

The metabolism of arsenic in humans:

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

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

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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.

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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.

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