Faculty of Life Sciences

Incidental Release of Radioactive Gaseous Waste to Atmosphere as a result of work with Volatile Radioactive Substances in Fume Cupboards.

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1 Introduction

1.1 Authorised Limit

On the 8th of May 2002 SEPA imposed a limit on the incidental release of radioactive gaseous waste to atmosphere from the Wellcome Trust Biocentre (WTB). The Certificate of Authorisation states: "In all the waste discharged in any one day, the total activity expressed in kilobequerels, of all the radionuclides, other than tritium, taken together divided by the total volume, expressed in cubic metres, of all the waste so discharged shall not exceed 0.4."

1.2 Interpretation

This means that the total kilobequerels (KBq) of radioactive gas (excluding tritium, which has it's own limit) released from the fume hood exit pipe in one day, divided by the total cubic meters of air expelled from the fume hood exit pipe in one day, must not exceed 0.4 KBq/m^3.

1.3 Implications

Records must be kept that demonstrate this limit is being complied with. This necessitates:

- Identification of experimental procedures involving the use of volatile radioactive substances undertaken within the WTB fume hoods.
- Calculation of the total cubic meters of air expelled from the fume hood exit pipe in one day.
- Calculation of the maximum KBq of radioactive gaseous waste that may be emitted in one day, based on the 0.4 KBq/m^3 limit and total cubic meters of air expelled.
- Quantitation and recording of potential maximum incidental radioactive gaseous waste release to atmosphere.

Each of these requirements is dealt with in the following section.

2 Observing the Limit

2.1 Identification of Experimental Procedures Involving the Use of Volatile Radioactive Substances

WTB Radiation Protection Supervisors (RPS) were made aware of the new limit and asked to submit details of experimental procedures involving the use of volatile radioactive material undertaken within their area. The issues were discussed at a Lab Manager's meeting held on 27/3/02 (as recorded in the minutes) and RPSs were notified by E-mail and memo. Non-WTB RPS were also made aware of the situation. See Appendix 1 for copies of correspondence.

Based on the response, it was concluded that only two procedures involving the use of volatile radioactive substances are routinely undertaken in the WTB, both in the supervised area on Floor 2:

i. Sodium 125-Iodide solution is used in the radio-iodination of proteins.
ii. 35-Sulfur labelled amino acid solution is used in cell labelling experiments.

Details of the experimental procedures and radioactive substances are given in section 2.4.

2.2 Calculation of the Total Volume of Air Expelled form the Fume Hood in One Day

George Morrison (University Estates and Buildings Mechanical Engineer) and David Hewick (University Radiation Protection Adviser) supplied data (see Appendix 2) upon which the following calculation is based.

The Supervised area on Floor 2 of WTB contains two separate fume hoods, one used for ^{125}I work and one for ^{35}S. Both have the same aperture width, sash height and face velocity. The following figures and calculations therefore apply to either hood.

Aperture Width = 0.91m

Aperture Height = 0.02m at minimum sash height
(assume sash is at minimum height for most of the day)

Aperture Area = Width x Height
= 0.91m x 0.02m
$=0.0182 m^2$

Face Velocity = 0.8m/s

Rate at which air is expelled
= Aperture Area x Face Velocity
= 0.0182 m^2 x 0.8m/s
= 0.01456 m^3/s

Volume of air expelled in one day
= rate x (60 x 60 x 24)s
= 0.01456m^3/s x 86400s
= 1258m^3

2.3 Calculation of the Total KBq of Radioactive Gas that May be Emitted in One Day

Total KBq that may be emitted in 1 day
= total m^3 of air expelled in 1 day x KBq allowed per m^3
= 1258m^3 x 0.4KBq/m^3
= 503KBq
Translated to μCi for the benefit of the end users
= 13.6μCi
Note: this is the amount that can be emitted from **each** hood.

2.4 Quantitation of Incidental Radioactive Gaseous Waste Release

2.4.1 Radio-Iodination Experiments

Radio-iodination experiments involve the use of Sodium 125-Iodide solution to produce ^{125}I labelled proteins.

The manufacturer of the Sodium 125-Iodide solution, Amersham, claims that the 125-Iodide remains as iodide whilst the pH of the solution is above 7.0 (see Appendix 3 for data sheet). The alkaline solution neutralises during storage, and especially upon opening, due to absorption of CO_2 from the atmosphere. The pH normally falls to 7.5 and remains so for at least 6 weeks, after which one must assume it may acidify and release volatile 125-Iodine. Storage at or below 0ºC also produces volatile 125-Iodine. To minimise the possibility of volatilisation stocks should be stored at room temperature and for no longer than 6 weeks.

The data from Amersham gives the impression that volatilisation is not a problem if the stocks are correctly stored, however, the release of molecular radio-iodine from 125-Iodide solutions[1], and ^{125}I labelled proteins[2], has been observed under experimental conditions. A report by O'Neill *et al* (see Appendix 4) concludes that the rate of volatilisation from Sodium 125-Iodide solution is less than the 3% per day (0.1% per hour) rate previously reported by Ramsey *et al*[2] for solutions of ^{125}I labelled proteins. We have opted to adhere to the upper value of 0.1% per hour in our calculations for both Sodium 125-Iodide and ^{125}I labelled protein solutions.

A typical radio-iodination experiment may involve aliquoting 1mCi from a 5mCi stock-pot of Sodium 125-Iodide solution into an iodogen tube, along with the protein to be labelled, for a 10 to 15 minute incubation. After incubation the reaction mixture is applied to a PD10 column which retains the unbound 125-Iodide. The ^{125}I labelled protein is eluted off, collected and then stored in a sealed tube for use in subsequent experiments. The column is capped, top and bottom, and disposed of.

The 5mCi stock is open to the atmosphere for a maximum of 1 minute, the 1mCi reaction mixture for a maximum of 15 minutes and the lower activity eluate

(activity varies but assume 250μCi for the purposes of this example) is exposed for approximately 15 minutes.

Based on the above times and using the 0.1% per hour volatilisation rate, the amount of 125-Iodine escaping to atmosphere via the fume hood, during a typical iodination procedure, can be calculated as follows:

5mCi x 1/60hr x 0.1%	= 0.000083mCi
	= 0.083μCi
Plus:	
1mCi x 15/60hr x 0.1%	= 0.00025mCi
	= 0.25μCi
Plus:	
250μCi x 15/60hr x 0.1%	= 0.0625μCi

Which gives a total of:
0.083μCi + 0.25μCi + 0.0625μCi = 0.3955μCi

Well within the 13.6 μCi limit.

The number of experiments carried out in any one day is also an important factor. Analysis of the previous 18 month's usage records indicates that typically only one radio-iodination experiment is carried out during a working day. In addition to this, 2 or 3 experiments involving existing stocks of labelled proteins may also be undertaken. At this frequency the limit will never be exceeded.

2.4.2 Use of 35-Sulfur Labelled Amino Acid Solutions in Cell Labelling Experiments

35-S labelled amino acid solutions are aliquoted out for use in cell labelling incubations carried out in a dedicated CO_2 incubator.

The primary product used is ICN's Trans ^{35}S-Label (see Appendix 5 for data sheet), a mixture of ^{35}S labelled L-Methionine (70%) and L-Cysteine (15 %) in 50mM L-Lysine/10mM βME buffer. ICN acknowledge the potential for formation of volatile decomposition products during product storage and cell labelling incubations. The user is referred to a Biotechniques paper by Miller[3], which deals mainly with the evolution of ^{35}S volatiles from tissue culture medium and the use of stabilizers to reduce the rate of release. Miller concludes a slow rate of release, averaging 12nCi/mCi/day (or 0.0012%), and demonstrates the efficacy of commercially used stabilizers in reducing, although not eliminating, the generation of volatile compounds. Useful advice is given on safe working practices (e.g. thawing and opening containers in a fume cupboard; using charcoal and/or water traps in incubators) and storage conditions (e.g. long term storage at –80°C or below; storing in small aliquots to avoid repeated freeze-thawing). However, the paper does not give figures for the release of ^{35}S volatiles from stock amino acid solutions.

A short letter to Nature by Meisenhelder and Hunter[4] also discusses the volatility problems encountered during use of ^{35}S labelled amino acids. They report a release of 1μCi of ^{35}S volatiles upon opening an 8mCi stock vial after thawing from frozen

(0.0125% loss to atmosphere). Further release during incubation with cell culture medium is also investigated. It is interesting to note that volatile material is released from culture medium regardless of whether cells are present, indicating a chemical/physical rather than metabolic process.

For the purposes of this exercise we can discount the nCi amounts of ^{35}S volatiles evolved during cell labelling incubations – they are safely absorbed into activated charcoal and water traps within the CO_2 incubator. Calculation of incidental release of gaseous ^{35}S waste to atmosphere will consider only the release of ^{35}S volatiles upon opening a vial within the fume cupboard, after thawing from frozen.

Example calculation:

7mCi stock vial is thawed and opened within the fume cupboard.
Assume 0.0125% of the total vial activity is lost to atmosphere:

= 7mCi x 0.0125%
= 0.000875mCi
= 0.875μCi

Well within the 13.6μCi limit.

The number of stock vial openings in any one day is also an important factor. Analysis of the previous 18 month's usage records indicates that up to 6 experiments may be carried out in a working day. This could result in the opening of 6 stock vials of varying activities, but none greater than 7mCi. At this frequency and level of activity, the limit will never be exceeded.

References

1. **Evans, G.J. et al.** 1993. "The Volatilisation of Iodine Species Over Dilute Iodide Solutions." Canadian J. of Chem. Engng 71: 761-765.

2. **Ramsey, N.W. et al.** 1980. "Loss of ^{125}I from Labelled Proteins." Br. J. Radiology 53: 357-358.

3. **Miller, Daniel M.** 1990. "Buffer Solutes as Stabilizers of ^{35}S-Amino Acids: A Study of Volatility, Radiochemical Purity and Biological Activity." Biotechniques 9, No.5: 592 - 596.

4. **Meisenhelder, Jill, Hunter, Tony**. 1988. "Radioactive Protein-Labelling Techniques." Nature 335: 120.