Iodine status on the island of Ireland

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An assessment of iodine status of teenage girls and infants on the island of Ireland

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Foreword

This research was prompted by a 2011 survey (1) which suggested that iodine deficiency is an emerging public health issue in the UK, including Northern Ireland. Given that recent data from the Republic of Ireland was not available the research was commissioned by *safe*food in order to provide information on the current iodine status on the island of Ireland.

The research looked at a number of indicators of iodine sufficiency in the island of Ireland population:

- A survey of the urinary iodine concentration of teenage girls, the biomarker advocated by the World Health Organisation to measure population status. The survey used similar methodology to that used in the UK survey to allow cross-survey comparability. The impact of diet and other factors affecting urinary iodine status was also explored.
- A review of the blood thyroid-stimulating hormone measures in infants.

A survey of the iodine concentration of dairy milks was also undertaken during the research. The findings from this work can be used to inform public policy.

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Acknowledgements

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Key findings

- Teenage girls aged from 14 to 15 years living on the island of Ireland (IOI) have adequate amounts of iodine in their bodies, according to World Health Organisation guidelines. This is the case even in summer months, when iodine status is expected to be at its lowest.

- Although the results show that this population group had adequate amounts of iodine, it is important to note that the median value was at the low end of the range identified as adequate by the World Health Organisation.

- Findings confirm previous reports that higher intakes of dairy products are associated with better iodine status. Participants (n=6) who reported consuming soya-based drinks had the lowest urinary iodine concentration although numbers were small. Urinary iodine concentrations were not associated with self-reported intake or fish or eggs. Self-reported consumption of white fish, oily fish and shellfish once a week or more was low (23%, 20% and 3%, respectively).

- The thyroid-stimulating hormone (TSH) blood-spot test results of infants born in Northern Ireland between 2000 and 2014 also indicate iodine sufficiency.

- Continual population-level monitoring of iodine and intake of dietary sources of iodine remains important – the WHO recommends that this be reviewed every 5 years.

- There is no indication that fortification of food with iodine is required to improve the iodine status of this population group.

- There was no difference in iodine concentrations between organic and conventional milk samples tested; however, the number of organic samples collected was small (n=22).

- There was no difference between iodine concentrations of milk samples collected in spring or summer months compared with those collected in autumn or winter months.

1 Background

Iodine is an essential trace element that is important for health. It is needed by the thyroid gland for the production of thyroid hormones - thyroxine and triiodothyronine. These are required for normal growth, metabolism – the conversion of food into energy, new cells, waste proudcts and so on – and for the development of a baby's brain during pregnancy and early life (2). Iodine deficiency disorders encompass a range of health consequences (Table 1) ranging from progressive thyroid enlargement i.e. goitre, as an adaption to acquire more iodine and to sustain production of thyroid hormones, to a range of effects arising from deficiency of the thyroid hormones, including defective reproduction, growth impairment, and neurodevelopmental damage (cretinism) (3). Approximately one in every 3,500 babies born in the Republic of Ireland (ROI) (4) and one in every 3,000 babies born in Northern Ireland (NI) (5, 6) has congenital hypothyroidism.

Table 1: Iodine deficiency disorders according to physiological group

Physiological group	Health consequences of iodine deficiency
All ages	Goitre Hypothyroidism
Foetus	Spontaneous abortion Stillbirth Congenital anomalies Perinatal mortality
Neonate	Endemic cretinism including mental deficiency with a mixture of mutism, spastic diplegia, squint, hypothyroidism and short stature Infant mortality
Child and adolescent	Impaired mental function Delayed physical development Iodine-induced hyperthyroidism
Adults	Impaired mental function Iodine-induced hyperthyroidism

It is well established that measuring the iodine content of urine is an effective indicator for iodine status. The World Health Organization (WHO) recommends that the assessment of iodine status at a population level should be based on the median concentration of iodine obtained from "spot" (single, randomly timed) urine samples taken from a group of at least 30 people (7). ("Median" means the value at the mid-point of a range of results is reported; for example, data for the fiftieth-highest iodine level out of 100 samples taken.) This median urinary iodine concentration (UIC) can be compared with established cut-offs to determine iodine intakes (Table 2 and Table 3).

Within Ireland and the United Kingdom (UK), for many years, iodine intakes were thought to be more than adequate. There is concern at a global level that some groups within the population such as vegans/vegetarians and weaning infants may not be consuming enough iodine, and therefore the levels in their bodies may be low (8). In the UK and Ireland, although severe iodine deficiency is unlikely, even mild deficiency can negatively affect health (9). Intakes in women of childbearing age are of particular importance, as low levels of iodine during pregnancy may affect the intelligence quotient (the "IQ") and reading skills of the child in later life (10).

More recently, a survey in 2011 reported iodine levels in over 700 teenage girls living across the UK (1). Results suggested that this group were considered iodine deficient. Of particular concern, the girls who took part in the survey that lived in Northern Ireland (NI) had the lowest levels of urinary iodine. Published data from the Republic of Ireland (ROI) also indicated mild deficiency in the general population (11) in 1999 and, more recently, in pregnant women (12, 13).

The American Thyroid Association recommends that regions develop strategies for ensuring adequate iodine intake pre-conception (that is, before becoming pregnant), and during pregnancy and lactation, according to regional dietary patterns and the availability of iodised salt (table salt with added iodine) (14). A review published in 2012 has also recognised iodine deficiency as a global public health problem affecting most countries, including developed countries and island nations (15). The review observed that about one-third of countries lack national estimates of the prevalence, or extent, of iodine deficiency in the population, therefore regular monitoring programmes are required.

Table 2: Epidemiological criterial for assessing urinary iodine concentrations of adults (excluding pregnant women and lactating women) and school-age children (6 years or older)[a]

Median urinary iodine (µg/L)	Iodine intake	Iodine status
Below 20	Insufficient	Severe iodine deficiency
20 to 49	Insufficient	Moderate iodine deficiency
50 to 99	Insufficient	Mild iodine deficiency
100 to 199	Adequate	Adequate iodine nutrition
200 to 299	Above requirements	May pose a slight risk of more than adequate iodine intake in this population
300 and above	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid diseases)

[a] Applies to adults, but not to pregnant and lactating women. *Source:* (16)

Table 3: Epidemiological criteria for assessing iodine nutrition based on the median or range in urinary iodine concentrations of pregnant women[a]

Population group	Median urinary iodine concentration (µg/L)	Iodine intake
Pregnant women	Below 150	Insufficient
	150 to 249	Adequate
	250 to 499	Above requirements
	500 and above	Excessive – in excess of the amount required to prevent and control iodine deficiency

For lactating women and children <2 years of age a median urinary iodine concentration of 100 µg/L can be used to define adequate iodine intake, but no other categories of iodine intake are defined. Although lactating women have the same requirement as pregnant women, the median urinary iodine is lower because iodine is excreted in breast milk). *Source:* (16)

Iodine is found in a range of foods. The richest sources are fish and dairy products (17). Within Ireland and the UK, milk and other dairy products tend to be the main sources of dietary iodine (13, 17, 18). The amount of iodine in foods varies according to the iodine content of the soil; the time of year (the iodine content of products such as milk is higher in winter than in summer (19, 20); and farming techniques, such as the use of cattle feed that contains iodine and using iodine as an antiseptic or cleaning agent. There is no formal iodine food fortification programme in place in the UK and Ireland, so iodine status entirely depends on dietary intakes of iodine-containing foods (13).

Although population-based UIC is considered the "gold standard" for assessment, it has also been suggested that neonatal (newborn baby) blood-spot TSH screening programmes could be another method of identifying population-level iodine deficiency (21, 22). Neonatal TSH values are considered reflective of iodine status. This is because of the relatively low iodine content of the neonatal thyroid and hence iodine turnover is much higher. When a newborn baby is iodine deficient, iodine turnover within the thyroid is increased further, resulting in hyperstimulation of TSH (23). The WHO have suggested that, within a population, a prevalence or frequency of less than 3% of neonates with TSH values above 5 milliunits per litre (mU/L) can be used to indicate iodine deficiency (24).

It has also been suggested that investigating trends over time in neonatal TSH results (for example, evidence of moderately raised TSH at the higher end of "normal", such as values above 2 mIU/L) may be a more suitable method of monitoring iodine status in mildly deficient populations or populations at the lower end of sufficiency (23, 25). Such a trend was recently identified in a study of TSH concentrations of babies born in the ROI between 1995 and 2006 (25). In this study, according to the WHO cut-off, the Irish female reproductive population was considered iodine sufficient during that period of time. However, a year-on-year tendency for higher TSH was observed. The authors suggested that this trend indicated the potential for this population group to become iodine deficient, and recommended the continued monitoring of blood-spot neonatal TSH levels.

2 Aims and objectives

This research project aimed to

- Provide an insight into the current iodine status on the island if Ireland and to identify the main dietary and other determinants of urinary iodine status
- Investigate trends in iodine status over time using the thyroid-stimulating hormone (TSH) test results of babies born throughout the island of Ireland between 2000 and 2014.

The objectives of the project were to

1. Assess the iodine status (by measurement of urinary iodine concentration) of 14- to 15-year-old females from 7 sample sites across the island of Ireland
2. Investigate the environmental availability of iodine, during each sampling phase
3. Examine dietary and other determinants of urinary iodine status
4. Examine regional and seasonal variation in the iodine content of milk throughout the island of Ireland
5. Measure neonatal blood TSH on the island of Ireland
6. Investigate trends over time in neonatal TSH results.

3 Methods

Survey of urinary iodine status and its main dietary and other determinants in girls aged 14 to 15 years

Study population

A cross-sectional survey, which takes a snapshot of data at a single point in time, was used to collect information on iodine status in females aged 14 to 15 years living on the IOI. The methodology was broadly based on WHO recommendations for assessing iodine status at a population level (16). This study focused on teenage girls aged 14–15 years, since those who might proceed to pregnancy in the short-to-medium term (and their off spring) are the most susceptible to the adverse effects of iodine deficiency.

Participants were recruited from 7 sample sites across Ireland: Belfast, Londonderry/Derry, Dublin, Cork, Galway, Sligo and Roscommon. Girls living in Belfast and Londonderry/Derry were resampled specifically to investigate potential seasonal variations in iodine status (with iodine status typically being lower in summer than in winter months (1, 26)).

Data collection took place between March 2014 and October 2015. Three sampling phases were undertaken:

- Phase 1 Spring/summer sampling in NI (March to June 2014)
- Phase 2 Autumn/winter sampling in NI (October to December 2014)
- Phase 3 Sampling in the ROI (January to May 2015 and October 2015).

Schools in Dublin, Cork, Roscommon and Sligo took part in the study between January and May 2015. Schools in Galway took part in October 2015.

To investigate seasonal differences in iodine status, 2 sampling phases took place in NI. Samples collected between March and June 2014 were considered as representative of spring/summer iodine status. Samples collected between October and December 2014 were considered as representative of autumn/winter iodine status.

During each sampling phase, schools with female pupils within each of the 7 sample areas – the study centres – were identified. The schools were initially invited by letter (addressed to the school Principal,

outlining the study aims and objectives) to take part in the research. A list of schools in each centre area was obtained either online or via contact with the appropriate educational authority. Schools which admitted girls were selected at random. All schools approached were followed up by telephone and email. Where a principal was unable to accommodate the researchers during the sampling phase, or where a response was not received, the school was replaced at random from the original list.

If a school agreed to take part, a researcher from Queen's University Belfast (QUB) travelled to the school and gave females aged 14 to 15 years an information session about the study, the role of the thyroid gland and the effects of iodine deficiency. Attendees were then given a pack to take home, including a consent form and information sheet.

Each girl who provided consent for the study was asked to provide an early morning urine sample (a "spot" sample). They were also asked to complete a food frequency questionnaire (FFQ) (adapted from Bath et al. (27)) and a demographic questionnaire (Appendix1). ("Demographics" describes the characteristics of a population, for example age, weight, smoker status, or any other information that may be of interest.)

The FFQ was designed to gather information on how often participants consumed iodine-rich foods including milk, dairy products and fish. Information on use of iodised salt, kelp or seaweed supplements and multivitamin/multimineral supplements was collected. The demographic questionnaire collected information on ethnic background, time of urine collection and the participant's date of birth.

Two hundred and twenty-two schools were approached to take part in the research (Table 4). Twenty-seven schools agreed to participate. The most commonly cited reasons for a school choosing not to take part in the research were a busy school schedule or that the school had recently taken part in another survey.

Table 4: Summary of recruitment to the study

Sampling area	Number of schools approached	Number of schools that took part	Number of study packs distributed	Number of study packs returned with consent	Proportion of packs returned with consent
Belfast	33 (15%)	6 (22%)	823	294	36%
Londonderry/Derry	2 (9%)	6 (22%)	498	131	26%
Dublin	47 (21%)	2 (7%)	223	97	43%
Cork	51 (23%)	5 (19%)	313	147	47%
Galway	40 (18%)	3 (11%)	160	72	45%
Roscommon	19 (9%)	1 (4%)	97	52	54%
Sligo	12 (5%)	4 (15%)	252	110	44%
Total	**222**	**27**	**2,366**	**903**[1]	**38%**

The study received ethical approval from the School of Medicine, Dentistry and Biomedical Sciences Research Ethics Committee (reference number: 13/42v2), for the Northern Irish study centres.

Ethical approval for the ROI study centres was sought and granted from regional ethics boards:

- Dublin – Royal College of Physicians of Ireland (reference number: RCPI RECSAF 27)
- Cork – Clinical Research Ethics Committee of the Cork Teaching Hospitals, University College Cork (reference number: ECM 3 [oo] 02109114)
- Galway, Roscommon and Sligo – Galway Regional Hospitals Clinical Research Ethics Committee (reference number: CA 1149).

Assessment of iodine status

Iodine status was measured by collecting spot urine samples. However, due to variation typically observed in UIC depending on the dilution of the urine sample, the concentration of urinary creatinine (a by-product of muscle metabolism) was used to correct UIC for the variable dilution among spot urine samples. This is a standard approach to many biochemical analyses conducted in urine, with the iodine content then explained as a ratio compared to creatinine.

[1] A total of 903 teenage girls participated in the survey. Of the 903 participants who provided the necessary consent, 901 provided a spot urine sample and 892 returned FFQs and demographic questionnaires.

Iodine assessment

In accordance with WHO guidance (16), median UIC of all samples was calculated and compared with established cut-offs to identify if the group was considered iodine sufficient.

The laboratory assay (a method of measuring or counting) for the measurement of iodine in urine was established in Belfast. Following method development undertaken between June and October 2014, a final standard operating procedure (SOP) was produced (Appendix 3).

Urinary iodine excretion was measured using a Multiplate® persulphate digestion method to remove interfering substances followed by Sandel-Kolthoff colorimetry (28), which monitors a colour change, with results expressed as micrograms per litre (µg/L). Samples were analysed in triplicate and the limit of detection was 10 µg/L. (The "limit of detection" is the level at which the presence of an organism or substance – in this case urinary iodine – can be distinguished.)

The Belfast laboratory was registered with the "Ensuring the Quality of Urinary Iodine Procedures" (EQUIP) quality assurance programme through the United States (US) Center for Disease Control (CDC), and was given an anonymous laboratory number (E-182). Quality assurance samples were received from EQUIP at a 2 time points throughout the project; these were subsequently analysed using our established assay procedure, the results of which were returned to EQUIP and a performance report was received by the Belfast laboratory (Appendix 5).

The iodine status of the group was determined by comparing the median UIC value with the WHO cut-off for iodine sufficiency in school-aged children (16).

Creatinine adjustment

Urinary creatinine was measured in QUB using an established assay (28) and SOP (Appendix 3). Creatinine concentration was determined using an ILAB 600 Chemistry Analyser (from Werfen, UK) using the Jaffe rate method, which measures the reaction of creatinine with picric acid in an alkaline environment. Measurement of creatinine allowed for results to be expressed in terms of an iodine–creatinine ratio.

Tap water sample collection, storage and analysis

To investigate the "environmental" availability of iodine (iodine that is not taken in from food or supplements), 5 millilitres (ml) of tap water was collected at each sample site – the schools – at the same time as urine sample collection. Tap water was collected in iodine-free containers and kept at minus 20 degrees Celsius (°C) until analysis was undertaken. Samples were analysed using inductively coupled plasma–mass spectrometry (ICP–MS), which is a process capable of detecting elements at very low concentrations.

Standard solutions

Iodine standard solutions of 10 parts per million (ppm) and 100 parts per billion (ppb) of iodine to water were prepared using an iodide standard (sourced from Inorganic Ventures, Christiansburg, US). Two stock standards of intermediate concentration, 10 ppm and 100 ppb were prepared. The concentrations of standard solutions were 0.1 ppb, 0.5 ppb, 1.0 ppb and 5.0 ppb. The tetramethylammonium hydroxide (TMAH) used was of ultrahigh purity grade (sourced from Sigma Aldrich, Dorset, UK).

Instrumentation

Samples were analysed using inductively coupled plasma–mass spectrometry (ICP–MS). The ICP–MS used was the Thermo Scientific™ iCAP™ Q (from Thermo Fisher Scientific, US). The limit of detection, calculated from the slope of the calibration curve, was 0.038 ppb-µg/L. The standard operating conditions are described in Table 5.

Table 5: Operating conditions of Inductively Coupled Plasma–Mass Spectrometer

Inductively coupled plasma conditions	
Radio Frequency	1,500 watts (W)
Nebuliser gas	0.925 litres per minute (L/min)
Auxiliary gas	0.8 L/min
Cool gas	14.0 L/min
Data acquisition	
Mass	Iodine only at mass 127
Dwell time	0.5 seconds (S)
Number of sweeps	120

Milk sample collection, storage and analysis

Within Ireland and the UK, milk and dairy products tend to be the main sources of dietary iodine (13, 17, 18). For this reason milk samples were tested for their iodine content.

Milk sample collection

In each of the 7 study centres, samples of 5 semi-skimmed milk brands were purchased between May 2014 and March 2015. Semi-skimmed milk was chosen for sampling as it is the most commonly consumed milk, and its iodine concentration has been reported to not differ from skimmed milk and full-fat milk (29).

During the planning phase of the study, the project team visited a number of supermarkets to see how many different milk samples were readily available to the consumer. In any supermarket, the maximum number of different semi-skimmed milk samples was 5. The brands chosen included own-brand supermarket milk, branded milk and organic milk. As the iodine content of milk has been reported to vary between winter and summer months, the approach of sampling every 2 months also allowed for potential seasonal differences in milk's iodine content to be investigated.

A milk collection protocol (a set of procedures and methods to be used) was developed (Appendix 4) and this, along with a milk sampling kit, was distributed to a researcher in each of 6 sample areas (Londonderry/Derry, Dublin, Cork, Galway, Sligo and Roscommon). The researcher based in NI was responsible for milk sampling in Belfast. Milk samples were stored at each centre in laboratory freezers at minus 20 °C, until sample collection was completed and transportation to QUB was arranged for storage and analysis.

Milk sample storage and analysis

In preparation for iodine content analysis by ICP–MS, all milk samples were freeze-dried overnight using a Martin Christ Alpha 1-4 LDplus freeze dryer. Following successful freeze-drying, fat was extracted from the samples by homogenisation in a 2:1 petroleum ether solution. ("Homogenisation" is a process that breaks fat down and suspends it evenly throughout a solution so that it does not float to the top.)

The freeze-dried fat-extracted milk samples were accurately weighed to 0.2 grams (g), and 1.0 ml of 5% TMAH solution (sourced from Sigma-Aldrich, US) was added to each sample to allow for an overnight digestion. Following the digestion process, 4.5 ml of standard deionised water was added to each sample and samples were placed in a microwave (Mars 6 240150 from CEM Microwave Technology, US) for a 50-minute cycle. To each sample, 10 microlitres (µl) of the internal standard, rhodium was added (Fluka® Analytical from Sigma-Aldrich, US) and samples were made up to 10 ml using standard deionised water.

All samples were centrifuged – placed in a machine and spun at very high speed so that different elements in the liquids separate from each other. The samples were then filtered using a 0.45µm Millipore Express® filter unit (Millex® sourced from Mercke Millipore, US) to ensure successful removal of any residual particulate (minute separated particles remaining in the solution). Finally, 3 ml of 0.5% TMAH solution was added to 3 ml of the filtered sample solution.

The digested, filtered samples were analysed for iodine concentration by ICP–MS (Thermo Scientific™ iCAP™ Q, from Thermo Fisher Scientific, US). Results were verified using a certified reference material (CRM), which is a substance used as a uniform standard to measure against and compare with sample

test results. The CRM used was Skimmed Milk Powder ERM-BD151 (sourced from European Reference Materials, Belgium), with a certified iodine content of 1.78 milligrams per kilogram (mg/kg).

Measurement of neonatal blood thyroid-stimulating hormone

This part of the research set out to analyse the population iodine status of infants using neonatal blood TSH as an indicator for of iodine deficiency. This approach aimed to utilise existing neonatal screening datasets.

Northern Ireland neonatal blood thyroid-stimulating hormone screening

As part of the national neonatal screening programme (30), all newborn babies born in NI have a blood-spot TSH measurement taken from a heel-prick blood sample provided on day 5, 6 or 7 after birth. For the purpose of this research access was obtained to samples taken between 2001 and 2014. As this research was limited to the anonymous, secondary use of information, previously collected in the course of normal care, the researchers sought and were granted ethical approval by the UK National Health Service (NHS) Research Ethics Committee (reference number: 14/SC/1331) and governance approval from the Belfast Health and Social Care Trust (reference number: 14113IY-AS). Within the Belfast Trust, blood TSH is measured by dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA), which is a sensitive immunometric assay, on an AutoDELFIA® analyser (from PerkinElmer, UK).

Republic of Ireland neonatal blood thyroid-stimulating hormone screening

As part of the national neonatal screening programme in the ROI, a heel-prick blood sample is taken between 72 and 120 hours after birth. All samples are sent to the National Newborn Screening Laboratory at the Children's University Hospital, Dublin, for analyses. Similar to the analysis of TSH levels in NI, blood TSH in ROI is measured by DELFIA on an AutoDELFIA® analyser.

Statistical analyses

Statistical analyses were conducted using the Statistical Package for the Social Sciences software (Version 21.0, SPSS, Chicago, US). The definition of statistical significance was set at p below 0.05.

Iodine status

Urinary iodine concentration values were not normally distributed, i.e. did not follow a bell-shaped curve. Following logarithmic transformation to try to make the distribution more normal, residuals followed a normal distribution. One-way analysis of variance (or "ANOVA", which tests for significant differences in the averages of at least 2 unrelated categories of data) was used to explore relationships between food groups from the FFQ and urinary iodine excretion.

For the purposes of statistical analyses, responses for dairy products (cream, yoghurt, dairy desserts, butter and cheese), fish (white fish, oily fish and shellfish), poultry and meat were combined and recoded. This is to reflect intakes for respondents who consume such food products “never”, “less than once a month”, “once in 2 weeks” or “greater than or equal to once a week”, as recorded in the FFQ.

One-way ANOVA was also used to investigate differences between sample site locations and any ethnicity differences in urinary iodine levels. Any significant results achieved from one-way ANOVA analyses were explored using “Duncan's new multiple range tests” (a system for making multiple comparisons of data).

Independent “t-tests”, which compare 2 sets of data, were used to explore potential seasonal variations (spring/summer sampling as against autumn/winter sampling), and the effects of multivitamin or mineral supplements, kelp or seaweed, organic milk and iodised salt consumption on UIC.

In keeping with previously published research, despite the use of “parametric” tests (which make certain assumptions about the parameters, or definitions, of the population to be studied), UIC was presented as median and interquartile range (IQR) values to allow interpretation and comparison with other studies. (This means that as well as the value at the mid-point of a range of data being reported, it is also given at one-quarter and three-quarters of the range; for example, data for the twenty-fifth and seventy-fifth highest UIC levels out of 100 tests taken).

Iodine tap water concentrations were not normally distributed. Distribution was not improved by logarithmic transformation, so non-parametric tests where used. “Spearmans rank correlation coefficients” were used to assess the relationship between tap water iodine content and urinary iodine excretion in each sample location. (These aim to show whether a specific value in the data is increasing as another also increases – or whether a value is increasing as the other decreases.) A Mann-Whitney test was used to explore potential seasonal variations in iodine concentration of tap water samples and a Kruskall-Wallis test was used to explore potential sampling site differences in iodine content of tap water samples.

Iodine concentrations of milk samples were not normally distributed, so data were transformed using the natural logarithm to allow for parametric testing. Independent t-tests were used to test differences between the iodine content of organic and conventional (non-organic) milks and to test potential seasonal differences (spring/summer collections as against autumn/winter collections) in the iodine concentrations of samples. An independent t-test was also used to investigate differences in iodine between branded and own-brand supermarket milks. One-way ANOVA (with Student–

Newman–Keuls correction to identify statistically significantly different groups for post-hoc analysis) was used to investigate differences in iodine concentration between sample sites.

Neonatal thyroid-stimulating hormone

"Time series" methods – analyses made over a period of time at certain intervals – were used to investigate the long-term trends and seasonality in the monthly TSH results from January 2000 to August 2014. The TSH results were categorised using cut-off points of greater than 2 mIU/L and greater than 5 mIU/L TSH for consistency with previous publications (25, 31).

Analysis of the long-term trends in the proportions of results exceeding these cut-offs was first conducted using Joinpoint Regression Program software (Version 4.2 – June 2015, Statistical Research and Applications Branch and Data Modeling Branch, Surveillance Research Program National Cancer Institute, Bethesda, Maryland, US), which was developed for surveillance of trends in cancer incidence.

"Joinpoint" illustrates long-term trends in data by fitting straight linear segments – lines – between time points on a graph that have been estimated from the data. For an example of what this looks like, see Figure 4. The programme provides a "permutation test" (a test that allows for different possible scenarios) to assess the number of linear segments, and the times at which they join, while taking into account the multiple testing issues inherent in the approach (32). The "standard error", the measurement of accuracy for the data predictions made, for the proportion (p) for each month was determined according to the usual formula for proportions, $SE(p)= \sqrt{[p*(1-p)/n]}$, where "n" is the number of samples, "r" is the number exceeding the cut-off point and "p" = "r" divided by "n".

Having established the long-term trends, residuals (the differences between estimated and actual data) were obtained and submitted to a "weighted" multiple regression analysis incorporating cosine and sine terms to represent months of the year. ("Weighting" emphasises the impact of a feature of the data, or of a set of data, in order to get an effect or result.) Weights were taken as the reciprocal of the squares of the standard errors, SE (p).

Robust standard error estimates were obtained in the Stata® software package (Stata Stastical Software: Release 12, College Station, Texas, US) to weaken possible exaggerated significance caused by autocorrelation (the correlation between the values of the same variables being based on related objects, which violates the assumption of independence) in the time series. Months were assigned cosine and sine values in accordance with their angles when arranged on the circumference of a circle (January at 15°, February at 45° and December at 345°). The amplitude of the seasonal component was estimated as $\sqrt{[b_{sin}^2 + b_{cos}^2]}$, and the peak angle as $\tan^{-1}(b_{sin}/b_{cos})$ with the quadrant determined by the signs of the regression coefficients, b_{sin} and b_{cos}.

4 Results

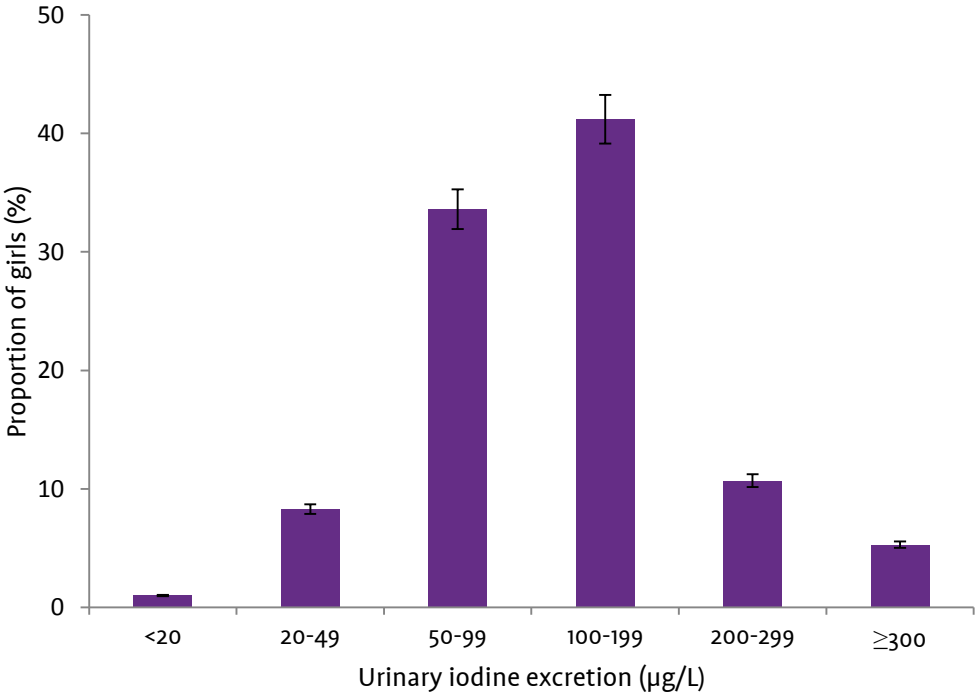
Urinary iodine concentrations and determinants of status in girls aged 14 to 15 years

Median population urinary iodine concentrations

Figure 1 shows the classification of the urinary iodine concentrations of the girls sampled.

- The median UIC within the study sample (n=901) was 111.2 µg/L.
- This population group had adequate amounts of iodine according to World Health Organisation guideline which specifies that the median urinary iodine concentration in the general population should be within the range 100–199 µg/L.
- Urinary iodine measurements indicative of iodine sufficiency (100 to 199 µg/L) were present in 41% of the population (n=371).
- Urinary iodine measurements indicative of mild iodine deficiency (50 to 99 µg/L) were present in 34% of the population (n=303).
- Moderate deficiency (20 to 49 µg/L) was present in 8% (n=75) of the population.
- Severe deficiency (below 20 µg/L) was present in 1% of the population (n=8).
- Participants with UIC above WHO requirements (200 to 299 µg/L) comprised 11% (n=96) of the population.
- Excessive intakes (300 µg/L or more) present in 5% (n = 48) of respondents

Figure 1: Proportion of participants classified within categories of iodine excretion according to established WHO cut-offs



* Below 20 µg/L = severe iodine deficiency; 20 to 49 µg/L = moderate iodine deficiency; 50 to 99 µg/L = mild iodine deficiency; 100 to 199 µg/L = adequate iodine; 200 to 299 µg/L = iodine above requirements; 300 µg/L or above = excessive iodine.

Median urinary iodine concentrations of teenage girls by sample site location

Median urinary iodine excretion differed significantly between the sample centres (Figure 2; "p" value less than 0.001).

- The lowest measurements recorded were in Galway (median 97.9 µg/L).
- The highest measurements recorded were in Belfast (125.3 µg/L).

Figure 2: Median urinary iodine concentrations of teenage girls by sample site location

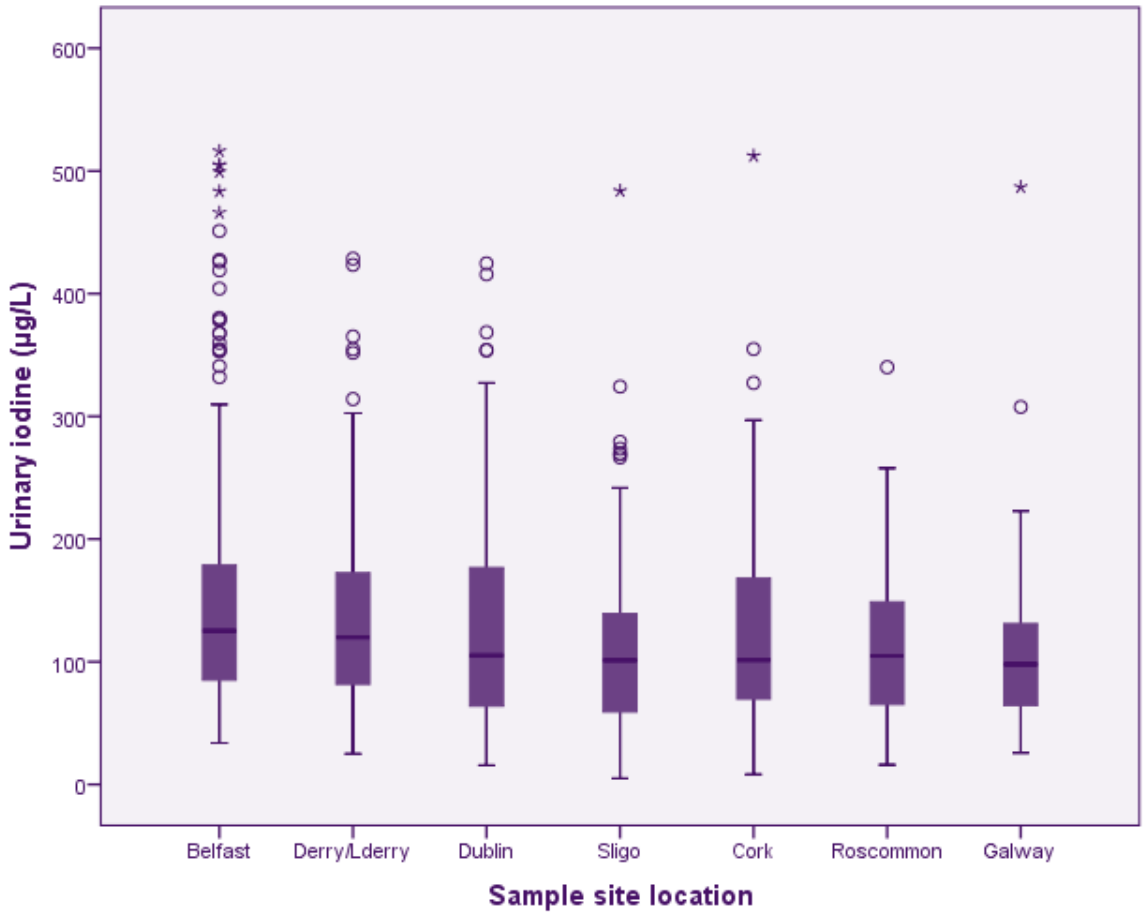


Table 6 shows the proportion of participants with mild iodine deficiency (urinary iodine below 100 µg/L) in each sample centre, as well as the subgroups with moderate (50 to 99 µg/L) and severe iodine deficiency (levels below 50 µg/L).

- The prevalence of iodine deficiency was highest in Galway, where 53% of participants had urinary iodine excretion less than 100 µg/L.
- Very low urinary iodine measurements (below 20 µg/L) compatible with severe iodine deficiency were found in 2 participants in Dublin, 1 in Cork, 4 in Sligo and 1 in Roscommon.

Table 6: Urinary iodine concentrations of teenage girls according to WHO cut-offs for deficiency by sample site location

Location	Number (n)	Participants with iodine deficiency (below 100 µg/L)	Participants with mild iodine deficiency (50 to 99 µg/L)	Participants with moderate to severe iodine deficiency (below 50 µg/L)
Belfast	294	103 (35%)	90 (31%)	13 (4%)
Londonderry/Derry	131	49 (37%)	41 (31%)	8 (6%)
Dublin	97	47 (49%)	36 (37%)	11 (11%)
Cork	146	71 (48%)	55 (37%)	16 (11%)
Galway	72	38 (53%)	28 (39%)	10 (14%)
Sligo	109	54 (49%)	36 (33%)	18 (17%)
Roscommon	52	24 (46%)	17 (33%)	7 (13%)

Urinary creatinine concentration was also measured in each spot sample to correct UICs for any intra-individual variation in daily urine volume produced, therefore data is presented as both UIC and as iodine–creatinine ratio in Table 7.

Table 7: Urinary iodine concentrations and urinary iodine–creatinine ratio split of teenage girls by sample site location

Location	Number (n)	Urinary iodine concentration µg/L Median (IQR)	Urinary iodine–creatinine ratio µg/g Median (IQR)
Belfast	294	125.31 (85.02 to 178.94)	89.31 (63.72 to 139.28)
Londonderry/Derry	131	119.84 80.52 to 172.43)	83.89 (59.97 to 121.06)
Dublin	97	105.27 (63.52 to 178.07)	87.28 (54.95 to 156.71)
Cork	146	101.37 (69.49 to 167.96)	86.00 (56.94 to 128.77)
Sligo	109	101.18 (58.91 to 139.48)	77.18 (44.85 to 132.07)
Roscommon	52	104.91 (64.30 to 149.56)	89.06 (55.82 to 135.15)
Galway	72	97.88 (63.49 to 133.81)	76.60 (49.88 to 97.14)

Median urinary iodine concentrations of teenage girls by season

Formal resampling was undertaken in the 2 sampling areas in NI (Belfast and Londonderry/Derry) to allow for investigation of seasonal effects on iodine status.

- Urinary iodine concentration was lower during spring and summer months (n = 228, median 116.95 µg/L, IQR 75.81 to 164.82) than in winter (n = 197, median 129.84 µg/L, IQR 90.91 to 194.18); p = 0.005.

Median urinary iodine concentrations according to dietary intake

Table 8 shows median urinary iodine concentrations according to self-reported consumption of dairy products and eggs.

- The most commonly consumed type of milk was cows' milk (n = 866, 96% of participants).
- Six participants were consumers of goat's milk, 6 were consumers of soya-based drinks, and 10 reported consuming "other types" of "milk" (such as almond or rice-based drinks).

- Three participants reported that they did not consume milk.
- Urinary iodine intake was associated with the type of milk consumed (p = 0.016), with those who reported using soya-based drinks displaying the lowest UIC (62.31 µg/L) and those who reported using goat's milk displaying the highest UIC (135.7 µg/L).
- One hundred and six participants reported using organic milk (12%). However, there was no difference in urinary iodine excretion between those who used organic milk as compared with those who reported consuming conventional milk (p = 0.884).
- Higher intakes of milk (p value below 0.001), cream (p = 0.027) and dairy-based desserts, e.g. custard, rice pudding (p = 0.004) were associated with higher median UIC.
- Urinary iodine levels were not associated with self-reported intakes of eggs, cheese, butter or yoghurt.

Table 8: Urinary iodine concentrations of teenage girls according to intake of dairy products and eggs

Food intake	Number of participants n (%)	Median urinary iodine concentration µg/L (IQR)	p value*
Cows' milk consumed per day (n = 864)			
None	50 (6%)	89.76 (63.00 to 116.05)[a]	
140ml	197 (23%)	92.37 (63.32 to 124.37)[a]	
140 to 279ml	230 (27%)	114.90 (72.95 to 157.68)[b]	
280 to 242ml	166 (19%)	119.85 (88.82 to 164.75)[b]	
425 to 570ml	110 (13%)	138.75 (80.62 to 203.97)[b,c]	
More than 570ml	111 (13%)	145.35 (102.91 to 242.71)[d]	
			Below 0.001

Cream (n = 885)			
Never, or less than once a month	571 (65%)	108.16 (68.81 to 161.69)[a,b]	
Once in two weeks	229 (26%)	114.90 (80.86 to 165.76)[b]	
Once a week or more often	85 (10%)	122.32 (92.83 to 195.58)[b,c]	
			0.027
Dairy desserts (n = 891)			
Never, or less than once a month	485 (54%)	104.47 (68.18 to 151.98)[a]	
Once in two weeks	235 (26%)	116.84 (75.44 to 172.36)[b]	
Once a week or more often	171 (19%)	127.74 (86.27 to 172.42)[b]	
			0.004
Cheese (n = 888)			
Never, or less than once a month	159 (18%)	105.32 (68.31 to 154.93)[a]	
Once in two weeks	107 (12%)	107.88 (69.79 to 152.69)[a]	
Once a week or more often	622 (70%)	114.13 (74.70 to 167.68)[a]	
			0.163
Eggs (n = 890)			
None	242 (27%)	105.42 (68.34-156.97)[a]	
One per week	245 (27%)	125.97 (89.88 to 178.41)[a]	
Two per week	213 (24%)	106.45 (69.67 to 159.43)[a]	

Three per week	101 (11%)	106.21 (66.87 to 156.74)[a]	
Four or more per week	89 (10%)	115.21 (69.39 to 150.10)[a]	
			0.139
Yoghurt			
Low fat (n = 887)			
Never/less than once a month	443 (49%)	106.44 (72.32 to 166.47)[a]	
Once in two weeks	133 (15%)	113.20 (61.78 to 153.62)[a]	
Once a week or more	311 (34%)	119.46 (77.30 to 166.61)[a]	
			0.209
Full fat or greek (n = 884)			
Never/less than once a month	520 (58%)	108.68 (71.91 to 163.67)[a]	
Once in two weeks	144 (16%)	114.70 (72.37 to 149.77)[a]	
Once a week or more often	220 (24%)	118.73 (70.14 to 169.52)[a]	
			0.761

*One-way ANOVA; different letters represent statistically significant differences in UIC for each FFQ category.

Table 9 shows median urinary iodine concentrations according to self-reported consumption of meat, poultry and fish.

- Eighty-three per cent of participants reported consuming meat once a week or more often.
- Ninety-two per cent reported consuming poultry once a week or more.
- Self-reported consumption of white fish, oily fish and shellfish once a week or more was low (23%, 20% and 3%, respectively).
- Urinary iodine levels were not associated with self-reported intakes of meat, poultry or fish (including white, oily or shellfish).

Table 9: Urinary iodine concentrations of teenage girls according to Intake of meat, poultry and fish

Food intake	Number of participants (n)	Median urinary iodine concentration µg/L (IQR)	p value*
Meat (n = 893)			
Never, or less than once a month	67 (7%)	118.64 (91.50 to 157.99)[a]	
Once in two weeks	74 (8%)	99.54 (71.05 to 146.58)[a]	
Once a week or more often	752 (83%)	112.82 (70.40 to 167.77)[a]	
			0.741
Poultry (n = 892)			
Never, or less than once a month	37 (4%)	126.93 (78.09 to 169.58)[a]	
Once in two weeks	26 (3%)	119.93 (75.74 to 152.22)[a]	
Once a week or more often	829 (92%)	110.019 (71.08 to 163.67)[a]	
			0.770
Fish			
White fish **(n = 892)**			
Never, or less than once a month	440 (49%)	106.20 (68.97 to 156.17)[a]	
Once in two weeks	251 (28%)	119.46 (74.56 to 167.93)[a]	

Once a week or more often	201 (22%)	117.31 (76.78 to 170.22)[a]	
			0.449
Oily fish **(n = 891)**			
Never, or less than once a month	534 (59%)	108.09 (70.20 to 163.99)[a]	
Once in two weeks	180 (20%)	115.40 (69.43 to 157.35)[a]	
Once a week or more often	177 (20%)	119.38 (81.88 to 170.94)[a]	
			0.152
Shellfish **(n = 888)**			
Never, or less than once a month	772 (86%)	110.01 (71.73 to 163.67)[a]	
Once in two weeks	87 (10%)	124.92 (80.40 to 176.55)[a]	
Once a week or more	29 (3%)	101.34 (58.41 to 155.96)[a]	
			0.328

*One-way ANOVA; different letters represent statistically significant differences in UIC for each FFQ category.

Eight hundred and eighty-three participants provided information on supplement use (Table 10).

- There was no significant difference in UIC in those who reported using a multivitamin or multimineral supplement compared with those who do not use these products (p = 0.070), although this did approach statistical significance.
- Self-reported supplement use was highest in Dublin (32%) and lowest in Roscommon (10%).
- Sixteen participants (2%) reported using kelp or seaweed supplements.
- 27 participants (3%) reported using iodised salt.

- There was no difference in urinary iodine excretion between those who reported using kelp or seaweed supplements (p = 0.405) or those using iodised salt compared with those who did not use these products (p = 0.643).

Table 10: Urinary iodine concentrations of teenage girls according to supplement, ionised salt and seaweed or kelp use

Intake	Number of participants (n)	Urinary iodine concentration µg/L Median (IQR)	p value*
Supplements (n = 881)			
Yes	191 (22%)	120.70 (83.00 to 170.22)[a]	0.070
No	690 (78%)	108.40 (69.00 to 160.66)[a]	
Kelp or seaweed (n = 888)			
Yes	16 (2%)	94.21 (59.65 to 143.45)[a]	0.405
No	872 (98%)	111.74 (71.71 to 164.32)[a]	
Iodised salt (n = 888)			
Yes	27 (3%)	101.53 (63.87 to 168.58)[a]	0.643
No	861 (97%)	111.20 (71.47 to 163.12)[a]	

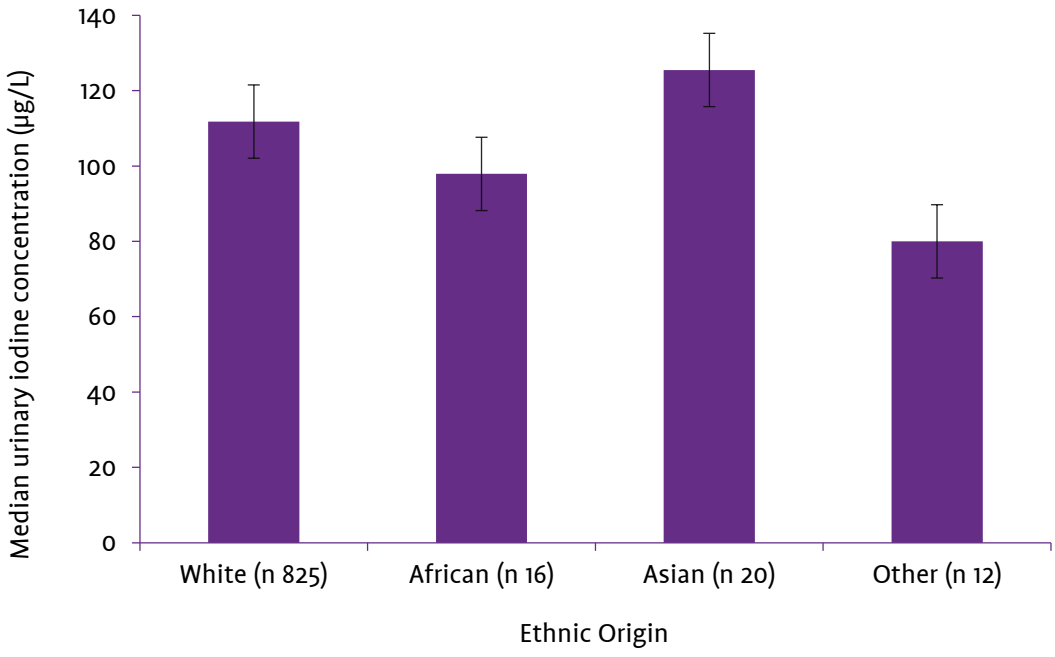
*Independent samples t-test; different letters represent statistically significant differences in UIC for each FFQ category.

Median urinary iodine concentrations of teenage girls according to ethnic origin

Ethnic origin was reported by 873 participants (Figure 3)

- Urine samples were provided by 825 participants of white ethnic origin (median urinary iodine excretion 111.8 µg/L).
- Sixteen participants were of African ethnic origin (median urinary iodine excretion 97.9 µg/L).
- Twenty participants were of Asian ethnic origin (median urinary iodine excretion 125.5 µg/L).
- Twelve participants belong to other ethnic groups (median urinary iodine excretion 80.0 µg/L).
- There was no difference in urinary iodine excretion between ethnic groups (p = 0.681), although numbers were small.

Figure 3: Urinary iodine concentrations of teenage girls according to ethnic origin



Tap water analysis

The iodine content of tap water samples collected during urinary iodine sample collection are shown in Table 11.

- There was no correlation between the iodine content of tap water samples collected and median UIC calculated for each school (that is, each sampling site; p = 0.321).
- For samples collected in NI, where seasonal resampling was undertaken, no seasonal differences in tap water content between samples collected in winter months (median iodine concentration 1.423 µg/L, IQR 0.566 to 2.724) and spring or summer months (median iodine concentration 1.519 µg/L, IQR 0.546 to 3.133; p = 0.671) were observed.
- Finally, there was no difference between sample site locations and iodine concentrations of tap water samples (p = 0.153).

Table 11: Tap water iodine and median urinary iodine excretion by sample site location

Sample site location	Number of tap water samples collected (n)	Iodine content of tap water µg/L Median (IQR)	Urinary iodine concentration µg/L Median (IQR)
Belfast	7	2.951 (0.901 to 3.410)	125.31 (85.02 to 178.94)
Londonderry/Derry	7	0.802 (0.478 to 2.137)	119.84 (80.52 to 172.43)
Dublin	2	0.589 (0.364 to 0.589)	105.27 (63.52 to 178.07)
Cork	5	1.796 (0.353 to 1.902)	101.37 (69.49 to 167.96)
Galway	3	2.563 (0.316 to 2.563)	101.18 (58.91 to 139.48)
Sligo	4	1.906 (1.901 to 2.148)	97.88 (63.49 to 133.81)
Roscommon	1	0.500	104.91 (64.30 to 149.56)

Iodine concentration of milk samples

A total of 190 milk samples were collected from the 7 study centres between May 2014 and March 2015. Of the 190 samples collected, 5 were excluded from the final dataset, resulting in a total of 185 samples for analysis. Four of the milk samples were excluded as they were not semi-skimmed milks (two were whole milks, 2 were skimmed milks) and the final sample excluded had no available information on the type or brand of milk.

- Of the remaining 185 samples, 22 were organic and 163 were conventional milks. There was no difference in iodine concentrations (p = 0.13) between organic (mean iodine concentration, 148 µg/100g) and conventional milk samples (mean iodine concentration 217 µg/100g); however, the number of organic samples collected was small (n = 22).
- There was also no difference between iodine concentrations of milk samples collected in spring or summer months (n = 101, mean iodine concentration, 222 µg/100g) compared with those collected in autumn or winter months (n = 84, mean iodine concentration, 191 µg/100g; p = 0.166).
- The iodine concentration of own brand supermarket milks (n = 52, mean iodine concentration 228 µg/100g) did not differ significantly from branded milks (n = 133, mean iodine concentration 200 µg/100g; p = 0.277).
- One-way ANOVA demonstrated that iodine concentrations of milk samples did differ significantly according to sample site location (Table 12). However, samples collected in Londonderry/Derry had a higher iodine concentration compared with those collected in Galway (p = 0.029) and Roscommon (p = 0.016). It is important to note that the sample site location may not correlate with the origin of the milk samples given the nature of the milk processing, distribution and retail chain on the island of Ireland.

Table 12: Iodine concentrations of milk by sample site location

Sample site location	Number of samples (n)	Mean iodine concentration (µg/100g)
Belfast	37	282[a,b]
Londonderry/Derry	13	339[b]
Dublin	30	221[a,b]
Cork	30	193[a,b]
Galway	19	146[a]
Sligo	29	215[a,b]
Roscommon	27	148[a]
Total	**185**	

[a, b] Values that do not share a letter are significantly different.

Neonatal blood thyroid-stimulating hormone as a proxy measure for iodine status in infants

Northern Ireland

A file of 354,403 neonatal TSH results for NI was received for analysis and the results were categorised using cut-off points of greater than 5 mIU/L and greater than 2 mIU/L. A small number of repeat results were removed and the rest were aggregated (collected together) according to the month of sampling. The average number of samples per month in the 176 months of the period being analysed was 2,010 (ranging between 1,528 and 2,329).

- Between the years of 2000 and 2014, 0.5% of newborn babies had blood TSH concentrations greater than 5 mIU/L and and 6.6% had concentrations greater than 2 mIU/L, indicating a population that is iodine sufficient according to WHO cut-offs (24) (Table 13).
- In no year over the collection period did the proportion of TSH concentrations greater than 5m IU/L rise above 1%.

Table 13: Percentage of neonatal blood thyroid-stimulating hormone values greater than 5 mIU/L and greater than 2 mIU/L from 2000 to 2014

Year	Neonatal blood TSH greater than 5 mU/L		Neonatal blood TSH greater than 2 mU/L	
	Percentage (%)	Number (n)	Percentage (%)	Number (n)
2000	0.6	124	7.5	1,643
2001	0.8	181	10.5	228
2002	0.7	147	8.1	1,747
2003	0.4	96	6.3	1,384
2004	0.6	136	6.7	1,549
2005	0.5	119	5.9	1,356
2006	0.5	118	5.3	1,299
2007	0.4	92	4.1	1,031
2008	0.4	96	4.8	1,257
2009	0.6	148	5.6	1,444
2010	0.5	136	6.7	1,766
2011	0.4	104	6.7	1,761
2012	0.5	134	6.9	1,784
2013	0.5	121	6.8	1,650
2014	0.7	107	9.8	1,580

The results of applying Joinpoint analyses are presented in Figure 4 for the 2 mIU/L cut-off and Figure 5 for the 5 mIU/L cut-off points. The programme fitted 4 linear components for the greater than 2 mIU/L time series plot and 3 linear components for the greater than 5 mIU/L time series plot.

Figure 4: Joinpoint plots of Northern Ireland neonatal blood thyroid-stimulating hormone monthly time series data for percentage of population with concentrations greater than 2 mIU/L

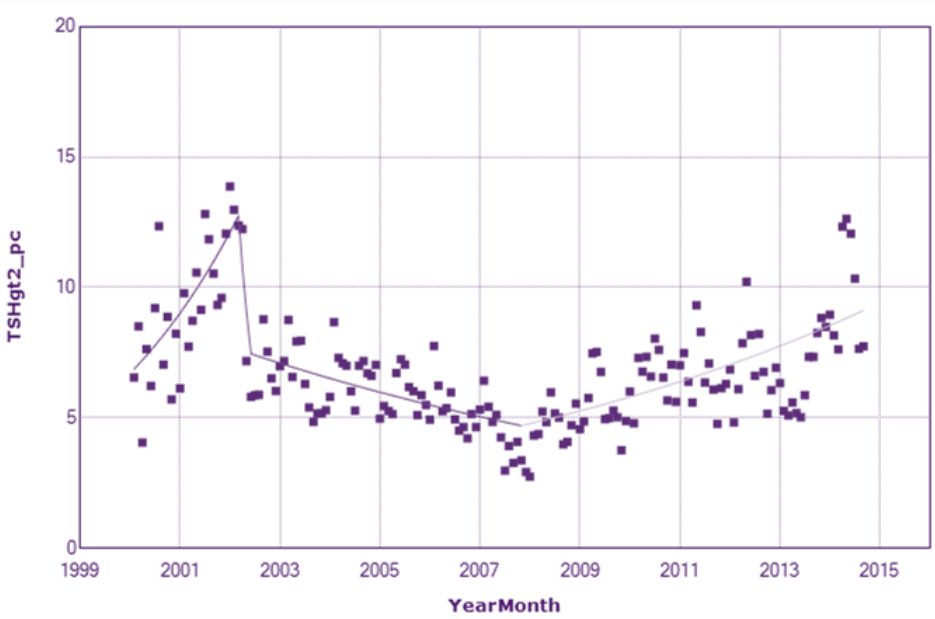
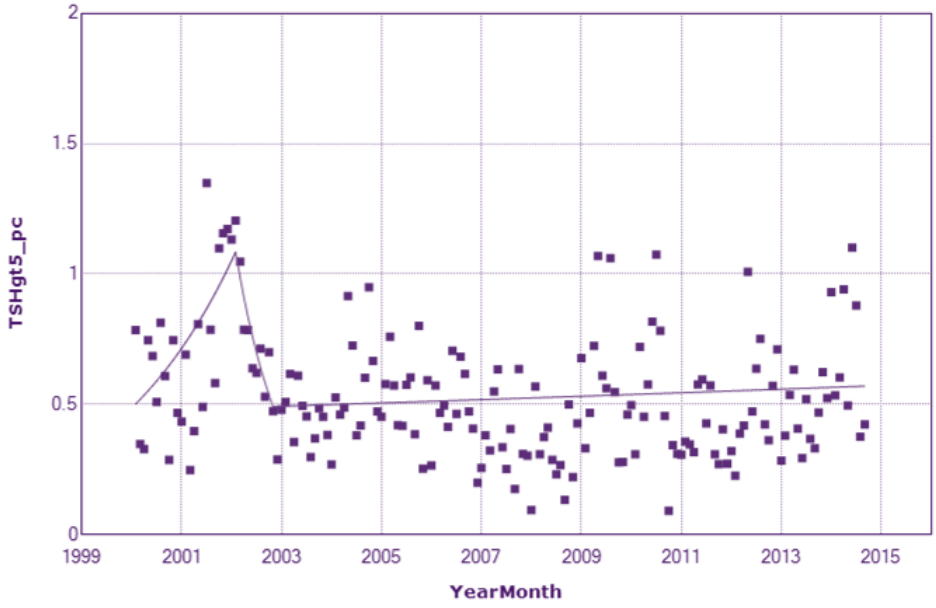


Figure 5: Joinpoint plots of Northern Ireland neonatal blood thyroid-stimulating hormone monthly time series data for percentage of population with concentrations above 5 mIU/L



Residuals from these linear "fits" were used as the dependent variables in multiple regressions incorporating sine and cosine terms to represent months of the year. The characteristics of the fitted annual cycles are summarised in Table 14 for the greater than 2 mIU/L cut-off point and Table 15 for the greater than 5 mIU/L cut-off. The results for unweighted and weighted regressions were similar.

- Both cut-offs gave peaks in May and June, but the amplitude for the 2 mIU/L and above cut-off point was greater at plus or minus 0.6% (plus or minus 0.5% in weighted analysis).
- There was a comparatively smaller amplitude for the greater than 5 mIU/L cut-off point of plus or minus 0.07% (plus or minus 0.08% in weighted analysis).
- Use of robust variance estimates in the multiple regression to mitigate the effects of autocorrelation did not materially alter the significance of the findings.

Table 14: Characteristics of fitted seasonal component for the cut-off point for neonatal blood thyroid-stimulating hormone concentrations greater than 2 mIU/L

Term	Coefficient, b (95% CI)	F statistic (df = 2,173)†	P†	Ampli-tude	Peak (month)
Unweighted					
Cosine	-0.156 (-0.431, 0.120)	7.92 (7.90)	Less than 0.001/Less than 0.001	Plus or minus 0.6%	164° (mid-June)
Sine	0.538 (0.261, 0.815)				
Weighted					
Cosine	-0.126 (-0.384, 0.133)	6.96 (6.52)	0.001/ 0.002	Plus or minus 0.5%	165° (mid-June)
Sine	0.476 (0.216, 0.736)				

† Robust variance estimate results in brackets

Table 15: Characteristics of fitted seasonal component for the cut-off point for neonatal blood thyroid-stimulating hormone concentrations greater than 5 mIU/L

Term	Coefficient, b (95% CI)	F statistic (df = 2,173)†	P†	Amplitude	Peak (month)
Unweighted					
Cosine	-0.048 (-0.090,-0.012)	5.17 (5.06)	0.007 (0.007)	Plus or minus 0.07%	136° (mid-May)
Sine	0.050 (0.008, 0.093)				
Weighted					
Cosine	-0.039 (-0.078, 0.00)	8.22 (7.07)	Less than 0.001 (0.001)	Plus or minus 0.08%	152° (early-June)
Sine	0.072 (0.033, 0.112)				

† Robust variance estimate results in brackets

The fitted cyclic pattern superimposed on the Joinpoint linear trends are depicted in Figure 6 for the greater than 2 mIU/L cut-off point and Figure 7 for the greater than 5 mIU/L cut-off point.

Figure 6: Northern Ireland neonatal blood thyroid-stimulating hormone monthly time series data for percentage of population with concentrations greater than 2 mIU/L with superimposed long-term trend and seasonal pattern

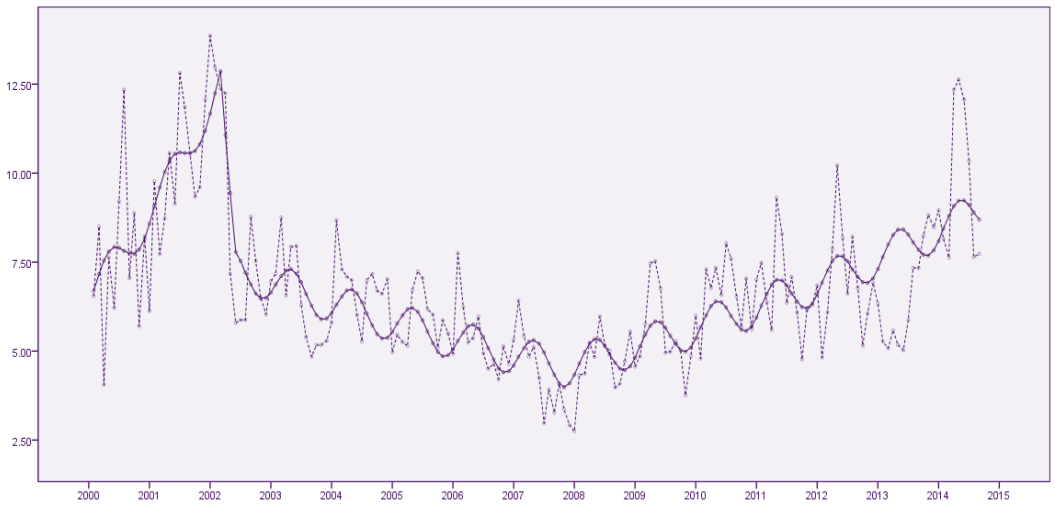
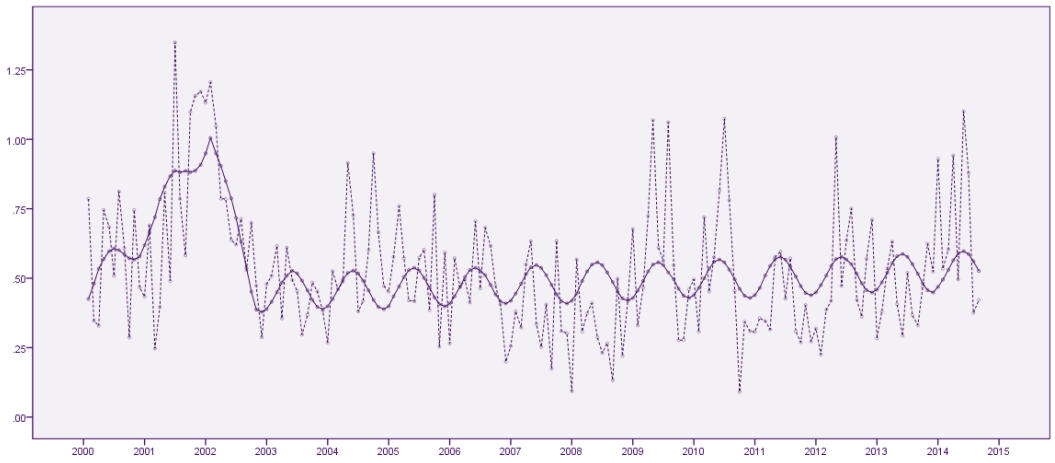


Figure 7: Northern Ireland neonatal blood thyroid-stimulating hormone monthly time series data for percentage of population with concentrations greater than 5 mIU/L with superimposed long-term trend and seasonal pattern



Republic of Ireland

Data was to be used from the national neonatal screening programme in the ROI. However, it did not prove possible to access this data.

5 Discussion

Survey of teenage girls' urinary iodine status

The results from this survey suggest that, as a population, 14-15-year-old females living on the IOI are iodine sufficient. Median UIC for the study sample was 111.2 µg/L.

These findings are in contrast with the findings of the 2011 UK survey that reported teenage girls to be iodine deficient. The observed median urinary iodine excretion reported here is 1.4 times larger than that recorded in the UK survey (1) (111.2 µg/L as against 80.1 µg/L), suggesting that teenage girls living in Ireland have considerably higher iodine status than their mainland UK counterparts.

The UK survey also reported the lowest levels of urinary iodine in those girls living in Belfast; but in contrast, in this survey, teenage girls living in Belfast had the highest level of iodine sufficiency of all the girls sampled (65% with levels above 100 µg/L, median UIC 125.3 µg/L). Within the current study, a similar population was targeted to that used in the UK survey to allow for comparison between the surveys. However, no data were collected in either survey in relation to socioeconomic background (family income, occupation and education, for example) or dietary information was self-reported. Because of this, there may be socio economic differences in the girls recruited for the 2 surveys.

The results of the current study did, however, reflect those from a recent pilot study undertaken in the UK among younger children aged 8 to 10 years that included a sample site in NI. Bath et al. (26) reported iodine sufficiency within this population group (median UIC of 144 µg/L), with children living in Omagh in NI having the highest urinary iodine excretion of the 3 locations sampled. These results, along with our own, suggest that iodine levels within children and teenagers living on the IOI are currently considered as sufficient.

Although the sample collection protocol allowed for an estimation of overall population status of iodine, and this assessment indicates sufficiency, based on median urinary iodine excretion, the values are still at the low end of the range. The urinary iodine measurements also suggest that 43% of participants had some degree of iodine deficiency (in contrast to 68% in the UK survey). This suggests that a reasonable proportion of the population may benefit from increasing their consumption of iodine-rich foods.

According to the WHO (16), an individual's UIC can vary daily, or even within the same day. However, within groups of individuals, these variations tend to "even out", providing a useful measure of the

iodine status of the population. Urinary iodine concentrations are, therefore, not useful for the diagnosis of individual iodine deficiency. For this reason, our urine collection protocol cannot be considered as a measure of individual iodine status, and therefore further surveys with more robust assessments of individual iodine status would be required before more detailed commentary could be provided regarding individual need for increased iodine intake.

These results did show a notable difference in iodine status between sample areas. Within 6 of the 7 areas, participants had a median UIC above 100 µg/L and Galway fell just short of the WHO definition of sufficiency with a median UIC of 97.9 µg/L.

Seasonal variation was considered in the 2 NI sample areas (Belfast and Londonderry/Derry). Despite iodine levels within these 2 areas being significantly lower during spring and summer (median UIC 116.95 µg/L) than in winter (median UIC 129.84 µg/L) throughout the seasons, the populations were still considered to be iodine sufficient.

Ethnic origin was reported by 873 participants, with the majority of participants (95%) being of white ethnic origin. No significant differences were observed in median urinary iodine excretion between ethnic groups; however, this result could in part be explained by the small number of participants from other ethnic backgrounds.

Results from dietary data also support findings from previous research (1, 26) suggesting higher UICs are observed with higher intakes of dairy produce. Within the current study, intakes of cows' milk, dairy-based desserts and cream were all positively associated with iodine status. Forty-five per cent of the sample reported consuming half a pint (280 ml) of milk per day or more, 19% consumed dairy desserts once a week or more often and 10% consumed cream once a week or more often. These self-reported levels of dairy consumption could in part explain the higher iodine levels observed in this study, compared with the UK survey (1).

However, due to the nature of the FFQ used within this survey, compared with that used in the UK survey, it is difficult to quantify exact differences in dairy consumption. For example, data on the consumption of cream and dairy desserts was recorded whereas the UK survey did not. Furthermore, the FFQ used in the UK survey quantified milk consumption by "cup" servings – for example, "I drink 1 cup of milk a day"; but in the current study, serving sizes were represented in "millilitre" and "pint" equivalents – for example, "I drink between one-quarter and one-half of a pint (140 to 279 ml) per day".

Results within the current study can, however, be compared to other studies that also reported milk consumption in "millilitres per day". For example, Bath et al. reported that 37% of children living in the UK (20), and 28% of pregnant women living in Surrey (33) consumed more than 280 ml of milk per day. This demonstrates that the self-reported quantities of milk consumed within this survey (45%

consumed half of a pint [280 ml] or more per day) are substantially higher compared to other studies investigating iodine status and milk intake within the UK.

In addition, milk consumption within the current cohort appeared higher than the national average within the UK and Ireland. Data from the UK National Diet and Nutrition Survey (34) (2008/2009 and 2011/2012) demonstrated that, on average, young adults aged 11 to 18 years reported consuming 103 ml of semi-skimmed milk per day. Milk consumption data from the ROI (taken from the National Teen's Food Survey 2005/2006 (35) demonstrated slightly higher intakes than the UK in females aged 13 to 17 of 144 ml per day.

Urinary iodine intake was also associated with the type of milk consumed. Those who reported using soya-based drinks displayed the lowest UIC (62.31 µg/L) and those who reported using goat's milk displayed the highest UIC (135.7 µg/L), although numbers consuming these alternatives to cows' milk were very small. Recent research conducted in the UK has suggested that most soya drinks are not fortified with iodine and could be considered as a poor source of iodine (36). Regular consumers of soya drinks may therefore be at greater risk of iodine deficiency; however, due to the small numbers of soya drink consumers within the current study (n = 6), further research is required before firm conclusions can be drawn.

In relation to organic milk consumption, there was no significant difference observed in urinary iodine excretion between those who reported consuming conventional milk as compared with organic milk. A recent study undertaken in the UK (37) suggested that organic milk contained 35.5% less iodine than conventional milk. The authors suggested that replacement of conventional milk by organic milk may increase the risk of becoming deficient in iodine, especially for pregnant or lactating women. Organic cows, historically, did not receive iodine in feed, which in part may explain why iodine levels have been reported as lower in organic milk. More recently this practice has changed, with the organic milk industry resuming the practice of enriching feed with iodine in 2014 (38). Further research is therefore required to establish whether the iodine content of organic milk has improved following this industry change. Results should be compared with representative population-level urinary iodine results to identify whether shopping choices contribute to iodine status.

Although non-consumers of white fish and oily fish had a lower iodine status than "high frequency" consumers (those who reported consuming 1 portion or more in a week), the differences in UIC were not significant. Furthermore, self-reported consumption of eggs, cheese, butter, yoghurt, meat or poultry was not associated with urinary iodine excretion.

Previous research has either not recorded supplement use or excluded individuals who were currently using iodine-containing supplements. However, as 1 in 4 people living on the IOI use some form of food supplement (39), it was felt that including a measure of supplement use (in this case self-

reported) was of interest when considering dietary iodine contribution. Approximately 20% of participants within the current study reported supplement use. In some areas, self-reported supplement use was even higher (32% was reported in Dublin).

Supplement use was not associated with iodine status. It may therefore be useful for future studies to include a measure of supplement use to allow for further investigation of the contribution of supplements to dietary iodine intakes. Participants in the current study were asked to record the brand and dose of supplement; however, in many cases this was not completed. It may be more practical to ask participants who do use supplements to provide the product label as part of study protocol in future research. Thus would provide a more objective measure of supplement contribution to dietary iodine than self-reporting.

Iodised salt use was low (3%) within the current study. This is in line with a recent UK survey that suggested that iodised household table salt is unlikely to contribute meaningful amounts to UK iodine intake (40). This is due to the fact that table salt is only a small percentage of total UK salt intake and that public health campaigns have encouraged reduced salt consumptions – both points that are relevant to the IOI. This implies that iodine intake in Ireland is very much dependent on food choice; therefore, particularly among teenagers, a balanced diet should continue to be encouraged, with the inclusion of healthy choices of iodine-rich foods.

As a secondary objective, this study set out to gain further insight into the observed trends in regional and seasonal variation of iodine intakes within this population by collecting samples of semi-skimmed milk on the IOI every 2 months for a one-year period. Analyses of the iodine content of the milk available for purchase demonstrated a significant difference in iodine concentrations of milk samples according to location. Samples collected in Londonderry/Derry had a significantly higher iodine concentration compared with those collected in Galway and Roscommon. This finding is in keeping with the results reported by Bath et al. (20), who observed regional differences in iodine concentrations of milk samples collected in the UK.). It is important to note however that the sample site location may not correlate with the origin of the milk samples given the nature of the milk processing, distribution and retail chain on the island of Ireland.

In contrast to the hypothesis and the results of previous studies, (20, 37, 41, 42) this study found no significant difference in iodine concentrations of organic milks as compared with conventional milks; however, the number of organic milk samples available for analysis was very small (n = 22). According to the Organic Milk Suppliers Cooperative (43), within the UK organic dairy market, organic milk is typically not well represented in shops and often only 1 brand is available. This is reflective of the limited availability of organic milks for purchase and use in the current study, despite best efforts to obtain a representative sample.

It has, however, been suggested that in the UK and Ireland, the production and consumption of organic milk is on the rise. In 2015 it was reported that in the UK 1 in 4 consumers now purchase organic milk (43). Furthermore, a 2002 report by the Irish Agriculture and Food Development Authority (Teagasc) (44), reported that between 1993 and 2001 there was a five-fold increase in the number of registered organic producers on the IOI. (This includes meat and vegetable production, not just the production of organic milk.) With a potential increase in organic milk consumption, there is a possibility that groups already vulnerable to insufficient iodine status (such as pregnant women and women of childbearing age) could become even more at risk of iodine deficiency.

In relation to potential seasonal variations within the current study, there were no differences observed in the iodine content of milk samples collected. It is typically assumed that winter milks have a higher iodine content than summer milks (due to the mineral-supplemented feed in cattle diets in the winter months); however, this was not reflected in our results. As this study did not collect specific details on the production of individual milk samples (to include variations in the type of feed, the housing conditions of cattle and the iodine content of soil between summer and winter milk production) it is difficult to provide an explanation for the null results observed for seasonal variations in the iodine content of milk samples collected.

In conclusion, as milk is one of the main sources of iodine within the UK and Ireland, further investigation of variations of geographical location and milk type (conventional, or non-organic, as against organic) is of interest. In particular, gathering information on specific farming differences within the milk production chain on the IOI is required to develop a greater understanding of the contribution of the food chain to iodine intakes.

Strengths of the study

The strengths of the present study include that this is the first report of iodine status in this age group in females living on the IOI. This group was of particular interest, considering the results of the recent UK survey (1) that suggested that adolescent girls living in the UK were deficient in iodine. To date, the current population iodine status in Ireland has remained relatively unknown, but concern has been expressed regarding the iodine status of pregnant women (12, 13). Therefore, identifying iodine status within a group that are likely to proceed to pregnancy in the short- to medium term was of particular importance.

There were difficulties faced during the recruitment process, such as schools being unable to facilitate the research due to a busy schedule or participants being reluctant to return a urine sample. Despite this, the sample size within the current study is comparable with, and indeed exceeds, that of the recent UK survey whose authors indicated that their numbers were large enough to estimate median

UIC at a population level. Furthermore, a similar methodology to that used in the UK survey was employed to allow for direct comparisons between surveys.

Limitations of the study

There are some limitations to the study that should also be noted. Despite using the WHO recommended method of collecting spot urine samples to estimate iodine status at a population level (16), it may have been more informative to collect 24-hour urine samples. This may have allowed further insight as to whether the higher iodine levels observed in the current study would be confirmed following adjustment for total urine volume.

This study did, however, measure creatinine concentrations in spot samples to correct UIC for intra-individual variation in daily urine volume. The measurement of creatinine as an indicator of iodine status is not recommended by the WHO because low creatinine excretion can be the result of malnutrition; however, results from previous research have suggested that presenting iodine levels both in terms of median urinary iodine excretion and as an iodine–creatinine ratio improves the ability to relate iodine status to dietary intake (27, 33). Therefore, the results of the current study are presented as both adjusted and unadjusted for urinary creatinine concentrations.

It should also be taken into consideration that, despite the research team's best efforts to undertake sampling between the months of March and June (in an attempt to keep seasonal variation in iodine status to a minimum), schools were not always able to participate within this timeframe. This resulted in sampling of some areas in winter months (outside of the formal resampling undertaken in the Northern Irish sites, during which seasonal comparison was deliberate); in particular, Galway samples were collected in October 2015. A similar scenario was reported within the UK survey (1), in which samples were also collected over a broader time period, highlighting the difficulties faced in recruiting for such studies in a school setting. For example, the Belfast cohort of 159 participants within the previous UK study showed deficiency in 85% of participants, whereas in the current study 35% of 103 participants were deficient.

The risk of recruitment bias, where schools are not truly sampled at random, due to response rates not being random, remains high in a study which relies on the cooperation and goodwill of schools. This is reflected within the current study, with a school uptake rate of 12% and subsequent participation uptake of less than 40%. As discussed previously, potential differences in the participants recruited within the current survey as compared with the UK survey (1) may in part explain variations in results obtained. Variables such as socioeconomic status or the presence of illness may have differed between participants within each survey; however, due to the nature of the study design (with a focus on capturing information on iodine-containing foods and limiting the

burden on participants), measurement of these factors was not included within the current or the UK survey.

In addition, as participants were recruited from different geographical locations (Great Britain and NI in the UK survey (1) as against the ROI and NI in the current survey), there are of course likely differences in a range of factors between these areas that could, in part, explain differences in urinary iodine measurements observed, over and above dietary intake differences. The likely differences include soil iodine content (and therefore iodine content found within the food chain), and farming practices.

In conclusion, the results of the current survey provide an improving picture of iodine sufficiency in teenage girls living on the IOI that may well translate through to later life stages such as pregnancy. However, it is important to note that, although the results suggest iodine sufficiency at a population level in teenage girls, this does not detract from the fact that 43% of participants displayed urinary iodine measurements indicative of deficiency.

Finally, considering the absence of a food or salt iodination programme (thus making iodine intakes entirely dependent on dietary intake) and the limited data available of iodine status at a population level on the IOI, regular monitoring of iodine status remains essential.

Neonatal thyroid-stimulating hormone data

The WHO classifies a population as iodine deficient when more than 3% of newborn babies have a blood TSH concentration greater than 5 mIU/L (24). Based on this classification, babies born in NI between January 2000 to August 2014 can be considered as iodine sufficient, with an average population frequency of 0.5% TSH levels greater than 5 mIU/L between 2000 and 2014. At no point during the years analysed did TSH rates within this Northern Irish cohort exceed 3% with levels greater than 5 mIU/L.

In comparison with previously published studies, the frequency of blood-spot TSH concentrations greater than 5 mIU/L in NI is substantially lower than reported levels of 1.5% of newborn babies in Wales and between 2.6% and 3.3% in Belgium. Furthermore, Burns et al. (25) reported frequencies of 2.4% to 3.6% in babies born in the ROI between 1995 and 2006.

As previously discussed, however, the validity of using a cut-off point of 3% with levels greater than 5 mIU/L in mildly iodine-deficient populations has been met with some criticism (23, 25, 31). For this reason, within the current study the percentage of TSH values that were greater than 2 mIU/L was also examined.

Over the 14-year examination period an overall frequency of 6.6% was observed with levels greater than 2 mIU/L; this is much lower than the 11% to 12% of subjects with a blood-spot TSH greater than 2

mIU/L observed in Wales (31) and the 21% to 40% observed in Belgium (23). Potential explanations for these differences could include variations in methodologies employed to measure TSH, the time the sample was taken and factors surrounding the pregnancy and birth of these babies. For example, within the Belgian cohort, various methods were employed to measure TSH levels over a 3-year period. Despite the authors' best efforts to standardise results, some methodological bias may have remained, resulting in a higher percentage of TSH values greater than 2 mIU/L observed as compared with those in the current study. It has also been suggested that the timing of blood sample collection can impact on results observed (45).

There was variation between the 3 studies as to when TSH samples were collected. For example, samples in the Welsh cohort were collected 5 to 8 days after birth; samples within the Belgian cohort were collected 3 to 5 days after birth; and samples within the current study were collected 5 to 7 days after birth. Guidance on when samples are collected appears to differ depending on national screening programme standards, therefore the timing of samples should be taken into consideration when comparing results across studies.

In addition, factors surrounding the pregnancy and birth of babies that may impact on TSH values are not always readily available for inclusion in studies. For example, birth weight (23) and the use of iodine-containing antiseptics are just 2 of the factors that have been associated with variations in TSH values (46). Therefore some of the differences observed between the current cohort and those within the Welsh (31) and Belgian populations (23) could be attributed to these factors.

Despite the overall frequency of blood TSH values greater than 2 mIU/L being substantially lower than previously reported values, within the current cohort in Northern Ireland, there was a tendency towards a year-on-year increase in the proportion of blood TSH values above 2 mIU/L. Frequency of values above 2mIU/L increased from 7.5% in 2000 to 9.8% in 2014. A similar trend was also observed in babies born in the ROI between 1995 and 2006, where a shift towards values at the higher end of the normal range (that is, greater than 2 mIU/L) was reported (25). The authors suggested that the greatest increase in the proportion of values above 2 mIU/L (which occurred in 2003) coincided with reports of a decline in maternal urinary iodine, thus supporting a proposed association between maternal dietary iodine intake and foetal thyroid function (25). However, it is difficult to be certain whether a similar scenario exists within our cohort as currently there is no published study investigating maternal urinary iodine intakes in pregnant women living in NI.

Some preliminary data from this research does, however, suggest that pregnant women in NI have low intakes of foods typically considered as rich iodine sources (46) (as measured using an FFQ during each trimester). Within this study, 57% of women surveyed reported consuming less than 280 mls of milk per day during their first trimester. Furthermore, self-reported fish intakes were low with only 13

out of 241 participants (5%) consuming fish more than once a week. The authors concluded that, within this particular cohort, diet alone may not be adequate to meet iodine recommendations in pregnancy. Therefore, when maternal urinary iodine excretion data during pregnancy does become available within a Northern Irish population, investigating any coinciding increase in mildly elevated TSH values may be beneficial in monitoring the possibility of deficiency arising.

Results of this study appear to be complementary to, or fit well with, those reported in the survey of iodine status in teenage girls living on the island of Ireland that identified iodine sufficiency. Furthermore, similar to previously published studies (23, 25), a seasonal trend in iodine status was observed among babies born in NI between 2000 and 2014. Babies born in spring or summer months (March to August) had significantly higher TSH levels (indicating lower maternal iodine stores) compared with those born in autumn or winter months (September to February). This highlights that dietary advice provided to pregnant women may require some form of seasonal adaption.

Although sufficient iodine intakes during any pregnancy are vital, women who are pregnant during summer months may be particularly vulnerable to deficiency. This could in part be due to previously reported seasonal variations in dairy produce (a principal source of dietary iodine in UK and Irish diets (13, 18)) with lower levels of iodine found in dairy foods, such as milk, in summer than in winter months (18, 19).

Strengths of the study

The strengths of this study include that it is the first to examine trends in neonatal TSH values of infants born in recent years in NI. Furthermore, results of the current study provide further evidence that can be used to query the effectiveness of using the WHO cut-off point – a rate of occurrence reaching 3% of TSH values above 5 mIU/L – to identify iodine sufficiency (43).

Limitations of the study

It should be noted that this study had also planned to investigate neonatal TSH trends within the ROI. This would have been particularly informative as, in comparison with NI (where there is little or no data available on the iodine status of pregnant women), within the ROI there has been an observed decline in median UI excretion in pregnant women in their first trimester and in non-pregnant women of childbearing age (12, 13). If it had been possible to access ROI TSH data, it would have allowed further insight into maternal urinary iodine status in more recent years and built on the previous study of TSH values in the ROI conducted in infants born between 1995 and 2006 (25). However, unfortunately it did not prove possible to access these data.

In conclusion, whilst using the WHO cut-off point of a rate of occurrence reaching 3% for levels greater than 5 mIU/L (24), the distribution of blood-spot TSH data from newborn babies in NI does not

support the hypothesis, or idea, that the population is iodine deficient. However, it has been suggested that this figure may not be sensitive enough to reveal mild iodine deficiency (which would be of greater concern in the UK and Ireland) and, as previously discussed, may be affected by other variables, such as birth weight (23) and use of iodine-containing antiseptics (47). Further studies are therefore needed to clarify TSH cut-offs associated with mild deficiency and to correlate neonatal TSH values with maternal urinary iodine levels in NI. Whilst the measurement of urinary iodine excretion is still considered the best indicator of iodine status, further evaluation of the feasibility and usefulness of readily available neonatal blood TSH values on the island of Ireland is of interest.

Despite the current study reporting iodine sufficiency for babies born in NI over the past 14 years, it is advisable to continue to review the blood-spot TSH data that is routinely collected on the island of Ireland to identify any emerging trends in iodine status, particularly those that would be considered indicative of early iodine deficiency.

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Appendices

Appendix 1: Food frequency questionnaire and demographic questionnaire

You or your parent/guardian(s) can contact the school or researchers at any time.

Dr Lesley Hamill, Postdoctoral Research Fellow, Institute of Clinical Science Block B, Grosvenor Road, Belfast BT12 6BJ. Tel: +44 (0) 28 90632764, +44 (0) 7841816946. E-mail: l.hamill@qub.ac.uk

Professor Jayne Woodside, Institute of Clinical Science Block B, Grosvenor Road, Belfast BT12 6BJ. Tel: +44 (0) 28 90632585. E-mail: j.woodside@qub.ac.uk

Food frequency questionnaire

Please provide your answers in pen

Study I.D. _____

Date: _____

- This is a **2 page questionnaire**
- Your answers are confidential and will only be used for this research
- Please answer every question, if you are unsure about how to answer please do the best you can
- This questionnaire is designed to tell us about how often **on average** you eat certain foods (containing iodine)

Please put a tick (✓) on **every** line							
Foods	On average how often have you eaten the following in the last year?						
Fish and meat	Never or less than once/month	Once in 2 weeks	Once a week	2-3 times per week	4-6 times per week	Once a day	More than once a day
White fish (fresh or frozen): e.g. cod, haddock, plaice, fish fingers, fish in batter							
Oily fish (fresh or canned) e.g. salmon, tuna, mackerel, sardines, kippers							
Shellfish, e.g. prawns, crab, mussels							
Meat, e.g. beef, pork, lamb							
Poultry, e.g. chicken, turkey							
Dairy products	Never or less than once/month	Once in 2 weeks	Once a week	2-3 times per week	4-6 times per week	Once a day	More than once a day
Cream: single, double, clotted							
Full fat or greek yoghurt							
Low fat yoghurt							
Dairy desserts (e.g. custard, rice pudding)							
Cheese e.g. cheddar, soft cheese							

Butter							
Milk							
What type of milk do you most often use?	Cows'		Soya	
	Goats'		Other (e.g. almond, rice drink)	
Do you use organic milk?	Yes		No	
How much milk do you drink each day? Include milk with tea, coffee, cereals etc.	None		Between ½-¾ pint (280-424 ml)	
	Less than ¼ pint (140 ml)		Between ¾-1 pint (425-570 ml)	
	Between ¼-½ pint (140-279 ml)		More than 1 pint	
Iodised salt				
Do you use iodised salt? Please note that this is labelled as "iodised"; it is **not** rock salt, sea salt or ordinary table salt	Yes		No	
Egg				
How many eggs do you eat per week?	None		Three	
	One		Four	
	Two		More than four	
Nutritional supplements				
Do you take kelp or seaweed supplements?	Yes		No	
Do you take a multivitamin and multimineral supplement?	Yes		No	
If yes, please give details below				
Brand (e.g. Sanatogen, Seven Seas)				
Dose (e.g. number of tablets per day)				

Demographic questionnaire

Please provide your answers in pen

Study I.D. _____

Date of urine collection _____

Time of urine collection _____

Fill out date and time of urine collection in the morning

What is your date of birth?

What is your current address (including postcode, if you have one)?

How long have you lived at this address?

If you have lived at a different address in the past 2 years please provide your previous address (including postcode, if there was one):

What is your ethnic background (tick most appropriate box)?

White ☐

African ☐

Asian ☐

Other ☐

Please provide further details if you have ticked other

Appendix 2: Standard operating procedure for urine and tap water sample collection and processing

This work is conducted within the Nutrition and Metabolism Group, Centre for Public Health, Institute of Clinical Science B, Grosvenor Road, Belfast. Processing and storage of the samples is carried out in Laboratory B, Lower Ground Floor, Pathology Building, Grosvenor Road, Belfast. Personnel working on this study must have undergone an induction including 'Basic Laboratory Skills' and 'Introduction to COSHH' with Dr Sarah Gilchrist and Christine Belton (Floor Managers for the Lower Ground Floor and Ground Floor Laboratories of Pathology respectively). Personnel also need to be familiar with the following (they each must have read this document and signed the necessary record, stored with Cyril McMaster, Chief technician for the Centre):

- PRA-002-B: CoSHH risk assessment for handling and processing urine [Lab B]
- TRA-002-B: TASK risk assessment for handling and processing urine [Lab B]

Section 1 - Contacting Schools

Schools are contacted in the first instance using the letter specifically for schools (ethically approved), which is addressed to the Head Teacher. Follow-up telephone calls are made a few days after letters are issued, and continue to be made until the Head Teacher or a member of staff who may have been asked to deal with it are reached. Often, documents may need to be emailed at their request – or if contacting the School via telephone proves difficult, an email may help. If the School is keen to participate in the study, set-up a suitable time for the study visits. In advance of the visits complete the following checklist with the teacher in charge.

- Have they got PowerPoint and will it be available for the first visit
- Will there definitely be a teacher present at all times
- Are the school happy with the incentive (each girl receives £5/5Euro voucher on completion)
- How many girls will be present – to prepare packs
- Would they like a copy of the presentation in advance

Prepare the appropriate number of packs – each pack needs to be numbered in advance. Each pack must contain

- A5 step-by-step instruction leaflet
- Letter for the parent/guardian
- Participant information leaflet and 2 x consent forms
- Combined food frequency questionnaire and demographic questionnaire

- Urine sample collection kit
- Red poly bag for return of the urine sample (for discretion)

Section 2 - School visits

- Contact the School the day before the study visit to confirm everything is still okay.
- Pack the boxes, with the packs into the car.
- Make sure the 'example' pack has been included, a laser pointer and that the presentation is ready on a memory pen or external hard drive.
- Bring along 2 labelled (with School name) sterilin's for the tap water sample collection.
- Bring along a batch of vouchers (make sure there are enough for the visit).

Day 1

- Arrive at the School well in advance of the study appointment and sign-in/register at reception.
- The teacher in charge will come along take you to where you need to be. Check with the teacher where the girls should return their packs to and if someone can give them their vouchers on return of their sample packs.
- Present to the girls, the 10 minute PowerPoint presentation and make sure they have ample time to ask questions – be clear about where they bring the packs back to in the morning.
- Give out the packs.
- Leave the empty black boxes behind (labelled 'Iodine Survey QUB') for the returns.
- Check what time is best to return the following day to collected returned packs. Perhaps break-time or afterwards might be best, to allow them up until then to make returns.
- Collect the 2 tap water samples from the School.

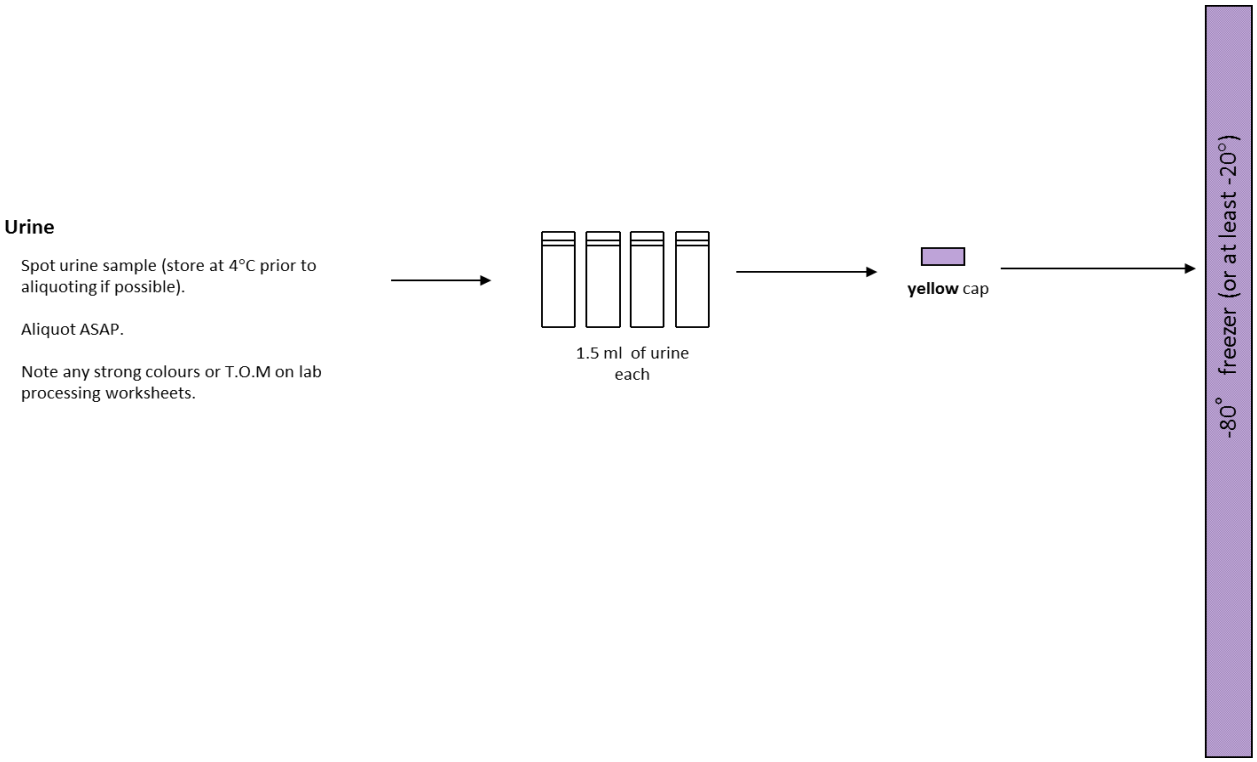
Day 2

- Arrive at the School at the given time.
- Collect the returns.
- Collect any un-used vouchers.

Section 3 - Sample collection and processing

- Once back at University, check each pack for urine sample, completed consent and completed questionnaire
- File the questionnaires
- Sign and file the consent forms (double checking for completeness)
- Log which sample numbers have come back in onto the appropriate excel file

- Make and print tube labels for each urine sample
- Label up o-rings and aliquot the samples (4 x 1.5 ml per participant)
- Make a note of strong colours, TOM and any sample where full 6 ml has not been aliquoted
- Freeze the samples in the next appropriate sartsted box, in Freezer number 38 in the Freezer Store Room (-80 °C T-Scan freezer)
- Fill out the lab processing worksheets (by hand, onto worksheets)
- Fill out the excel file HTA log
- Dispose any duplicate consents by blanking out any names in black marker and shredding
- Freeze the 2 labelled tap water samples in -20 °C T-Scan freezer (lab B)
- Contact the school with a final number recruited and many thanks



Appendix 3: Standard operating procedure for measurement of iodine in human urine samples

This work is conducted within the Nutrition and Metabolism Group, Centre for Public Health, Institute of Clinical Science B, Grosvenor Road, Belfast. The laboratory work is carried out in Laboratory A, B, C and D, Lower Ground Floor, Pathology Building, Grosvenor Road, Belfast. Personnel working on these experiments must have undergone an induction including 'Basic Laboratory Skills' and 'Introduction to COSHH' with Dr Sarah Gilchrist and Christine Belton (Floor Managers for the Lower Ground Floor and Ground Floor Laboratories of Pathology respectively). Personnel also need to be familiar with the following (they each must have read this document and signed the necessary record, stored with Cyril McMaster, Chief technician for the Centre)

- PRA-002-B: CoSHH risk assessment for handling and processing urine [Lab B]
- TRA-002-B: TASK risk assessment for handling and processing urine [Lab B]

Section 1 - Samples must be treated and stored appropriately

- It is very important the urine sample is clean i.e. has not been dip sticked by a doctor/nurse in a practice or hospital.
- The urine sample needs to be collected into a clean container but the container doesn't need to be sterilised.
- Samples need to be processed and stored as quickly as is possible – ideally on the day of collection. However, if this is not possible, the iodine should be stable for 1-2 days.
- Store the samples in either -20 °C or -80 °C freezers. If the samples are not on a monitored freezer it would be good practice to split them between two freezers. Iodine will be stable in frozen samples.
- In 24-hour urine samples measure and record the total volume of urine collected. In spot samples this is not necessary.
- Screen the urine sample and make a note on the laboratory record of colour – in particular strongly coloured urines may give abnormally low iodine results. Make a note if there is a lot of precipitation for example (although precipitate does not normally interfere with the assay) or blood in the sample.
- To measure urinary iodine, samples will be analysed in triplicate. The assay requires 50 µl per run i.e. 3 x 50 µl = 150 µl in total. Currently we store 4 x 1.5 ml samples from a spot collection.
- Ideally a fresh aliquot of urine will be used for the iodine assay. However, ideally a second aliquot of urine will be available for the creatinine assay. The creatinine assay is run as a single replicate requiring 12 µl of urine sample per run. If urine sample is limited, both assays can be performed from a single aliquot.

- The World Health Organisation (WHO) does not recommend iodine results urine to be standardised for either creatinine or volume. We will analyse the samples and express the results in µg/L. However, we will also standardise results for creatinine and for volume (in 24-hour collections) and therefore if at publication, reviewers require results to be standardised for either creatinine or volume, the results will be readily available.

Section 2 - Preparation in advance of assay

Make sodium hydroxide, 0.875 mol/L (to make up arsenious acid solution)

Chemical name: Sodium hydroxide
Formula: HNaO
Molecular Weight: 40 g/mol

- Weigh 17.5 g of sodium hydroxide into a large beaker (i.e. one which holds approx. 500 ml)
- Add approximately 400 ml of deionised H_2O into the beaker, along with a stirring bar
- Stir on a plate until all of the pellets have dissolved (note this may take quite some time) – make sure it is covered and there is a sign placed along with it, so other lab users are aware of what it contains !
- When dissolved, place the solution into a 500 ml volumetric flask and top up with deionised H_2O to the meniscus
- Invert a few times (with a stopper in) and place in a well labelled bottle – make sure the bottle is plastic if possible. There is no expiry date for this solution.

For each batch of aresnious acid, 100 ml of this is required so this will make 5 batches

Make sulphuric acid, 1.75 mol/L [to make up ceric ammonium sulphate solution

Chemical name: Sulphuric acid
Formula: H_2SO_4
Molecular Weight: 98.08 g/mol

- The acid is by Sigma-Aldrich product no 258105 and under the FAQ section on Sigma-Aldrich UK, they stipulate that the molarity is 18 M i.e. concentrated, so it can be used neat (!WITH CARE!) for the asenious acid solution
- To make 1.75 mol/L for the ceric ammonium solution it needs to be diluted by 10.3x
- 500 ml is required, each time the ceric ammonium sulphate solution is being made – so LH suggests making a large batch of just over 2.5 L (thus would allow for 5 batches)
- Remember: always add acid to water

- Carefully measure exactly 2,325 ml of H_2O into a clean Winchester (*LH suggests washing the Winchester in the dishwasher on a high heat, drying in the oven, and leaving to cool beforehand*)
- Set the base of the Winchester in a large igloo filled with ice, in a fume hood.
- Within the fume hood, measure (carefully) 250 ml of conc. sulphuric acid.
- Slowly add the conc. sulphuric acid to the water.
- Once made – LABEL WELL and store in a locked, acid cabinet, ready for use.

Make arsenious acid solution

Chemical name: Arsenic (III) oxide

Formula: ASO_2O_3

Molecular Weight: 197.84 g/mol

Work in a fume hood with double gloves, goggles and face shield at all times

! This solution takes approximately 2-3 days to dissolve!

- Mix 5 g arsenic trioxide with 100 ml 0.875 mol/L sodium hydroxide solution (using volumetric flask – take good care) – mix well – note that this may not dissolve
- CAREFULLY ! add 16 ml of concentrated (18 M sulphuric acid) (3 X 5 ml + 1 x 1 ml), swill after each addition and allow to cool thoroughly before adding the next [while keeping count]
- Once the full 16 ml has been added, leave it to stir on a magnetic plate and weigh out 12.5 g of sodium chloride
- Place the sodium chloride in a 500 ml volumetric flask and add the now cooled solution
- Top up to 500 ml with deionised water
- Leave this to stir (with a sign – it is important other lab personnel are made aware of how dangerous this mixture is CARCINOGENIC, TERATOGENIC, MUTAGENIC) until completely dissolved (may take 2/3 days)
- Once dissolved, filter it (using the filter system in Lab B, LGF) before placing into a DARK bottle with 6 months expiry date on
- Store in fume hood at RT
- Any excess solution needs to be disposed of into a glass waste bottle, in the fume hood which needs to be taken away and treated by a chemical company
- To rinse any glassware (including the filter glassware), fully fill a sink in the laboratory with water, and rinse glassware into it i.e. thus diluting and flushing the chemical as much as possible, as it is not allowed to enter drains
- Dispose of into designated waste bottle

Note this makes 500 ml in total – need 100 µl per sample i.e. 9.6 ml per 96 well plate – 500 ml will make 50 plates

Make ceric ammonium solution

Chemical name: Ammonium cerium (IV) sulphate dihydrate

Formula: $H_{16}CeN_4O_{16}S_4.2H_2O$

Molecular Weight: 632.55 g/mol

- Work in a fume hood with double gloves and goggles at all times
- Dissolve 6 g ammonium cerium (IV) sulphate dihydrate in a beaker along with 1.75 mol/L sulphuric acid – mix well on a stir plate (with a flea)
- Once dissolved completely, top up to 500 ml in a volumetric flask – taking care!
- Transfer to a DARK bottle, label well and store @ RT, in fume hood (6 months expiry date on)
- Dispose of into designated waste bottle

Note this makes 500 ml in total – need 50 µl per sample i.e. 4.8 ml per 96 well plate – 500 ml will make 10 plates

Make standards

Chemical name: Potassium Iodate

Formula: KIO_3

Molecular Weight: K = 39.10, I = 129.91, O = 15.999*3 = 47.997 = total 214.00 g/mol

- Dissolve 168.8 mg potassium iodate in 100 ml H_2O (using volumetric flask – take good care) – mix well
- Top stock (label clearly with 1000 mg/**L** iodine standard)
- Dilute this stock 1,000 fold i.e.:
    - Take a 1 L volumetric flask & fill almost to the meniscus with H_2O
    - Add 1 ml (with a pipette) of 1000 mg/L iodine standard
    - Mix well (label clearly with **1000 µg/L** iodine standard)
- Do not mix up the 1000 mg/L & 1000 µg/L solutions – these can both be stored long term, in the cold room.

[Calcn check: 1+999 = 1000/1 = 1000 x diln]

- Serial dilute the **1000 µg/L** solution to achieve a standard curve as follows:
- **500 µg/L =** 600µl H_2O + 600µl **1000µg/L** MIX WELL (in o-ring)
- **200 µg/L =** 1200µl H_2O + 300µl **1000µg/L** MIX WELL (in o-ring)
  100 µg/L = 900µl H_2O + 100µl **1000µg/L** MIX WELL (in o-ring)
- **50 µg/L =** 900µl H_2O + 100µl **500µg/L** MIX WELL (in o-ring)
- **20 µg/L =** 900µl H_2O + 100µl **200µg/L** MIX WELL (in o-ring)
- **10 µg/L =** 900µl H_2O + 100µl **100µg/L** MIX WELL (in o-ring)

If desired, this curve can be made in a large batch, and stored in the cold room, long term

Make ammonium persulphate *** FRESH DAILY ***

Chemical name: Ammonium persulphate

Formula: $H_8N_2O_8S_2$

Molecular Weight: 228.20 g/mol

SOP

- Weigh 6 g ammonium persulfate into a small glass vial
- Pipette 20 ml of deionised water into the glass vial (4 x 5 ml)
- Mix well by inverting
- Cover in tin foil
- Dispose of into designated waste bottle (at the end of each day)

Make spike mix for recovery sample

- Spike the low urine QC sample with a final concentration of 50 µg/L and 100 µg/L in each plate
- While making the standards, make a batch of spike mix for each of these as well
- For the 50 µg/L spike, make a batch of 250 µg/L stock (250 µg/L = 9 ml H_2O + 3 ml 1000µg/L) into a small glass vial
- For the 100 µg/L spike, make a batch of 500 µg/L stock (500 µg/L = 6 ml H_2O + 6 ml 1000µg/L) into a small glass vial
- To assess recovery 40 µl of the low urine QC will be spiked with 10 µl of the appropriate spike mix

Section 3 - Running a plate according to Ohashi *et al.*, 2000

- First thing each day switch the oven on in lab d, to heat up (100 °c)
- -Make up the fresh ammonium persulfate solution
- -Lift out the high and low-iodine qc urine samples to defrost (stored in lab d, at -20 °c) and the standards (stored in lab d, at 4°c)

On the bench top

- Pipette the calibrators, QC samples and 'unknown' urine samples (50 µL each) into the wells of a polypropylene plate (PP) (labelled plate 1) [nb it needs to be polypropylene to withstand the oven]
- To each well in the plate, add 100 µL of ammonium persulfate solution
- Set the PP plate into the sealing cassette, close the cassette tightly
- Place in the pre-heated oven for exactly 90 min at 100°C [use a stopwatch to time 90 min]

- Collect ice in a tray (a few minutes before the plate is due out of the oven), and leave beside the oven for the cooling phase
- After digestion, remove the cassette carefully from the oven, with oven gloves and place the cassette onto the ice for 20 min [use a stopwatch to time 20 min], being careful not to 'cover' the plate in ice – allowing to cool to RT, to stop the digestion
- Open the cassette and transfer 50 µL aliquots of the resulting digests to corresponding wells of a clean plate (labelled plate 2)

In the fumehood

- To each well in the fresh plate, add 100 µL of arsenious acid solution and 50 µL of ceric ammonium sulfate solution
- Note the ceric needs to be added quickly (within 1 min)
- Allow the reaction mixture to sit for exactly 20 min at RT [use a stopwatch to time 20 min]
- Measure the absorbance at 405 nm with a microplate reader

Note according to WHO

- 50-99 µg/L mild deficiency
- 20-49 µg/L moderate deficiency
- < 20 µg/L severe deficiency
- UK Vanderpump et al study = median excretion of 80.1 µg/L

Appendix 4: Milk sampling collection standard operating procedure

Persons responsible for milk collection

An individual will be designated responsible for the collection of milk samples at each of the 7 sampling sites on the island of Ireland – Belfast, Londonderry/Derry, Cork, Galway, Sligo, Roscommon and Dublin. The post-doctoral researcher, based in Queen's University Belfast, working on the Iodine study, will post the following items to each party responsible for sampling once a postal address has been received by email:

- 50 x plastic 15 ml storage tubes
- 1 x cardboard storage box per site (labelled by post-doctoral researcher Lesley with site e.g. Belfast, Cork etc)
- Permanent marker pens

Sampling schedule

Milk samples will be purchased every 2 months as set out in the sampling timetable (Table 16). Starting at the end of April 2014, post-doctoral researcher will send a reminder email to purchase milk samples. The post-doctoral researcher will do so before each sampling month as a reminder.

Table 16: Milk sampling timetable

May 2014
July 2014
September 2014
November 2014
January 2015
March 2015

Sampling procedure

- Visit 1 supermarket within the first week of each month (where possible). Depending on what is available on the day, please purchase up to 5 different semi-skimmed milk samples (e.g. supermarket brand, local brand, organic brand, national brand). Collect and keep the receipt for the purchase.
- Place a single sample of each of the milks collected into a 15 ml plastic storage tube. Label the tube in permanent marker with a letter, starting with A for the first sample and continuing sequentially though the alphabet.
- Fill the tube up, but not right to the top, to allow some room for expansion in the freezer.
- Place the tubes into the cardboard storage box and keep in a freezer at -20 °C. A domestic freezer is perfectly suitable.
- Keep and enjoy the remainder of the milk.
- At the end of the sampling period, any one person will have a maximum of 30 samples (5 milks x 6 collection time points). The box provided allows for storage of up to 49 samples. The dimensions of the box are 152x152x130 mm, and so therefore should not take up too much room in the freezer.
- Fill out the collection information for each milk into Table 17 where there is space for a maximum of 30 samples. Please follow the examples given in the table.
- Once sampling has been completed i.e. in March 2015, the post-doctoral researcher will make contact with each of the persons responsible for milk collection, to arrange collection/delivery of the samples for iodine analysis in Belfast, and reimbursement of costs.

Table 17: Information to be collected for milk samples

Milk sample label	Milk sample details	Where purchased	Date of purchase
Example A	Tesco NI semi-skimmed milk	Tesco, Antrim	03.04.2014
Example B	Cravendale semi-skimmed milk	Tesco, Antrim	03.04.2014
Example C	Yeo Valley semi-skimmed milk	Tesco, Antrim	03.04.2014
Example D	Dale farm semi-skimmed milk	Tesco, Antrim	03.04.2014
Example E	Dale farm organic semi-skimmed milk	Tesco, Antrim	03.04.2014
A			

Appendix 5: Results from the Ensuring Quality in Urinary Iodine Procedures (EQUIP) programme

The QUB laboratory participates in the Centers for Disease Control and prevention (CDC) EQUIP scheme (Ensuring the Quality of Urine Iodine Procedures) program[2]. Samples of unknown iodine content are shipped to the laboratory, assayed and data returned to the co-ordinating centre. Table 18 and

Table 19 confirm that QUB laboratory values for these unknown samples were within the expected range thus providing quality assurance for the assay.

Table 18: Ensuring Quality in Urinary Iodine Procedures (EQUIP) Round 39 (October 2014)

Sample ID	UI 100623	UI 100654	UI 100675	UI 100695
CDC target value (µg/L)	89.20	437.00	184.39	49.10
Mean E-182 (µg/L)	93.2	428.5	183.4	51.1
Acceptable range	66.10 to 111.15	371.45 to 502.55	147.51 to 221.27	34.37 to 63.83

Table 19: Ensuring Quality in Urinary Iodine Procedures (EQUIP) Round 41 (June 2015)

Sample ID	UI 100618	UI 100657	UI 100668	UI 100671
CDC target value (µg/L)	398.80	359.19	136.70	34.20
Mean E-182 (µg/L)	347.9	325.4	138.6	35.9
Acceptable range	66.10 to 111.15	371.45 to 502.55	147.51 to 221.27	34.37 to 63.83

[2] https://www.cdc.gov/labstandards/equip.html

*safe*food:

7 Eastgate Avenue, Eastgate, Little Island, Co. Cork

7 Ascaill an Gheata Thoir, An tOiléan Beag, Co. Chorcaí

7 Aistyett Avenue, Aistyett, Wee Isle, Co. Cork

Tel: +353 (0)21 230 4100 ***Fax:*** +353 (0)21 230 4111

Email: info@safefood.eu ***Web:*** www.safefood.eu