

BACKGROUND CONTRIBUTION IN LIQUID SCINTILLATION COUNTING FOR DETERMINATION OF ORGANIC BOUND TRITIUM (OBT) IN URINE SAMPLES

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ABSTRACT. Tritium may accumulate in plants, organisms, or humans as organic bound tritium (OBT). Due to the longer retention time of OBT in the body relative to free tritium, evaluating the OBT contribution in the tritium dose estimation is of interest. The OBT content in urine can be determined by liquid scintillation counting (LSC). When using this method, the main background contributions are from the ⁴⁰K activity and chemiluminescence effects. These contributions were quantified for the standard process used for urine samples and for other samples containing organic compounds. The ⁴⁰K contribution was found to be negligible in normal urine samples, but the chemiluminescence effect was significant. When using concentrated urine samples or other organic compounds, significant errors may occur due to chemiluminescence if no background correction is performed.

INTRODUCTION

Tritium released to the environment as tritium oxide (HTO), molecular tritium (HT), or as volatile organic bound tritium (OBT) may accumulate in plants, organisms, or humans as OBT. HTO is the main link of tritium binding to live organisms, and its penetration to extra- and intracellular water is quite rapid. The kinetics of HTO in the body follow that of body water. Tritium atoms from HTO can replace the hydrogen atoms in organic compounds; thus, a variable portion of tritium may remain bound in the tissues as OBT (Trivedi et al. 2000). OBT will be retained in the human body much longer than tritiated water; the biological half-life is 4 times greater than for HTO (Potter 2004), and therefore, the relative contribution to the radiation dose will be higher. The portion of OBT in the body can be evaluated by its content in urine.

OBT activity determination is not performed routinely, mainly due to the time-consuming work needed when dealing with urine samples. In a separate work (Gonen et al., forthcoming), we tested a direct counting method that is simpler and faster in determining OBT in urine by liquid scintillation counting (LSC). Its principle is based on subtracting the tritiated water phase activity (HTO) from the total activity in the urine phase (OBT+HTO). Direct OBT determination by LSC has the advantage of being fast and accurate. However, this method has some disadvantages due to contributions of different effects to the background level, which may influence the measurements and increase the minimum detection level. The main contributors to the background are chemiluminescence and photoluminescence, as well as ⁴⁰K, which is present in urine samples.

Chemiluminescence originates in chemical reactions between additives or the specimen and components of the liquid scintillator. Kearns (1972) showed that the spectrum of the chemiluminescence light pulses overlaps with both ³H and ¹⁴C spectra. Thus, chemiluminescence cannot be excluded by increasing the baseline discriminator level, nor corrected by the channels-ratio method. However, the light pulses from chemiluminescence may be discriminated electronically, by coincidence. Newer liquid scintillation spectrometers are based on this principle, but it may not be completely effective when the luminescence is attributed to multiphoton events. Sample preparation by combustion may be employed if all other means of eliminating chemiluminescence fail (Peng 1977). Many substances emit light upon photo-activation, which is known as photoluminescence. It differs

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from chemiluminescence in that the light-emitting substance can be repeatedly photo-activated, whereas chemiluminescence is a direct result and a one-time effect due to chemical reaction. Chemiluminescence and phosphorescence decay with time (in hours to days at room temperatures, but in hours only if the liquid scintillator is refrigerated [Department of Energy Handbook 1994]).

The potassium content of our body is about 0.2–0.3% (Forbes 1994) and is present in urine samples. ^{40}K is a natural contaminant, and its isotopic abundance is 0.0118%. This isotope is well known as one of the causes of human internal radiation exposure (Ehmann 1991). ^{40}K emits beta radiation at a much higher energy than tritium, but as the beta spectra are continuous, the interaction in the scintillator produces also small pulse heights that may contribute to the count rate while determining tritium in urine samples.

The objective of this work is to determine the background contributions of the main interference effects when determining the OBT content in urine samples by LSC with a Quantulus 1220TM. This was performed by preparing samples with known contents of interfering agents. In addition, to determine the actual interfering effects, urine samples of 56 workers potentially exposed to tritium were also analyzed.

MATERIALS AND METHODS

The Counting System

For the LSC measurements in this work, we used a Quantulus 1220 (Wallac Oy, Finland) operated at Soreq Nuclear Center. This is a low-background system that uses passive and active shielding. The passive shield is an asymmetric lead shield with a maximal thickness of 20 cm above the measuring position. The active shielding is obtained by an asymmetric liquid scintillator guard viewed by 2 photomultipliers, which envelopes the counting chamber. Rejection of cosmic rays occurs by electronically inhibiting anticoincidence pulses from the guard and the main beta detector. An additional coincidence unit operates between the 2 photomultipliers viewing the measured sample.

Chemiluminescence is a single photon reaction; therefore, it will not trigger the fast coincidence discriminator between the 2 photomultipliers viewing the measured sample. However, at high count rates there may be a considerable contribution due to random coincidence. As the random coincidence is the sum of only 2 photons, the pulse height will be low, in the range of the tritium region, contributing to the background of tritium counting. The random coincidences are monitored by the Quantulus 1220 using delayed coincidence; thus, chemiluminescence and other random contributions are determined directly. The random coincidence counts are stored in Half 1 of the first multichannel (MCA1).

Sample Preparation

As mentioned earlier, we determine the OBT content in urine samples via a direct method based on subtracting the tritiated water phase activity (HTO) from the total activity in the urine phase (OBT+HTO). Total activity of the soluble tritium phase (OBT+HTO) in urine was determined by mixing 2 mL of urine with 14 mL of liquid scintillator (Ultima GoldTM XR) and 4 mL of nano-pure water (18.2 ohms) containing practically no tritium. These volumes are based on recommended ratios by the scintillator manufacturer. The activity of HTO was determined from 20-mL urine samples, after mixing them with 3 g of active charcoal, which absorbed all organic compounds. The mixtures were filtered through Whatman paper filters (no. 41). The use of active charcoal was based on known protocols, which we verified (Gonen et al., forthcoming). Samples were prepared

by mixing 2 mL of the filtrate with 14 mL of liquid scintillation (Ultima Gold XR) and 4 mL of tritium-free water.

All samples were shaken to homogenize the ingredients, stored in a refrigerator (4–8 °C) for at least 12 hr prior to counting, and counted in the Quantulus 1220 for 45 min. In order to perform corrections for the quenching effect, a set of standard quenched samples was prepared by adding various volumes of the quenching agent CCl₄ to vials containing a fixed amount of ³H in a standard volume of liquid scintillator. Tritium-free water was added to complete the 20-mL total volume for each vial.

Blanks were prepared by mixing 14 mL of scintillator with 6 mL of tritium-free water. The average count rate of 35 blanks was 1.6 cpm, and the count rate was subtracted from the count value of each sample. Since the quench conditions of the samples studied in this work varied over a wide range, we used the maximal tritium window up to channel 335, which gave a typical figure of merit (FM) value of about 330. Based on the background value mentioned above and using the equation adopted by the American Standard Recommendation ANSI-N13.30 (Health Physics Society 1996), the minimum detectable activity for a 45-min counting time is 0.058 Bq/sample.

THE CONTRIBUTION OF ⁴⁰K

The distribution of the ⁴⁰K spectrum can be seen in Figure 1. In order to evaluate this contribution to the ³H quantitative determination, samples containing pure KCl, tritium-free water, and scintillator material were prepared and counted. The potassium solutions were prepared from KCl powder of analytical grade (Frutarom, Israel). As no other materials were present, the count rates in the ³H region were attributed solely to ⁴⁰K. According to the analysis of spectra from these samples, 4.27 ± 0.98% from the spectrum area is in the ³H region. Thus, the contribution from ⁴⁰K to the equivalent ³H activity can be determined by 4.27% from the integral area in the higher region.

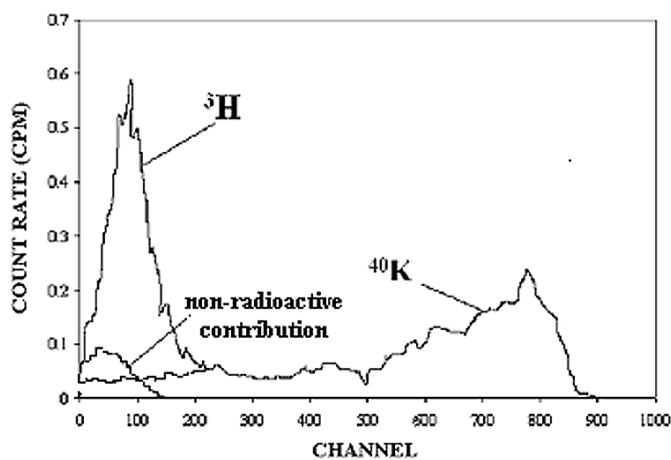


Figure 1 Equivalent ³H spectra of different background contributions

Different ⁴⁰K concentrations were prepared by using a 3M KCl solution. Table 1 presents the different proportions of the KCl solution and water volumes to prepare 20-mL aqueous solution standards. The K content in each standard and the corresponding content in 1600 cc (the average daily urine output adopted in the ICRP-89 standard [International Commission on Radiological Protec-

tion 2002]) are also given. The normal K output in the daily urinary excretion is several grams, and we tested a higher range of 50–90 g/day in order to obtain better statistical accuracy. From each standard, 2-mL samples were mixed with the scintillator to prepare the samples, which were counted as normal tritium samples. The counting results are presented in Table 1; the results represent the interference expected from the ^{40}K contribution. As there is no actual ^3H present, the results are a translation of the interference to ^3H activity; therefore, we define the values as “equivalent ^3H activity.” This definition will be used for other background contributions as well. The weight (in the 20-mL samples) to ^3H equivalent activity ratios are also given the table, and are constant with the KCl amount, indicating no disturbing effects for the KCl quantities shown.

Table 1 KCl solutions in 20-mL scintillator samples, the ^3H equivalent activities, and their standard deviations measured by the Quantulus 1220 system.

3M KCl volume (mL)	KCl in sample (g/20 mL)	KCl in 1600 cc (g)	Equivalent ^3H activity (Bq/mL)	Weight/activity ratio
2.79	0.625	50	0.022 ± 0.004	28.4
3.35	0.75	60	0.025 ± 0.004	30.0
4.47	1.00	80	0.034 ± 0.005	29.4
5.03	1.125	90	0.039 ± 0.005	28.8

In order to evaluate the contribution of ^{40}K in real cases, 56 actual urine samples were also analyzed. The samples were treated with active charcoal as described before, to remove all organic materials present, and were prepared according to the procedure for HTO determination. Teflon[®] vials (with aluminum-lined caps) containing low ^{40}K amounts were used. All samples were counted in the Quantulus 1220 for ^3H content. The results obtained are presented in Figure 2, where the samples were arranged for the sake of clarity in increasing order of measured activity. The equivalent ^3H activity obtained varies from <0.004 to ~ 0.014 Bq/cc. This wide range is expected, as the material's balance in the body depends to a great extent on personal metabolic and physiological properties and on diet. Normal K output, as reported by Eastham (1975), varies from 1.4 to 3.5 g/day (or per 1600 mL, which is the daily urinary excretion of an average person according to ICRP-89). The activity of ^{40}K , calculated from its abundance of 0.0118% and its specific activity of 5.65×10^{-6} Ci/g (Browne 1986), will be in the range from 0.02 to ~ 0.05 Bq/mL. The contribution in the ^3H region is expected to be ~ 0.001 to 0.002 Bq/mL, which is generally lower than the range of results in this study. The differences are probably due to the fact that the samples in this work were spot samples taken in the morning and are more concentrated than daily excretion averages.

The average limit of detection of OBT when using the direct subtraction method that we employed for a group of workers not classified as occupationally exposed to “tritium” was evaluated in a separate work (Gonen et al., forthcoming) as 0.052 Bq/cc. The “equivalent ^3H activity” due to the presence of ^{40}K in actual urine samples is lower than this value, indicating that in normal urine samples, ^{40}K presence does not contribute significantly to the ^3H background. However, by integrating the spectrum in the region higher than the ^3H region and using the area ratio between the ^3H region and the total spectrum, even these small contributions can be subtracted.

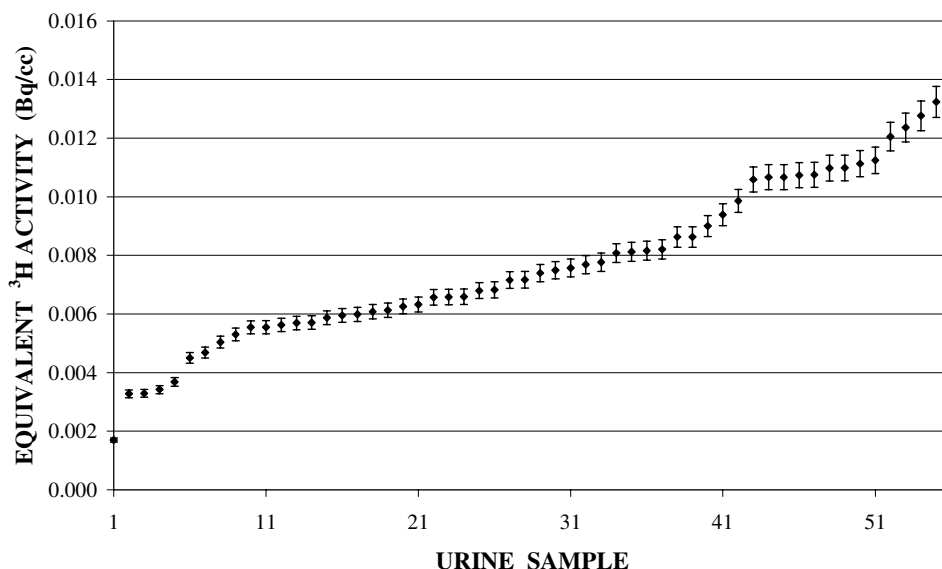


Figure 2 Equivalent ^3H activities due to ^{40}K present in the urine samples

THE CONTRIBUTION OF NON-RADIATION EFFECTS

Organic Matter in Urine

Organic matter contribution is attributed to random effects not filtered electronically by the coincidence units. The contribution includes chemiluminescence, electronic noise from the photomultipliers, and random coincidences between beta decay events (which depend on the sample activity). Determination of the OBT content is performed by subtracting the HTO counting (after removal of organic matter) from the original urine counting (which includes the organic matter). It can be assumed that the contribution of electronic noise and random coincidences not due to chemiluminescence is similar in these 2 samples and will be canceled by subtraction; thus, the main contribution will be of chemiluminescence due to the presence of organic matter only in the “full urine” sample.

We previously mentioned that the contribution by random coincidence effects is monitored in the Quantulus 1220 system, and the spectrum information is available for each counting in the SP11 section. The shape of the spectrum can be seen in Figure 1. We used this information to investigate the contribution of organic matter in the “full urine” samples used for ^3H determination. We investigated first the effect in the urine samples of the 56 workers potentially exposed to tritium. The random coincidence contributions obtained for the different urine samples are presented in Figure 3, where the samples were arranged in increasing order of measured activity. The equivalent ^3H activity obtained varies from negligible values to about 0.1 Bq/cc, with most values in the range of up to 0.06 Bq/cc.

To evaluate the significance of these results, the urine samples of the 56 workers potentially exposed to tritium were analyzed in 2 ways: by subtracting the random coincidence contribution and by neglecting it. Figure 4 presents the OBT results for the 56 urine samples for both cases, arranged in increasing order of measured activity. One can clearly see that there is a measurable difference between the 2 groups of results, and the random coincidence contribution cannot be neglected. The significance of not correcting for the random coincidence contribution becomes more acute if we try

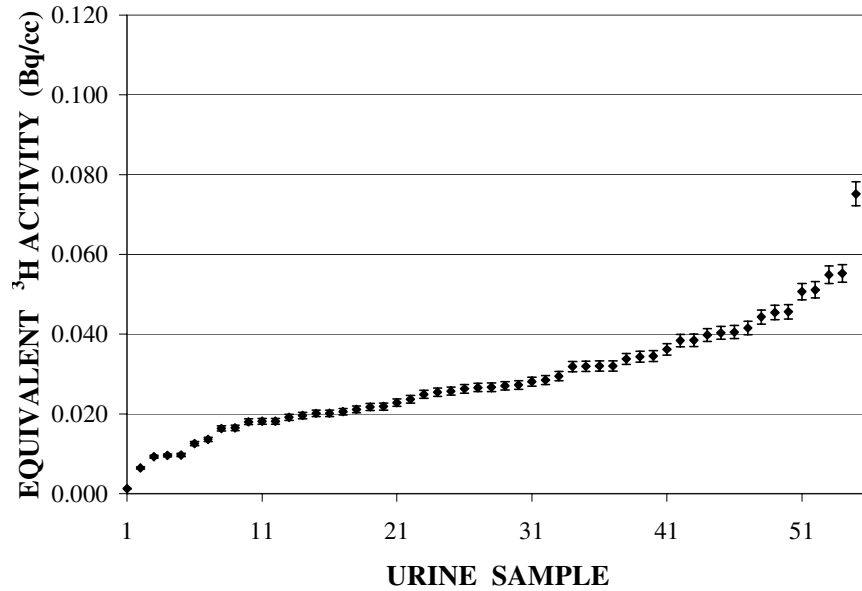


Figure 3 Equivalent OBT activities due to random coincidence effects

to evaluate the percentage of OBT from the total tritium content in the urine samples. Figure 5 presents this evaluation for the group of 56 workers. If the random coincidence contribution is neglected, OBT is found to be present in most of the samples. If the counts are corrected for random coincidence contribution, for most of the samples the OBT content is below the detection limit, and OBT can be identified positively in only ~30% of the samples. It is most probable that the positive determination of OBT in ~70% of the samples is not real, but is due to the uncorrected random coincidence contribution, which is interpreted as OBT content.

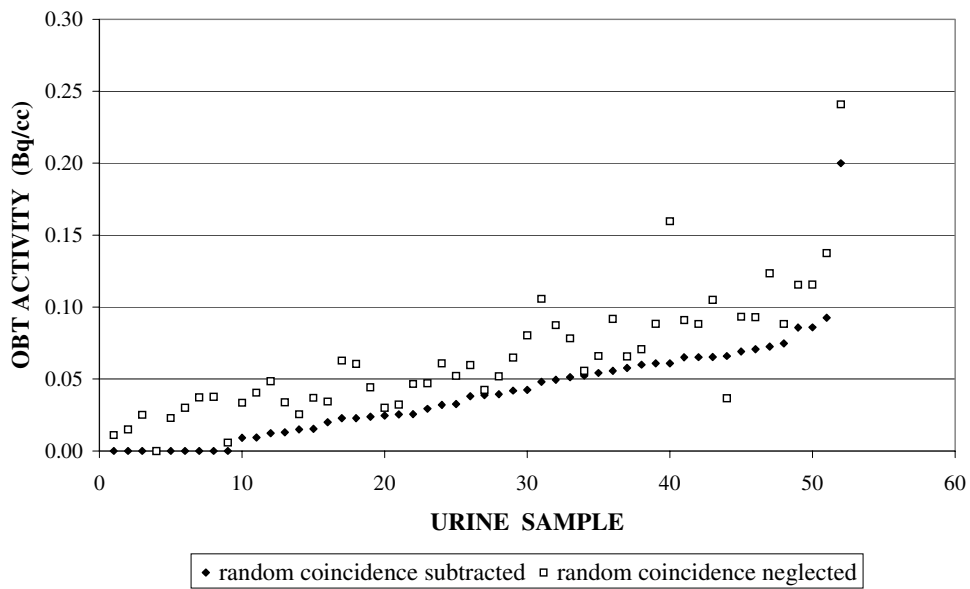


Figure 4 OBT activities in urine samples determined with and without subtraction of random coincidence effects

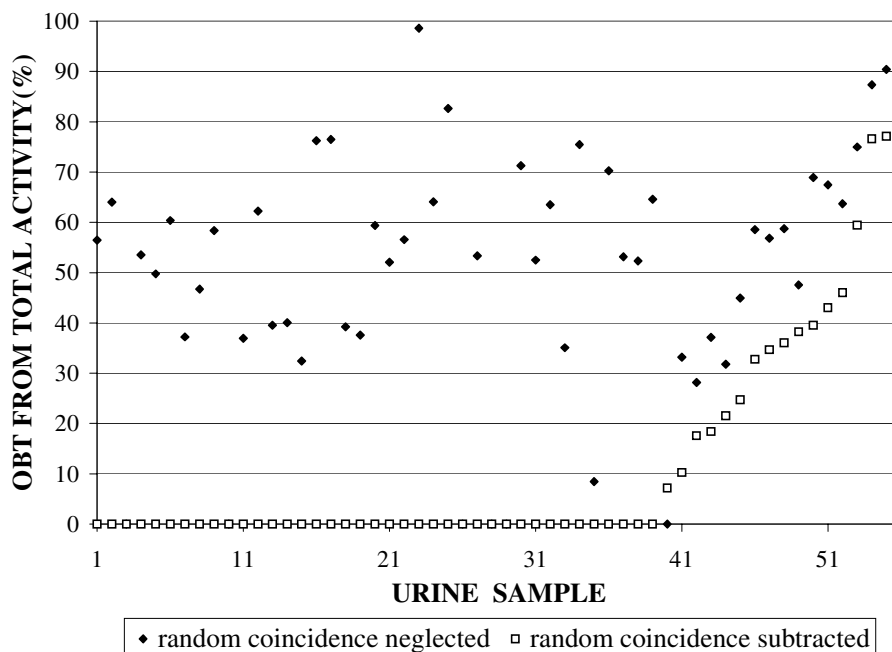


Figure 5 OBT percent from the total tritium activity in urine as determined with and without subtraction of random coincidence effects.

The concentration of organic components in urine changes from person to person and fluctuates during the day. Some processes may require concentration of the urine sample; therefore, it is of interest to investigate the chemiluminescent effect also for concentrated urine. A series of experiments was performed in which 500-mL urine samples were concentrated by low-rate thermal evaporation at 40 °C with slow stirring to 50 mL. Different amounts of concentrated ($\times 10$) urine were divided between standard counting vials, to which equal OBT spikes of 63 Bq were added (Fructose-1- ^3H , made by Dupont NEN, Belgium). The samples were homogenized with different amounts of liquid scintillator, according to the manufacturer's instructions of permissible dilutions. The vials containing the different solutions were counted in the Quantulus 1220 system. Equivalent tritium activities were evaluated by the procedures described in this work. Results are given in Table 2 of the chemiluminescent contributions for the samples containing different volumes of $\times 10$ concentrated urine. The table also contains results of other organic compounds, as discussed in the next section. The maximum urine volume in the samples was 3.2 mL, for which the limit of the quench correction curve was reached. For the whole volumes' range, the equivalent ^3H activity of the random effects contribution increased from the background value to about 0.51 Bq/sample. The OBT spike activities could be evaluated for all cases within the expected $\pm 5\%$ uncertainty of the target value of 63 Bq. As the spike activity used was relatively high, no significant interference of the chemiluminescence background was measured, but for a spike 1/10 of that used, the background would have been about 10% from the measured value, stressing the importance of taking into consideration the background contribution.

Other Organic Compounds

The main purpose of this work was to quantify the background effects in urine samples, which are the main interest in a radio-toxicological laboratory, but sometimes there may be the need to mea-

sure the OBT content in other organic compounds. For example, OBT content in oil vapors, which may be inhaled by workers, can be significant. In this section, the background effects were evaluated for some organic compounds, including glucose solutions and oils. The samples were prepared by adding different amounts of checked materials to standard counting vials, to which equal OBT spikes of 60 Bq were added (Fructose-1-³H, Dupont NEN, Belgium), and were filled to 20 mL with different amounts of liquid scintillator. The vials containing the different solutions were counted in the Quantulus 1220. For all samples, care was taken to remain in the range of the quench curve, which permitted proper quench correction. The random coincidence contributions obtained for the different materials and amounts, including $\times 10$ concentrated urine, are presented in Table 2. These contributions are translated as fictitious activities, which are background of the tritium evaluated activities. As mentioned earlier, the main contribution to these background counts is due to chemiluminescence, and this process seems to be quite significant when some organic compounds are counted by LSC.

Table 2 Background-equivalent ³H activities and their standard deviations in 20-mL scintillator samples containing different volumes of organic compounds.

CONCENTRATED URINE ($\times 10$)		50% GLUCOSE SOLUTION		VACUUM PUMP OIL	
Compound volume (mL)	Random effect contribution (Bq/sample)	Compound volume (mL)	Random effect contribution (Bq/sample)	Compound volume (mL)	Random effect contribution (Bq/sample)
0.05	0.018 \pm 0.004	0.2	0.013 \pm 0.004	0.05	0.013 \pm 0.004
0.1	0.062 \pm 0.009	0.4	0.004 \pm 0.002	0.1	0.018 \pm 0.005
0.2	0.063 \pm 0.009	0.8	0.005 \pm 0.002	0.2	0.017 \pm 0.005
0.4	0.078 \pm 0.011	2.0	0.010 \pm 0.003	0.4	0.013 \pm 0.004
0.8	0.240 \pm 0.025	4.0	0.011 \pm 0.004	0.8	0.022 \pm 0.005
1.6	0.410 \pm 0.042	6.0	0.007 \pm 0.003	1.6	0.022 \pm 0.005
3.2	0.510 \pm 0.070				

CANNABIS OIL		OLIVE OIL	
Compound volume (mL)	Random effect contribution (Bq/sample)	Compound volume (mL)	Random effect contribution (Bq/sample)
0.05	0.010 \pm 0.003	0.05	0.018 \pm 0.004
0.10	0.012 \pm 0.004	0.10	0.042 \pm 0.007
0.15	0.047 \pm 0.009	0.15	0.100 \pm 0.010
0.20	0.052 \pm 0.01	0.20	0.130 \pm 0.013
0.25	0.055 \pm 0.01	0.25	0.190 \pm 0.015
0.30	0.053 \pm 0.01	0.30	0.240 \pm 0.017
0.40	0.077 \pm 0.01	0.40	0.470 \pm 0.025
0.45	0.120 \pm 0.020	0.45	0.730 \pm 0.033
0.50	0.180 \pm 0.025	0.50	0.820 \pm 0.035

According to Table 2, one can see that the different compounds have significantly different background contributions. The 50% glucose solution seems to produce a very low effect, and even when the highest volume was added, the contribution was not much different from background. The oils,

especially the olive oil, seem to provide the highest background. Even a small addition of 0.5 mL is enough to produce a light amount equivalent to that of about 1 Bq of ^3H . It seems that the background contribution is not linear with the added volume. For the oils, a certain supralinearity can be observed for the greater volumes, while for concentrated urine a sublinearity may be present. The volume limits for the different organic compounds were set to obtain an acceptable error in determining the OB T spike activities. For the ranges given in Table 2, the spike activity could be evaluated for all cases within $\pm 5\%$ uncertainty of the target value of 60 Bq.

CONCLUSIONS

Background effect is normal for every measurement, and its contribution must be evaluated when determining accurate and correct results. The background contributions were determined for the LSC process used in our laboratory for OB T determination in urine samples, based on subtracting the tritiated water phase activity (HTO) from the total activity in the urine phase (OB T+HTO).

The ^{40}K contribution, which is a natural contaminant and is present in all urine samples, was determined. The partial area of the pulse-height spectrum corresponding to the tritium range is about 4% from the total spectrum area obtained in LSC. This interference is negligible in normal urine samples, as determined from the analysis of 56 urine samples of workers potentially exposed to tritium.

The main contribution to the background is attributed to random coincidence effects in the photomultipliers that view the LSC sample. Among these effects, chemiluminescence is the principal one; thus, the presence of organic matter is the main origin for disturbing the background. As organic matter is present in urine, this effect must be evaluated and accounted for in processes where "full urine" counting is performed. This can be done by using sophisticated LSC counters that store the information of the random coincidence occurrences.

The background contribution due to random coincidence effects was significant in urine samples of workers analyzed for OB T content. The effect may become more critical when using processes in which the urine is concentrated or when measuring other organic compounds. However, even for high chemiluminescent materials such as oils, accurate estimation of the tritium content may be performed by limiting the organic compound volume added to the scintillator. The background contribution, however, must be monitored as it may be significant, especially for low-level counting.

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