# DISINFECTION BY-PRODUCTS IN DRINKING WATER AND GENOTOXIC CHANGES IN URINARY BLADDER EPITHELIAL CELLS

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This is to confirm that I have made the following contributions to the conduct of the study presented in this thesis. The works was undertaken through the National Centre for Epidemiology and Population Health of the Australian National University. Contributions made by external individuals or organisations have been acknowledged in the methods chapter and in the acknowledgements of this thesis.

Geethanjali P. Ranmuthugala

**Candidate's contributions to the conduct of the study**

Major role in re-designing the study
Designed, planned and coordinated pilot studies
Submitted amended ethics application
Modified questionnaire
Selection of study sample
Planning recruitment of study subjects to study
Planning and involvement in fieldwork
Construction of data entry methods
Data cleaning
Data analysis

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## Abstract

There is much debate on the carcinogenic potential of disinfection by-products (DBP) in chlorinated water supplies. Until recently, epidemiological studies have been limited in their ability to examine accurately the risk of cancer with exposure to environmental carcinogens. This has largely been due to the long latency periods associated with cancer development, and the difficulties in accurately estimating chronic exposure. Although there is evidence, from predominantly case-control studies, of increased bladder cancer with exposure to chlorinated water supplies, the evidence is inconclusive.

In an attempt to determine the carcinogenic potential of trihalomethanes (THMs) in chlorinated water, this study utilises DNA damage to bladder cells, evident as micronuclei, as a pre-clinical outcome measure. Using a pre-clinical marker helps overcome some of the limitations associated with long latency periods. The study improves on previous studies by estimating exposure to DBP at an individual level, and takes into consideration ingestion, inhalation and dermal exposure.

A cohort study was undertaken in three Australian communities. The Bungendore (NSW) water supply was not chlorinated thereby providing a community unexposed to DBPs from chlorinated water. Canberra (ACT) and Adelaide (SA) had intermediate and relatively higher (but still within NHMRC guideline levels) of DBPs in the reticulation system. Trihalomethane levels in reticulated water (external dose) and in urine (internal dose) were used as exposure indices. As well, intake dose was computed by adjusting external dose for individual variations in ingestion and bathing. The primary outcome measure was the prevalence of micronuclei in bladder epithelial cells. A DNA index derived from flow cytometry was also used to estimate DNA damage in bladder cells. Associations between exposure and outcome were estimated using Poisson regression models, having identified and adjusted for interaction effects and confounders.

A total of 529 participants were eligible to participate, of which 348 (65.8%) completed all aspects of the study. Analysis was limited to the 228 participants

(65.53% of those who completed the study) who had slides suitable for micronuclei scoring. One hundred and forty three (63%) of the 228 participants were from the exposed communities, while 85 (37%) were from the unexposed community. This sample exceeded the estimated 50 per group required to detect a relative risk of 1.4, with a significance level of 0.05 and 80% power.

External dose for total THM for the two chlorinated (exposed) communities ranged from 37.75 to 157.25 μg/l. Intake dose estimated by fluid intake diary ranged from 2.9 to 469.5 μg/l, while a retrospective questionnaire estimated intake dose to range from 0 to 409.4 μg/l. Internal dose (urine levels) of total THM for the same two communities ranged from 0 to 6.82 μg/l. Adjusted risk estimate for DNA damage to bladder cells (using the micronuclei assay) when total THM was assessed by available dose was 1.0002 (0.997 to 1.003), by intake dose estimated by fluid intake diary was 1.0001 (0.998 to 1.002), by intake dose estimated by questionnaire was 1.001 (0.999 to 1.003), and by internal dose was 1.05 (0.89 to 1.24). Using DNA index from flow cytometry as the outcome measure also did not identify significant associations, except when exposure was assessed as available dose of total THM (RR=1.0042; 1.0003 to 1.0081).

The results suggest that THM levels are not significantly associated with DNA damage to bladder cell. This supports suggestions of THMs being non-genotoxic. Further work is required to assess the relationship between THM and the more mutagenic compounds, and to assess the carcinogenicity of the more mutagenic compounds at concentrations occurring in drinking water.

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