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Analytical Method Development for Tritium in Tree Transpirate from the Little Forest Burial Ground.

by

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1. Introduction

The Little Forest Burial Ground (LFBG) is a near-surface low-level nuclear waste repository located within the buffer zone surrounding the Lucas Heights Research Laboratories of ANSTO (Figure 1). Tritium (^3H , 'T'), as tritiated water (HTO), was one of the radioactive substances placed into the trenches located within the LFBG (Isaacs and Mears, 1977). This material will behave conservatively in regard to any seepage from the site of deposition. As such, it should be a good indicator of groundwater movement at the site.

Water is a vital requirement of plants. Hence, it was proposed that samples from herbs and trees may be useful to assess the biologically available HTO and also provide an indication of a potential exposure for environmental dose assessment, not only for ^3H but also for the other radionuclides potentially migrating with the water from the trenches.

As part of the initial draft plan for a vegetation survey in the LFBG (Twining and Creighton, 2007) the following two null hypotheses were established:

- H_{0a} *That there is no significantly higher concentration of specific contaminants in foliage of trees growing over, or adjacent to, the pits than there is in the foliage of the same species growing away from the pits;*
- H_{0b} *That there is no correlation between contaminant levels in the seepage plume and surface vegetation.*

These hypotheses are to be tested using the acquired data. However, as part of the process of applying HTO in transpire as a monitoring tool, some method development has been required. This report covers all aspects of that development and provides a recommended approach to acquiring such data and recording the information.

2. Methodology

2.1. Sampling

As part of the initial investigation for the project, a site inspection was undertaken. On the basis of observations made at that time, a number of trees and shrubs over, and adjacent to, the burial pits were identified for use in the initial sampling runs. These plants, comprising a gum tree (*Eucalyptus* sp; Eu 1), two *Acacia* sp (Ac 1 and 2), two Turpentine trees (*Syncarpia* sp; Sy 1 and 2), a *Leptospermum* sp shrub (Le 1) and a *Banksia serrata* tree from which two branches were sampled (Ba 1.1 and 1.2) are identified in Figure 2. Apart from proximity, the other major selection criterion was ease of access to leafy tree limbs as this affects the ability to safely and reproducibly sample the vegetation.

Initial samples were collected in July 2007. Subsequent sets of samples using the same trees, and as far as possible the same branches, were collected approximately every 3 months until March 2009. In October 2007 and subsequently, samples were collected from more branches on the trees identified as Ba 1, Sy 1 and Ac 2 (Figure 2). The additional sampling was to better estimate the variability within any tree.

Transpire sampling has been a fairly straight-forward procedure. The leaves at the distal end of a limb (in the case of trees or large shrubs) or limbs (in the case of smaller shrubs) were enclosed in a large plastic bag with the open end being taped closed around the branch (or branches) with cloth tape. The sampling bags are clear

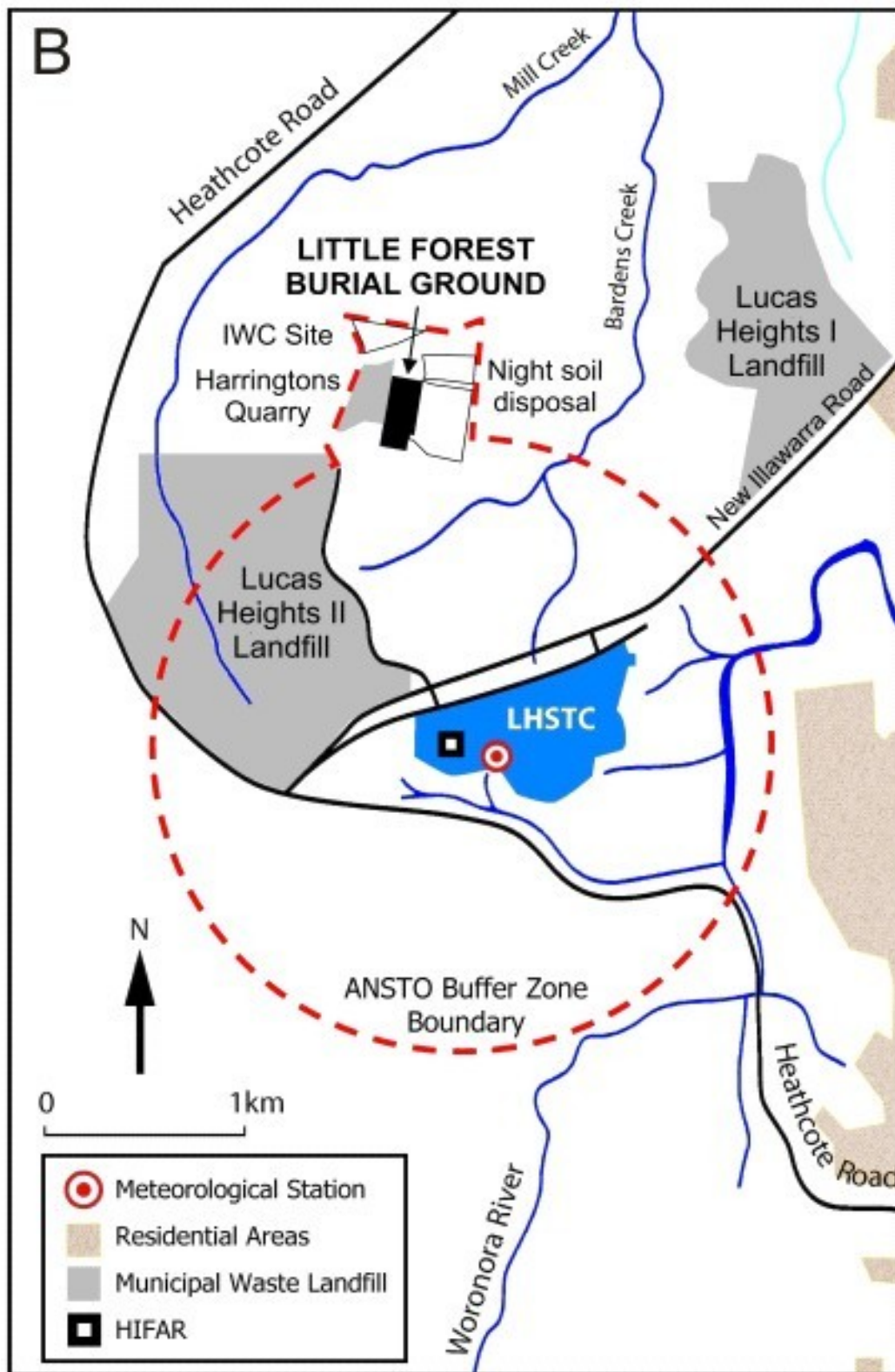
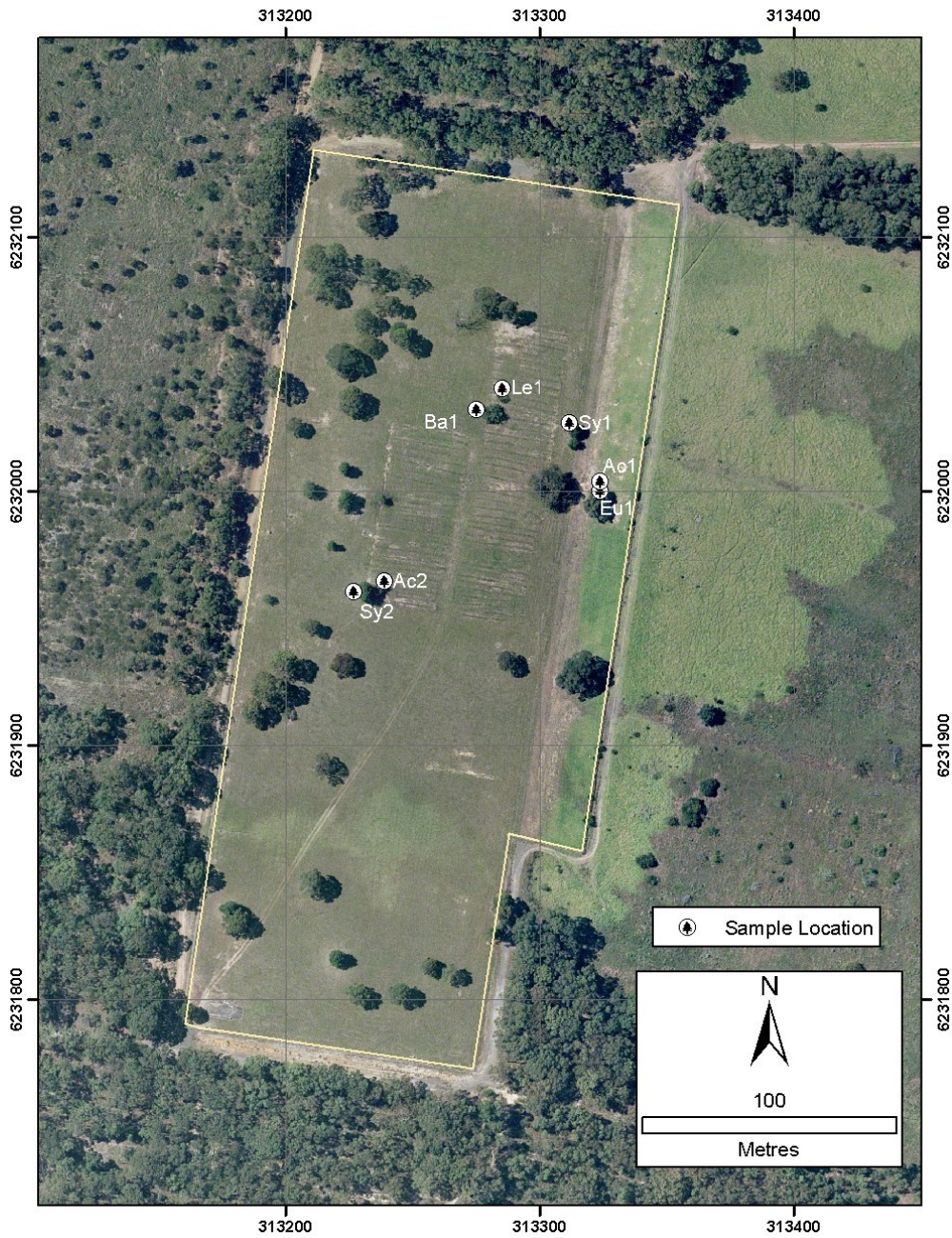


Figure 1. Location of the Little Forest Burial Ground in relation to the ANSTO site.

LFBG



Transpirate
Sample Locations

Figure 2. Plants from which transpirate were initially collected. These comprise the longest set of on-going samples collected over recent times.

PVC, approximately 48cm wide by 85 cm long when flat. These were found to enclose sufficient leaves to provide an adequate volume of transpire over a typical sampling period. Branches towards the northern side of the tree were preferentially selected as this maximises sun exposure and hence potential transpiration. For the same reason, sampling preferentially occurred on clear, sunny days. Rainy days were avoided due to the increased risk of contamination, as well as the reduced amount of transpiration. Bagging was undertaken in the morning and the sample collected, after a few hours, in the afternoon of the same day. Before bagging, any surface moisture from dew or precipitation was removed as far as was practicable by shaking the branch. Any residual surface moisture, if present, was considered a minimal contamination on the basis of the volumes collected later in the day.

When collected, the bag and limb were tapped and shaken to condense and coalesce any water to the bottom of the bag, prior to opening. Transpire samples were collected in new, dry, HDPE screw mouth plastic bottles. When necessary, more than one bottle was used, with the bottles being labelled accordingly. The contents from multiple bottles were combined for subsequent analyses on the sample. Contaminants such as leaves, twigs, insects, seeds, etc were excluded as far as possible during collection. Initially, filtration was performed after the samples were returned to the laboratory but, more recently, dual stage 1 and 0.45 μm polypropylene membranes attached to 50 mL disposable syringes were used in the field to filter the samples at the time of collection. On occasions, more than one filter was required per sample. Samples returned from the LFBG were stored at 4°C until processed further.

2.2. Analytical method development

Generating measurements of ^3H activity from water has two technical aspects: sample preparation and Liquid Scintillation Counting (LSC). This section of the report will deal with each aspect separately.

2.2.1. Sample preparation

The standard methodology for preparing water samples for tritium analysis at ANSTO's Low Level Laboratory (Building 34) follows the International Standards Organisation method 9698 (ISO, 1989). A quick guide has been prepared and is attached as Annex 1. The method basically comprises a distillation step to remove impurities that may cause chemical and colour quenching in the scintillation process. However, the minimum sample volume required for this approach is 200 mL. From our first collection (July 07), only 3 of the 8 sample volumes were sufficient for that procedure. Hence, our first assessment was to compare the results that were obtained by using the standard method with those obtained by simple 0.45 μm filtration of the transpire samples. It was initially believed that the transpire would be of sufficiently high quality that filtration alone would be adequate for the purposes of LSC.

All eight samples were filtered through 0.45 μm membrane filters to provide 5 mL aliquots for LSC. Further, 250 mL from each of the three larger-volume samples was processed according to ISO 9698. The distillation process recommends that the first 50-75 mL of distillate be discarded, and that the subsequent 100 mL be retained as the sample for analysis, whilst retaining some water in the boiling flask. This method would seem to provide a potential for fractionation of the water sample due to the higher mass of HTO compared to non-tritiated water. This may lead to low tritium concentrations in the earlier distilled fraction and high tritium in the residual. In an

attempt to evaluate potential fractionation of the distilled samples, the initial distillate and a late distillate were also retained to compare with the intermediate 100 mL sample.

From our second collection (October 07), five of the 19 samples were of sufficient volume for distillation. Hence, the approach outlined above was repeated for these later samples.

By the time of our third sampling run (December 07) we had become aware of commercially available ion-exchange columns (Eichrom Tritium columns, Eichrom Technologies, Incorporated) suitable for HTO samples (Eichrom, 1996). Each column contains three extraction resins. The Diphonix™ resin removes cations, the AG 1X8 resin removes anions and the XAD-7 resin removes organically bound ¹⁴C and ³H as well as other organic molecules. The columns require sample volumes of only 25 mL to collect 20 mL for LSC. For samples collected during this campaign, we compared the results of samples prepared using simple 0.45 µm membrane filtration with those using the columns. All but one (Ac 2.5) of the 12 samples in December 07 were passed through the columns.

The basic procedure for using the columns is as follows:

- Mount the column on an appropriate stand to ensure the column remains vertical and to allow for easy collection of drainage.
- Remove the plug and end cap to drain and discard the filling solution (columns should not be allowed to dry out).
- Using a funnel, use 10 mL of Milli-Q water to flush the remaining filling solution. Allow to drain and discard.
- Using a 0.2 µm pre-filter, pass 25 mL of sample through the column, discarding the first 5 mL.
- Retain the last 20 mL for LSC.

All subsequent 3-monthly samples were prepared using the dual stage membrane filtration at collection, refrigerator storage and the column technique.

2.2.2. *Liquid Scintillation Counting*

2.2.2.1. *Scintillation Cocktail and Vials*

Aliquots of prepared water samples were added to organic scintillation cocktails within 22 mL capacity super polyethylene LSC vials. The ratio of water to solvent should be sufficiently high to maximise counts due to radioactive decay, but should be sufficiently low to ensure that the water is intimately mixed with the organic scintillant and that there is adequate transfer of the ionisation induced by the radiation to the scintillant for photon generation and detection by the counter. Most commercially-available LS cocktails can tolerate up to 12 mL of water per vial before scintillation performance starts to substantially degrade.

For the first and second sets of samples, 5 mL of water (distilled or filtered) was added to 11 mL of Ultima Gold-XR (July) or Ultima Gold-LLT (October) scintillant in HDPE scintillation vials (Packard). For the third and fourth sets, 10 mL of sample was used with 11 mL of Ultima Gold-LLT.

We subsequently assessed the impact of using different scintillant cocktails and volume ratios by comparing the counting efficiency of standard tritium additions within each run. Figure of merit calculations were made of the various combinations (see results below). These observations confirmed that efficiency was effectively

reduced when using 10mL of sample compared to 5 mL. Hence, for the fifth & subsequent sets, 5 mL of sample was used with 11 mL Ultima Gold-XR, Ultima Gold-LLT or Ultima Gold-uLLT.

2.2.2.2. *Detectors, Backgrounds and Efficiency*

The first set of samples was counted on the Packard TriCarb 2700TR LSC (in ANSTO's Low Level Laboratory at Building 34). Six, 20 minute counting cycles were run, with the first run being ignored for purposes of calculations. This avoids any short-term chemiluminescence, which can increase background counts that may be potentially induced in the scintillant as a consequence of being exposed to fluorescent lighting. Blanks comprised either distilled tap water (Building 34) or dead water (i.e. from a source with no tritium) and these were run for background comparison. It was subsequently noted that the count rates for nominally dead water were higher than those for distilled blanks. This was found to be due to chemicals present in the water causing chemiluminescence. Subsequently, the dead water has been distilled to remove such contaminants and the problem has been resolved. Tritium standards for use in that instrument were run with the LFBG samples and blank to assess efficiency. Three standards were included with nominal activities of 5.55, 5.41 and 5.47 Bq/vial. Based on these activities, the detection efficiency for the run was 25%.

For the subsequent sets, the samples were measured on the Packard TriCarb 2900TR LSC (in Building 21A) using up to 31 cycles selecting channels over an energy range from 2-18.6 keV. Blanks were run and standard tritium solutions were used to assess counting efficiency. For each of these sets, at least two efficiency standards were prepared. These comprised either 0.1, 0.5 or 1.0 mL of NBS 20 which had a specific activity of 631.5 dpm/g on 3 September 1998. In October 2007 an extra set of standards comprising 0.03 and 0.06 mL of NBS 20 were also run to provide low activity comparisons with the sample data. Detection efficiencies were around 25% and minimum detection levels were in the order of 10-20 Bq/L.

It became clear over time that it was important to undertake a 'normalisation' and calibration process for the LSC before each run. Not doing so could, on occasions, substantially influence the detection efficiency. Normalisation and calibration involves running a set of sealed standards comprising a blank, a ^3H sample and a ^{14}C sample that were supplied with the instrument, through a single cycle of 1 hr counts.

Another observation made during the collation of the results over time, was an unexpected variability in detection efficiency potentially related to variation in count rates for the background sample within each run and differences in the water volume in blank samples. To account for this between-series variability, a final count of all samples was conducted in Building 21A following field sampling in March 2009. All available samples (recent and previously stored) that were prepared using the column technique were re-analysed using identical vials, counter setup, scintillation cocktail (same type and batch but more than 1 bottle was used), sample/scintillant volume ratio, etc.

2.2.2.3. *Quench Correction*

The final assessment made on the individual results is of the quenching factor. Quenching occurs in LSC as a result of three potential factors, all of which reduce the counting efficiency. These are: chemical quenching, by which the transfer of the ionisation derived from radioactive decay to the scintillant, or the performance of the

scintillant itself, is inhibited; colour quenching, where the light emitted by the scintillant is absorbed to varying degrees before it reaches the detector resulting in a spectral shift to different wavelengths (lower energies); and self-absorption, where the mass of the sample itself absorbs either the radiation or the induced scintillation.

A number of different approaches are available for assessing quench. These comprise: a spectral approach using either the shift in the peaks of the isotopes of interest in the sample (SIS) or the shift in the spectrum generated within the sample by the close application of an external gamma-emitting standard (transformed Spectral Index of the External standard or tSIE); establishing a quench curve by counting a series of standards (quench set) that each contain equal activity with varied amounts of a quenching agent; and an internal standard method whereby the sample is counted initially and thereafter a small aliquot of standard tritium with known activity is added and the sample recounted.

The SIS method was not used as the samples were at low activity levels and strong spectra are optimal for this approach. As part of our method development the tSIE, the quench curve and the internal standard method were assessed. The tSIE is generated automatically as part of the counting procedures in the instruments used. On Packard instruments, it is based on the detected shift in the spectrum induced by gamma emissions from ^{133}Ba as it is placed beside the sample. Quench curves (tSIE versus efficiency) were established by counting sets of samples containing increasing masses of either *Eucalyptus* oil (as a chemical quenching agent) or food dye (as a colour quenching agent). The internal standard method was assessed using a standard addition of ~0.1 g of NBS 20 solution (a secondary National Bureau of Standards ^3H standard prepared at ANSTO), measured gravimetrically to each of the samples which were then recounted on the same instrument to determine relative efficiency.

3. Results and Discussion

3.1. Effect of Distillation on ^3H Fractionation

The results of LSC on distilled samples from the first and second runs are shown in Table 1. Across two different sampling periods and analytical runs there is no significant difference between the various distillation fractions for any one sample. Thus, the distillation process does not significantly alter the proportion of HTO in the collected water from the original sample within the errors of the analysis.

Table 1. Comparisons of ^3H activity in distillation fractions to assess the possibility of heavier isotopes concentrating in later distillations. Errors are based on Poisson distributions of total counts from 6 x 20 min counting cycles.

Sample		1 st fraction		2 nd fraction ^a		3 rd fraction	
Month	I.D.	Bq/L	2 s.d.	Bq/L	2 s.d.	Bq/L	2 s.d.
July	Sy 1.1	202	12	208	12	221	12
	Le 1.1	83	9	101	9	93	9
	Ac 2.1	152	11	168	11	165	11
October	Ba 1.1	127	5	134	5	141	5
	Sy 2.1	433	7	451	7	451	7
	Ac 1.1	23	4	19	4	19	4
	Sy 1.4	239	6	251	6	251	6
	Sy 1.5	270	6	273	6	273	6

a. The second fraction only is normally collected for LSC analysis.

However, when ranked against fraction collected, there is a trend towards higher ^3H activity which is evident in at least 5 of the 8 comparisons. This is shown in

Figure 3 where the ^3H activities have been normalised to the average across all distillations and then plotted against fraction collected. The samples which do not show the trend are 3 of the 4 least active samples (Table 1) and thereby prone to larger proportional error. Hence, whilst there are no significant differences between results for each of the fractions, distillation may give rise to fractionation that could become apparent should analytical errors be reduced. The potential effects of distillation on sample fractionation should be considered with some caution in future analyses.

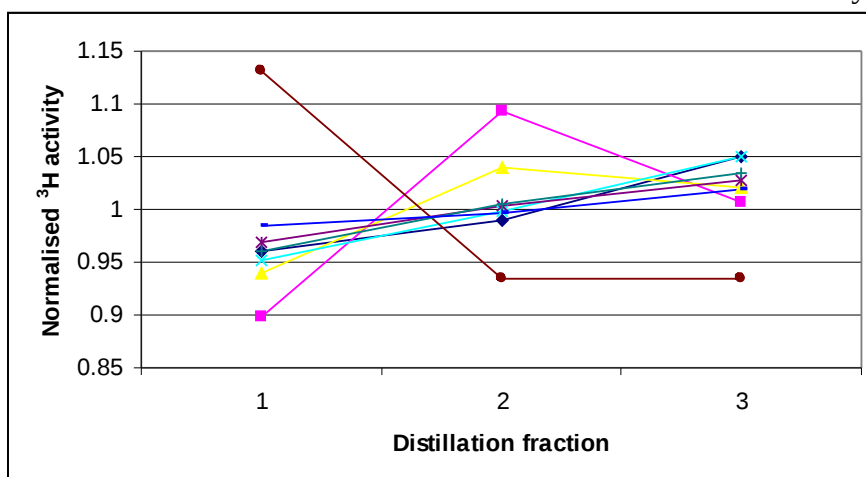


Figure 3. Tritium activity in water distilled from transpirate samples collected in July and October 2007 from 4 species, normalised to average activity for each sample and plotted against fraction collected.

3.2. Comparison of Results following Distillation and Filtration

In Table 2 there is a comparison of the results for ^3H activity in samples that were prepared by simple filtration or both filtration and distillation.

Table 2. Comparison of ^3H activity results for samples collected in July and October 2007 that were processed by filtration and distillation as well as by filtration alone. Errors are based on Poisson distributions derived from 6 x 20 min cycles. Instances of differences greater than ± 2 s.d. between the treatments are shown in bold italics.

Sample		Filtered and Distilled ^a		Filtered		ratio ^b
Month	I.D.	Bq/L	2 s.d.	Bq/L	2 s.d.	
July	Sy 1.1	208	12	208	12	1.00
	Le 1.1	101	9	88	9	0.87
	Ac 2.1	168	11	168	11	1.00
October	Ba 1.1	134	5	112	9	0.84
	Sy 2.1	451	7	360	12	0.80
	Ac 1.1	19	4	19	7	1.00
	Sy 1.4	251	6	204	10	0.81
	Sy 1.5	273	6	226	11	0.83

a. Quoted activities are for 2nd fraction samples.

b. Ratio of the activity concentration in the filtered sample over that in the filtered and distilled sample.

There is evidence in Table 2 that sample preparation by filtration and distillation together, as distinct from filtration only, leads to substantially different results within any sample run. Typically, the distilled sample reported higher ^3H activity than the filtered sample. From this it is evident that, in some cases, there are dissolved

substances in the transpirate that give rise to some degree of chemical and/or colour quenching in the filtered sample. In all cases where the filtered sample produced a lower result, the ratio of the effect was fairly consistent (0.8-0.9). At present, reasons for this consistency are unknown. Nonetheless, it is apparent that filtration alone is inadequate as a sample preparation process.

3.3. Comparison of Eichrom Tritium column and Filtration methods

Table 3 contains comparative results for ^3H activity in samples prepared by filtration alone or by filtration followed by ion-exchange column extraction. In most samples (all but 2), the measured activity concentration was higher after passing the sample through the Eichrom Tritium column. Exceptions to this observation were restricted to low activity samples. On average the filtered sample results are 0.88 that of the column results, a similar result to the improvement noted for distillation above. This implies the presence of quenching agents remaining within the samples that have undergone filtration alone. On this basis, use of the columns is preferred to simple filtration.

Table 3. Comparison of ^3H activity results for samples collected in June 2008 and prepared using either filtration only or filtration followed by elution through an Eichrom Tritium column. Errors are based on Poisson distributions derived from 6 x 20 minute counting cycles.

Sample		Column			Filtered			ratio ^a
Month	I.D.	Bq/L	2 s.d.	tSIE ^b	Bq/L	2 s.d.	tSIE ^b	
Jun 08	Ac1.1	4	13	308	6	13	301	1.36
	Ac2.1	66	14	305	47	13	298	0.70
	Ac2.2	83	14	305	69	14	299	0.83
	Ac2.3	57	14	303	38	13	293	0.67
	Ac2.5	11	13	300	11	13	296	0.98
	Ba1.1	40	13	298	35	13	301	0.88
	Ba1.2	33	13	298	32	13	295	0.96
	Eu1.1	7	13	298	-2	13	273	
	Le1.2	39	13	301	28	13	265	0.71
	Sy1.1	131	15	301	125	15	289	0.96
	Sy2.1	109	15	302	78	14	274	0.71
	Sy2.2	-1	13	306	9	13	292	

a. Ratio of the activity concentration in the filtered sample over that in the column sample.

b. Values of tSIE are included for later comparison.

3.4. Quench correction

Quench correction by three methods was assessed: the internal standard method, tSIE, and quench curves.

3.4.1. Internal Spike method

The internal standard method was assessed using samples collected in June 2008. Table 4 contains the results for ^3H activity measurements obtained after correcting for internal spike and can be compared with the results in Table 3 which were the uncorrected values.

None of the results obtained using the internal standard method was significantly different from those obtained earlier. The internal standard method is widely accepted as the best approach to evaluating quench in the LSC samples in that each sample

provides an estimate of its own degree of quenching. However, the method is more demanding of labour and resources. It requires double handling of samples, double the counting and data interpretation times and it depletes the limited supplies of standard reference material. Given that the Eichrom Tritium column generates statistically similar results, then the column method is recommended for on-going and routine monitoring purposes.

Table 4. Comparison of ^3H activity results for samples given in Table 3 after correcting for detection efficiency using an internal standard. Errors are based on Poisson distributions for 6 x 20 min counting cycles.

Sample		Column				Filtered			
Month	I.D.	Bq/L	2 s.d.	tSIE	spike eff. ^a	Bq/L	2 s.d.	tSIE	spike eff. ^a
Jun 08	Ac1.1	4	13	309	0.23	6	13	299	0.22
	Ac2.1	72	20	307	0.21	45	15	297	0.24
	Ac2.2	78	19	309	0.25	72	20	300	0.22
	Ac2.3	60	18	308	0.22	36	14	294	0.25
	Ac2.5	10	12	308	0.25	12	14	294	0.21
	Ba1.1	43	16	307	0.22	33	14	305	0.25
	Ba1.2	34	14	308	0.23	37	17	298	0.20
	Eu1.1	7	14	308	0.22	-3	15	271	0.19
	Le1.2	42	16	309	0.21	33	17	263	0.19
	Sy1.1	143	33	307	0.21	145	35	288	0.20
	Sy2.1	121	29	306	0.21	86	23	271	0.21
	Sy2.2	-1	13	309	0.22	11	17	297	0.18

a. The spike efficiency is the increase in net sample counts (total less background) divided by the total disintegrations of the added spike over the counting period.

3.4.2. tSIE

The correlation between spike efficiency determined for the internal standard correction and the tSIE of the same samples collected over two periods (Dec 07 and Jun 08) is shown in Figures 4a and b.

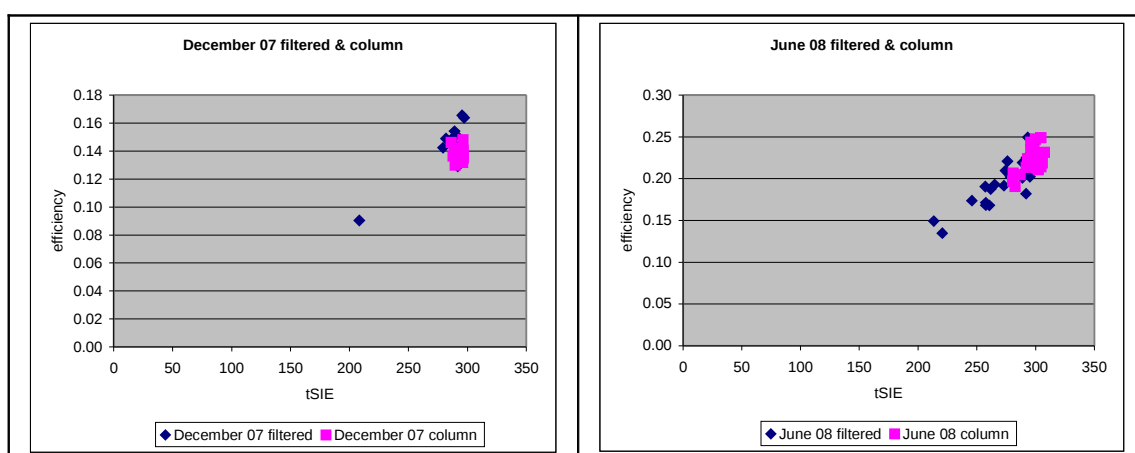


Figure 4. Relationship between sample tSIE values and efficiency determined using the internal spike method as a measure of sample quenching: a) all December 2007 data; b) all June 2008 data. Note the markedly reduced variability in Tritium column data in comparison with the same samples that only underwent filtration.

From the figures it is apparent that the tSIE does give a lower value for more quenched samples. That relationship is also consistent despite the fact that counting efficiency was different within the two counting cycles. From this, tSIE seems to be a robust and reliable measure of quenching in transpirate samples prepared for ^3H analysis.

Figure 4 also presents evidence for the improvement in precision imparted by using the Eichrom Tritium columns for sample preparation. Removal of contaminants in the transpirate sample gives rise to markedly reduced variability in the degree of quenching between samples as measured by the range of tSIE values derived. This is discussed further below.

3.4.3. Quench curves

The transpirate samples were collected from native Australian species. Hence it was assumed that some chemical quenching of the sample counts may be due to the presence of natural oils. To evaluate that possibility, counting efficiency was assessed over a range of distilled dead water samples to which was added an aliquot of ^3H standard and an increasing mass of *Eucalyptus* oil purchased from a local supermarket. The resulting chemical quench curve is presented in Figure 5.

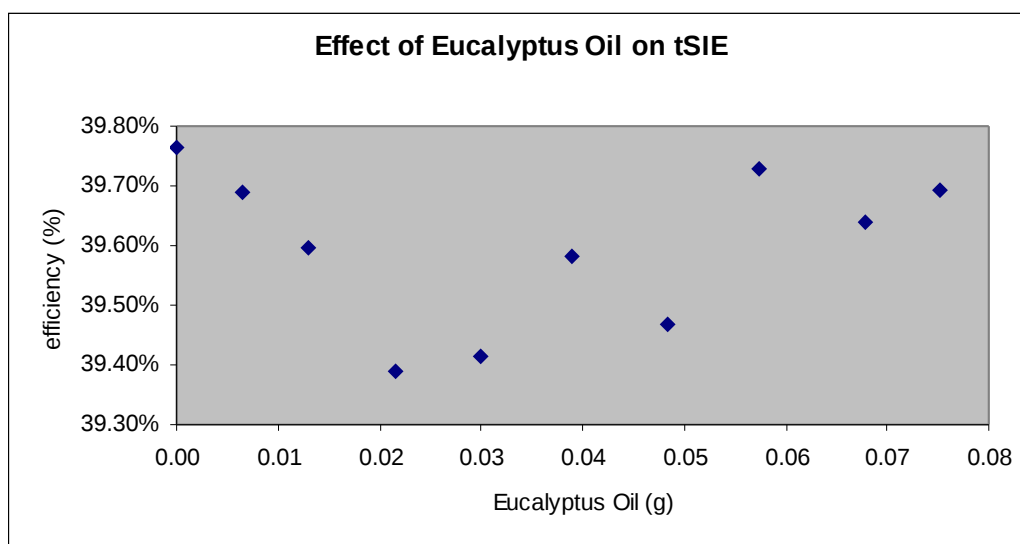


Figure 5. The relationship between Eucalyptus oil contamination and counting efficiency.

It is apparent that there is no consistent relationship between the concentration of *Eucalyptus* oil in a sample and the degree of quenching. As can be seen from the limited range on the y-axis, there is hardly any change in detection efficiency across the range of contamination applied and the little variability observed is not correlated with amount added. From this it can be assumed that there is little chemical quenching in these samples.

Colour was another probable cause of quenching given the presence of tannins in the transpirate. Commercially available yellow food dye was used to produce a colour quenching curve given in Figure 6.

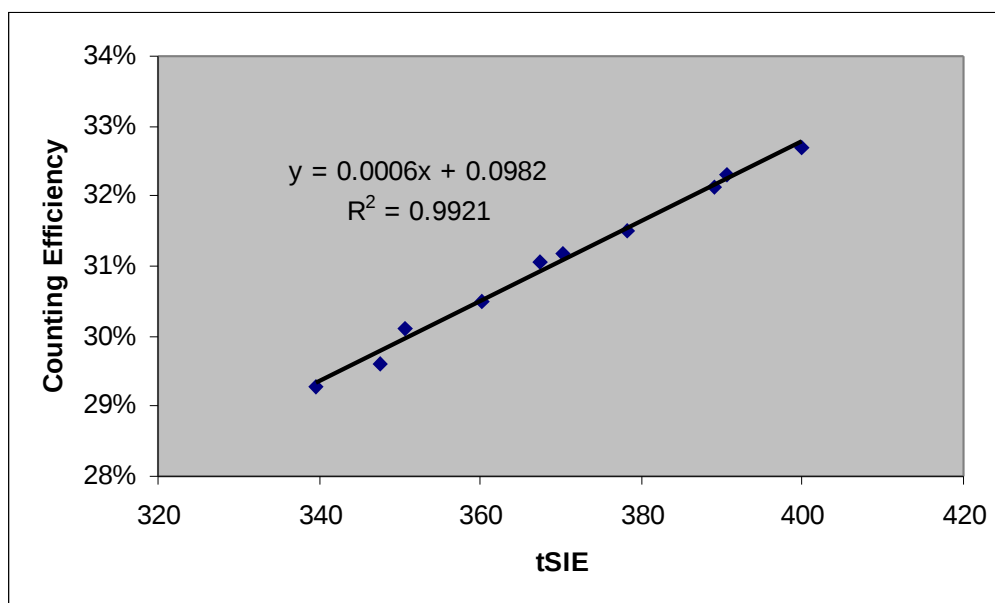


Figure 6. Quench correction curve generated using food dye across typical tSIE range for transpirate samples.

The colour quenching curve demonstrates a highly significant relationship between counting efficiency and the tSIE values derived from the quenched samples. Hence, colour quench curves are a reasonable measure to use to correct net counts from ^3H in transpirate samples into activity concentrations. As can be seen from this example, the range of efficiency differences within any single cycle of transpirate measurements can be small (4% or less) but not insignificant.

With each LSC instrument is supplied a Packard™ Ultima Gold™ quench set. This can be used to establish a quench correction curve to be automatically applied to any counting protocol based on tSIE values. However, the current set are out of date and this approach also assumes that the quenching agent and standard set are representative of the working samples. Because of these uncertainties, we have preferred our own quench correction set.

3.5. Effect of Sample Volume and Scintillant Cocktail on Counting Efficiency

A number of different sample preparation recipes were used during method development as a consequence of access to different materials and equipment. To assess the relative merits of the approaches used, a single counting set was used to compare the results obtained for the various options under constant conditions. The variables assessed were the type of scintillation cocktail and the ratio of cocktail to water in the vial. Detection efficiency for each condition was determined by comparing counts in a spiked sample with an equivalent un-spiked blank. The summary results are shown in Table 5.

The results in Table 5 clearly show that the mass of sample used is the major factor determining the relative efficiency of the sample count. This is achieved by both reduced background counts and increased detection efficiency as shown by the figure of merit (FOM). The 10 mL samples developed much lower tSIE values than the 5 mL samples. This indicates markedly increased quenching with sample mass, and concomitantly reduced efficiency. This effect is somewhat off-set by the fact that with extra sample there is more activity present. However the trade-off is in favour of

the lower volume as smaller extrapolations are needed to arrive at the best estimate of the activity concentration within the sample in that case.

Table 5. Effect of sample mass and scintillation cocktail on counting efficiency.

Scintillant Type	10 mL of sample			5 mL of sample		
	tSIE	Efficiency	FOM*	tSIE	Efficiency	FOM*
Ultima Gold XR	273	11%	12.57	380	28%	66.45
Gold Star	242	13%	13.14	313	22%	38.11
Ultima Gold uLLT	302	19%	24.66	400	30%	87.78

* FOM – Figure of Merit (efficiency²/background)

In relation to the type of scintillant, Ultima Gold uLLT provided substantially better outcomes than the other two cocktails, Gold Star being the least favoured. On this basis Ultima Gold uLLT is the preferred cocktail for future use.

3.6. Background variability

Over the course of the method development, one of the major problems in obtaining consistent results was the degree of variability obtained for net background counts. To try to assess any patterns in that fluctuation, a background sample comprising 5 mL of distilled dead water in 11 mL of Ultima Gold uLLT cocktail was counted for 20 min cycles for a continuous period of three weeks. The results are displayed in Figure 7.

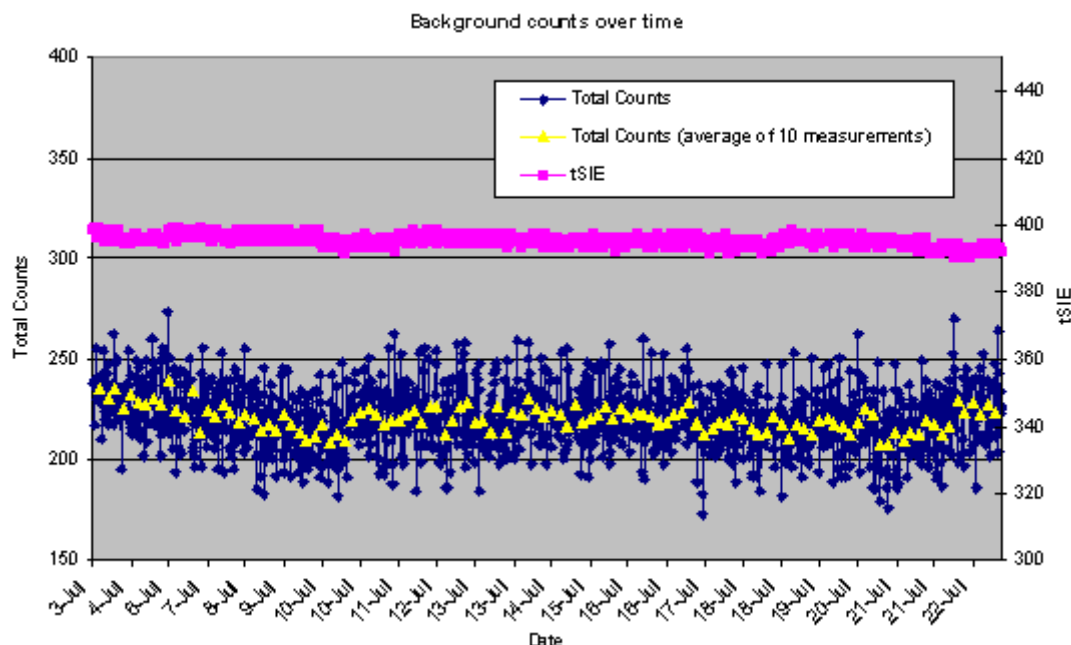


Figure 7. Variation in background count rate and tSIE over a period of 3 weeks.

The results in Figure 7 demonstrate a remarkable variability. The 20 minute count varies over a range from 173 – 273. The overall difference covers more than 50% of the maximum value. This is particularly remarkable given that there were no other radioactive samples within the LSC at that time to induce variability as a consequence

of potential proximity. The 10 sample (approx 3 hour) lagging average also indicates some irregular trends within the data although there is no consistent temporal pattern. However, the use of multiple count averages has reduced the range of background estimates from 208 - 239, a much improved outcome. At this stage the variability remains unexplained and an on-going area of concern for low-level ^3H counting.

The tSIE also seems to trend towards lower values in Figure 7, possibly indicating degradation in the scintillation cocktail over time. However, this trend is not significantly linear, a regression explaining only approximately 30% of the variance in the data. In any case the decline, if present, is only in the order of 10 in 400 and hence not substantial.

As a practical outcome, the reduced variability obtained by using multiple counts confirms the need to run as many counting cycles as sample number and equipment demand permits so as to obtain more reliable measures of background and sample variability. Given the reduction observed in this data set, 10 cycles could be considered reasonable.

4. Summary and Conclusions

Method development has been required to improve techniques for ^3H analysis of transpire samples over the initial sampling period. Despite transpired water being the result of an evaporative process, the means of collecting the sample results in the inclusion of dissolved materials that give rise to quenching, probably both chemical and colour quenching. An instrument-based method of evaluating the quenching factor, tSIE, in conjunction with a colour quenching correction curve seems adequate for evaluating ^3H activity in transpired water.

Distillation of the filtered samples can provide an adequate means of removing the material causing the quenching; however, the volumes of transpired water collected are often insufficient for distillation. The Eichrom Tritium column requires less water and gives similar results to those obtained by either distillation or by use of the internal standard method for quench correction. The internal standard method gives accurate results, however it is constrained by increased resource and time needs. On this basis, the Eichrom Tritium column method is considered the best option for routine on-going sample processing and analysis.

It was also apparent that consistency was required in relation to sample volume and scintillation cocktail used. Increased sample volume provided more tritium for analysis but this advantage was more than off-set by reduced efficiency and increased quenching. A 5 mL aliquot of sample or blank water mixed with 11 mL of Ultima Gold uLLT cocktail was considered the best recipe.

High variability was observed in background count rates and there was no consistent pattern that could be discerned to explain this. The best method to minimise this problem is to perform as many counting cycles as practically possible. A minimum of 10 x 20 min cycles is suggested.

A final recommended technique for future analyses of ^3H in transpire is presented in Annex 2.

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5. References

Eichrom Technologies Incorporated (1996). OTW02 Analytical procedures: Tritium in water. Eichrom Technologies Inc., Darien IL.

Isaacs S. and Mears K. (1977). A study of the burial ground used for radioactive waste at the Little Forest Area near Lucas Heights New South Wales. Australian Atomic Energy Commission report AAEC/E427, December 1977.

ISO (1989). Water Quality - Determination of tritium activity concentration - liquid scintillation counting method. ISO 9698. International Standards Organisation.

[Twining J. and Creighton N. \(2007\). Draft hypothesis setting and related initial sampling plan for biota in the LFBG. Radwaste Science Project.](#)

ANNEX 1.

B34 Tritium Distillation – Quick Guide – July 2007

Place 200-250 mL water sample into a round-bottomed boiling flask.

Add the following reagents:

- 500 mg sodium carbonate
- 400 mg hydrated sodium thiosulfate (or 250 mg if anhydrous)

Note: the sodium thiosulfate converts any iodine in the sample to iodide, whilst the sodium carbonate makes the sample alkaline, keeping the iodine in solution.

Place the boiling flask on the electric heating mantle and carefully fit a splash-head, condenser column and a 50 mL collection flask (use the blue clamp for the 50 mL flask).

Turn on the heating mantle (use max setting, 10).

Turn on the water pump. This circulates water through the condenser jacket to condense the distilled water vapour.

- Collect the first 50-75 mL and discard (this fraction is not representative of the sample).
- Collect the next 100 mL of distillate – even if you don't need it all. This is the distilled sample for subsequent analyses.
- DO NOT let the flask boil dry as H₂S will be generated and glassware could crack. There should be 50-100 mL of sample remaining, which can be discarded when cool.

Note: Using this procedure (based on ISO 9698) there should be no significant isotopic fractionation.

Turn off the heating mantle and cooling water.

Wash the glassware and rinse the boiling flask with 2M nitric acid if necessary. Rinse subsequently with tap or distilled water and place in the drying oven until dry.

PREPARING LSC VIALS

Label lid only of 20 mL polyethylene LSC vial.

Add 5 mL of distilled sample and 11 mL of Ultima Gold-XR LSC cocktail using the volumetric dispenser supplied.

Tighten lid and shake vigorously.

Wipe clean the surface of the vial. Do not wear plastic gloves as this can induce static charge which may increase background.

Store for 24 hours in the dark to reduce potential chemiluminescence induced by exposure to sunlight or fluorescent lights.

LSC CASSETTE – Use protocol #__ (Check with lab manager for current number)

Position 1 (LHS nearest flag) = Background sample (e.g. distilled water blank);

Position 2 onwards = sample vials;

Last positions, load the 3 tritium standards;

Complete details on “tritium counting record” sheet.

Push flag across to left and place cassette(s) into LSC counter on RHS.

Use mouse to activate the computer screen and select GREEN “start” button. The cassette will move to the rear of the LSC and the first vial will be lowered for counting.

Results will print out when all programmed cycles have been completed.

ANNEX 2.

Recommended Analytical Method for Analysing Tritium in Tree Transpirate at ANSTO.

Sampling

- Collect transpirate on warm sunny day from an easily accessible branch on the north side of a tree or shrub using a large, clean, dry PVC bag enclosing foliage by taping the bag opening firmly closed around the supporting branch. Leave for at least 4 hours.
- Filter the collected sample (30 mL minimum) in the field using a dual stage (1 and 0.45 μm) membrane filter on a 50 mL syringe into a clean, dry, HDPE sample bottle, labelled appropriately.
- Return to the laboratory and store in a cool room until further processing is required. Allow sample to return to room temperature at that time.
- Record sample details and ID into the electronic logbook. [LFBG Electronic Logbook](#)

Eichrom Tritium Column

- Mount the column on an appropriate stand to ensure the column remains vertical and to allow for easy collection of drainage.
- Remove the plug and end cap to drain and discard the filling solution (columns should not be allowed to dry out).
- Using a funnel, use 10 mL of Milli-Q water to flush the remaining filling solution. Allow to drain and discard.
- Using a 0.2 μm pre-filter, pass 25 mL of sample through the column, discarding the first 5 mL.
- Retain the last 20 mL for use in LSC and clearly label the container with sample ID.

Preparation of LSC sample, blanks and standards

- Weigh approx. 5 mL of sample into a new, clean, 20 mL super polyethylene scintillation vial. Record weight.
- Pipette 11 mL of Ultima Gold uLLT scintillation cocktail into the vial, cap and shake well.
- Label lid of vial with sample ID using permanent marker.
- Blanks are made up using 5 mL of distilled dead water that has been passed through the Tritium column. Distilled dead water is currently available on request from the low-level Tritium laboratory in Building 21B.
- More than two ^3H standards should be prepared as 5 mL samples using traceable reference solutions that approximately cover the expected activity of the samples. The additions should be measured gravimetrically.
- Record all details in your laboratory notebook. This will include Eichrom batch numbers; standards and masses; purification and preparation dates; cocktail batch and type; sample IDs and any other observations.
- Set aside all vials in the dark (to minimise chemiluminescence) and count in the near future.

Colour Quench Curve

- This is only required periodically, perhaps every 6-12 months. The decision to redo the curve will be made by comparing the quench corrected activity estimates of the standards with the nominal activities. If the values are outside tolerances the curve should be re-run.
- Weigh approximately 5g of traceable tritium standard into 10 vials. Record weights.
- Add increasing amounts of yellow food colouring (from 0.005 to 0.5g was used here but this may need to be checked when using other dyes) ensuring that the first vial has no dye added.
- Complete preparation as above.
- Use the Packard TriCarb in Building 21A and create a new quench set counting protocol with reference to the instrument manual.

Liquid Scintillation Counting

- Use the Packard TriCarb 2900TR LSC in Building 21A.
- Apply at least 10 counting cycles. A 20 minute per sample counting cycle is recommended.
- Select counting channels over an energy range from 2-18.6 keV.
- Link the counting protocol with the previously acquired quench correction protocol (above).
- When editing the counting protocol, adding sample ID and mass data, corresponding to position in the counting carousel, will enable the LSC to provide corrected activity concentrations.
- The counting protocol should also include an output of the raw count data in .csv format (this format needs to be specified) to enable manual calculation if necessary. For more details refer to the instrument manual.

Data Download and Transfer

- The LSC instrument is not networked but has a CD/W drive.
- Data from the counting cycle can be copied to any disk and the LSC will add a folder to the list if data already exist on that disk.
- The data can then be uploaded into Project folders on the network to ensure that the data is safely stored.
- Preferred folder naming format is YYYYMMDD. See examples here [Tritium Results](#)

Calculation of ³H Activity Concentration

- This will be done automatically using the approach above. However, if manual calculation is required for any reason (eg standards results not being accurate) use the following method.
- Open this template [Tritium calculation template.xls](#) and follow the detailed instructions.
- The final results can be found on the “Calc data” worksheet.