

Chapter 6

Questions regarding the Occurrence of Unwanted Luminescence in Liquid Scintillation Samples

B. Scales

*Imperial Chemical Industries Ltd., Pharmaceuticals Division,
Alderley Park, Cheshire, England*

INTRODUCTION

The continuing increase in the use of soft β -emitters, and the relatively high cost of the necessary counting equipment often makes it imperative that relatively unsophisticated equipment is purchased; the total number of counting heads available being much more important than the 'storage capacity' of a machine. We have available ten automatic liquid scintillation counters. The order in which we purchased these instruments and some of their more useful features are shown in Table 1. The rapid increase in the number of counting heads must gladden the hearts of manufacturers of liquid scintillation counting equipment, but it can bring unforeseen problems to the users of that equipment.

All manufacturers attempt to satisfy the most stringent requirements of their customers and, besides stability, two of the most important requirements are:

1. a high counting efficiency, and
2. a low background count.

As is the case with most other workers with soft β -emitters, the ability to create troublesome chemiluminescence was acquired soon after our entry into the field of liquid scintillation counting, but with experience we learned that in order to have acceptably low background counts, certain methods of sample preparation were best treated with extreme caution, or better still, completely ignored.

Occasionally, some workers with isotopes can accept a relatively high and fluctuating background count, but when the activity of the isotopes is limited as in tissue residue studies, or when metabolism studies or sub-micro analytical procedures are being developed using organic materials of relatively low specific activity, a low and stable background is desirable.

It is over the last two years, when different makes of equipment have become available and new techniques have appeared, that further problems have become apparent. Those problems which fit into the context of this paper are all connected with the presence of undesirable luminescence, i.e. an increase in background count.

Some of our problems have been solved; this paper attempts to show how they arose, and how they were overcome.

Table 1. Availability of automatic liquid scintillation counters.

Model	Purchased	Sample capacity	Temperature °C	Facilities
Packard 314EX	/60	100	2-4	Lister
Packard 314EX	9/61	100	2-4	Lister
Packard 3314	6/62	200	2-4	Lister, b.g., A.E.S.
Packard 3124	10/64	200	2-4	Lister, b.g., A.E.S.
Packard 3320	7/65	200	2-4	Lister, b.g., A.E.S.
Philips LSA	12/69	420	18	Teletype, Computer
Packard 3320	3/70	200	2-4	Teletype, b.g., A.E.S.
Packard 2002	1/71	100	ambient	Lister, b.g.
Packard 2002	1/71	100	ambient	Lister, b.g.
Intertechnique SL30	3/71	200	8	Teletype, b.g., A.E.S.

Lister = Lister printer

b.g. = background subtract

A.E.S. = Automatic external standardization facilities

Vial caps of different manufacture

The first problem was concerned with the phosphorescence of a certain make of vial cap. It was noticed that after a series of 50 to 60 samples had been counted overnight for 10 min each in a Packard 3320 the blank samples in the series gave background counts of the order of 150 c.p.m., i.e. from 4 to 5 times the normal count of 30 c.p.m. The sealed blanks gave normal background counts when assayed at the most diagnostic isotope settings i.e. tritium in toluene and carbon-14 in toluene, and tube noise was satisfactory.

After some time it was realized that the batch of vials being used was not from our usual source. Our new Philips Liquid Scintillation Analyzer was temporarily out of order (due to our own negligence) and these high background samples had been prepared in the last of the batch of Philips vials sent with the original purchase order. After some time we discovered that the caps of these vials had a phosphorescence which although discarded by the Philips electronics, was strong enough to pass through the coincidence system of our refrigerated Packards, when operating under normal counting conditions (see Table 2). This phosphorescence decayed very slowly at reduced temperatures (0 to 4°C).

The phosphorescence in the Philips caps was activated slightly by strip lighting and very much more so by sunlight. Irradiation by ultraviolet light at 256 nm (Chromatolite) caused gross activation of phosphorescence in both Philips and Packard caps.

Our problem had been that:

1. Carrying the vials along corridors fitted with strip lighting was sufficient to activate the phosphorescence of one make of cap and not another, and
2. This was discarded by one machine and not by another.

We tried to prevent the problem from recurring by standardizing on the type of vial and cap which appeared to suit all machines; we chose Packard vials, but I am now informed that the problem with the Philips caps was an isolated one which was rapidly overcome.

Effects of temperature on different vial caps

A second problem with the luminescence of vial caps came soon after the purchase of two room temperature machines. The vial caps which had been proved to be acceptable

Table 2. Effect of ultraviolet-induced luminescence of caps on background counts (c.p.m.).

Instrument	Cap	Time in instrument (h)				
		0	2	6	24	48
Philips L.S.A.	A	31.5	31.7	30.2	31.0	30.9
	B	29.5	30.6	30.6	30.7	30.1
Packard 3320	A	156.5	154.7	132.9	129.2	131.9
	B	28.1	29.0	27.7	27.5	29.3

Vials were low potassium-40 supplied by Packard.

Cap A supplied by Philips.

Cap B supplied by Packard.

Vials contained 20 ml toluene containing 0.6% butyl P.B.D.

All samples were counted for 10 min periods, under optimum gain conditions for carbon-14.

in all our refrigerated (controlled temperature) machines gave rise to high and unstable background counts in the two ambient temperature instruments.

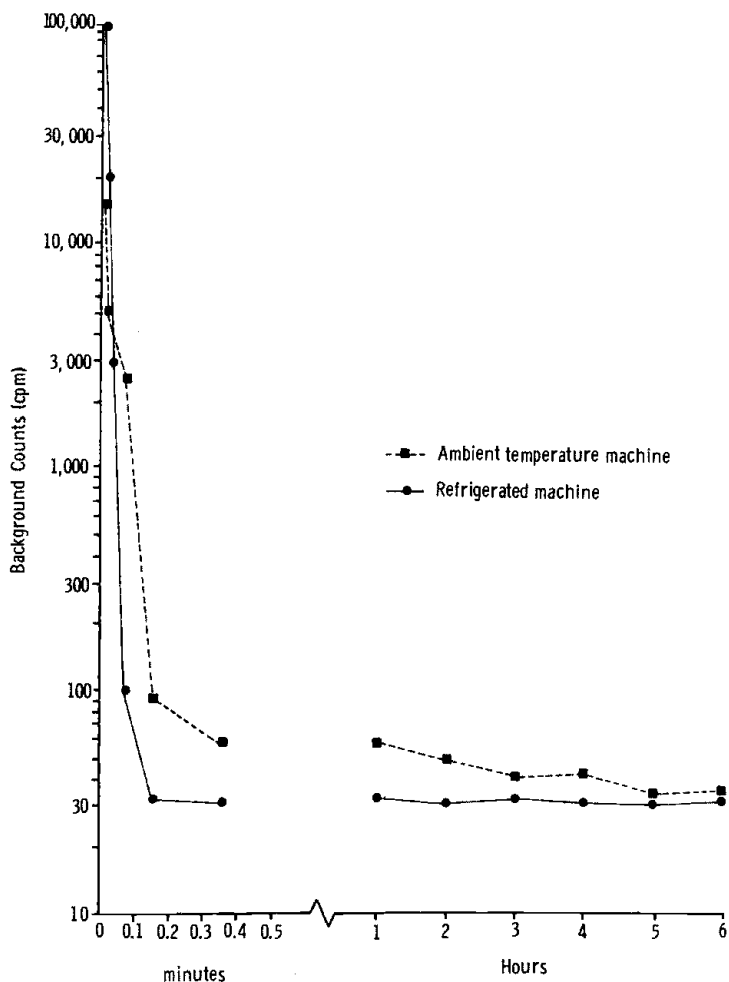
The possibility of static was ruled out, and we eventually found that the sunlight, of which we had more than usual during February and March, was the chief cause of the problem. The sunlight, shining although somewhat weakly across the laboratory, was sufficient to give rise to initially 2000 c.p.m. above background. This decayed with at least two component half-lives (see Fig. 1). One half-life was of the order of seconds so that the immediate counting rate on entering the counting chamber was of the order of 30000 to 100000 c.p.m. This was not new to us; it is often seen in a refrigerated machine placed in bright sunlight. The other half-life was much longer (of the order of 2 to 3 h) and it was only observed in the ambient temperature machines where it lifted the background count by 20 to 30 c.p.m.

In this problem, therefore, there were two machines with identical electronics, yet at room temperature a long-lived component of the light-induced phosphorescence was sufficiently intense to pass the coincidence system and double the background count in the tritium channel. These problems can be solved by:

1. Increasing the threshold of the tritium and carbon-14 windows. This resulted in a lowering of the tritium efficiency from 45% to 25%, and of the carbon-14 efficiency from 88% to 84% before a normal background was achieved.
2. Reducing all sources of ultraviolet light to a minimum by using tungsten lighting, shields over fluorescent lighting and by the use of window blinds. This we feel is absolutely essential especially in the counting room.
3. Most successfully, when re-using vials – this is done on average about ten times before they are discarded – they are re-capped with cheap disposable plastic caps from the Metal Box Company. Cat. No. 1A. 22R3. These soft plastic caps do not have a cork-tin foil insert, but rely on an annular lip of plastic for the seal. Because of the lack of a tin foil insert, there is in our experience a slight decrease in counting efficiency of from 1 to 1.5% for both tritium and carbon-14. More importantly we have been unable to induce any phosphorescence in these caps which can be detected in any of our machines, even after intense irradiation with ultraviolet light at 265 nm or 365 nm.

Triton X-100

A further problem which would not have been readily apparent if we had not pur-



During the initial 0.50 minute period of high luminescence, the average count rate during the various time intervals was calculated from the visually observed accumulation of pulses.

After 1 minute when the background was relatively stable, the average count rate was calculated from 20 minute counting intervals.

Fig. 1. Chemiluminescence of dioxane systems in refrigerated and ambient temperature machines.

chased ambient temperature machines concerned the use of Triton X-100. Although Patterson and Greene¹ state that a high background can be obtained with some batches of Triton X-100, this had never been our experience when using refrigerated equipment. However, under optimum ¹⁴C-counting conditions the conventional Triton X-100–water systems were found to give backgrounds of 125 to 250 c.p.m. in the ambient temperature instruments. Background counts of 250 c.p.m. dropped with a half-life of about five days

Table 3. Effect of different batches of Triton X-100 on background counts (c.p.m.).

Instrument Packard	Temp.	Batch	Time in instrument (days)						
			1	3	5	8	10	15	22
2002	Ambient	New	253.5	201.1	135.7	110.3	85.5	62.1	42.2
		Old	33.3	32.8	32.9	32.3	33.0	31.9	32.7
3320	2-4°C	New	34.8	34.3	33.6	33.9	33.2	33.4	32.1
		Old	32.0	29.1	28.7	29.1	31.1	30.8	31.8

Samples were 10.0 ml of a mixture of toluene-Triton X-100 (2:1) containing 0.6% butyl P.B.D. Batch-New contains a high proportion of luminescent impurity. Batch-Old contains acceptably low amounts of the impurity. All samples were counted for 100 min each under conditions for optimum carbon-14 counting.

(see Table 3). Careful examination of the background counts obtained from the same samples in the refrigerated machine showed that they were about 10 to 15% higher than expected. Also a decay of luminescence was detectable, but it occurred with a half-life of two to three weeks, and it was not therefore noticeable when using the refrigerated machine that a faulty batch was in use. The normal, acceptable background counts obtained on both refrigerated and room temperature machines with good quality Triton X-100 are shown in Table 3 for comparison.

The luminescence from this impurity was such that its contribution to the background of the tritium channel could only be removed by increasing the threshold to such a level that the ^3H -counting efficiency was reduced by 2/3. The ^{14}C -counting efficiency was also reduced by about 1/6 before its background became normal.

This fluorescent impurity could not be removed by absorption onto silica as suggested by Patterson and Greene.¹ The fluorescent impurity appears to be a contaminant occurring during manufacture since it does not form on keeping Triton X-100 in darkness or in sunlight and, when present, its proportion appears to remain constant. It would, therefore, appear that selection of batches of Triton X-100 prior to purchase is the best way of ensuring the quality.

Dioxane

Until recently, in common with many other workers we used dioxane-naphthalene - PPO-POPOP or -butyl PBD scintillators for aqueous samples containing tritium, carbon-14, calcium-45 or iodine-131. For this purpose the dioxane was purchased in 25 gallon drums from BASF. This product has a very low water content (less than 0.1%) and usually needs no special purification, even for ^3H -counting, especially as the dioxane-based scintillators were used at the rate of 20 l/week. Occasionally, if extra high purity material is required, in order to decrease the amount of chemiluminescence produced by somewhat alkaline solutions, we obtain a very good clean-up by a crude zone-melting procedure, carried out by alternately freezing and thawing winchesters of dioxane. This is done quite simply by wheeling trolleys loaded with the winchesters into the cold room (0 to 4°C) for freezing overnight; any liquid impurities are decanted the following morning and the solid contents then thawed out during the day. The process is then repeated until no liquid impurities are apparent. This results in good quality dioxane which is very satisfactory for liquid scintillation counting with little effort and hazard. However, the ready and often uncritical acceptance of Triton X-100 by many workers

resulted in a decreased usage of dioxane and as a result of the increased peroxide formation we started getting high backgrounds due to chemiluminescence, even at neutral pH (6.5 to 7.5). Once again this luminescence was only apparent in ambient temperature machines in which, of course, the luminescence decayed fairly rapidly. Thus at neutral pH it was not noticeable after 30 min. At pH 8.5 or greater, even in refrigerated machines it can take at least 2 to 3 days for acceptable background counts to be obtained and often much longer. The addition of antioxidants such as B.H.T. or recommended sulphhydryl reagents and chelating agents such as diethyl dithiocarbamate,² although reducing this chemiluminescence also significantly reduced counting efficiency in the tritium channel.

Injection tubes for GLC

The problems discussed above were not isolated problems; they did in fact occur within a period of two months. The final problem I will mention was encountered at the same time as the problems of luminescence with vial caps, Triton X-100 and peroxide formation in dioxane, so that at one time we had at least three unwanted phenomena taking place in any one vial.

This last problem occurred when we were investigating various analytical techniques intended for use eventually with non-radio-labelled drugs undergoing toxicological and clinical evaluation. The technique in question involved a series of extractions and back-extractions from biological fluids, TLC of the extracts and after elution from the plates, the formation of a derivative for GLC. The application of the samples to the Pye GLC machine was by use of a 'solid injector'.

It is imperative in such a complicated method which has to be carried out on the nanogram level that each stage works efficiently and that any slight variation can be understood and overcome. To this end, the use of radio-labelled materials is invaluable even though the count rate can be quite low with specific activities in the 2 to 20 $\mu\text{C}/\text{mg}$ range.

Whilst checking for possible losses of a drug during evaporation of 30 μl of solvent from the Pye solid injection tubes, and also checking that drug placed in the tubes was volatilized in the pre-heater of the GLC column, it was observed that the small glass 'solid injection' tubes, when placed in a vial containing scintillator, could give rise to anything from 0 to 15000 c.p.m. in the tritium channel and 0 to 5000 c.p.m. in the carbon-14 channel. This count occurred in all machines irrespective of temperature and scintillation medium. The tubes had no phosphorescence of their own and in dioxane-water required the presence of naphthalene, or better, naphthalene-butyl PBD for maximum luminescence. It is inconceivable that the tubes have a high potassium-40 content, since the energy spectrum is not that of potassium-40 and the count is too high for natural potassium-40.

Some batches of injection tubes when cleaned in dilute hydrochloric acid and methanol had a normal tritium and carbon-14 background, but those cleaned for us in Haemasol and water, to give tubes which were satisfactory for GLC use, occasionally but not always gave this high background of from 1000 to 15000 c.p.m. We were therefore initially very suspicious of the use of Haemasol. Attempts to induce this prolonged luminescence by baking Haemasol and methanol onto GLC injection tubes and liquid scintillation vials have not been very successful. All we have achieved is a count of 400 c.p.m. which decays over a 30 min period. We have subsequently tried to remove

this low luminescence from the injection tubes by acid washing and so far have been quite unsuccessful. The problem of high luminescence from some of these glass injection tubes remains a mystery.

CONCLUSION

During this talk I have repeatedly stated that these problems showed up when ambient temperature machines were used. I am not suggesting that such machines are a bad buy as do many of our users, but I would rather suggest that they did bring to light sloppy techniques, and that much more care is required in liquid scintillation counting than many workers are prepared to admit.

REFERENCES

- 1 M. S. Patterson and R. C. Greene, *Anal. Chem.* **37**, 854 (1964).
- 2 *Chemical Notes*: A summary of current practices in sample preparation for liquid scintillation counting – March 1970. Issued by the Packard Instrument Company.

DISCUSSION

Anon: Comment: We have had considerable problems with materials in plastic bottles leaching out into solvents and producing excessively high background counts. Recently, different batches of solid scintillators, when dissolved in solvent were found to produce a very high, long-lived luminescence. This was eventually traced to the use of scoops made out of plastic bottles which were allowed to remain in contact with the organic scintillators.

F. E. L. ten Haaf: Comment: I should like to make a brief comment on the fluorescent vial caps as I happen to know the inside story about them. The vials and the caps are made for us by another manufacturer and delivered to us in batches of a few hundred thousand at a time.

We designed them rather carefully and the material for the caps has been given due consideration. Several series were delivered and found to be good, but suddenly, without consulting us, the manufacturer changed the material for the caps. Before this was discovered a few thousand vials had been delivered to customers and I am fully aware that, although we replaced them with good ones, this has caused you and other users considerable embarrassment. I can assure you that we will try very hard not to make a habit of it.