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National Diet and Nutrition Survey

Assessment of salt intake from urinary sodium in adults (aged 19 to 64 years) in England, 2018 to 2019

Appendices

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Public Health England, Wellington House, 133-155 Waterloo Road, London SE1 8UG
Tel: 020 7654 8000
www.gov.uk/phe
Twitter: @PHE_uk
Facebook: www.facebook.com/PublicHealthEngland

Prepared by: Robert Ashford,[1] Kerry Jones,[2] Dave Collins,[3] Kate Earl,[1] Stephanie Moore,[3] Albert Koulman,[2] James Yarde,[1] Beverley Bates,[1] Polly Page,[3] and Gillian Swan[4]

[1] NatCen Social Research, [2] National Institute of Health Research Biomedical Research Centre Nutritional Biomarker Laboratory (NIHR BRC NBL) at the MRC Epidemiology Unit (University of Cambridge), [3] National Institute of Health Research Biomedical Research Centre Diet, Anthropometry and Physical Activity Group (NIHR BRC DAPA) at the MRC Epidemiology Unit (University of Cambridge), [4] Public Health England

For queries relating to this document, please contact: gillian.swan@phe.gov.uk

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Appendix A: Methodology: 2018/19 England Sodium Survey (adults aged 19 to 64 years)

A.1 Sample design and sample size

In line with previous urinary sodium surveys, the sample size calculation was based on the comparison of dietary salt intake between surveys. As the distribution of the 24-hour urinary sodium excretion and salt intake data is skewed, the sample size calculations reflect a reduction in salt intake on the log scale (or analogously a percentage reduction in geometric mean salt intake).[i] A 0.5 g reduction in salt intake was deemed scientifically meaningful; this equates to a 7% reduction from the level seen in 2014.[1] Data from the 2011[2] and 2014[1] sodium surveys were used to estimate the average variability in salt intake (standard deviation (SD) = 0.435 on the natural log-transformed salt intake scale). In order to achieve 80% power and assuming a statistical significance level of 5%, a sample size of 565 complete 24-hour urine collections representative of the population of England aged 19 to 64 years was required to detect a 7% reduction in salt intake between surveys.

Participants were sampled from the Health Survey for England (HSE) 2017 cohort.[ii] The HSE sample was designed to be representative of the population living in private households in England. Full details of the HSE sample design are reported in the Health Survey for England 2017 Methods Report.[3]

To be eligible for the 2018/19 England Sodium Survey, HSE 2017 participants had to be within the required age range (that is, aged between 19 and 64 years at the time of 2018/19 England Sodium Survey), have agreed to be recontacted about future research and have provided a telephone number for recontact.

A.2 Participant selection

All HSE 2017 households containing eligible individuals (see section A.1) were included in the sample for this survey. In households with 1 or 2 eligible adults, all were included. In households containing 3 or more eligible adults, 2 were randomly selected. The England Sodium Survey 2014 sample[1] was skewed towards older adults; so in order to

[i] See chapter 2, section 2.7 for detail of the survey's statistical methodology.
[ii] The previous two standalone sodium surveys in England (2011 and 2014) used a Random Digit Dialing sample design.[1,2]

increase the number of younger adults in the 2018/19 England Sodium Survey, participant selection was carried out as follows:

1. In households where all adults were aged 19 to 34 years and in households where all adults were aged 35 years or older, up to 2 adults were selected with equal probability.
2. In households containing at least 1 adult aged 19 to 34 years and at least 1 adult aged 35 years or older, 2 adults were selected with unequal probability by increasing the probability of selection for those aged 19 to 34 years by a factor of 1.35 (that is, younger adults were 35% more likely to be selected than those aged 35 years or older)[iii].

The final sample, issued to NatCen's Telephone Unit (TU), comprised 3,575 selected participants[iv] in 2,540 households.

A.3.1 TU training

Prior to starting work on the survey, TU interviewers attended a half-day training session which covered the background and purpose of the survey and their role in recruiting participants. Interviewers were also given detailed written project instructions covering the aims of the survey, methodology and fieldwork procedures.

The survey was referred to in the field as the 'Diet and Health Study' to minimise the risk of participants changing their diets. Telephone interviewers (and nurses) were briefed not to mention salt but instead to say that we were interested in measuring electrolytes such as potassium in the diet.

A.3 Participant recruitment

The 3,575 individuals identified from HSE 2017 as being eligible were sent an advance letter inviting them to take part in the 2018/19 England Sodium Survey. Participants were recruited by NatCen's TU interviewers.

Individuals were batched into households to avoid unnecessary repetition of contact attempts by TU interviewers. The TU interviewers attempted to contact households using the telephone number(s) provided during the HSE 2017 interview. Once contact had been made with a household, eligibility/willingness was determined for all selected

[iii] As the England Sodium Survey 2014 sample was also skewed towards women, oversampling men for the 2018/19 survey was also considered. However, it was determined that oversampling men (as well as younger adults) would result in a lower effective sample size.

[iv] Twenty individuals were originally identified but had to be removed from the issued sample due to incomplete contact details.

participants. If successfully contacted, a Computer Assisted Telephone Interviewing (CATI) script was used to introduce the survey and check the eligibility of the selected participants. Individuals were asked if they had moved address[v] or were pregnant/breastfeeding (women only) since the original HSE 2017 interview. The TU interviewers also ascertained potential language barriers to the individual being able to provide informed consent. Agreement was then sought for a nurse to contact the selected participant(s) in order to arrange a home visit for collection of the 24-hour urine sample(s). Each household that agreed to take part received a letter thanking them for their agreement to take part in the survey and informing them that the nurse would be in touch shortly to arrange a visit.

A.4 Nurse training

All nurses attended a full-day briefing. The briefings covered all elements of the survey including aims, background and methodology, fieldwork procedures and documentation, the CAPI (Computer Assisted Personal Interview) questionnaire and a practical demonstration of the equipment used to collect urine and the despatch procedures. To ensure that all nurses followed the same, standard protocol, training in the weighing and sub-sampling of the urine collection was provided. Further, nurses were accredited to ensure they could accurately measure the mass of the filled urine container using the spring balance. Nurses were given detailed written project instructions covering the aims and objectives of the survey, fieldwork procedures and methodology.

A.5 Nurse contact and first visit

The nurse made initial contact with the participant(s) via telephone. The nurse then visited participating households twice: the first visit to explain the collection protocol and provide the participant(s) with the collection equipment and the second visit to take a subsample of the urine collection.

The purpose of the first nurse visit was to:

- encourage the participant(s) to take part and answer any questions they may have had
- ensure the participant was eligible
- provide the participant(s) with detailed leaflets about PABA and the urine collection instructions (see appendix D)
- obtain written consent and deliver the equipment

[v] For fieldwork management reasons, individuals who had moved since their HSE 2017 interview were not followed up at their new address.

- randomly allocate a date, via the CAPI programme, for when the participant(s) would carry out the 24-hour urine collection
- provide the labelled Urine Collection Sheet
- book an appointment for the second visit (usually the day, or the day after, the 24-hour urine collection had finished)

The nurse completed an appointment card for the participant(s) to serve as a reminder of when the nurse would return to pick up the urine sample(s).

A.6 Urine collection protocol

After obtaining written consent (see appendix D), the nurse instructed participants in the 24-hour urine protocol. Participants were asked to collect all urine during a 24-hour period starting from the second morning urine pass of the 24-hour collection day and ending with the first urine passed the following morning. The nurse used the CAPI programme to randomly allocate a day of collection for the participant(s). If the allocated date was unsuitable for the participant, CAPI would allocate an alternative start day. Participants often preferred to do their collection on a weekend day but in order to give an even representation across the week nurses asked participants to collect on a Monday to Friday if a weekday was the day allocated by CAPI, explaining that diet may differ between weekdays and weekends. Women were guided to collect their urine when they were not menstruating, however samples were accepted if collected during menstruation.

To perform the 24-hour collection, participants were provided with:

- a 5-litre capacity screw cap plastic container (jerry can) to serve as the collection container for urine
- a 2-litre capacity screw cap plastic container for collections made away from the home. This was also used as an overflow container should the participant fill the 5-litre jerry can
- a 1-litre plastic jug, kept inside a re-sealable plastic bag when not used
- a funnel kept inside a re-sealable plastic bag when not used
- a plastic carrier bags for transporting the equipment away from home
- an aide-memoire safety pin for the participant to pin the under- and outer- garments together during the period of the collection to remind that the specimen of urine about to be passed should be collected
- 3 PABA tablets to be taken to verify completeness of the 24-hour collection
- coloured stickers to distinguish equipment between 2 participants in the same household

Participants were instructed to pass urine into the 1 litre plastic jug, and then pour the sample into the 5 litre collection container using the funnel provided. Plastic bags were provided for participants to carry the equipment (including the smaller 2 litre collection container) if they were not at home for some of the collection period. Participants were asked to take 1 PABA tablet at 3 evenly spaced intervals throughout the day of the collection. Participants were still eligible to take part if they were willing to carry out the 24-hour urine collection but did not want to (or could not) take PABA.[vi] Before leaving the household, the nurse recorded the participant details, the agreed start date of the 24-hour collection and whether the participant had consented to take PABA tablets on a Urine Collection Sheet (see appendix D). This sheet was then completed by the participant during the collection period. They were required to record the time they took the PABA tablets, the start and finish times of their urine collection, any missed urine passes or spillages, and any medication or supplements taken during the collection period.

A.7 Second nurse visit

The second nurse visit took place on the day or the day after the 24-hour urine collection was completed. The nurse collected 2 sub-samples from the 24-hour urine sample and disposed of the remaining urine and equipment.

To do this the nurse was supplied with:

- Salter Brecknell ElectroSamson digital hand-held scales for weighing the urine collection container (set to kg)
- 2 x 10 ml Sarstedt Urine syringe and 2 extension tubes for urine monovettes for aliquoting urine
- disposable gloves, apron, disposable work mat for handling the urine
- jiffy bag and packaging material for despatching the samples
- participant-specific pre-printed labels for the filled monovettes.

The container with the 24-hour collection was weighed twice by the nurse and the weight recorded on the despatch sheet and in the CAPI programme. The nurse then thoroughly mixed the urine by repeated inversion of the container before carrying out the sub-sampling procedure into labelled monovettes. Then the nurse discarded the remaining urine. The nurse also checked that the Urine Collection Sheet was complete (asking the participant for any missing information), paying particular attention to the start and end time, missed collections or spillages or missed PABA tablets and any medications/ supplements taken during the collection period. This information was entered into CAPI. The nurse then packaged and posted the samples, Urine Collection Sheet, PABA blister pack and despatch paperwork to the laboratory at MRC Epidemiology Unit.

[vi] Participants who were allergic to vitamin preparations, hair dyes or sunscreen lotions were not asked to take PABA.

A.8 Assessment of completeness of urine collection

Para-aminobenzoic acid (PABA) was used in the 2005/06 England Sodium Survey and in all UK government sodium surveys since. The use of PABA in the validation of 24-hour urine collections was established by Bingham and Cummings and has since been used widely in studies and surveys to assess completeness of 24-hour urine collections.[4]

The useability of a urine collection was determined by an algorithm (see appendix C). The PABA test for completeness of 24-hour urine collection relies on the assumption of complete absorption and urinary excretion of PABA metabolites.

Where participants reported taking the three 80 mg PABA tablets at appropriate intervals and urine collection time was within 20 to 28 hours, 24-hour collections were considered to be complete if they contained between 70% and 103% of the PABA. Urine collections with a PABA recovery < 70% were considered incomplete, while those with a PABA recovery greater than 103% were considered unfeasibly high and therefore unreliable. Complete collections (those with a PABA recovery of between 70 and 103%) were included in the results, while collections deemed incomplete (<70%) or unfeasibly high (>103%) were not included in further calculations of salt intakes. No correction factors were applied to the sodium data based on PABA recovery.

Individuals who could not take or elected not to take PABA but recorded they had completed a 24-hour urine collection with no missed collections or spillages were deemed to have a complete 24-hour collection and were included in the results. In addition, participants who elected to take PABA but reported that they did not take all 3 PABA tablets yet still completed a 24-hour collection between 23 and 25 hours (with no missed urine passes) were also included. If individuals could not or did not take all 3 PABA tablets and if collection times were outside 23 to 25 hours, they were excluded from further data analysis and were not included in the results.

A.9. Weighting

There were 3 stages to the weighting. These are described in detail in the following sections but in summary, the steps were to:

- account for differences in the willingness of HSE 2017 participants to consent to being contacted about future research (see section A.1)
- combine this weight with another set of weights which corrects for unequal selection probabilities of individuals within households
- make an adjustment for different levels of non-response to this survey

The 3 points at which non-response could occur were accounted for with the resulting weights then calibrated to ONS mid-year population estimates for 2018[5] by age, sex and region.

A.9.1 Consent weights

Individuals from HSE 2017 who would be in the required age range (19 to 64 years) at the time of the 2018/19 England Sodium Survey and who consented to be contacted for follow-up research were eligible to be sampled.

The aim of the consent weights was to reduce bias caused by differences in the likelihood of individuals consenting to be re-contacted compared to those who did not give consent to be re-contacted.

To estimate the probability that an individual consented to be contacted for follow-up research, logistic regression modelling was undertaken.[vii] Individual level characteristics used as explanatory variables were: region, sex, age group, occupational classification (NS-SEC), cigarette smoking status, and household composition.

The results from the model were then used to estimate the probability of consent to be contacted for follow-up research. The consent weights (wt1) were calculated as the reciprocals of these for each of those in the sample frame (within the required age range) who consented to recontact.[viii]

A.9.2 Selection weights

All households containing at least 1 eligible individual (that is, in the required age range and who consented to be contacted for follow-up research) were included in the sample for the 2018/19 England Sodium Survey. A maximum of 2 individuals could be sampled from each household. In households with 1 or 2 eligible individuals, all such individuals were sampled with certainty (that is, they were selected with a probability of 1).

In larger households, where there were more than 2 eligible individuals, 2 were selected at random, with probability proportionate to an assigned value. The probability of selection for an individual in such households was determined by their age (that is, whether aged 19 to 34 years or aged 35 to 64 years). The probability of selection for households containing 3 or more eligible adults was:

- that all eligible adults were in the same age group (that is, all aged 19 to 34 years or all aged 35 to 64 years): 2[ix] / number of eligible adults in the household

[vii] The model was weighted by the HSE 2017 interview weight (wt_int) to account for differences in the achieved HSE 2017 interview sample compared to the population. For further details, refer to the HSE 2017 Methods Report.[3]
[viii] wt1 = 1 / Probability individual consented to recontact.
[ix] Within each household containing more than 1 eligible individual, the sum of probabilities is always equal to 2.

- to compensate for lower response rates amongst younger adults, those aged 19 to 34 years in mixed-age households (that is, containing adult(s) aged 19 to 34 years and aged 35 to 64 years) received a probability of selection boosted by a factor of 1.35
- the remaining probability of selection was then split equally between those aged 35 to 64 years
- for example, that in a household with 2 eligible participants aged 19 to 34 years and 2 eligible participants aged 35 to 64 years, those aged 19 to 34 years had a probability of selection of 0.675 and those aged 35 to 64 years had a probability of selection of 0.325[x]

The selection weights (wt_sel) were calculated as the inverse of the probability of selection. This was then combined with the HSE 2017 interview weights (wt_int) and the consent weights (wt1) to produce wt2.[xi]

A.9.3 Non-response weights and calibration

The 3 points at which non-response could occur were identified and accounted for in this stage of survey weighting. At each step, the probability of response was modelled using logistic regression, as follows:

1. **Household-level non-response**: the outcome variable at this step was whether a nurse visit to the household was arranged.[xii] Effectively, this gives the probability that an individual agreed to a nurse visit, or belonged to a household where the other sampled individual agreed to a nurse visit. The explanatory variables used to model this were: region, sex, age group, occupational classification, cigarette smoking status and Index of Multiple Deprivation (IMD) quintile. The inverse of the estimated probability that an individual's household was issued to a nurse was then combined with wt2 to produce the nurse visit weights (wt3)[xiii]
2. **Urine sample provided**: not all individuals issued to a nurse provided a urine sample. At this step, the outcome variable was whether *any* urine sample was provided.[xiv] The explanatory variables used here were: region, sex, age group, occupational classification, cigarette smoking status, IMD quintile, and population density quintile. The inverse of the estimated probability *any* urine sample was

[x] If the probability of selection was equal, p(equal) = 2/4. By boosting the probability for those aged 19 to 34 years by a factor of 1.35, P(young) = 2/4*1.35 = 0.675 each. P(old) = (2-2*0.675)/2 = 0.325 each.
[xi] This was used as the entry weight for the non-response stage of weighting and was calculated as follows: wt2 = wt_int x wt1 x wt_sel
[xii] The model was weighted by wt2, which accounts for the previously discussed sources of bias.
[xiii] wt3 = wt2 x (1 / Probability individual's household issued for nurse visit).
[xiv] The model was weighted by wt3 to account for bias preceding this step.

provided was then combined with the preceding weights (wt3) to produce the "*any* urine sample" weights (wt4)[xv]

3. **Useable urine sample given (useable sample weight only)**: not all of the urine samples collected were deemed useable for analysis. Here, whether a *useable* urine sample was provided was used as the outcome variable.[xvi] This was modelled against the following: region, sex, age group, occupational classification, cigarette smoking status, and household composition. The inverse of the estimated probability a *useable* urine sample was provided was then combined with the preceding weights (wt4) to produce the "*useable* urine sample" weights (wt5)[xvii]

Once the useable urine sample weights (wt5) had been produced, these were calibrated to ONS mid-year population estimates for 2018[5] to produce the final weights (finwt_validsamp). As such, the weighted profile of participants who provided a useable urine sample matches the profile of the population by age, sex and region. The distributions of the population and weighted and unweighted achieved sample are shown in table 12.

A.10. Statistical methodology

For data which follow a symmetric bell-shaped (Normal) distribution, the 'typical value' is best summarised using an arithmetic mean (by adding the values and dividing by the number of values). A logarithmic transformation of positively skewed data will make the distribution less skewed, enabling appropriate use of the arithmetic mean. To represent the 'typical value' of positively skewed data in the original unit (grams per day), back-transformation can be applied to the arithmetic mean of the log-transformed data; this value is known as the geometric mean.

[xv] wt4 = wt3 x (1 / Probability individual provided *any* urine sample).
[xvi] The model was weighted by wt4 to account for bias preceding this step.
[xvii] wt5 = wt4 x (1 / Probability individual provided *useable* urine sample).

Appendix B: Analytical methods and quality control procedures

B.1 Introduction

This appendix describes the analytical methods used to measure urinary analytes for the 2018/19 England Sodium Survey. The principles and procedure are detailed for each method and details of quality control materials and quality assurance processes are provided. The quality of the laboratory analyses is underpinned by rigorous monitoring and maintenance of all equipment, staff training, adherence to standard operating procedures, participation in external quality assurance and good laboratory practice. The quality control and cross-validation data described below were reviewed by Dr Elaine Gunter (Specimen Solutions, LLC), external quality advisor to the NDNS RP and the 2018/19 England Sodium Survey.

B.2 Urinary sodium and potassium analysis

B.2.1 Principles

Sodium and potassium[xviii] concentrations were measured using ion selective electrode (ISE) technologies. The analyser used was a Cobas C111 bench-top clinical chemistry analyser (Roche Diagnostics Ltd, Burgess Hill, UK).

The sodium and potassium ion selective electrodes (ISE) have a selective membrane in contact with both the test solution and a reference solution with known and fixed ion concentration. The electrical potential (electromotive force, EMF) difference between the 2 solutions is used to determine the ion concentration in the test solution. The technology is reliable, robust, selective and sensitive.

B.2.1.1 Procedure for analysing sodium and potassium

Analysis of urinary sodium and potassium was performed according to standard operating procedure.[xix] Samples were thawed on a roller mixer and centrifuged at 3,500g for 10 minutes. After daily analyser calibration, analysis was performed in batches of 7 samples together with 1 of 2 quality control (QC) materials. Sample IDs were read with

[xviii] Urinary potassium concentration was also measured. Data is not presented in this report but will be available via the UK Data Service.
[xix] NIHR BRC NBL-SOP-012 ‘Urine electrolyte analysis using Roche Cobas C111 v01’.

barcode scanners. Reference material for internal quality assurance (QA) was run at the start of each day of analysis and after calibration (see below for details).

B.2.1.2 Internal QC

Internal, commercially-prepared QC materials (Bio-Rad Urine Chemistry Controls 1 & 2 (product codes 397 and 398, Bio-Rad Laboratories Ltd, Hertfordshire, UK)) were run with each batch of 7 samples, alternating levels between each batch, to monitor between-batch precision.

Batches were accepted provided the QC result was within the manufacturer's range and also within the more stringent range determined within the laboratory (+/- 2 standard deviations of the mean value). If a repeat QC result was again out of range then the analyser was re-calibrated and the samples from that batch re-run.

Table B.1. Internal QC for sodium and potassium during the 2018/19 England Sodium Survey

	Internal QCs for sodium		Internal QCs for potassium	
	Bio-Rad 397	Bio-Rad 398	Bio-Rad 397	Bio-Rad 398
n	116	107	116	107
Mean (mmol/L)	81.3	174.7	30.3	65.8
SD (mmol/L)	1.2	1.6	0.5	1.8
% CV	1.4	0.9	1.5	2.8

B.2.1.3 Internal QA

There is no suitable urine matrix certified reference material available at the required concentrations of sodium or potassium to determine accuracy of the applied method. Therefore, commercial aqueous calibration solutions were used. For sodium, products at 2 pre-prepared levels were selected (product codes HI-7083M and HI-7088M, Hanna Instruments Ltd, Bedfordshire, UK) that were produced in accordance with ISO 3696/BS3978 using high purity salts, deionised water, certified weight-checked balances, and Class A glassware in a controlled environment. Reported values are traceable to NIST Standard Reference Material. For potassium, a 100 mmol/L high purity standard (HI-4014-01, Hanna Instruments Ltd, Bedfordshire, UK) that was diluted with ultrapure water (resistivity of 18.2 MΩ.cm) was used. Prior to the start of the 2018/19 survey, percentage inaccuracy for sodium, calculated from 63 runs, was 1.2% and 0.2% for the low and high internal QA, respectively.

Table B.2. Internal QA for sodium and potassium during the 2018/19 England Sodium Survey

	Sodium		Potassium		
	Low	High	Low	Mid	High
n	19	19	20	19	20
Target, mmol/L	51.4	100	25	50	100
Mean (SD), mmol/L	51.7 (0.5)	100.6 (0.5)	25.8 (0.4)	49.4 (1.1)	96.8 (2.5)
%CV	1.1	0.5	0.4	1.7	2.5
%inaccuracy (SD)[a]	0.6 (1.1)	0.6 (0.5)	3.1 (1.8)	-1.2 (2.2)	-3.2 (2.5)

[a] Calculated as (measured-target)/target*100

B.2.1.4 External QA

The National Institute of Health Research Biomedical Research Centre Nutritional Biomarker Laboratory (NIHR BRC NBL) is a member of the UK NEQAS for urinary sodium and potassium. This scheme sends samples quarterly to participating hospital and analytical laboratories in the UK. Results allow comparison of our own laboratory performance and, more broadly the scheme aims to improve harmonisation between manufacturers and laboratories. NEQAS samples are urine or artificial matrices spiked to simulate the range of concentrations found in human urine. Table B.3 summarises the NIHR BRC NBL performance prior to and during analysis (between February and June 2019) of sodium and potassium for the survey.

Table B.3. Performance against NEQAS for sodium and potassium during the 2018/19 England Sodium Survey

	Sodium	Potassium
n	27	27
% bias from ALTM[a]	-0.2	-4.4
SD of % bias from ALTM	4.9	2.8

[a] All laboratory trimmed mean

B.3 Urinary *para*-aminobenzoic acid (PABA) analysis

B.3.1 Principle

PABA is used for the validation of 24-hour urine collections. The test relies on the assumption of complete absorption and complete urinary excretion of PABA within 24 hours. Major metabolic products of PABA are *p*-aminohippuric acid (PAHA), *p*-acetamidobenzoic acid (PAABA) and *p*-acetamidohippuric acid (PAAHA). For analysis, these metabolites are hydrolysed back to PABA under alkaline conditions and the resultant PABA concentration determined by high performance liquid chromatography (HPLC) with UV detection at 290 nm. The ratio of PABA peak area to internal standard

peak area was compared to that of a calibration curve to determine PABA concentration in mg/L. An internal standard is added before the hydrolysis stage to compensate for any losses throughout the sample preparation procedure.

B.3.2 Procedure

Analysis of urinary PABA was performed according to standard operating procedure.[xx]

Samples were thawed on a roller mixer and then hydrolysed by addition of sodium hydroxide after addition of the internal standard (3-hydroxybenzoic acid). The samples were then heated at 110°C for 2 hours and subsequently left to cool. Samples were then neutralised with orthophosphoric acid before being filtered using a syringe attached to a 0.45 µm PTFE filter into vials for analysis on the HPLC with UV detection. The PABA HPLC method is based upon the method described by Jakobsen *et al.* (1997) modified to use methanol instead of acetonitrile as the mobile phase.[6] The ratio of PABA peak area to internal standard peak area was compared to that of a calibration curve to determine PABA concentration in mg/L.

Samples were run weekly with 3 levels of urine QC material and a hydrolysis check QC material (to assess completeness of hydrolysis) at the beginning and end of every batch. Standards were analysed with every batch.

B.3.3 Assessing completeness

The reference range for PABA excretion indicating a complete 24-hour urine collection, was 70 - 103% of the 240 mg dose (mean ± 2 standard deviations). This was established by a methodological study (Cox *et al.*, 2018) conducted at the MRC Elsie Widdowson Laboratory in 49 adults.[7] This study determined that for the current analytical HPLC method, the appropriate cut-off for completeness in healthy adults is 70% (mean -2 standard deviations) incorporating both biological and analytical variation. PABA above this range could indicate either inadequate mixing of the urine before sampling or inaccurate recording of the volume, and therefore an incorrect 24-hour sodium excretion result. Ingestion of PABA in dietary supplements would preclude assessment of completeness of urine collections by this method. Urines with PABA recovery more than 103% were excluded from the dataset.

24-hour PABA excretion is calculated by multiplying the PABA concentration in mg/L by the 24-hour volume in litres. This is then expressed as a percentage of the 240 mg PABA dose given to the participants and compared with the reference range above (see appendix C).

[xx] NIHR BRC NBL-SOP-007 ‘Measurement of PABA in urine by HPLC with UV detection’.

B.3.3.1 Internal QC

There is no commercially available QC material for PABA so in-house urine QC material was prepared from a single donor and pooled urine samples from participants who had consumed PABA to give 3 different levels of QC material. All 3 levels were analysed alongside the participant samples at the beginning and end of each batch (typically 30 to 40 urine samples). Completeness of hydrolysis was monitored by inclusion of a sample containing PAHA at the beginning and end of each batch. PAHA is hydrolysed to PABA in a 1:1 ratio so a known concentration of PAHA should yield the same concentration of PABA if hydrolysis is complete.

Batches were accepted provided the QC result was within range determined within the laboratory (+/- 2 standard deviations of the mean value). If more than 1 urine QC result or the hydrolysis check were out of range, then the batch was re-run. Table B.4 summarises the internal QC data for PABA analysis.

Table B.4. Internal QC for PABA during the 2018/19 England Sodium Survey

	Old QC1	New QC1	QC	QC3	PAHA (268 nmol/L)
n	33	40	53	54	53
Mean (nmol/L)	75.8	66.2	33.1	203.1	268.8
SD (nmol/L)	3.0	2.5	1.5	4.3	4.6
%CV	4.0	3.7	4.5	2.1	1.7

B.3.3.2 External QA

There is no external QA scheme for PABA.

B.4 Cross-over studies between laboratories and methods

Cross-over studies were performed for sodium and PABA to evaluate performance at NIHR BRC NBL against the results obtained in the 2014 England Sodium Survey for which samples were originally measured in 2014/15 by MRC Human Nutrition Research[xxi] and were kept frozen at -70°C until re-analysis.

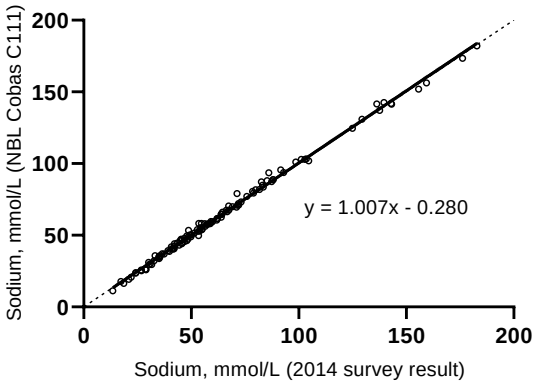
[xxi] In 2016, following restructuring and refocusing of its research interests, MRC Human Nutrition Research was renamed the MRC Elsie Widdowson Laboratory (MRC EWL). This took effect from 01 September 2016.

B.4.1 Sodium

NIHR BRC NBL used a Roche Cobas C111 analyser (the same instrument model as used to derive the method-specific factors) to measure urinary sodium in the 2018/19 England Sodium Survey. Internal precision and accuracy of this analyser at NIHR BRC NBL are described above in section B.2.1.2.

In order to evaluate performance of the Roche Cobas C111 relative to data from the 2014 England Sodium Survey, a re-analysis of 120 samples from the 2014 survey was performed on the Roche Cobas C111 instrument and compared against results obtained from the Siemens Dimension Xp and analyser that had been adjusted by the method-specific factor[xxii,1] Agreement was excellent and provides confidence in the ability to compare the 2018/19 survey data with previous survey results (figure B.1).

Figure B.1. Deming regression of urinary sodium measured at NIHR BRC NBL in 2019 against corrected data reported in the 2014 England Sodium Survey



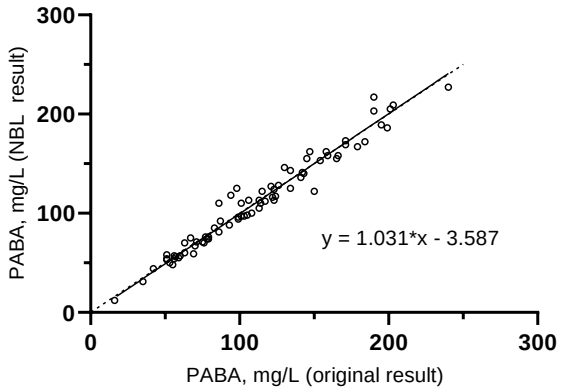
n=120. Open circles, observed data points; solid line, Deming regression line; dashed line, line of equality.

B.4.2 PABA

PABA was re-analysed at NIHR BRC NBL using the same method and equipment as in the previous surveys. Agreement between the original results from 2014 and re-analysis at NIHR BRC NBL was excellent and provides confidence that the assay performance is consistent with that during the 2014 survey data (figure B.2).

[xxii] At the time of the 2014 England Sodium Survey adjustments were applied using factors to take account of method-specific analytical biases to urinary sodium concentrations from some of the previous surveys. This was to harmonise data to facilitate comparability across the different survey assessments which used different analytical methods for sodium analysis.

Figure B.2. Deming regression of urinary PABA measured at NIHR BRC NBL in 2019 against original data analysis for the 2014 England Sodium Survey



n=76. Open circles, observed data points; solid line, Deming regression line; dashed line, line of equality.

Appendix C Determination of 24-hour urine collection completeness

C.1 Method used in the 2018/19 England Sodium Survey

Known values:

- 24-hour urine volume
- number of PABA tablets taken
- number of collection hours
- number of missed collections
- PABA concentration

First calculate the PABA %

- PABA 24hr = PABA Concentration * 24hr Urine Volume
- PABA % = PABA 24hr * 100 / 240[xxiii]

A sample completeness indicator is then determined using the following algorithm. Note that the calculated PABA % is only used if the participant claimed to have taken all 3 PABA tablets.

If [Number of PABA tablets taken] = 3 then
If [PABA %] is between 70 and 103 then
Completeness = “Complete by PABA (took 3 PABA)”
Else If [PABA %] is greater than 103 then
Completeness = “Over by PABA (took 3 PABA)”
Else If [PABA %] is less than 70 then
Completeness = “Incomplete by PABA (took 3 PABA)”

Else If [Number of PABA tablets taken] = 0, 1, or 2 then
If [Number of missed collections] = 0 then

If [Collection time] between 23 and 25 hours then
Completeness = “Complete by claim (took 0, 1 or 2 PABA)”
Else If [Collection time] less than 23 hours then
Completeness = “Incomplete by claim (took 0, 1 or 2 PABA)”
Else If [Collection time] greater than 25 hours then

[xxiii] The division by 240 is because the participant is asked to take 3 x 80 mg PABA tablets.

Completeness = “Over by claim (took 0, 1 or 2 PABA)”
Else If [Collection time] not specified then
Completeness = “Collection time not known (took 0, 1 or 2 PABA)”
Else If [Number of missed collections] greater than 0 then

Completeness = “Incomplete by claim (took 0, 1 or 2 PABA)”

Appendix D: Field documents

See separate document.

References

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[7] Cox, L., Guberg, K., Young, S., Nicholson, S., Steer, T., Prentice, A., Page, P. (2018). Validation of the use of p-aminobenzoic acid to determine completeness of 24 h urine collections in surveys of diet and nutrition. *European journal of clinical nutrition,* 72(8), 1180-1182.