, J., Zhang, L., Lu, D., Guan, R.., & Zhang, Y. (2016). The bioaccessibility of polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs) in cooked plant and animal origin foods. *Environment international, 94*, 33-42.

Sirot, V., Tard, A., Venisseau, A., Brosseaud, A., Marchand, P., Le Bizec, B., & Leblanc, J. C. (2012). Dietary exposure to polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls of the French population: results of the second French Total Diet Study. *Chemosphere*, *88*(4), 492-500.

Takaki, K., Wade, A. J., & Collins, C. D. (2015). Modelling the bioaccumulation of persistent organic pollutants in agricultural food chains for regulatory exposure assessment. *Environmental Science and Pollution Research*, 1-9.

Tang, H. P. O. (2013). Recent development in analysis of persistent organic pollutants under the Stockholm Convention. *TrAC Trends in Analytical Chemistry, 45*, 48-66.

Wang, H. S., Zhao, Y. G., Man, Y. B., Wong, C. K., & Wong, M. H. (2011). Oral bioaccessibility and human risk assessment of organochlorine pesticides (OCPs) via fish consumption, using an invitro gastrointestinal model. *Food Chemistry, 127(4)*, 1673-1679.

Wang, W., Huang, M. J., Zheng, J. S., Cheung, K. C., & Wong, M. H. (2013). Exposure assessment and distribution of polychlorinated biphenyls (PCBs) contained in indoor and outdoor dusts and the impacts of particle size and bioaccessibility. *Science of the Total Environment, 463*, 1201-1209.

Xia, Z., McClements, D. J., & Xiao, H. (2017). Influence of Lipid Content in a Corn Oil Preparation on the Bioaccessibility of β-Carotene: A Comparison of Low-Fat and High-Fat Samples. *Journal of Food Science, 82(2)*, 373-379.

Xing, G. H., Yang, Y., Chan, J. K. Y., Tao, S., & Wong, M. H. (2008). Bioaccessibility of polychlorinated biphenyls in different foods using an in vitro digestion method. *Environmental pollution, 156(3)*, 1218-1226.

Yu, Y. X., Li, J. L., Zhang, X. Y., Yu, Z. Q., Van de Wiele, T., Han, S. Y., ... & Fu, J. M. (2009). Assessment of the bioaccessibility of polybrominated diphenyl ethers in foods and the correlations of the bioaccessibility with nutrient contents. *Journal of agricultural and food chemistry, 58(1)*, 301-308.

Yu, Y. X., Huang, N. B., Zhang, X. Y., Li, J. L., Yu, Z. Q., Han, S. Y., & Fu, J. M. (2011a). Polybrominated diphenyl ethers in food and associated human daily intake assessment considering bioaccessibility measured by simulated gastrointestinal digestion. *Chemosphere, 83(2)*, 152-160.

Yu, Y., Pang, Y., Zhang, X., Li, C., Yu, Z., & Fu, J. (2011b). Optimization of an in vitro method to measure the bioaccessibility of polybrominated diphenyl ethers in dust using response surface methodology. *Journal of Environmental Sciences, 23(10)*, 1738-1746.

Zapadlo, M., Krupčík, J., Kovalczuk, T., Májek, P., Špánik, I., Armstrong, D. W., & Sandra, P. (2011). Enhanced comprehensive two-dimensional gas chromatographic resolution of polychlorinated biphenyls on a non-polar polysiloxane and an ionic liquid column series. *Journal of Chromatography A, 1218(5)*, 746-751.

Zhang, R., Zhang, Z., Kumosani, T., Khoja, S., Abualnaja, K. O., & McClements, D. J. (2016). Encapsulation of β-carotene in Nanoemulsion-Based Delivery Systems Formed by Spontaneous Emulsification: Influence of Lipid Composition on Stability and Bioaccessibility. *Food Biophysics, 11(2)*, 154-164.

Zou, L., Zheng, B., Zhang, R., Zhang, Z., Liu, W., Liu, C., ... & McClements, D. J. (2016). Influence of Lipid Phase Composition of Excipient Emulsions on Curcumin Solubility, Stability, and Bioaccessibility. *Food Biophysics, 11(3)*, 213-225.

Supplementary material

Table S1. Elution order of PCBs using the Rtx-Dioxin2/BPX-50 column set. $^{1}t_R$ (s) and $^{2}t_R$ (s) are the retention time in seconds on respectively the first and the second dimension.

Compound	Congener	WHO-TEF	$^{1}t_R$ (s)	$^{2}t_R$ (s)
2,4,4'-Trichlorobiphenyl	28		1580	3,17
2,2',5,5'-Tetrachlorobiphenyl	52		1610	3,35
2,2',4,5,5'-Pentachlorobiphenyl	101		2085	3,562
3,4,4',5-Tetrachlorobiphenyl	81	0,0003	2265	3,69
3,3',4,4'-Tetrachlorobiphenyl	77	0,0001	2320	3,76
2',3,4,4',5-Pentachlorobiphenyl	123	0,00003	2385	3,72
2,3',4,4',5-Pentachlorobiphenyl	118	0,00003	2415	3,704
2,3,4,4',5-Pentachlorobiphenyl	114	0,00003	2465	3,865
2,2',4,4',5,5'-Hexachlorobiphenyl	153		2490	3,7
2,3,3',4,4'-Pentachlorobiphenyl	105	0,00003	2545	4,01
2,2',3,4,4',5'-Hexachlorobiphenyl	138		2630	3,931
3,3',4,4',5-Pentachlorobiphenyl	126	0,1	2730	3,88
2,3',4,4',5,5'-Hexachlorobiphenyl	167	0,00003	2795	3,832
2,3,3',4,4',5-Hexachlorobiphenyl	156	0,00003	2915	4,02
2,3,3',4,4',5'-Hexachlorobiphenyl	157	0,00003	2930	4,12
2,2',3,4,4',5,5'-Heptachlorobiphenyl	180		2960	3,97
3,3',4,4',5,5'-Hexachlorobiphenyl	169	0,03	3125	3,939
2,3,3',4,4',5,5'-Heptachlorobiphenyl	189	0,00003	3280	4,073

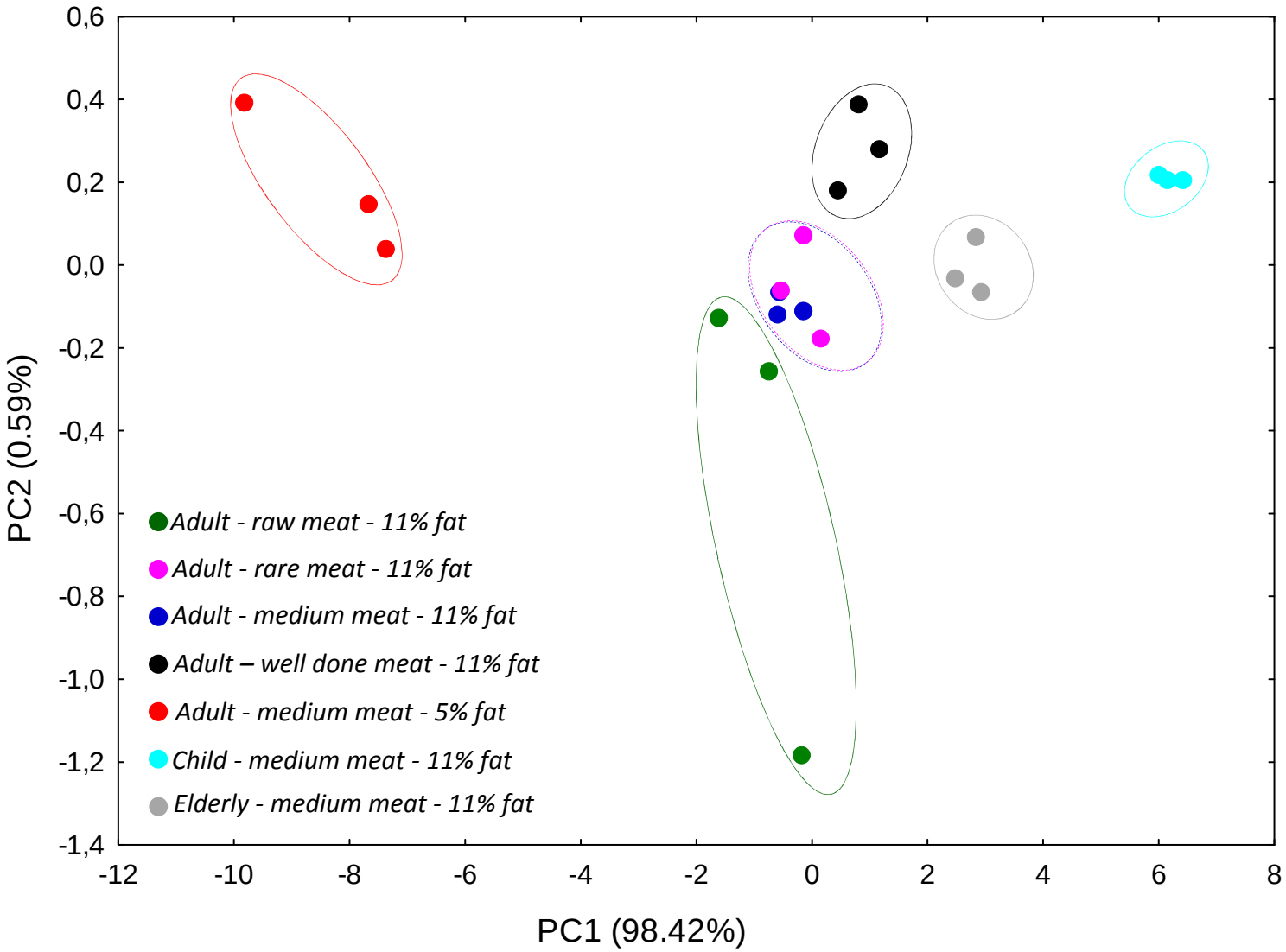

Fig S1. Discrimination of bioaccessibility (%) of PCBs in meat according to intensity of meat cooking before digestion, level of meat fat and age of consumer

Complément de discussion sur l'étude de l'impact de la digestion sur la bioaccessibilité des contaminants chimiques de la viande :

Cette troisième partie montre l'importance de prendre en compte la bioaccessibilité des contaminants chimiques lors des analyses de risque. Chez l'adulte et pour une viande à 11% de matière grasse cuite à 70°C à coeur, seul un quart des PCB contenus dans cette matrice peut être libéré dans le tractus digestif et potentiellement passer la barrière intestinale pour exercer des effets toxiques. Ces données permettent donc de relativiser significativement le risque lié à la présence des PCB dans les aliments même s'il n'est pas exclu que les PCB non bioaccessibles puissent perturber les étapes ultérieures de la digestion et éventuellement, exercer leurs effets toxiques.

Les résultats ont également indiqué que la teneur initiale de la viande en matière grasse et les variations physiologiques liées à l'âge du consommateur influencent significativement la bioaccessibilité. En effet, la bioaccessibilité augmente lorsque la teneur en matière grasse de la viande diminue. Il semblerait cependant exister un seuil minimal de matière grasse en dessous duquel la bioaccessibilité reste constante. Par ailleurs, la bioaccessibilité est significativement plus faible chez les enfants et les personnes âgées que chez les adultes. Ces deux critères (teneur en matière grasse de la viande et âge du consommateur) devront donc être pris en compte dans les analyses de risque. En revanche, la cuisson ne semble pas représenter un facteur de variation significatif de la bioaccessibilité des PCB. Il sera ultérieurement nécessaire de comparer les résultats obtenus pour les PCB avec d'autres familles de contaminants aux propriétés physico-chimiques différentes.

Les expériences de digestion *in vitro* de ce travail ont été réalisées à partir d'un modèle statique. Ce modèle, choisi par Minekus *et al.* (2014) afin de proposer un protocole standardisé, permet notamment de limiter les risques de contamination du digesteur par certains contaminants. Il serait cependant intéressant de pouvoir disposer de données obtenues avec des systèmes de digestion dynamiques, plus représentatifs de la digestion chez l'Homme. En outre, l'obtention de données *in vivo* sur des modèles animaux permettrait d'évaluer la pertinence des résultats obtenus *in vitro* et potentiellement de les valider.

Conclusion générale

L'ensemble des résultats obtenus au cours de ces travaux de recherche démontrent l'intérêt de prendre en compte les procédés technologiques (notamment la cuisson) mais également les processus physiologiques (intervenant lors de la digestion) lors de la réalisation des analyses en vue d'une évaluation du risque relatif aux contaminants chimiques de la viande.

Ce travail de thèse a notamment démontré que l'impact de la cuisson est très variable selon les familles de contaminants chimiques et, au sein d'une même famille, selon les différentes substances étudiées. Alors que des pertes de plus de 50% ont été décrites pour certains contaminants (sulfamethoxazole, dieldrin, sulfotep, phorate), aucune perte significative n'a été observée pour d'autres substances (tetracycline, doxycycline, aldrin, lindane, DDE, DDD). Ce travail met également en évidence des différences significatives même dans le cas de contaminants aux propriétés physico-chimiques proches, comme les PCB et les PCDD/F : avec une cuisson standard (70°C à cœur), des pertes moyennes de 30% ont été observées pour les PCB alors qu'aucune perte significative en PCDD/F n'a pu être détectée. Au-delà des variations liées au contaminant, il est intéressant de noter que les pertes observées lors de la cuisson varient également selon l'intensité du traitement thermique. Ainsi, pour les PCB, les pertes moyennes sont de 18% avec une cuisson douce (50°C à cœur) et de 48% avec une cuisson intense (85°C à cœur). De la même façon, pour le DDT, les pertes moyennes sont de 42% avec une cuisson douce et 68% avec une cuisson intense. Cette observation est à mettre en perspective par rapport aux recommandations de l'OMS pour une cuisson de la viande hachée à 70°C à cœur. En effet, les habitudes de cuisson étant très variables selon les consommateurs, nos résultats pourraient conclure à émettre l'hypothèse que des conditions de cuisson très intenses (type cuisson au grill ou au barbecue) induisent des pertes en contaminants très significatives. Cependant, il est important de rappeler qu'en même temps qu'elles diminuent les teneurs en micropolluants, ces conditions de cuisson intenses favorisent la formation de composés néoformés toxiques comme les hydrocarbures aromatiques polycliques (HAP) (Giri *et al.*, 2015) ou les amines aromatiques hétérocycliques (AAH) (Meurillon & Engel, 2016). Enfin, il faut également prendre en compte la diversité des pratiques de dégustation et notamment la possibilité de consommer également le jus de cuisson.

En étudiant en parallèle le devenir lors de la cuisson de composés thermorésistants et de composés thermosensibles, nous avons pu confirmer que différents phénomènes

concourent à expliquer les pertes en contaminants observées lors de la cuisson. Si nos résultats indiquent qu'une expulsion dans le jus de cuisson est l'explication la plus plausible pour les substances thermorésistantes, le phénomène de dégradation thermique est également en partie responsable de la perte en contaminants thermosensibles, et dans ce cas l'identification des produits de dégradation devient indispensable pour une complète évaluation du risque. L'étude de la thermodégradation du sulfaméthoxazole pris comme modèle dans notre travail a montré que l'utilisation du radiomarquage pouvait alors permettre d'accéder rapidement à l'évaluation des pertes, ainsi qu'à la quantification absolue des produits de dégradation sans avoir recours à des méthodes nécessitant l'accès à des composés standards de référence et au développement de méthodes de quantification dédiées. Bien que très pertinentes, ces études de thermodégradation ne peuvent cependant être menées qu'au cas par cas et restent des méthodes coûteuses et lourdes à mettre en œuvre en raison notamment de l'utilisation de composés radiomarqués. Par ailleurs, quelque soit l'approche utilisée, des études toxicologiques ultérieures sont nécessaires afin de pouvoir déterminer l'incidence de cette thermodégradation en comparant le risque chimique résultant de l'ensemble des produits de la réaction à celui qui est lié à la présence de la molécule mère.

Les résultats relatifs à l'impact de la digestion sur la bioaccessibilité des contaminants chimiques de la viande montrent qu'en moyenne, chez l'adulte, seul un quart des PCB contenus dans une viande à 11% de matière grasse et cuite suivant les recommandations de l'OMS était bioaccessible. Tandis que le barême temps/température de la cuisson ne semble avoir qu'une influence modeste sur la bioaccessibilité des PCB (26% *vs* 23% respectivement pour les viande crue et cuite) et uniquement en cas de cuisson intense, la teneur en matière grasse des aliments étudiés est un facteur clé (26% *vs* 48% lorsque la teneur en matière grasse de la viande passe de 11% à 5%). Dans un contexte d'évaluation du risque, ces données sont d'autant plus intéressantes que les PCB sont des contaminants lipophiles qui ont tendance à s'accumuler dans la fraction lipidique. Les aliments riches en lipides ont donc tendance à avoir une teneur en PCB plus élevée que les aliments pauvres en matière grasse. Les résultats de nos études de bioaccessibilité viennent contrebalancer cette caractéristique. En effet, bien que les aliments riches en lipides ont tendance à contenir d'avantage de PCB, ces derniers se révèlent moins bioaccessibles selon nos conclusions. Nos résultats apportent donc un nouvel éclairage sur les différents facteurs à prendre en compte pour une évaluation du risque la plus complète possible, intégrant non seulement la nature et la teneur en contaminants des denrées,

mais également leur devenir lors de processus de transformation avant (cuisson) et après (bioaccessibilité, métabolisme) consommation.

Au-delà de ces considérations, les résultats de notre travail montrent que les variations liées à l'âge des consommateurs doivent également être prises en condidération lors des études de bioaccessibilité. Les résultats ont en effet montré que la bioaccessibilité des PCB est plus faible chez les personnes âgées (17%), et plus encore chez les enfants (8%), que chez les adultes (28%).

Ces données montrent que l'étape de digestion ne doit pas être négligée dans l'évaluation des risques, notamment chez les sujets très jeunes, puisque nos observations indiquent que la digestion semble constituer un facteur pouvant moduler l'exposition interne aux contaminants de la viande. Ces informations pourraient également être collectées pour ajuster les recommandations alimentaires en fonction de la composition des matières premières, du type de cuisson employée et des populations de consommateurs (enfants, adultes ou personnes âgées).

Dans le but de travailler dans des conditions permettant une comparaison et une mise en perspective objective des résultats, les expérimentations décrites dans ce travail reposent notamment sur la préparation de viandes hachées intentionnellement surchargées. Le protocole proposé a permis de disposer tout au long de ce travail d'un lot d'échantillons de viandes contaminées homogène. Cette pratique a ainsi permis de limiter la variabilité liée à la composition de la matrice, et notamment à sa teneur en lipides qui est un facteur de variation des pertes en contaminants liposolubles au cours de la cuisson mais également un facteur de variation de la bioaccessibilité. L'utilisation d'une matrice identique tout au long de cette étude a permis d'obtenir des résultats reproductibles, comparables entre eux et ainsi de pouvoir tirer des conclusions générales. Néanmoins, afin de valider la portée des résultats obtenus sur une viande surchargée, ceux-ci ont été systématiquement confrontés à des mesures réalisées sur des échantillons naturellement contaminés. Cette évaluation systématique a nécessité l'utilisation de systèmes analytiques ultrasensibles permise par la collaboration avec les Laboratoires Nationaux de Référence impliqués dans le projet (ANSES Maisons-Alfort, ANSES Fougère, ONIRIS-LABERCA). En effet, contrairement aux échantillons intentionnellement contaminés pour lesquels la concentration en contaminants peut être contrôlée et volontairement portée à des niveaux permettant une détection aisée, les résidus de contaminants des échantillons naturellement contaminés peuvent être présents à des

niveaux de concentrations très faibles, accessibles uniquement aux équipements analytiques les plus performants.

De façon générale, les résultats obtenus durant ce travail de thèse permettent de reconsidérer le risque lié aux contaminants chimiques des matrices carnées par rapport aux données disponibles jusqu'à présent. Dans cette optique, une collaboration menée dans le cadre du WP4 du projet SOMEAT avec l'unité MetaRisk de l'INRA devrait permettre de proposer un nouveau modèle d'évaluation des risques liés aux contaminants chimiques de la viande qui prendra en compte les modulations induites par la cuisson et la digestion. Par ailleurs, l'importance des effets modulateurs mis en évidence pourrait justifier d'élargir les mesures de bioaccessibilité à d'autres familles de contaminants (contaminants hydrophiles dont on peut attendre des comportements différents de celui des PCB lors de la digestion, contaminants instables en milieu acide lors de la digestion…) mais également d'élargir cette étude à d'autres matrices alimentaires qui constituent des produits majeurs de notre alimentation. Ce type d'étude est encouragé par les agences de sécurité sanitaire qui souhaitent évaluer au mieux le risque chimique lié à notre alimentation. Enfin, à plus long terme, un troisième phénomène modulateur en plus de la cuisson et de la digestion devra être pris en compte en évaluation des risques. Il s'agit de l'effet « cocktail » qui fait référence à l'impact sanitaire hyper- ou éventuellement hypo-additif d'un mélange de plusieurs contaminants toxiques. Dans le cadre du WP3 de SOMEAT, l'équipe de R Rahmani (TOXALIM) a développé de nouvelles stratégies analytiques pour étudier qualitativement ces phénomènes grâce à des approches de type cellomique. Ces recherches devront être poursuivies dans les années à venir pour rendre possible la quantification systématique de ces effets cocktail et leur prise en compte en évaluation des risques.

Références bibliographiques

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Aaslyng, M. D., Bejerholm, C., Ertbjerg, P., Bertram, H. C., & Andersen, H. J. (2003). Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food quality and preference, 14(4)*, 277-288.

Abou-Arab, A. A. K. (1997). Effect of Ras cheese manufacturing on the stability of DDT and its metabolites. *Food chemistry, 59(1)*, 115-119.

Abou-Arab, A. A. K. (1999). Behavior of pesticides in tomatoes during commercial and home preparation. *Food chemistry, 65(4)*, 509-514.

Abou-Raya, S. H., Shalaby, A. R., Salama, N. A., Emam, W. H., & Mehaya, F. M. (2013). Effect of Ordinary Cooking Procedures on Tetracycline Residues in Chicken Meat. *Journal of Food & Drug Analysis, 21(1)*.

AFNOR-NF EN 13805 (2014). Foodstuffs - Determination of trace elements - Pressure digestion, Saint Denis, France.

Amiard, J. C., Amiard-Triquet, C., Charbonnier, L., Mesnil, A., Rainbow, P. S., & Wang, W. X. (2008). Bioaccessibility of essential and non-essential metals in commercial shellfish from Western Europe and Asia. *Food and Chemical Toxicology, 46(6)*, 2010-2022.

ANSES. Etude de l'alimentation totale française 2 (EAT2) Tome 1 Contaminants inorganiques, minéraux, polluants organiques persistants, mycotoxines, phyto-estrogènes, Juin 2011, Edition scientifique.

ANSES. Etude de l'alimentation totale française 2 (EAT2) Tome 2 Résidus de pesticides, additifs, acrylamide, hydrocarbures aromatiques polycycliques, Juin 2011, Edition scientifique.

Barontini, F., Cozzani, V., & Petarca, L. (2001). Thermal stability and decomposition products of hexabromocyclododecane. *Industrial & engineering chemistry research, 40(15)*, 3270-3280.

Bayarri, S., Conchello, P., Arino, A., Lazaro, R., & Herrera, A. (1994). DDT, DDT metabolites, and other organochlorines as affected by thermal processing in three commercial cuts of lamb. *Bulletin of environmental contamination and toxicology, 52(4)*, 554-559.

Bayen, S., Barlow, P., Lee, H. K., & Obbard, J. P. (2005). Effect of cooking on the loss of persistent organic pollutants from salmon. *Journal of Toxicology and Environmental Health, Part A, 68(4)*, 253-265.

Bendig, P., Blumenstein, M., Hägele, F., & Vetter, W. (2012). Hydrodebromination of decabromodiphenyl ether (BDE-209) in cooking experiments with salmon fillet. *Journal of agricultural and food chemistry, 60(34)*, 8521-8527.

Berge, P., Ratel, J., Fournier, A., Jondreville, C., Feidt, C., Roudaut, B., Le Bizec, B., & Engel, E. (2011). Use of volatile compound metabolic signatures in poultry liver to back-trace dietary exposure to rapidly metabolized xenobiotics. *Environmental science & technology, 45(15)*, 6584-6591.

Blanchet-Letrouvé, I., Zalouk-Vergnoux, A., Vénisseau, A., Couderc, M., Le Bizec, B., Elie, P., Herrenknecht, C., Mouneyrac, C., & Poirier, L. (2014). Dioxin-like, non-dioxin like PCB and PCDD/F contamination in European eel (Anguilla anguilla) from the Loire estuarine continuum: spatial and biological variabilities. *Science of the Total Environment, 472*, 562-571.

Björklund, E., Nilsson, T., & Bøwadt, S. (2000). Pressurised liquid extraction of persistent organic pollutants in environmental analysis. *TrAC Trends in Analytical Chemistry, 19(7)*, 434-445.

Björklund, E., Müller, A., & von Holst, C. (2001). Comparison of fat retainers in accelerated solvent extraction for the selective extraction of PCBs from fat-containing samples. *Analytical chemistry, 73(16)*, 4050-4053.

Bordajandi, L. R., Gómez, G., Abad, E., Rivera, J., Fernández-Bastón, M. D. M., Blasco, J., & González, M. J. (2004). Survey of persistent organochlorine contaminants (PCBs, PCDD/Fs, and PAHs), heavy metals (Cu, Cd, Zn, Pb, and Hg), and arsenic in food samples from Huelva (Spain): levels and health implications. *Journal of agricultural and food chemistry, 52(4)*, 992-1001.

Brinch, U. C., Ekelund, F., & Jacobsen, C. S. (2002). Method for spiking soil samples with organic compounds. *Applied and Environmental Microbiology, 68(4)*, 1808-1816.

Carlson, D. L., & Hites, R. A. (2005). Polychlorinated biphenyls in salmon and salmon feed: global differences and bioaccumulation. *Environmental science & technology, 39(19)*, 7389-7395.

Chevallier, E., Chekri, R., Zinck, J., Guérin, T., & Noël, L. (2015). Simultaneous determination of 31 elements in foodstuffs by ICP-MS after closed-vessel microwave digestion: Method validation based on the accuracy profile. *Journal of Food Composition and Analysis, 41*, 35-41.

Collins, C. D., Craggs, M., Garcia-Alcega, S., Kademoglou, K., & Lowe, S. (2015). Towards a unified approach for the determination of the bioaccessibility of organic pollutants. *Environment international, 78*, 24-31.

Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs.

Commission Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

Conchello, M. P., Herrera, A., Arino, A., Lazaro, R., & Bayarri, S. (1993). Effect of several kitchen treatments on hexachlorocyclohexane residues in ovine meat. *Bulletin of environmental contamination and toxicology, 51(4)*, 612-618.

Coulibaly, K., & Smith, J. S. (1993). Thermostability of organophosphate pesticides and some of their major metabolites in water and beef muscle. *Journal of Agricultural and Food Chemistry, 41(10)*, 1719-1723.

Coulibaly, K., & Smith, J. S. (1994). Effect of pH and cooking temperature on the stability of organophosphate pesticides in beef muscle. *Journal of Agricultural and Food Chemistry, 42(9)*, 2035-2039.

Crisp, T. M., Clegg, E. D., Cooper, R. L., Wood, W. P., Anderson, D. G., Baetcke, K. P., ... & Touart, L. W. (1998). Environmental endocrine disruption: an effects assessment and analysis. *Environmental health perspectives, 106(1)*, 11.

Dadorama, S. (1996). Amino acids, peptides, and proteins. In O. R. Fennema (Ed.), *Food chemistry* (3rd ed., pp. 321–429). New York, NY, USA: Marcel Dekker Inc.

Daghrir, R., & Drogui, P. (2013). Tetracycline antibiotics in the environment: a review. *Environmental chemistry letters, 11(3)*, 209-227.

Darko, G., & Acquaah, S. O. (2007). Levels of organochlorine pesticides residues in meat. *International Journal of Environmental Science & Technology, 4(4)*, 521-524.

Dasgupta, S., Banerjee, K., Patil, S. H., Ghaste, M., Dhumal, K. N., & Adsule, P. G. (2010). Optimization of two-dimensional gas chromatography time-of-flight mass spectrometry for separation and estimation of the residues of 160 pesticides and 25 persistent organic pollutants in grape and wine. *Journal of Chromatography A, 1217(24)*, 3881-3889.

Dean, J. R., & Ma, R. (2007). Approaches to assess the oral bioaccessibility of persistent organic pollutants: a critical review. *Chemosphere, 68(8)*, 1399-1407.

Devesa, V., Macho, M. L., Jalón, M., Urieta, I., Muñoz, O., Súñer, M. A., López, F., Dinoraz Vélez, D., & Montoro, R. (2001). Arsenic in cooked seafood products: study on the effect of cooking on total and inorganic arsenic contents. *Journal of Agricultural and Food Chemistry, 49(8)*, 4132-4140.

Domingo, J. L. (2010). Influence of cooking processes on the concentrations of toxic metals and various organic environmental pollutants in food: a review of the published literature. *Critical reviews in food science and nutrition, 51(1),* 29-37.

Dupont, D., Mandalari, G., Molle, D., Jardin, J., Léonil, J., Faulks, R. M., ... & Mackie, A. R. (2010). Comparative resistance of food proteins to adult and infant in vitro digestion models. *Molecular nutrition & food research, 54(6),* 767-780.

Engel, E., Ratel, J., Blinet, P., Chin, S. T., Rose, G., & Marriott, P. J. (2013). Benchmarking of candidate detectors for multiresidue analysis of pesticides by comprehensive two-dimensional gas chromatography. *Journal of Chromatography A, 1311,* 140-148.

Engel, E., Ratel, J., Bouhlel, J., Planche, C., & Meurillon, M. (2015). Novel approaches to improving the chemical safety of the meat chain towards toxicants. *Meat science, 109,* 75-85.

Environmental Protection Agency (EPA) (2003). EPA Method 8000C, Determinative Chromatographic Separations, Revision 3, Washington, DC, USA.

Ersoy, B., Yanar, Y., Küçükgülmez, A., & Çelik, M. (2006). Effects of four cooking methods on the heavy metal concentrations of sea bass fillets (Dicentrarchus labrax Linne, 1785). *Food Chemistry, 99(4),* 748-751.

Fedeniuk, R. W., Shand, P. J., & McCurdy, A. R. (1997). Effect of thermal processing and additives on the kinetics of oxytetracycline degradation in pork muscle. *Journal of Agricultural and Food Chemistry, 45(6),* 2252-2257.

Fischer, L. J., Thulin, A. J., Zabik, M. E., Booren, A. M., Poppenga, R. H., & Chapman, K. J. (1992). Sulfamethazine and its metabolites in pork: Effects of cooking and gastrointestinal absorption of residues. *Journal of agricultural and food chemistry, 40(9),* 1677-1682.

Fisher, B. E. (1999). Most unwanted. *Environmental health perspectives, 107(1),* A18.

Focant, J. F., Sjödin, A., & Patterson, D. G. (2003). Qualitative evaluation of thermal desorption-programmable temperature vaporization-comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the analysis of selected halogenated contaminants. *Journal of Chromatography A, 1019(1),* 143-156.

Focant, J. F., Reiner, E. J., MacPherson, K., Kolic, T., Sjödin, A., Patterson, D. G., ... & Cochran, J. (2004a). Measurement of PCDDs, PCDFs, and non-ortho-PCBs by comprehensive two-dimensional gas chromatography-isotope dilution time-of-flight mass spectrometry (GC× GC-IDTOFMS). *Talanta, 63(5),* 1231-1240.

Focant, J. F., Sjödin, A., & Patterson, D. G. (2004b). Improved separation of the 209 polychlorinated biphenyl congeners using comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry. *Journal of Chromatography A, 1040(2),* 227-238.

Focant, J. F., Sjödin, A., Turner, W. E., & Patterson, D. G. (2004c). Measurement of selected polybrominated diphenyl ethers, polybrominated and polychlorinated biphenyls, and organochlorine pesticides in human serum and milk using comprehensive two-dimensional gas chromatography isotope dilution time-of-flight mass spectrometry. *Analytical chemistry, 76(21),* 6313-6320.

Focant, J. F., Eppe, G., Scippo, M. L., Massart, A. C., Pirard, C., Maghuin-Rogister, G., & De Pauw, E. (2005a). Comprehensive two-dimensional gas chromatography with isotope dilution time-of-flight mass spectrometry for the measurement of dioxins and polychlorinated biphenyls in foodstuffs: Comparison with other methods. *Journal of Chromatography A, 1086(1),* 45-60.

Focant, J. F., Pirard, C., Eppe, G., & De Pauw, E. (2005b). Recent advances in mass spectrometric measurement of dioxins. *Journal of Chromatography A, 1067(1),* 265-275.

Furusawa, N., & Hanabusa, R. (2002). Cooking effects on sulfonamide residues in chicken thigh muscle. *Food research international, 35(1),* 37-42.

García-Bermejo, A., Ábalos, M., Sauló, J., Abad Holgado, E., González, M. J., & Gómara, B. (2011). Feasibility of triple-quadrupole GC–MS/MS as an alternative technique to GC–HRMS for the analysis of PCDD/Fs and DL-PCBs in food and feedstuff samples, *Organohalogen Compounds, 73*, 653–656.

Gatellier, P., & Santé-Lhoutellier, V. (2009). Digestion study of proteins from cooked meat using an enzymatic microreactor. *Meat science, 81(2),* 405-409.

Giri, A., Khummueng, W., Mercier, F., Kondjoyan, N., Tournayre, P., Meurillon, M., ... & Engel, E. (2015). Relevance of two-dimensional gas chromatography and high resolution olfactometry for the parallel determination of heat-induced toxicants and odorants in cooked food. *Journal of Chromatography A, 1388*, 217-226.

Gratacós-Cubarsí, M., Fernandez-García, A., Picouet, P., Valero-Pamplona, A., García-Regueiro, J. A., & Castellari, M. (2007). Formation of tetracycline degradation products in chicken and pig meat under different thermal processing conditions. *Journal of agricultural and food chemistry, 55(11),* 4610-4616.

Guerra, A., Etienne-Mesmin, L., Livrelli, V., Denis, S., Blanquet-Diot, S., & Alric, M. (2012). Relevance and challenges in modeling human gastric and small intestinal digestion. *Trends in biotechnology, 30(11)*, 591-600.

Hassani, M., Lazaro, R., Perez, C., Condon, S., & Pagan, R. (2008). Thermostability of oxytetracycline, tetracycline, and doxycycline at ultrahigh temperatures. *Journal of agricultural and food chemistry, 56(8)*, 2676-2680.

Hawker, D. W., & Connell, D. W. (1988). Octanol-water partition coefficients of polychlorinated biphenyl congeners. *Environmental science & technology, 22(4)*, 382-387.

He, M., Ke, C. H., & Wang, W. X. (2010). Effects of cooking and subcellular distribution on the bioaccessibility of trace elements in two marine fish species. *Journal of agricultural and food chemistry, 58(6)*, 3517-3523.

Herrero, M., Ibáñez, E., Cifuentes, A., & Bernal, J. (2009). Multidimensional chromatography in food analysis. *Journal of chromatography A, 1216(43)*, 7110-7129.

Hoff, R. B., Pizzolato, T. M., Peralba, M. D. C. R., Díaz-Cruz, M. S., & Barceló, D. (2015). Determination of sulfonamide antibiotics and metabolites in liver, muscle and kidney samples by pressurized liquid extraction or ultrasound-assisted extraction followed by liquid chromatography–quadrupole linear ion trap-tandem mass spectrometry (HPLC–QqLIT-MS/MS). *Talanta, 134*, 768-778.

Hoh, E., Mastovska, K., & Lehotay, S. J. (2007). Optimization of separation and detection conditions for comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans. *Journal of Chromatography A, 1145(1)*, 210-221.

Hori, T., Nakagawa, R., Tobiishi, K., Iida, T., Tsutsumi, T., Sasaki, K., & Toyoda, M. (2005). Effects of cooking on concentrations of polychlorinated dibenzo-p-dioxins and related compounds in fish and meat. *Journal of agricultural and food chemistry, 53(22)*, 8820-8828.

Howsam, M., Verweij, R. A., & van Straalen, N. M. (2003). A solvent-free method for spiking terrestrial algae (Desmococcus spp.) with pyrene for use in bioassays. *Chemosphere, 53(3)*, 253-262.

Ibrahim, A., & Moats, W. A. (1994). Effect of cooking procedures on oxytetracycline residues in lamb muscle. *Journal of agricultural and food chemistry, 42(11)*, 2561-2563.

Ingelido, A. M., Brambilla, G., Abballe, A., Domenico, A., Fulgenzi, A. R., Iacovella, N., ... & Felip, E. D. (2012). PCDD, PCDF, and DL-PCB analysis in food: performance evaluation

of the high-resolution gas chromatography/low-resolution tandem mass spectrometry technique using consensus-based samples. *Rapid Communications in Mass Spectrometry, 26(3)*, 236-242.

Ishiwatari, N., Fukuoka, M., & Sakai, N. (2013). Effect of protein denaturation degree on texture and water state of cooked meat. *Journal of Food engineering, 117(3)*, 361-369.

Ismail-Fitry, M. R., Jinap, S., Jamilah, B., & Saleha, A. A. (2008). Effect of deep-frying at different temperature and time on sulfonamide residues in chicken meat-balls. *Journal of Food & Drug Analysis, 16(6)*.

Jadán-Piedra, C., Clemente, M. J., Devesa, V., & Vélez, D. (2016). Influence of Physiological Gastrointestinal Parameters on the Bioaccessibility of Mercury and Selenium from Swordfish. *Journal of agricultural and food chemistry, 64(3)*, 690-698.

Jan, J., & Malnersic, S. (1982). Effects of some food additives and fat content in meat on lindane transformation to nonpolar compounds during cooking. *Journal of Agricultural and Food Chemistry, 30(6)*, 1253-1256.

Jones, K. C., & De Voogt, P. (1999). Persistent organic pollutants (POPs): state of the science. *Environmental pollution, 100(1)*, 209-221.

Kang, Y., Yin, Y., Man, Y., Li, L., Zhang, Q., Zeng, L., & Wong, M. H. (2013). Bioaccessibility of polychlorinated biphenyls in workplace dust and its implication for risk assessment. *Chemosphere, 93(6)*, 924-930.

Kishida, K. (2007). Quantitation and confirmation of six sulphonamides in meat by liquid chromatography–mass spectrometry with photodiode array detection. *Food Control, 18(4)*, 301-305.

Kondjoyan, A., Oillic, S., Portanguen, S., & Gros, J. B. (2013). Combined heat transfer and kinetic models to predict cooking loss during heat treatment of beef meat. *Meat science, 95(2)*, 336-344.

Kotz, A., Malisch, R., Wahl, K., Bitomsky, N., Adamovic, K., Gerteisen, I., ... & Winterhalter, H. (2011). GC-MS/MS determination of PCDD/Fs and PCBs in feed and food–comparison with GC-HRMS. *Organohalogen Compouds, 73*, 688-691.

Kühne, M., Hamscher, G., Körner, U., Schedl, D., & Wenzel, S. (2001). Formation of anhydrotetracycline during a high-temperature treatment of animal-derived feed contaminated with tetracycline. *Food chemistry, 75(4)*, 423-429.

Kulp, K. S., Fortson, S. L., Knize, M. G., & Felton, J. S. (2003). An in vitro model system to predict the bioaccessibility of heterocyclic amines from a cooked meat matrix. *Food and Chemical Toxicology, 41(12),* 1701-1710.

Lan, C. C., HWANG, B. S., & Tu, M. F. (2001). Effect of microwave and roast treatment on the degradation of sulfamethazine residue in tilapia meat. *Journal of Food and Drug Analysis, 9(2).*

Levi, C. S., & Lesmes, U. (2014). Bi-compartmental elderly or adult dynamic digestion models applied to interrogate protein digestibility. *Food & function, 5(10),* 2402-2409.

Li, L., Xie, S., Cai, H., Bai, X., & Xue, Z. (2008). Quantitative structure–property relationships for octanol–water partition coefficients of polybrominated diphenyl ethers. *Chemosphere, 72(10),* 1602-1606.

Malisch, R., & Kotz, A. (2014). Dioxins and PCBs in feed and food—review from European perspective. *Science of the Total Environment, 491*, 2-10.

Marques, A., Lourenço, H. M., Nunes, M. L., Roseiro, C., Santos, C., Barranco, A., ... & Cencic, A. (2011). New tools to assess toxicity, bioaccessibility and uptake of chemical contaminants in meat and seafood. *Food Research International, 44(2),* 510-522.

Marriott, P., & Shellie, R. (2002). Principles and applications of comprehensive two-dimensional gas chromatography. *TrAC Trends in Analytical Chemistry, 21(9),* 573-583.

Maulvault, A. L., Machado, R., Afonso, C., Lourenço, H. M., Nunes, M. L., Coelho, I., ... & Marques, A. (2011). Bioaccessibility of Hg, Cd and As in cooked black scabbard fish and edible crab. *Food and Chemical Toxicology, 49(11),* 2808-2815.

Megson, D., Kalin, R., Worsfold, P. J., Gauchotte-Lindsay, C., Patterson, D. G., Lohan, M. C., ... & O'Sullivan, G. (2013). Fingerprinting polychlorinated biphenyls in environmental samples using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. *Journal of Chromatography A, 1318*, 276-283.

Metian, M., Charbonnier, L., Oberhänsli, F., Bustamante, P., Jeffree, R., Amiard, J. C., & Warnau, M. (2009). Assessment of metal, metalloid, and radionuclide bioaccessibility from mussels to human consumers, using centrifugation and simulated digestion methods coupled with radiotracer techniques. *Ecotoxicology and Environmental Safety, 72(5),* 1499-1502.

Meurillon, M., & Engel, E. (2016). Mitigation strategies to reduce the impact of heterocyclic aromatic amines in proteinaceous foods. *Trends in Food Science & Technology, 50*, 70-84.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., ... & Dufour, C. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. *Food & function, 5(6)*, 1113-1124.

Morgan, K. J., Zabik, M. E., & Funk, K. (1972). Lindane, dieldrin and DDT residues in raw and cooked chicken and chicken broth. *Poultry science, 51(2)*, 470-475.

Moya, J., Garrahan, K. G., Poston, T. M., & Durell, G. S. (1998). Effects of cooking on levels of PCBs in the fillets of winter flounder. *Bulletin of environmental contamination and toxicology, 60(6)*, 845-851.

Muresan, C., Covaci, A., Socaci, S., Suharoschi, R., Tofana, M., Muste, S., & Pop, A. (2015). Influence of Meat Processing on the Content of Organochlorine Pesticides. *Journal of Food Processing & Technology, 6(11)*, 1.

Nachman, K. E., Baron, P. A., Raber, G., Francesconi, K. A., Navas-Acien, A., & Love, D. C. (2013). Roxarsone, inorganic arsenic, and other arsenic species in chicken: a US-based market basket sample. *Environmental Health Perspectives, 121(7)*, 818.

Noël, L., Leblanc, J.C., Guérin, T. (2003). Determination of several elements in duplicate meals from catering establishment using closed vessels microwave digestion with inductively coupled plasma mass spectrometry detection: estimation of daily dietary intake. *Food Additives and Contaminants, 20*, 44–56.

Northcott, G. L., & Jones, K. C. (2000a). Spiking hydrophobic organic compounds into soil and sediment: a review and critique of adopted procedures. *Environmental toxicology and chemistry, 19(10)*, 2418-2430.

Northcott, G. L., & Jones, K. C. (2000b). Developing a standard spiking procedure for the introduction of hydrophobic organic compounds into field-wet soil. *Environmental toxicology and chemistry, 19(10)*, 2409-2417.

Oomen, A. G., Sips, A. J., Groten, J. P., Sijm, D. T., & Tolls, J. (2000). Mobilization of PCBs and lindane from soil during in vitro digestion and their distribution among bile salt micelles and proteins of human digestive fluid and the soil. *Environmental science & technology, 34(2)*, 297-303.

Oomen, A. G., Hack, A., Minekus, M., Zeijdner, E., Cornelis, C., Schoeters, G., ... & Sips, A. J. (2002). Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. *Environmental science & technology, 36(15)*, 3326-3334.

Oomen, A. G., Rompelberg, C. J. M., Van de Kamp, E., Pereboom, D. P. K. H., De Zwart, L. L., & Sips, A. J. A. M. (2004). Effect of bile type on the bioaccessibility of soil contaminants in an in vitro digestion model. *Archives of Environmental Contamination and Toxicology, 46(2)*, 183-188.

Oroszvári, B. K., Rocha, C. S., Sjöholm, I., & Tornberg, E. (2006). Permeability and mass transfer as a function of the cooking temperature during the frying of beefburgers. *Journal of food engineering, 74(1)*, 1-12.

Page, D., Van Stratum, E., Degrou, A., & Renard, C. M. G. C. (2012). Kinetics of temperature increase during tomato processing modulate the bioaccessibility of lycopene. *Food chemistry, 135(4)*, 2462-2469.

Perelló, G., Martí-Cid, R., Llobet, J. M., & Domingo, J. L. (2008). Effects of various cooking processes on the concentrations of arsenic, cadmium, mercury, and lead in foods. *Journal of Agricultural and Food Chemistry, 56(23)*, 11262-11269.

Perelló, G., Martí-Cid, R., Castell, V., Llobet, J. M., & Domingo, J. L. (2009). Concentrations of polybrominated diphenyl ethers, hexachlorobenzene and polycyclic aromatic hydrocarbons in various foodstuffs before and after cooking. *Food and chemical toxicology, 47(4)*, 709-715.

Perelló, G., Martí-Cid, R., Castell, V., Llobet, J. M., & Domingo, J. L. (2010). Influence of various cooking processes on the concentrations of PCDD/PCDFs, PCBs and PCDEs in foods. *Food Control, 21(2)*, 178-185.

Periago, M. J., Bravo, S., García-Alonso, F. J., & Rincón, F. (2013). Detection of key factors affecting lycopene in vitro accessibility. *Journal of agricultural and food chemistry, 61(16)*, 3859-3867.

Petroske, E., Zaylskie, R. G., & Feil, V. J. (1998). Reduction in polychlorinated dibenzodioxin and dibenzofuran residues in hamburger meat during cooking. *Journal of Agricultural and Food Chemistry, 46(8)*, 3280-3284.

Planche, C., Ratel, J., Mercier, F., Blinet, P., Debrauwer, L., & Engel, E. (2015). Assessment of comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry based methods for investigating 206 dioxin-like micropollutants in animal-derived food matrices. *Journal of Chromatography A, 1392*, 74-81.

Poquet, L., & Wooster, T. J. (2016). Infant digestion physiology & the relevance of in vitro biochemical models to test infant formula lipid digestion. Molecular *Nutrition & Food Research, 60*, 1876-1895.

Püssa, T. (2013). Toxicological issues associated with production and processing of meat. *Meat science, 95(4),* 844-853.

Quan, C., Shi, Y., Wang, C., Wang, C., & Yang, K. (2014). p, p'-DDE damages spermatogenesis via phospholipid hydroperoxide glutathione peroxidase depletion and mitochondria apoptosis pathway. *Environmental toxicology.*

Rawn, D. F., Breakell, K., Verigin, V., Tittlemier, S. A., Del Gobbo, L., Diamond, M., ... & Sit, D. (2013). Impacts of cooking technique on polychlorinated biphenyl and polychlorinated dioxins/furan concentrations in fish and fish products with intake estimates. *Journal of agricultural and food chemistry, 61(4),* 989-997.

Reid, B. J., Northcott, G. L., Jones, K. C., & Semple, K. T. (1998). Evaluation of spiking procedures for the introduction of poorly water soluble contaminants into soil. *Environmental science & technology, 32(20),* 3224-3227.

Reig, M., & Toldrá, F. (2008). Veterinary drug residues in meat: Concerns and rapid methods for detection. *Meat science, 78(1),* 60-67.

Rémond, D., Shahar, D. R., Gille, D., Pinto, P., Kachal, J., Peyron, M. A., ... & Tomás-Cobos, L. (2015). Understanding the gastrointestinal tract of the elderly to develop dietary solutions that prevent malnutrition. *Oncotarget, 6(16),* 13858.

Rose, M. D., Farrington, W. H., & Shearer, G. (1995). The effect of cooking on veterinary drug residues in food: 3. sulphamethazine (sulphadimidine). *Food Additives & Contaminants, 12(6),* 739-750.

Rose, M. D., Bygrave, J., Farrington, W. H., & Shearer, G. (1996). The effect of cooking on veterinary drug residues in food: 4. Oxytetracycline. *Food Additives & Contaminants, 13(3),* 275-286.

Rose, M., Thorpe, S., Kelly, M., Harrison, N., & Startin, J. (2001). Changes in concentration of five PCDD/F congeners after cooking beef from treated cattle. *Chemosphere, 43(4),* 861-868.

Saito, K., Sjödin, A., Sandau, C. D., Davis, M. D., Nakazawa, H., Matsuki, Y., & Patterson, D. G. (2004). Development of a accelerated solvent extraction and gel permeation chromatography analytical method for measuring persistent organohalogen compounds in adipose and organ tissue analysis. *Chemosphere, 57(5),* 373-381.

Sallam, K. I., & Morshedy, A. E. M. A. (2008). Organochlorine pesticide residues in camel, cattle and sheep carcasses slaughtered in Sharkia Province, Egypt. *Food chemistry, 108(1),* 154-164.

Sandy, C., Fürst, P., Bernsmann, T., & Baumeister, D. (2011). Determination of polychlorinated biphenyl congeners in foodstuffs and animal feed using a Triple Quadrupole GC-MS/MS Instrument. *Organohalogen Compounds, 73(1370)*, e1371.

Santé-Lhoutellier, V., Astruc, T., Marinova, P., Greve, E., & Gatellier, P. (2008). Effect of meat cooking on physicochemical state and in vitro digestibility of myofibrillar proteins. *Journal of Agricultural and Food Chemistry, 56(4)*, 1488-1494.

Schecter, A., Dellarco, M., Päpke, O., & Olson, J. (1998). A comparison of dioxins, dibenzofurans and coplanar PCBs in uncooked and broiled ground beef, catfish and bacon. *Chemosphere, 37(9)*, 1723-1730.

Shen, H., Starr, J., Han, J., Zhang, L., Lu, D., Guan, R., ... & Zhang, Y. (2016). The bioaccessibility of polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs) in cooked plant and animal origin foods. *Environment international, 94*, 33-42.

Sibanda, M. M., Focke, W. W., Labuschagne, F. J., Moyo, L., Nhlapo, N. S., Maity, A., ... & Brindley, G. W. (2011). Degradation of insecticides used for indoor spraying in malaria control and possible solutions. *Malaria journal, 10(1)*, 1.

Sirot, V., Tard, A., Venisseau, A., Brosseaud, A., Marchand, P., Le Bizec, B., & Leblanc, J. C. (2012). Dietary exposure to polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls of the French population: results of the second French Total Diet Study. *Chemosphere, 88(4)*, 492-500.

Skibniewska, K. A. (2003). Diet monitoring for assessment of human exposure to environmental pollutants. *Environment international, 28(8)*, 703-709.

Snedeker, S. M. (2001). Pesticides and breast cancer risk: a review of DDT, DDE, and dieldrin. *Environmental Health Perspectives, 109(1)*, 35.

Sporring, S., & Björklund, E. (2004). Selective pressurized liquid extraction of polychlorinated biphenyls from fat-containing food and feed samples: Influence of cell dimensions, solvent type, temperature and flush volume. *Journal of Chromatography A, 1040(2)*, 155-161.

Srogi, K. (2008). Levels and congener distributions of PCDDs, PCDFs and dioxin-like PCBs in environmental and human samples: a review. *Environmental Chemistry Letters, 6(1)*, 1-28.

Stachiw, N. C., Zabik, M. E., Booren, A. M., & Jabik, M. J. (1988). Tetrachlorodibenzo-p-dioxin residue reduction through cooking/processing of restructured carp fillets. *Journal of agricultural and food chemistry, 36(4),* 848-852.

Suedel, B. C., Rodgers, J. H., & Clifford, P. A. (1993). Bioavailability of fluoranthene in freshwater sediment toxicity tests. *Environmental Toxicology and Chemistry, 12(1),* 155-165.

Sukul, P., & Spiteller, M. (2006). Sulfonamides in the environment as veterinary drugs. *Reviews of environmental contamination and toxicology* (pp. 67-101). Springer New York.

Sun, H., Ge, X., Lv, Y., & Wang, A. (2012). Application of accelerated solvent extraction in the analysis of organic contaminants, bioactive and nutritional compounds in food and feed. *Journal of Chromatography A, 1237,* 1-23.

Takaki, K., Wade, A. J., & Collins, C. D. (2015). Modelling the bioaccumulation of persistent organic pollutants in agricultural food chains for regulatory exposure assessment. *Environmental Science and Pollution Research,* 1-9.

Tang, H. P. O. (2013). Recent development in analysis of persistent organic pollutants under the Stockholm Convention. *TrAC Trends in Analytical Chemistry, 45,* 48-66.

Tornberg, E. (2005). Effects of heat on meat proteins–Implications on structure and quality of meat products. *Meat science, 70(3),* 493-508.

Torres-Escribano, S., Denis, S., Blanquet-Diot, S., Calatayud, M., Barrios, L., Vélez, D., ... & Montoro, R. (2011). Comparison of a static and a dynamic in vitro model to estimate the bioaccessibility of As, Cd, Pb and Hg from food reference materials Fucus sp.(IAEA-140/TM) and Lobster hepatopancreas (TORT-2). *Science of the total environment, 409(3),* 604-611.

Tranchida, P. Q., Franchina, F. A., Dugo, P., & Mondello, L. (2016). Comprehensive two-dimensional gas chromatography-mass spectrometry: Recent evolution and current trends. *Mass spectrometry reviews, 35(4),* 524-534.

Trovó, A. G., Nogueira, R. F., Agüera, A., Fernandez-Alba, A. R., Sirtori, C., & Malato, S. (2009a). Degradation of sulfamethoxazole in water by solar photo-Fenton. Chemical and toxicological evaluation. *Water research, 43(16),* 3922-3931.

Trovó, A. G., Nogueira, R. F., Agüera, A., Sirtori, C., & Fernández-Alba, A. R. (2009b). Photodegradation of sulfamethoxazole in various aqueous media: persistence, toxicity and photoproducts assessment. *Chemosphere, 77(10),* 1292-1298.

Vallack, H. W., Bakker, D. J., Brandt, I., Broström-Lundén, E., Brouwer, A., Bull, K. R., ... & Koch, R. (1998). Controlling persistent organic pollutants–what next?. *Environmental Toxicology and Pharmacology, 6(3)*, 143-175.

Van den Berg, M., Birnbaum, L., Bosveld, A. T., Brunström, B., Cook, P., Feeley, M., ... & Kubiak, T. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environmental health perspectives, 106(12)*, 775.

Van der Lee, M. K., Van der Weg, G., Traag, W. A., & Mol, H. G. (2008). Qualitative screening and quantitative determination of pesticides and contaminants in animal feed using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. *Journal of chromatography A, 1186(1)*, 325-339.

Vree, T. B., Van der Ven, A. J. A. M., Verwey-van Wissen, C. P. W. G. M., Kolmer, E. V. E. B., Swolfs, A. E. M., Van Galen, P. M., & Amatdjais-Groenen, H. (1994). Isolation, identification and determination of sulfamethoxazole and its known metabolites in human plasma and urine by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications, 658(2)*, 327-340.

Wang, Y., & Hong, C. S. (1999). Effect of hydrogen peroxide, periodate and persulfate on photocatalysis of 2-chlorobiphenyl in aqueous TiO 2 suspensions. *Water Research, 33(9)*, 2031-2036.

Wang, H. S., Man, Y. B., Wu, F. Y., Zhao, Y. G., Wong, C. K., & Wong, M. H. (2010a). Oral bioaccessibility of polycyclic aromatic hydrocarbons (PAHs) through fish consumption, based on an in vitro digestion model. *Journal of agricultural and food chemistry, 58(21)*, 11517-11524.

Wang, P., Zhang, Q., Wang, Y., Wang, T., Li, X., Ding, L., & Jiang, G. (2010b). Evaluation of Soxhlet extraction, accelerated solvent extraction and microwave-assisted extraction for the determination of polychlorinated biphenyls and polybrominated diphenyl ethers in soil and fish samples. *Analytica Chimica Acta, 663(1)*, 43-48.

Wang, H. S., Zhao, Y. G., Man, Y. B., Wong, C. K., & Wong, M. H. (2011). Oral bioaccessibility and human risk assessment of organochlorine pesticides (OCPs) via fish consumption, using an invitro gastrointestinal model. *Food Chemistry, 127(4)*, 1673-1679.

Wang, W., Huang, M. J., Zheng, J. S., Cheung, K. C., & Wong, M. H. (2013). Exposure assessment and distribution of polychlorinated biphenyls (PCBs) contained in indoor and outdoor dusts and the impacts of particle size and bioaccessibility. *Science of the Total Environment, 463*, 1201-1209.

Watkins, P. J., Rose, G., Salvatore, L., Allen, D., Tucman, D., Warner, R. D., Dunshea, F. R., & Pethick, D. W. (2010). Age and nutrition influence the concentrations of three branched chain fatty acids in sheep fat from Australian abattoirs. *Meat science, 86(3),* 594-599.

Weber, R., Gaus, C., Tysklind, M., Johnston, P., Forter, M., Hollert, H., ... & Moccarelli, P. (2008). Dioxin-and POP-contaminated sites—contemporary and future relevance and challenges. *Environmental Science and Pollution Research, 15(5),* 363.

Whitlock Jr, J. P. (1999). Induction of cytochrome P4501A1. *Annual review of pharmacology and toxicology, 39(1),* 103-125.

Wnuk, A., Rzemieniec, J., Litwa, E., Lasoń, W., Krzeptowski, W., Wójtowicz, A. K., & Kajta, M. (2016). The Crucial Involvement of Retinoid X Receptors in DDE Neurotoxicity. *Neurotoxicity research, 29(1),* 155-172.

Xia, Z., McClements, D. J., & Xiao, H. (2017). Influence of Lipid Content in a Corn Oil Preparation on the Bioaccessibility of β-Carotene: A Comparison of Low-Fat and High-Fat Samples. *Journal of Food Science, 82(2),* 373-379.

Xing, G. H., Yang, Y., Chan, J. K. Y., Tao, S., & Wong, M. H. (2008). Bioaccessibility of polychlorinated biphenyls in different foods using an in vitro digestion method. *Environmental pollution, 156(3),* 1218-1226.

Xu, D., Grizzle, J. M., Rogers, W. A., & Santerre, C. R. (1996). Effect of cooking on residues of ormetoprim and sulfadimethoxine in the muscle of channel catfish. *Food research international, 29(3),* 339-344.

Yu, Y., Han, S., Zhang, D., Van de Wiele, T., Lu, M., Wang, D., ... & Fu, J. (2008). Factors affecting the bioaccessibility of polybrominated diphenylethers in an in vitro digestion model. *Journal of agricultural and food chemistry, 57(1),* 133-139.

Yu, Y. X., Li, J. L., Zhang, X. Y., Yu, Z. Q., Van de Wiele, T., Han, S. Y., ... & Fu, J. M. (2009). Assessment of the bioaccessibility of polybrominated diphenyl ethers in foods and the correlations of the bioaccessibility with nutrient contents. *Journal of agricultural and food chemistry, 58(1),* 301-308.

Yu, Y. X., Huang, N. B., Zhang, X. Y., Li, J. L., Yu, Z. Q., Han, S. Y., ... & Fu, J. M. (2011a). Polybrominated diphenyl ethers in food and associated human daily intake assessment considering bioaccessibility measured by simulated gastrointestinal digestion. *Chemosphere, 83(2),* 152-160.

Yu, Y., Pang, Y., Zhang, X., Li, C., Yu, Z., & Fu, J. (2011b). Optimization of an in vitro method to measure the bioaccessibility of polybrominated diphenyl ethers in dust using response surface methodology. *Journal of Environmental Sciences, 23(10)*, 1738-1746.

Zabik, M. E., & Zabik, M. J. (1995). Tetrachlorodibenzo-p-dioxin residue reduction by cooking/processing of fish fillets harvested from the Great Lakes. *Bulletin of environmental contamination and toxicology, 55(2)*, 264-269.

Zapadlo, M., Krupčík, J., Kovalczuk, T., Májek, P., Špánik, I., Armstrong, D. W., & Sandra, P. (2011). Enhanced comprehensive two-dimensional gas chromatographic resolution of polychlorinated biphenyls on a non-polar polysiloxane and an ionic liquid column series. *Journal of Chromatography A, 1218(5)*, 746-751.

Zhang, R., Zhang, Z., Kumosani, T., Khoja, S., Abualnaja, K. O., & McClements, D. J. (2016). Encapsulation of β-carotene in Nanoemulsion-Based Delivery Systems Formed by Spontaneous Emulsification: Influence of Lipid Composition on Stability and Bioaccessibility. *Food Biophysics, 11(2)*, 154-164.

Zheng, J., Chen, K. H., Yan, X., Chen, S. J., Hu, G. C., Peng, X. W., Yuan, J. G., Mai, B. X., & Yang, Z. Y. (2013). Heavy metals in food, house dust, and water from an e-waste recycling area in South China and the potential risk to human health. *Ecotoxicology and environmental safety, 96*, 205-212.

Zhou, W., & Moore, D. E. (1994). Photochemical decomposition of sulfamethoxazole. *International journal of pharmaceutics, 110(1)*, 55-63.

Zou, L., Zheng, B., Zhang, R., Zhang, Z., Liu, W., Liu, C., ... & McClements, D. J. (2016). Influence of Lipid Phase Composition of Excipient Emulsions on Curcumin Solubility, Stability, and Bioaccessibility. *Food Biophysics, 11(3)*, 213-225.

Résumé :

Au cours de leur élevage, les animaux sont exposés à divers contaminants chimiques, qui peuvent s'accumuler au niveau des tissus animaux et être retrouvés *in fine* dans les produits carnés consommés par l'Homme, représentant alors un risque pour la santé des consommateurs. L'évaluation de ce risque repose classiquement sur la mesure de teneur en contaminants de la viande fraîche alors que plusieurs travaux suggèrent que seule une fraction de ces contaminants est effectivement assimilée par le consommateur du fait des transformations technologiques comme la cuisson et physiologiques comme la digestion. L'objectif de cette thèse est d'étudier ces effets modulateurs de la cuisson et de la digestion sur les contaminants chimiques de la viande et leur bioaccessibilité.

En s'appuyant sur le développement d'une méthode multirésidus GC×GC-TOF/MS pour le suivi de 206 polluants environnementaux et sur des collaborations avec des Laboratoires Nationaux de Référence, le premier objectif de ce travail était d'étudier l'impact de la cuisson sur un large spectre de contaminants chimiques d'une viande intentionnellement contaminée. Les résultats montrent que la cuisson n'a pas d'impact significatif sur la teneur en PCDD/F et en métaux lourds de la viande alors que des pertes significatives (d'autant plus importantes que l'intensité de cuisson est élevée) en PCB, antibiotiques et pesticides ont pu être détectées. Ces pertes ont pour origine soit une libération dans le jus de cuisson des contaminants thermorésistants, soit une dégradation sous l'effet de la cuisson des contaminants thermosensibles, comme cela a été observé pour un antibiotique, le sulfaméthoxazole, pour lequel un schéma de dégradation thermique est proposé.

Le second objectif de ce travail était d'évaluer la bioaccessibilité des contaminants chimiques de la viande en s'appuyant sur un protocole standardisé de digestion *in vitro* statique. Dans le cas des PCB, les résultats obtenus indiquent que la bioaccessibilité dans la viande est relativement faible (26%). La teneur initiale de la viande en matière grasse et les variations physiologiques liées à l'âge du consommateur influencent significativement ces valeurs de bioaccessibilité alors que la cuisson de la viande a moins d'influence sur la bioaccessibilité.

A terme, les résultats obtenus durant ce travail devraient conduire à proposer de nouvelles procédures d'évaluation des risques liés aux contaminants chimiques de la viande, prenant en compte les modulations induites par la cuisson et la digestion.

Mots clés : Contaminants chimiques, Cuisson, Bioaccessibilité, Viande, Evaluation du risque, Sécurité sanitaire des aliments.

Abstract:

Livestock animals are exposed to various chemical contaminants during breeding. These contaminants are rapidly transferred from the environment to animal edible tissues, thus representing a public health risk. This risk is classically assessed based on the level of contaminants in raw meat. However, due to technological processes such as cooking or physiological transformations such as digestion, only a fraction of meat contaminants can be absorbed by the body. The purpose of this PhD thesis is to investigate the modulating effect of cooking and digestion on chemical contaminants in meat and on their bioaccessibility.

Thanks to a GC×GC-TOF/MS multiresidue method developed in this study for the analysis of 206 environmental pollutants and to collaborations with French National Reference Laboratories, the first aim of this work was to assess the effects of pan cooking on a broad range of chemical contaminants in spiked meat. Cooking did not impact the level of PCDD/Fs and heavy metals in meat whereas significant losses (more important as cooking conditions were more intense) of PCBs, antibiotics and pesticides were observed. These losses may originate from juice expelling of heat-resistant compounds or from degradation by breakdown of thermolabile compounds such as the sulfamethoxazole antibiotic.

The second aim was to assess the bioaccessibility of chemical contaminants in meat based on a standardized *in vitro* static digestion. For PCBs, results showed that their bioaccessibility was low (26%). Both the meat fat content and the age of consumer significantly affect this bioaccessibility value. In contrast, meat cooking was shown to have less influence on PCB bioaccessibility. The ultimate goal of the project will be to improve chemical risk assessment procedures taking into account the changes induced by cooking and digestion on micropollutants in meat.

Keywords: Chemical contaminants, Pan cooking, Bioaccessibility, Meat, Risk assessment, Food safety.