RADIOACTIVE CONTAMINATION CONTROL PROGRAMS IN RADIOACTIVE INSTALLATIONS OF BIOLOGICAL RESEARCH

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INTRODUCTION

Radioactive compounds are nowadays widely used in Biological Research. There is a great quantity of analytic radioisotopic techniques since they yield evident advantages like specificity, versatility and sensitivity in comparison with non radioactive protocols. Nevertheless, it is obvious that manipulation of radioactive unsealed sources may generate risks for exposed workers.

The aim of our work has been to evaluate the probability and incidence of several contamination types that can occur. In order to achieve this purpose, we have make an exhaustive analysis of the most common radioisotopic techniques (table I) used in our Research Centers. Also, we have taken into account physical characteristics and radiotoxicity of the radioisotopes used in such techniques.

Type of mollecule	Most Common techniques	Labeled compouns mainly used
Nucleic acids	Northern, Southern, "in situ" hybridization	nucleotids-^{32}P, -^{33}P
	Sequencing	nucleotids-^{35}S, -^{32}P, -^{33}P
	Activity RNA-polimerase based methods	nucleotids-^{32}P, -^{33}P
	Footprinting, bandshift	nucleotids-^{32}P, -^{33}P
	Activity of genetic promoters	chloramphenicol, acetyl CoA-^{14}C
	DNA synthesis	Thyimidine-^{3}H
Proteins	Western blot, radioimmunoassays	Na^{125}I
	Binding hormone-receptor assays	ligands-^{32}P, -^{35}S, -^{125}I, Na^{125}I
	Enzymatic kinetics	several-^{32}P, -^{35}S, -^{125}I, -^{3}H, -^{14}C
	Proteins synthesis	methionine, cysteine-^{35}S
Carbohydrates and lypids	Action mechanism of steroydic hormones	ligands-^{14}C, -^{3}H
	Metabolism of lypids, cholesterol and carbohydrates	cholesterol, precursors-^{14}C, -^{3}H

Table I: Radioisotopic techniques most commonly used in Biological Research

The main factors that we have considered in relation to the probability and incidence of possible contamination are: maximum activity used per assay (assays carry out "in vivo" or "in vitro"), physicochemical characteristics of radioactive compounds used, and of his main and residual compounds; technical complexity (manipulation type and equipment needed). This information has been quite useful to set up the contamination control program that we expose below.

MATERIALS AND METHODS

In order to determine contamination levels in the different techniques indicated below, we have performed the following measures:

-Surface contamination: surfaces and diverse material.

-Enviromental contamination: coal filter, coal samples used during the manipulation of volatile compounds (labeled with S-35 or I-125 and/or I-131), liquid effluents.

-Personal contamination: external (protection clothes and skin) and internal (these measures haven't been done because of not having the necessary resources).

The measuring time depends on the kind of equipment or surface to measure. The distance between the monitor and the surface or equipment has been about 1 cm.

In the radioisotopic techniques using P-32, I-131 and/or I-125 the measures have always been directly taken.

In the case of S-35, C-14 and P-33, the measures have been both direct and indirect, and always indirect measurement in case of H-3.

The equipments used in the direct measurements were:

-BERTHOLD:LB-122 and 123.

-ROTEM: Ram-Gene1, Ram-Surf, Ram DA-3 with PM-10 ang GM-10 probes

-MINI-INSTRUMENTS: E, EP-15 and 44A.

The indirect measurements have been performed by smearing with filter paper wet in decontaminant solution are afterwards counting in a liquid scintillation counter.

We have evaluated the factors considerated in the introduction and according to them, we have been able to provide the specific hazards of each technique .All these data together with bibliographic references,have decided the necessary protection protocols.

* Radiolabelling of biological molecules with I-125 or I-131

* *Asociated risk.*

Volatility of radioactive iodo during the labelling experiment. This can produce personal, external and internal, contamination and superficial contamination.

* *Protection measures.*

- Storing the labelling compounds at room temperature.
- Avoid the use of strong oxidants.
- Work always into a fume hood with charcoal filters.
- Use the Hamilton syringes for dispense the radioactive compounds.
- Take advice to put the plates or filters of active charcoal into the fume hood and into the work place to prevent airborne contamination.

* Radiolabelling of proteins with S-35 amino-acids.

**Asociated risk..*

Releasing of ^{35}S--labeled volatile sub-products. Although this compounds have not yet been identified, likely candidates are methyl-mercaptan (CH_3-^{35}SH) as the major volatile product of radiolytic decay of methionine, with hydrogen sulfide H_2S and sulfur dioxide as minor components. Netherless, some authors suggest that less volatile sub-products are released from ^{35}S-cysteine than from ^{35}S-methionine.

Release of volatile radioactive compounds from ^{35}S-amino acids happens during storage, protein-labelling experiments and incubations.

These compounds are readily soluble in water, for this reason the incubators used in cell culture could become highly contaminated.

* *Protection measures .*

- Stored at -80º the 35S-aminoacids in dry ice
- Traw the products in a fume hood with charcoal filters
- Place the filters of activated charcoal in the incubator (this filter does not affect the CO_2 equilibrium in the incubator).
- The water inside incubator should be changed after each labelling.
- Use a sufficient volumen of tissue culture media (75µCi/ml).
- Add an effective stabilizer agent to the tissue culture media (tricina 50 mM). This agent should not be toxic.

*Radiolabelling of biological molecules with H-3

* *Asociated risk.*

- The tritiated compounds used for biological research wiith high risk of contamination are tritiated water, tritiated thymidine and some organic compounds .
- The biological risk highly depends of the tritiated compounds used. Tritiated thymidine presents a high risk of internal contamination because these compounds are quickly metabolized into the genetic material .
- The tritiated water (especially in high specific activity) causes contamination of the frost in freezers when this compounds is stored.
- Tritium gas, tritiated water and some tritiated organic solvents pass through the walls of plastic containes easily.
- Compounds stored in aqueous solution may give rise to tritiated water by exchange which can then result in contamination of the frost in freezers.

* *Protection measures .*

- Experiments with tritiated water or tritiated compounds must be carried on in a fume cupboards (with charcoal filters) to prevent airborne contamination.
- The refrigerators and incubators where tritiated compounds are stored should be checked to detect possible contaminations .
- Sealed glass vials in wich labelled compounds have been stored for long periods should be opened with great care, preferably in a fume cupboard or glove box.
- Tritiated water should be stored at room temperature in sealed containers.
- Wear two pairs of gloves.

CONCLUSIONS

Due to the analysis carried out on the application of radionuclides in biological research we have established a program for contamination control and surveillance and we have set up contamination Reference Levels in these radioactive installations:

REFERENCE LEVELS	Bq/cm^2
Surface contamination	1
Skin	0,37

REFERENCES

- Bolton, A.E. "Radioiodination techniques". The Radiochemical Centre Amersham England, Review 18. Amersham, Bucks. England.(1977).
--Galanek, MS. "Radiation Safety in biological research laboratories". Occup.Med,6 , 2, 255-269.(1991)
- Shapiro,J. "Radiation Protection. A guide for scientists and physicians". Harvard University Press(1990)
-- ICPR Publication 60. Pergamon Press, Oxford, England.(1990)
- D.M. Miller."Buffer solutes as stabilizers of 35S-aminoacids: a study of volatility, radiochemical purity and biological activity".Biotechniques, Vol.9, Nº 5, 592-596 ,(1990)
- Meisenhelder, J. and T. Hunter. "Radioactive protein labeling techniques". Nature 335, 120,(1989)
- Klein, R.C., V.Party and E.L.Gershey. "Safety in the laboratory". Nature, 341, 288, (1989)
- Smith, I., V. Furst and J. Holton. "Hazards of sulfur". Nature 337, 696,,(1989)
- ISO 7503-2 Evaluation de la contamination de surface,partie 2: contamination de surface par le tritium. (1988)
- P. E. Ballance, A.G. Richards and R.N. Thomas. Tritium: Radiation Protection in the laboratory. (1994).
-Safe Handling of Tritium, Review of Data and Experience. Technical Reports Series Nº 324. (1991)