The metabolism of arsenic in humans:

Bioaccessibility in the gastrointestinal tract, diffusion across lipid membranes and biotransformations in liver cells

Teresa González de Chávez Capilla

This thesis is submitted in fulfilment of the requirements for the degree of *Doctor of Philosophy*

January 2018
Institute for Applied Ecology
University of Canberra
Australia

To all the brave women before me who fought
for my right to get an education in science

Acknowledgements

First and most importantly I would like to thank my primary supervisor Simon Foster. He has done a great job in supervising and guiding me during my first steps in what I hope to be a career in academia. Thank you for encouraging me to be independent and to learn from my mistakes, for your patience and positive attitude, and for teaching me so much.

Bill Maher and Tamsin Kelly, my secondary supervisors, thanks for your meticulous edits to improve my publications and thesis drafts. Tamsin, thanks for your assistance while analysing my samples by LC-MS-MS. Bill, I truly appreciate your involvement and wise advice on different aspects of my project, and of course your unusual sense of humour that has made many days better.

Thanks to the HPLC-ICP-MS for giving me such a hard (and delightful) time and to Simon again for introducing me to the wonderful universe of troubleshooting. Thanks to Frank Krikowa for all his support and help when dealing with equipment failure, despite constantly reminding me how jinxed I am.

Thanks to all those who have let me use their facilities or borrow equipment to complete my experiments. Thanks to Alison Shield for letting me use the cell culture room in the Pharmaceutical Laboratory at University of Canberra and to Sue Mahat-Basnet for teaching me how to culture cells. Thanks to Ashraf Ganem for giving me access to the Organic Synthesis Laboratory at University of Canberra to synthesise some of my standards and to Frady Gouany for taking me to the NMR facilities at the Australian National University and helping me with the analysis of my compounds there. Thanks to Marco Casarotto for allowing me to use the Zetasizer in his laboratory at the John Curtin School of Medical Research, Australian National University. Thanks to Mona Beshai for volunteering to help with some of my experiments and to Andrew O'Reilly-Nugent for IT support with the software required to complete one of my chapters. Thanks to James Grech for his assistance with the LC-MS-MS in the Forensics Laboratory at University of Canberra. Thanks to Richard Carne for his patience and help when ordering consumables and to Rubén Ramírez for letting me borrow small equipment for my experiments.

Thanks to the University of Canberra for awarding me the W. J. Weeden Scholarship that has covered my tuition fees and living allowance during my first three years of PhD. Many thanks to the Institute for Applied Ecology (IAE) for the Top up Scholarship to further support my living expenses during these three years; for covering my tuition fees during my fourth year of PhD; for the Extension and Writing Scholarships that have helped me to survive during this last year; and for the travel allowance that has covered my expenses in several conferences during my candidature.

Within the IAE I would particularly like to thank the Ecochemistry group for all the feedback, general support and enjoyable moments sharing international dishes. I would also like to thank Barbara Harris, who has been highly helpful in every administrative task required; to Bernd Gruber and Janine Deakin for having been excellent IAE HDR Coordinators; and particularly to Janine for being such an inspirational role model for women in science.

Without the Dungeon crew and their peculiar sense of humour this journey would have not been the same. Thanks to Rod, Yaz, Jill and Eman for being such great office mates. Our minutes of 'personal reflection' for meditation, headstands, email jokes and 'coffeeee!' have been priceless. Specially thanks to Rod and Yaz for always being there. Together with Rod and Yaz, I would also like to thank my other IAE HDR student fellows Jonas, Andrew, Will, Margi, Al, Sal and Adrian for the funny times making beer and movies to relieve our PhD stress. Thanks to Will and Yaz for that Agatha Christie's novel as potential reference material for my project in arsenic. I will read it someday. Special thanks to Jonas, not only as a colleague but as my partner, who has been, and is, simply the best.

To everyone that has supported me during these four years, thank you.

No quiero acabar sin incluir al otro lado del mundo, en donde la gente que más me importa me ha demostrado que la distancia no se mide en kilómetros. Muchísimas gracias a mis padres por haberme dado tanto, pero sobre todo por facilitarme la educación que me ha hecho llegar hasta aquí y por inculcarme los valores de honestidad e integridad que tan necesarios son en el mundo de la investigación. Gracias a mis hermanos, en especial a Mauro, a mis abuelos y al resto de mi familia por su apoyo

incondicional. Aurora, Guille (y Cebolla), Matías, Susana, Suheda, Laura, Tahir, Jens, Valentina, Ruggero, Borja, Vero, Marta, Paula, Silvia, Bruno, Miriam, y Chloe: muchas gracias por las conversaciones y risas por Skype y por los mails que han hecho de mis desayunos la mejor manera de empezar el día. Amigos y familia como vosotros hacen que estar tan lejos sea más llevadero.

A todos los que me han apoyado durante estos cuatro años, muchas gracias.

ABSTRACT

Arsenic is ubiquitous in the environment and widely available to humans through their diet. Arsenic toxicity strongly depends on the chemical species absorbed and metabolised once inside an organism. The current regulations to prevent overexposure to arsenic are primarily based on inorganic arsenic species, disregarding more than 30 arsenic species also present in food. There are many studies on the effects of inorganic arsenic on human health, however, only recently the focus has shifted towards the consequences of both endogenic and exogenic complex organic arsenic species. Although little is known about general organic arsenic metabolism, recent publications have shown that some of these species are toxic to humans. Even so, health and environmental organisations still require more information to update the evidence based policies that control arsenic permitted levels in food.

The aim of this thesis was to provide new insights into the poorly understood metabolic pathways of these arsenic species in humans.

Arsenic transformations in the gastrointestinal tract determine the nature of the arsenic species available for metabolism in cells. The studies in *Chapters 2* and *3* investigated the bioaccessibility and degradation of the organic arsenic species commonly found food. Physiological based extraction tests of rice, seaweed, fish and krill oil were performed, as well as of the pure standards of the major arsenic species present in these foodstuffs (arsenic glutathione complexes, arsenosugars, arseno-fatty acids and arseno-hydrocarbons), to assess the effect of the food matrix on arsenic bioaccessibility. It was found that around 80 % of arsenic in these foods is bioaccessible after gastrointestinal digestion. Hydrolysis and demethylation of arsenic glutathione complexes and arsenosugars standards was observed, however, no transformations occurred to arsenosugars present in seaweed. Arseno-fatty acids and arseno-hydrocarbons in krill oil were also found to be bioaccessible. Demethylation of methylarsonic acid and dimethylarsinic acid from rice occurs increasing the amount of inorganic arsenic species available for metabolism in cells.

Arsenic metabolism depends on the ability of different arsenic species to traverse biological membranes. Simple diffusion provides an alternative route to mediated

transport mechanisms that can increase the amount of arsenic available for metabolism in cells. In *Chapter 4*, octanol-water and liposome-water partition coefficients were used to investigate the ability of arsenous acid, arsenate, methylarsonate, dimethylarsinate, thio-methylarsonate, thio-dimethylarsinic acid, arsenotriglutathione and monomethylarsonic diglutathione to diffuse through the lipid bilayer of cell membranes. Molecular modelling of arsenic species aided in the interpretation of results. All arsenic species with the exception of arsenate, methylarsonate and thio-methylarsonate were able to diffuse through the lipid bilayer of liposomes, with liposome-water partition coefficients between 0.04 and 0.13. The highest partition coefficients corresponded to trivalent and thio-arsenic species, which are known to exert higher toxicities than oxo-pentavalent arsenic species in humans.

Arsenous acid and arsenic acid are known carcinogens and their metabolism in mammals has been extensively studied. They are known to undergo methylation in humans, although the existing proposed pathways to explain the process are still under debate. The study in *Chapter 5* focused on the metabolism of arsenate and arsenous acid in HepG2 cells after 24 h exposure. Particular attention was paid to the species produced and their distribution within the different cellular organelles. It was found that microsomes and mitochondria are the main sites for the metabolism of these arsenic species in liver cells.

Arseno-fatty acids and arseno-hydrocarbons are common in seaweed, fish and crustaceans and have been reported to be toxic to human cells. As they were found to survive the physiological conditions of the gastrointestinal tract, *Chapter 6* investigated the biotransformations of arseno-fatty acids and arseno-hydrocarbons in HepG2 cells. Arsenic speciation of subcellular fractions after 24 h exposure showed that both arsenic species are degraded to dimethylarsinoyl propionic acid in mitochondria and peroxisomes.

A synthesis of the outcomes from this thesis is presented in *Chapter 7*. The key arsenic species for future research in human health studies are discussed. The work presented within this thesis will aid toxicologists and epidemiologists to approach arsenic related topics more effectively.

Contents

List of publications

Chapter 2

Teresa Chávez-Capilla, Mona Beshai, William Maher, Tamsin Kelly, Simon Foster, Bioaccessibility and degradation of naturally occurring arsenic species from food in the human gastrointestinal tract, Food Chemistry, 2016, 212, 189-197.

Chapter 4

Teresa Chávez-Capilla, William Maher, Tamsin Kelly, Simon Foster, Evaluation of the ability of arsenic species to passively diffuse across cell membranes using octanol-water and liposome-water partition coefficients, Journal of Environmental Sciences, 2016, 49, 222-232.

List of figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

List of tables

Chapter 1

Chapter 3

Chapter 4

Chapter 5

Chapter 6