safe and secure

a guide to working safely with radiolabelled compounds

Contents

Introduction

Radiochemicals are widely use in a variety of scientific applications. Amersham Biosciences' many years of experience in the manufacture and use of radiolabelled compounds provides a valuable source of information on how to work safely with radioactivity.

This guidebook provides basic information about the manufacture of radiolabelled compounds as well as guidelines regarding the methods of handling and suitable storage conditions. More detailed information and instructions may be found in the relevant data files and product specification sheets provided with the products.

Regulations

Before ordering or using radioactive materials, customers must take whatever actions are necessary to ensure that they are complying with their national or state regulations governing the use of such materials.

These regulations are additional to and do not replace local rules, instructions or training in the establishment, or advice from local Radiation Protection Advisors, which define actions and behavior in the work-place.

In most countries (except the USA) regulations are closely related to the International Atomic Energy Agency (IAEA) regulations and codes of practice.

In the UK, the principal legislation governing the keeping, use and disposal of radioactive substances is the Radioactive Substances Act 1993 (RSA), the Health and Safety at Work Act 1974 (HSWA) and the Ionizing Radiation Regulations 1999 (IRR).

Customers outside the UK must comply with all relevant local regulations and should contact their local regulatory authority for advice. In the absence of national Guidance Notes or Codes of Practice, it is recommended that the UK system be followed whenever practicable. If in doubt about how to proceed. contact your adviser on radiation protection or Amersham Biosciences Customer Services.

Before obtaining any radioactive substances or undertaking work with ionizing radiation in the UK for the first time, a person or organization must:

• Register by obtaining a certificate of registration and an authorization for disposal (if required) from the Department of the Environment (RSA Section 1) or, in Northern Ireland, the Department of Economic Development (DED).

- Notify the Health and Safety Executive (HSE) or, in Northern Ireland, the DED, of the intention to carry out the work, at least 28 days before commencing the work.
- Appoint a suitably qualified and experienced Radiation Protection Adviser (RPA), having given the HSE at least 28 days prior written notice of the intended appointment. Having appointed an RPA, his advice must be sought on observation of the regulations and safety aspects of the work.

In particular, the RPA must be asked to advise on:

- The selection and training of Radiation Protection Supervisors (RPSs) to supervise the work.
- The drawing up of written systems of work and local rules for the work to be done.
- Appropriate training for the person(s) carrying out the work.
- Appropriate dosimeters, dose-rate meters and contamination monitors.
- Hazard assessments.
- Contingency plans for dealing with any reasonably foreseeable accident, occurrence or incident involving radioactive substances.

Working safely with radioactive materials

The safety requirements for any of the less toxic nuclides (e.g. carbon-14 and tritium) may be less complex and less restrictive than the Regulations or Codes of Practice appear to indicate. This does not mean that these materials may be treated casually.

Compounds labelled with low-energy beta-emitters may be handled safely in the small quantities found in most research and teaching laboratories, with only modest precautions.

These quantities represent no greater hazard than working with many other laboratory chemicals.

It is important to follow the code of good laboratory practice in addition to specific precautions relating to the particular radionuclides used (e.g. when handling high-energy beta-emitters, such as phosphorus-32 or compounds labelled with gamma emitting isotopes).

Although radiation protection can be a complex subject it is possible to simplify it to ten golden rules, which should always be observed.

Ten golden rules

Types of monitoring and non-radioactive hazards

Regular monitoring of working areas is essential. Monitoring should take the form of contamination surveys and dose rate surveys where applicable. Any abnormal levels should be dealt with immediately. Monitoring may be considered under two headings.

Environmental monitoring

Working areas should be regularly monitored for contamination using the correct detector. Thin end-window Geiger counters are suitable for carbon-14, phosphorus-32, and phosphorus-33, and other medium- to high-energy betaemitters, but not for tritium. Tritium contamination is extremely difficult to monitor directly. The best practical method is to take wipes (with paper disks). Assessment of activity on the wipes can then be carried out by liquid scintillation counting or gas flow proportional counting.

Detection of X-rays and gamma-emitters is more efficient using a scintillation detector than a Geiger counter.

Dose rate surveys should be performed at regular intervals using a suitable instrument. Dose rates should be kept as low as reasonably achievable (ALARA) in the working environment by use of local shielding and minimization of quantities held.

Personal monitoring

The techniques used for checking contamination of the skin and clothing are broadly the same as those used for environmental monitoring.

Care must be taken when using high-energy beta-emitters, such as phosphorus-32, as low levels of contamination can give high skin dose.

Tritium can be readily absorbed through the skin.

The wearing of radiation monitoring films or thermoluminescent dosimeters for persons handling carbon-14, tritium, or sulfur-35 is normally considered unnecessary. Low-energy beta radiation from these nuclides cannot penetrate the covering material of the dosimeters. If other nuclides are to be handled (e.g. phosphorus-32) it may be necessary to wear extremity and body radiation monitoring dosimeters (refer to local rules). Biological monitoring for tritium and carbon-14 may be carried out by scintillation counting of urine samples. Breath samples can also be taken to detect carbon-14 labelled carbon dioxide.

Non-radioactive hazards

The chemical hazards associated with handling radiochemicals are relatively insignificant compared with the radioactive hazard because of the very small quantities normally present. Under certain circumstances, it may be necessary to take account of any hazard arising from the solvent present. The precautions taken to minimize radioactive hazards in the laboratory will also minimize chemical hazards. There are no substitutes for properly trained staff and good laboratory practices.

Types of shielding

The appropriate choice of shielding depends on the type of radioactive emission. Two types of shielding are available: one for phosphorus-32, the other for iodine-125.

Phosphorus-32

Products for safer handling of phosphorus-32 are constructed from heavy gauge, 10-mm optical-quality acrylic. This material has a very low "Z-number", reducing the energy of the "bremsstrahlung" produced and effectively absorbing all beta emissions from phosphorus-32.

Iodine-125

To attenuate γ-emissions, a high "Z-number" shielding (e.g. lead) is required. All Amersham Biosciences iodine-125 shielding provides both transparency and appropriate shielding by using a 12-mm lead acrylic co-polymer. This material has virtually all the normal chemical and physical properties, including transparency, of conventional acrylic. It has a lead equivalence value of 0.5 mm, providing protection against all iodine-125 emissions.

Note: Accessory products are designed to be compatible with the range of safety products from Amersham Biosciences, but they DO NOT provide any protection against radioactive emissions in themselves.

Physical properties of common radionuclides

Three important physical properties determine the usefulness of a radionuclide as a tracer (also see Table 1).

1. Half life

Radionuclides routinely used as tracers (e.g. 32P, 33P, 35S, 125I, tritium, and ¹⁴C) have half-lives ranging from several days to thousands of years. Such half-lives, and the related specific activities of such radionuclides make them ideally suited to applications such as DNA synthesis studies, radioimmunoassays, binding studies, drug screening, and ADME* work.

In contrast, when the half-life of a radionuclide is very short, any compound labelled with it will be difficult to prepare, use, and measure within the time of decay. For certain important elements there are no radionuclides that have half-lives long enough for practical use (e.g. oxygen-15 has a half-life of 2 min and nitrogen-13 a half-life of 10 min).

2. Modes of decay

A number of different modes of decay result in the emission of different types of ionizing radiation (i.e. alpha particles, beta particles, X- and gamma rays, and neutrons). The most commonly used radiolabelled materials decay by emission of beta particles and/or X- or gamma rays.

Applications using alpha-emitters are limited in biological research.

3. Energy of decay

The energy of decay, combined with the mode, determines how the nuclide can be measured. Beta-emitters can be measured by liquid scintillation counting, autoradiography, and, provided the energy of emission is not too low, thin end-window ionization counting. Gamma-emitters are usually measured by solid scintillation methods.

Table 1 lists some physical properties of important radionuclides.

^{*}ADME = adsorption, distribution, metabolism, excretion (elimination).

Table 1 Physical properties of some radionuclides

* Electron capture is a radioactive transformation in which the nucleus absorbs an electron from an inner orbital.

† A milliatom (matom) is the atomic weight of the element in milligrams.

The half-life and beta energy values are taken from *Radionuclide and Radiation Protection Handbook* **Vol. 98 No 1**. (D. Delacroix *et al*.), Nuclear Technology Publishing, (2002).

The maximum specific activity (S_{max}) values (Ci/g) are calculated as follows:

$$
S_{\text{max}} = \frac{356}{T} \text{ Ci/matom}
$$

where T is the half-life in years.

The following pages give more detailed information regarding the most commonly used radioisotopes.

Properties, decay tables, and special considerations

Iodine-125

The data provided is general information that gives a basic understanding of radiation safety. You must, however, consult your local radiation safety expert to ensure that you comply with all national regulations and local rules.

Special considerations

- Freezing or acidification of solutions containing iodide ions can lead to formation of volatile elemental iodine.
- Active aerosols can be produced by opening a vial of high radioactive concentration of iodine-125.
- Some iodo-compounds can penetrate surgical gloves, two pairs or polythene alternatives are recommended.
- In the event of a suspected intake, the thyroid may be blocked by the administration of potassium iodate or potassium iodide under appropriate supervision.
- Spills of iodine-125 should be stabilized with alkaline sodium thiosulfate solution before commencing decontamination.
- Vials should be opened and used in ventilated enclosures.
- Always follow the ten golden rules (see page 7).

Iodine-125 decay table

Carbon-14

The data provided is general information that gives a basic understanding of radiation safety. You must, however, consult your local radiation safety expert to ensure that you comply with all national regulations and local rules.

Special considerations

- Some organic compounds may be absorbed through surgical gloves.
- Avoid the generation of ${}^{14}CO_2$ which could be inhaled.
- Always follow the ten golden rules (see page 7).

Sulfur-35

The data provided is general information, which gives a basic understanding of radiation safety. You must, however, consult your local radiation safety expert to ensure that you comply with all national regulations and local rules.

Special considerations

- Vials should be opened and used in ventilated enclosures.
- Avoid generation of sulfur dioxide or hydrogen sulfide which could be inhaled.
- Radiolysis of ³⁵S-labelled amino acids may lead to the production of labelled volatiles which could contaminate internal surfaces and reaction vessels.
- Always follow the ten golden rules (see page 7).

Sulfur-35 decay table

Chromium-51

The data provided is general information that gives a basic understanding of radiation safety. You must, however, consult your local radiation safety expert to ensure that you comply with all national regulations and local rules.

Special considerations

In general, chromium-51 does not require any special precautions over and above those necessary for any radionuclides of this energy of emission.

Chromium-51 decay table

Phosphorus-32

The data provided is general information that gives a basic understanding of radiation safety. You must, however, consult your local radiation safety expert to ensure that you comply with all national regulations and local rules.

Special considerations

- Lead shielding can be used to reduce the dose from bremsstrahlung.
- Always follow the ten golden rules (see page 7).

Phosphorus-32 decay table

Phosphorus-33

The data provided is general information that gives a basic understanding of radiation safety. You must, however, consult your local radiation safety expert to ensure that you comply with all national regulations and local rules.

Special considerations

In general, phosphorus-33 does not require special precautions over and above those necessary for any beta-emitting radionuclides of this energy of emission.

Phosphorus-33 decay table

Calcium-45

The data provided is general information that gives a basic understanding of radiation safety. You must, however, consult your local radiation safety expert to ensure that you comply with all national regulations and local rules.

Special considerations

In general, calcium-45 does not require any special precautions over and above those necessary for any beta-emitting radionuclides of this energy of emission. The majority of calcium-45 is deposited in the bone and is retained with a long biological half-life.

Calcium-45 decay table

Tritium

The data provided is general information that gives a basic understanding of radiation safety. You must, however, consult your local radiation safety expert to ensure that you comply with all national regulations and local rules.

Special considerations

- Due to its low beta-energy, tritium is difficult to monitor directly, and therefore regular swabbing and counting of the work area is advisable.
- Tritium compounds can be absorbed readily through the skin.
- The biological half-life of ingested or absorbed radioactivity is dependent on the chemical form in which the isotope is found and can vary from a few days to several weeks, giving rise to different doses in affected personnel.

Should there be any evidence of contamination it is recommended that the radiation protection adviser be consulted immediately.

• Always follow the ten golden rules (see page 7).

Handling solutions of radiochemicals

Due thought must also be given to the safe handling of solutions of radiochemicals, as well as to the isotope itself and the properties of the solvent in which it is dissolved. Users should check that the gloves they plan to use offer satisfactory protection against splashes of the solvent. Otherwise this could lead to penetration of the radioisotope through the glove. Glove manufacturers provide data on the solvent resistance of the various types of gloves they supply, and such information is useful when handling radioactive, as well as non-radioactive, substances in solution.

Measurement of radioactivity

A number of methods are available for the accurate measurement of radioactivity. The most important are:

Liquid scintillation counting

This is used for the measurement of beta-emitting nuclides (e.g. tritium, sulfur-35, phosphorus-33, and carbon-14). The technique involves dissolving the sample containing the radionuclide in a suitable scintillation solution and use of a liquid scintillation counter. The solution (or "cocktail") normally consists of an aromatic organic solvent containing a fluor* together with a detergent to make the whole solution miscible when counting aqueous samples. The energy of the emitted beta-particles is transferred, via the solvent, to the primary fluor and sometimes to a secondary fluor which then emits energy as light photons. These photons are detected using a photomultiplier. Only a small proportion of the available energy is liberated as light, the residue being dissipated as vibrational and rotational energy in the solvent.

**A fluor is a compound that converts molecular excitation energy into light photons.*

Gamma counting

The method most commonly used for the measurement of electromagnetic radiation from gamma or X-ray emitting nuclides uses a solid scintillator. The technique is based on the radiation being converted into light photons that are then counted by a photomultiplier. No solvent is used. The radiation from the sample (e.g. a^{-125} I-labelled product) interacts directly with the solid scintillator, usually a crystal of sodium iodide.

Cerenkov counting

When beta particles are emitted they leave the nucleus at speeds approaching that of light in vacuo. However, in the surrounding medium the speed of light is slower than in vacuo and consequently the passage of the particles through the medium causes shock waves from which light photons are emitted. This light may be detected by a photomultiplier to give an indication of the radioactivity present. For the light to be emitted in a solution the energy of the particles has to be greater than a certain minimum energy, which is related to the refractive indexes of the solvent. The minimum energy required to produce light in an aqueous solution is 0.263 MeV. Consequently, only those radionuclides with comparatively high-energy beta emissions, such as phosphorus-32, may be counted by this method.

Quantitation of autoradiographs

Labelled polymer reference sources, in the form of calibrated radioactive standards (Microscales) can be used in conjunction with autoradiography to quantitate and check film responses.

Table 2. The radionuclides used in the quantitation of autoradiographs and their appropriate Microscales

The spatial distribution of radiolabelled compounds within thin (10–25 µm) sections of tissue can be detected by the blackening of the emulsion on films adjacent to the specimen. On developing the film, the pattern of optical densities within the resulting autoradiogram is proportional to the amount of radioactivity, and these can be measured by comparison with the calibrated radioactive scale, co-exposed with the sections of radiolabelled tissue. The optical density values, within discrete regions of an autoradiographical image, can be converted into molar quantities by interpolation from the standard curve using the values from Microscales.

SI unit of radioactivity

The Système international d'unitès (SI) are a consistent set of units for use in all branches of science. The general conference on weights and measures acting on the recommendation of the International Commission on Radiation Units and Measurements (ICRU) has adopted special unit names for SI units in connection with radioactivity.

The SI unit of radioactivity is the becquerel (Bq).

1 Bq = 1 disintegration per second = 2.7×10^{-11} curies (Ci)

1 Ci = 3.7×10^{10} Bq = 37 GBq

therefore:

1 mCi = 3.7×10^7 Bq = 37 MBq

1 Ci = 3.7×10^{10} Bq = 37 GBq

Table 3. Relationships between SI units and non-SI units

†For radiation protection purposes, the ICRP recommends the following values (see Table 4) of effective quality factor: (the higher the factor, the more damaging the radiation)

Table 4. ICRP recommended quality factor values

Table 5. Common prefixes for SI units

Table 6. Becquerel/curie conversion chart

Examples

Specific activity

The specific activity of a labelled compound is a measure of the radioactivity per unit mass, and is commonly expressed in terms of µCi/mg, mCi/mg, Ci/mmol, or Bq/mmol. When there is sufficient mass of a radiolabelled compound for a small sample to be accurately weighed and counted by liquid scintillation counting, the specific activity is calculated (e.g. µCi/mg). The conversion from µCi/mg to mCi/mmol is simply carried out by multiplying by the molecular weight and dividing by 1000. When the specific activity is greater than 1 Ci/mmol, there is often insufficient material present to be weighed. In such cases the specific activity may be calculated by relating the radioactive concentration (determined by liquid scintillation counting) to the chemical concentration, and then converting the figure obtained to Ci/mmol (Bq/mmol). The chemical concentration is commonly determined by UV spectroscopy, or an appropriate colorimetric method (comparing color density of the unknown strength solution with that of a range of known strengths).

When the degree of labelling exceeds 10% at one or more positions, mass spectrometry can be used. This is very frequently the method of choice for carbon-14 and tritium-labelled compounds.

Typical values of specific activities are:

Specific activity required for tracer experiments

The specific activity of a labelled compound required for a tracer experiment is normally determined by the application.

Carbon-14 labelled compounds of specific activities 0.37–2.2 GBq/mmol, 10–60 mCi/mmol are used for many ADME* studies.

Tritium labelled compounds of specific activities 0.37–3.15 TBq/mmol, 10–85 Ci/mmol, and iodine-125 labelled compounds of specific activities 11.1–74 TBq/mmol, 300–2000 Ci/mmol are commonly used for radioimmunoassays and protein-binding studies

Sulfur-35 is used to label the most commonly used amino acids in protein biosynthesis studies to specific activities of > 37 TBq/mmol, 1000 Ci/mmol, although products with lower specific activities are available.

Phosphorus-33 labelled nucleotides are employed in nucleic acid labelling procedures related to DNA sequencing and *in situ* hybridization. Specific activities of ~ 92.5 TBq/mmol, 2500 Ci/mmol are common.

Phosphorus-32 labelled nucleotides are employed in nucleic acid labelling procedures and have specific activities between 0.11–222 TBq/mmol, 3–6000 Ci/mmol, depending on the application.

On occasions, the actual specific activity of a given batch of a particular product may vary from the specific activity range quoted in the catalog. If this variation is greater than \pm 10% of the quoted catalog range the batch of material will not be released for sale without prior notification to the customer.

For the most up-to-date specific activity ranges, please refer to our Web catalog at **www.radiochemicals.com**

*ADME = adsorption, distribution, metabolism, excretion (elimination).

Relationship between specific activity, radioactive concentration, and chemical concentration for radiochemicals.

There are several ways to relate these features of a radiochemical: Some applications may require a lower specific activity than that of the commercially available radiolabelled compound.

*Radioactive concentration is the activity per unit volume of solvent in which the radionuclide or radiochemical is dissolved (e.g. 37 MBq/ml [1 mCi/ml]). It should not be confused with molar specific activity, which relates to the radioactive solute in the solution.

As an example, thymidine has a MW of \sim 242. For ¹⁴C-labelled thymidine at 60 mCi/mmol, a 250 μCi vial will contain:

> 0.25 [mCi] 60 [molar SA, mCi/mmol] \times 242 mg $= 1$ mg. 60 [molar SA, mCi/mmol]

Contrast this with a ³H-labelled thymidine at 60 Ci/mmol, where a 250 μCi vial will contain only:

$$
= \begin{pmatrix} 0.25 \text{ [mCi]} \\ 60 \text{ 000 [molar SA, mCi/mmol]} \end{pmatrix}
$$

\n× 242
\n= 0.001 mg = 1 µg.

Note: These calculations are approximate, since the molecular weight of the labelled thymidine is taken as 242, rather than using the actual figure of 244 for the ¹⁴C-labelled thymidine (242 + 2) or 246 for the ³H-labelled thymidine (242 + 4) where the effect of the respective isotope is included.

Dilution to a lower specific activity

Reduction of the specific activity is carried out by addition of a calculated weight (normally in solution) of the unlabelled (carrier) compound. The amount of carrier compound to be added can be calculated from the expression:

$$
W = Ma \left[\frac{1}{A'} - \frac{1}{A} \right]
$$

where $W = weight (in mg) of carrier compound to be added$ $M =$ molecular weight of compound a = total activity (in GBq, mCi) in sample A = actual molar specific activity (in GBq/mmol, mCi/mmol) of compound A' = desired molar specific activity (in GBq/mmol, mCi/mmol) after dilution

If the compound supplied is a non-volatile solid, it should be dissolved in a purified solvent and a standard solution of carrier should be prepared in the same solvent.

If the compound supplied is already in solution, the standard carrier solution is then added to the radioactive solution and mixed thoroughly before use.

Example:

To reduce the specific activity of 185 MBq, (5 mCi) of tritiated thymidine from 1.11 TBq/mmol, 30 Ci/mmol to 37 GBq/mmol, 1 Ci/mmol.

> $W = 242 \times 5 (1/1000 - 1/30 000)$ = 1.17 mg of unlabelled thymidine to add.

Consider that 185 MBq (5 mCi) of the above compound has been supplied in 5 ml of solution. Make up a standard solution of unlabelled thymidine by dissolving, for example, 20 mg (accurately weighed) in 10 ml of water (sterile and pyrogen free if necessary). Then add 0.585 ml of this standard solution (equivalent to 1.17 mg of carrier) to the solution of the radioactive thymidine. The specific activity of the thymidine will now be reduced to 37 GBq/mmol (1 Ci/mmol). Note however, that the radioactive concentration is changed from 37 MBq/ml (1 mCi/ml) to 33 MBq/ml (0.9 mCi/ml) because the solution volume has risen from 5 ml to 5.585 ml.

Radiochemicals that are gases or volatile liquids at room temperature are normally diluted with carrier using special equipment such as a calibrated vacuum manifold system. Unless such equipment is readily available, it is advisable to obtain these radiochemicals already diluted to the specific activity needed for the experiment.

To minimize the rate of self-radiolysis of radiochemicals, they should be stored at the lowest molar specific activity consistent with experimental requirements (see also the storage information on page 45).

Nomenclature

Amersham Biosciences employs the 'square-brackets-preceding' nomenclature system used by the Chemical Society, the American Chemical Society, and most biochemical journals.

The symbol for the isotope is placed in square brackets and immediately precedes that part of the name to which it refers.

Examples are:

L-3-Phosphatidyl[N-methyl-¹⁴C]choline,1,2-dipalmitoyl L-3-Phosphatidylcholine, 1,2-di[1-¹⁴C]palmitoyl

Arabic numerals, Greek letters, and prefixes placed within the square bracket are used to indicate the position of isotopic labelling.

Examples are:

[8-14C]Adenosine 5'-triphosphate Adenosine 5'-[γ-³²P]triphosphate [adenine-U-¹⁴C]Adenosine 5'-triphosphate

Uniform labelling

The symbol U is used with carbon-14 compounds to denote labelling that is distributed with statistical uniformity in either all carbon atoms in the structure or in those parts which are labelled.

Examples are:

L-[U-¹⁴C]Phenylalanine: Produced biochemically from $^{14}CO_{2}$ as the only source of carbon-14.

L-[ring-U-14C]Phenylalanine: Made chemically over several steps from [U-14C]Benzene.

The position of carbon-14 is indicated by \bullet

General labelling

The symbol G is used for a number of tritium-labelled compounds in which the labelling is distributed generally, but not necessarily uniformly, over the molecule (e.g. [G-3H]Benzo[a]pyrene, which is prepared by an exchange procedure).

Tritium nuclear magnetic resonance spectroscopy (tritium-NMR) is used to verify experimentally, in a non-destructive way, the distribution of the tritium label within the compound. The tritium-NMR results for generally labelled Benzo[a]pyrene (TRK662), prepared by platinum catalyzed exchange in 70% acetic acid containing tritiated water, are interesting in that they show that the sterically hindered 10- and 11-positions are only weakly labelled (\sim 4% of tritium in each position). Although batch-to-batch variation may occur, there is usually greater than 10% of the tritium label in positions 4, 6, 9, and 12. Labelling in the remaining positions is about equal.

Specific labelling

Specific labelling denotes labelling confined to defined positions within the molecule (e.g. 1,2,6,7-³H]Testosterone and [4-¹⁴C]Testosterone).

The symbol (n) is used to designate nominal positions of labelling, that is, the positions where tritium atoms are expected to be located based upon the method of preparation*. Where more than 95% of the tritium label has been verified (normally by tritium-NMR) as being at designated positions the (n) is dropped.

A position of labelling is included if 15% or more of the tritium is located in that position. Where more than one positon of labelling is specified, equal distribution of the label is not implied. The evidence on patterns of labelling obtained by chemical degradations can be misleading and requires critical examination. However, tritium-NMR is non-destructive and gives unequivocal labelling pattern information.

**The labelling process for many compounds involves the use of a metal transfer catalyst and tritium gas. This can cause exchange labelling at other C-H positions in addition to the expected labelling position(s). Tritium-NMR can be used to check the actual positions labelled and the % labelling at each position.*

Purity of radiochemicals

Radiochemical purity

The radiochemical purity (RCP) of a compound is defined as the percentage of the total radioactivity present in the specified chemical form. The methods of analysis used to assess radiochemical purity include high-performance liquid, paper and thin-layer chromatography, gas-liquid and column chromatography, electrophoresis, and enzymatic degradation. The techniques and chromatographic systems used are designed to separate likely impurities formed in the synthesis, as well as by decomposition, and are based on Amersham Biosciences' many years of experience in the analysis and storage of radiochemicals.

All of our labelled compounds are synthesized, analyzed and stored to ensure that the user receives only material of high RCP. Many compounds are supplied at greater than 98% RCP and materials for special use (e.g. labelled substrates for the assay of enzymes) will normally have an initial RCP of greater than 99%. For unstable compounds, or those labelled with isotopes of short half-life, the aim is to supply compounds at the highest possible RCP and certainly greater than 90%.

Chiral chromatography is often used to determine the optical purity of amino acids and other chiral compounds.

Fig 1. Analytical HPLC chromatogram of L-[4,5-3H]Leucine (TRK510). $RCP = 99.5\% \pm 0.5$ to 95% confidence.

Fig 2. Analytical HPLC chromatogram of R-(+)-7-Hydroxy-[3H]DPAT (TRK1001) active with active racemate.

Chemical purity

Purification processes that provide compounds of high radiochemical purity tend also to give products of high chemical purity, although this is far more difficult to quantitate, particularly for compounds at high specific activity because they are often present at very low chemical concentration in solution. With some biochemical studies, chemical impurities may act as enzyme inhibitors and may also adversely affect the stability of the radioactive compounds. Therefore it is even more important to supply these compounds at a high level of chemical purity. This is achieved by recrystallizing the compound following the chromatographic separation. This is only applicable to compounds of low specific activity that are present in sufficient weight for crystallization to be effective (e.g. it is much more common with carbon-14 compounds than for those labelled with other isotopes). Chemical purity is determined by ultra-violet or infrared spectrophotometry, proton-NMR, mass spectrometry, or by quantitative gas-liquid and high-performance liquid chromatographic procedures, as appropriate. For compounds supplied as solids further proof of chemical identity may be obtained from the melting point or titration, and for liquids the refractive index may be used.

Biological purity

Biological assays are performance indicators designed to complement the analytical techniques used to determine RCP. Biological testing provides a measure of the suitability of radiochemicals for use in certain biological systems and gives an indication of the absence of possible inhibitors and of the acceptability of blank values. Such tests of biological suitability are carried out routinely on batches of certain products (e.g. α-³²P-labelled deoxynucleotides and 35S-labelled amino acids).

Such compounds may be tested for incorporation into DNA using a nick translation reaction or a random-priming reaction. Amino acids labelled with carbon-14, tritium, or sulfur-35 may be tested for incorporation into protein using a standardized lysate system. These tests are in addition to chromatographic or other analyses normally carried out on the products.

Suitability for injection

The labelled compounds supplied by Amersham Biosciences are, unless otherwise specified, for research purposes only. Many solutions are supplied sterilized in order to minimize microbiological decomposition on storage, but are not recommended or intended for diagnosis of disease in humans or animals. They should not be used internally or externally in humans.

Packaging

The vast majority of radiochemicals are available from stock, ready for immediate dispatch. It is recommended that all radioactive shipments are opened and checked as soon as possible after receipt to ensure that the product is stored at the appropriate temperature and with the recommended shielding (if appropriate).

Total activity supplied

Solutions of Amersham Biosciences' radiolabelled compounds are generally dispensed at low concentrations (e.g. microgram levels per milliliter). To counteract losses due to adsorption of the compound onto the walls of the container; radiolabelled compounds are routinely over-dispensed by 5–10%. This also ensures that the quantity ordered is available for use.

Documentation

Most consignments of radiolabelled compounds (e.g. ¹⁴C, ³H, ¹²⁵L, ³²P, ³³P, ³⁵S) are accompanied by a product specification sheet. This gives essential technical information on the preparative method, molar specific activity, radiochemical and chemical purity, specificity of labelling, decomposition rate under defined storage conditions, and the likely impurities present in the material. (Due to the nature of radiolytic decomposition it is not possible to identify all impurities as they do not exist in the non-radioactive material.) Safety warnings and handling precautions are also provided for each product supplied.

Types of packaging

Labelled compounds are supplied by Amersham Biosciences in glass ampoules or vials. Each packaging system used reflects the characteristics of the different isotope, providing safety during shipment and storage while also ensuring the maximum stability of the compounds. The following vials and ampoules are used extensively and can be opened easily and safely using recommended procedures.

1. The duoseal and dimple vial (type P11 and P87)

The primary seal provides security against leakage during transit and comprises a Teflon-faced rubber disk secured by a crimped metal overseal. The product in solution may be removed directly, either in whole or in part, through the disk using a syringe with a standard 50-mm needle (sterilized, if necessary).

Alternatively, the overseal and disk may be removed and aliquots withdrawn with a pipette. The vial may be re-closed using the screw cap, which incorporates its own Teflon-faced rubber disk.

Fig 3. Schematic of the duoseal and dimple vials.

Note: When removing toluene-containing solutions the crimped metal seal and the associated rubber disk must be completely removed. The vial may be re-closed, if necessary, with the screw cap, which contains a Teflon-faced rubber disk. Most 125I-labelled compounds are now supplied in vials with a modified cap, eliminating the requirement for a crimped, metal overseal.

2. The peel-seal vial (type P17)

The inert-coated aluminum foil is non-porous to gaseous compounds. Removal of the aluminum seal allows access to the product in the vial. When the cap is replaced the Teflon-faced rubber disk in the screw cap becomes the new seal.

Fig 4. Schematic of the peel-seal vial (type P17).

3. Amersham Biosciences' microvial (type P15)

Fig 5. Schematic of Amersham Biosciences' microvial (type P15).

This vial is used for the supply of sodium [125] liodide and sodium [131] liodide solutions.

The packaging consists of two parts: an outer container of blue plastic with a steel tube molded inside to minimize exposure to radiation, and an inner plastic vial fitted with a conical glass insert which contains the radioactive solution. The cap of the vial has a specially coated rubber seal and is tightened pneumatically to a predetermined torque to provide an efficient seal. To achieve maximum benefit from the additional shielding it is recommended that iodination reactions are carried out in the conical vial without removing it from the outer container. Before opening the vial it is recommended that the container is centrifuged to bring all the liquid into the bottom of the cone. When using small capacity centrifuges it may be necessary to remove the capped vial from the plastic container.

Opening the vial

- 1. Unscrew the outer blue cap, invert it and push it onto the vial cap (Fig 5a).
- 2. Twist the blue cap anti-clockwise while pushing down to lock the vial onto the lug in the base of the container (Fig 5b).
- 3. Lift away the vial cap in the blue top. The vial is now ready for use in an iodination reaction. If necessary the vial can be removed from the outer container by lifting it out directly with forceps or tongs (Fig 5b).

Removing the capped vial from the outer container

The vial with its cap still in place can be lifted free of the outer container by pushing the inverted blue top onto the vial cap, twisting clockwise and lifting. Forceps or tongs can then be used to separate the vial from the blue top (Fig 5c).

4. The Customer Designed Container

The Customer Designed Container (CDC) has been specifically designed to provide optimum safety and convenience. It achieves this by providing full secondary containment during shipping and, consequently, a secure outer container in the laboratory.

CDCs are color-coded depending on the isotope with which the product within the inner vial is labelled.

Fig 6. Schematic of the Customer Designed Container.

5. Amersham Biosciences' Redivial

Fig 7. Illustration of the Redivial. **Fig 8.** Components of the Redivial and Customer Designed Container (CDC).

This vial is used for all 32P- and 33P-labelled nucleotides with a radioactive concentration of 74 MBq/ml (2 mCi/ml) or greater. Redivue 32P- and 33Plabelled nucleotides shipped at ambient temperature are supplied in the Redivial with a splashguard. Ambient shipment of nucleotides demands a secure vial design to ensure that the contents of the vial are not distributed over the inside surface of the whole vial during transit. The splashguard (if fitted*) covers the bottom of the v-insert, ensuring that the radioactive solution remains in the enclosed space, so minimizing contamination of the inner surfaces of the vial and cap during ambient shipment.

*Standard 32P- and 33P-labelled nucleotides shipped on dry ice do not require a splashguard.

Opening the vial and removing the splashguard

The vial base contains a recess that fits on a projection located in the bottom half of the outer container, which prevents the vial from turning. The cap is unscrewed by turning it anti-clockwise while the vial remains secured on the projection and within the shielding.

The one-use disposable splashguard (if fitted) is easily removed using a laboratory pipette and tip: typically \sim 1 μ l adheres to the surface of the guard and is disposed of as radioactive waste.

6. The break-seal ampoule (type P1)

Fig 9. Schematic of the break-seal ampoule.

Radioactive compounds that are gases or volatile liquids at room temperature are normally supplied in borosilicate glass break-seal ampoules, type P1, which are sealed under vacuum. Many compounds are present only in the vapor phase, so that the ampoule may appear to be empty. These ampoules can be attached to a vacuum manifold system, in which the contents are transferred to a receiving vessel following breakage of the seal by a previously inserted magnetic hammer (see Method B). If the ampoule contents are intended to be diluted with a liquid carrier or converted into a solution at room temperature, it is not always necessary to use a vacuum transfer system so a simpler method (Method A) may be employed. Method B is recommended in preference to Method A where loss of expensive materials during transfer may be significant.

Methods of opening P1 ampoules

Method A: dilution or dissolution of ampoule contents

- 1. Support the ampoule vertically in a clamp, after carefully introducing a magnetic hammer (approximately 6-mm diameter) so that it rests on the hook of the break-seal. If the solvent used is likely to react with iron the hammer should be glass-covered or PTFE coated.
- 2. Place the solvent (or solution) in the space above the break-seal and close the tube by means of a ground-glass cap. Note that there must be enough solvent to cover the top of the hook, and that not all the solvent will pass into the ampoule.
- 3. Using a magnet, raise the hammer about 5–10 cm above the break-seal, remove the magnet and allow the hammer to fall and break the seal. The solution passes quickly into the ampoule since it is normally under reduced pressure. Leave for at least 15 min to allow the mixture to equilibrate.
- 4. Make a scratch mark around the ampoule just below the level of the breakseal, using a glass cutting knife or sharp file. Hold a heated (red-hot) glass rod against the scratch mark until a crack develops along the mark. The ampoule is then easily opened.

Note: Care is needed in this final stage if flammable solvents are involved.

Method B: transfer under vacuum

- 1. Carefully introduce a magnetic hammer (a soft iron cylinder approximately 6-mm diameter, 20-mm long) into the adapter so that it rests on the glass hook of the seal. If the ampoule contents are likely to react with iron then the hammer should be glass-covered, or a PTFE-covered stirrer-bar can be used (see Fig 10).
- 2. Attach the ampoule to the vacuum line via the ground glass joint.
- 3. Cool the contents of the ampoule by placing the ampoule in a Dewar flask containing solid CO₂, liquid nitrogen, or some other suitable refrigerant. Open stopcocks to remove air and moisture from the system. Close them after a hard vacuum is obtained and test the system for leaks.
- 4. Cool the receiver by insertion into a Dewar flask containing a refrigerant and remove the cold bath from around the ampoule.
- 5. Using a magnet, raise the hammer about 5–10 cm above the break-seal then remove the magnet, thus allowing the hammer to fall and break the seal.
- 6. Open the stopcocks above the receiver and above the ampoule to allow the ampoule contents to distil rapidly into the receiver. For efficient transfer of the material, it is most important to maintain good vacuum conditions. The transfer of material may be facilitated by warming the ampoule (e.g. with a hair-dryer). Some low boiling gases, such as methane or carbon monoxide, require the use of a Toepler (or similar) pump for complete transfer.
- 7. Close the stopcock above the receiver.

Fig 10. Breaking the seal in a break-seal ampoule using a magnetizable hammer.

7. Sealed glass ampoules (types P2, P2A, P2B, and P5)

A number of radiochemicals in the form of freeze-dried solids, weighed solids or non-volatile liquids, and compounds dissolved in certain organic solvents are supplied in Pyrex glass ampoules sealed under vacuum or inert gas.

- 1. Hold the ampoule vertically with the neck up, and gently tap the ampoule until the contents are in the rounded (bottom) end. If the product is liquid or in solution, gentle centrifuging (500–1000 rpm) for a few minutes will ensure that the contents are at the lower end of the ampoule. If the compound has a high vapor pressure, cooling the end of the ampoule in a refrigerant will minimize loss on opening, but care must be taken for moisturesensitive compounds to ensure that no moisture enters the ampoule when opened.
- 2. Scratch the serration with a sharp file or glass-cutting knife.
- 3. Snap off the neck of the ampoule at the serration. Take care to avoid scattering the contents of ampoules sealed under vacuum.
- 4. Take care to avoid shaving small pieces from the plastic cap (provided for resealing P2 ampoules) with any sharp edges on the ampoule, as these may drop into the ampoule.

Note: Once an ampoule has been opened, the radiochemical is no longer being stored under optimum conditions and the rate of decomposition may increase. If possible, the air in the ampoule should be replaced by nitrogen before closing it. Care must be taken to prevent loss of the protecting solvent by evaporation from opened ampoules; the plastic cap is porous and solvents can evaporate through it over a period of a few days, especially at room temperature.

Fig 11. Dimensions of sealed glass ampoules (P2, P2A, P2B, and P5).

Recovery of non-volatile radiochemicals from solution

Non-volatile radiochemicals supplied in a solvent, such as toluene, aqueous ethanol, or ethanol, can be transferred to a suitable vessel and recovered by passing a gentle stream of dry nitrogen over the surface of the solution while the vessel is warmed in a water bath at 30–35 °C. The last traces of solvent may be removed under reduced pressure.

Freeze-drying techniques can also be used for radiochemicals supplied in aqueous solutions, and are essential for compounds that are sensitive to chemical decomposition by heat or oxygen. However it should be noted that many compounds, particularly those labelled with tritium, will be less stable in the solid state and should be dissolved immediately in the recommended solvent if further storage is intended. Alternatively, an aliquot of the radiochemical in solution can be taken and the solvent changed, leaving the remainder of the product in the recommended solvent in which it was supplied.

Fig 12. Removal of solvent from a non-volatile radiochemical using dry nitrogen.

Storage and stability of radiochemicals

The vast majority of radiolabelled compounds supplied by Amersham Biosciences are analyzed by HPLC, which provides a more accurate and discriminating measurement of radiochemical purity than other techniques. The stability of each radiochemical is constantly under review to ensure that only the highest quality material is available.

For products that have higher rates of decomposition, batches are produced more frequently to ensure that only material of the highest purity is in stock. Alternatively such products may be supplied in special formulations and at lower temperatures designed to minimize their decomposition rates.

Rates of decomposition

Chemical decomposition occurs as a natural process in all compounds during storage. However, this type of decomposition may be accelerated by free radicals, which are formed as a result of the energy released in radioactive decay. Consequently the decomposition rate of a radiolabelled compound will be determined by a combination of chemical and radiolytic decomposition processes. The observed decomposition rates of radiochemicals due to self-irradation during storage is more pronounced with compounds of high molar specific activity but measures are taken to minimize these effects.

Recommended conditions for storage of each compound are given in the product specification sheet, together with an estimate of the rate of observed decomposition under the conditions specified. It must be emphasized that the value given relates only to the compound as it is supplied and when it is stored under the recommended conditions. Even slight deviations from these conditions may result in more rapid decomposition. In general, compounds should be stored at low temperatures and in the dark. Solutions should be stored unfrozen at 1 mCi (37 MBq)/ml or below. When instability dictates that solutions of compounds must be stored frozen, it is wise to avoid freeze-thaw cycles.

Stocks of radiolabelled compounds are stored prior to shipment under conditions designed for maximum stability. The temperature of storage depends on the particular compound being stored, typical values being + 20 °C (room temperature), $+ 2$ to $+ 4$ °C (refrigerator temperature), $- 20$ °C (freezer temperature), - 80 °C (dry ice or - 80 °C freezer), and approximately - 140 °C (the vapor above liquid nitrogen).

Avoiding microbial contamination

Many radiochemicals (e.g. carbohydrates and amino acids) are excellent substrates for microbial growth. To minimize loss by such action, aqueous solutions of radiochemicals are sterilized by filtration before dispensing. Clean working procedures should be employed to reduce the risk of microbial

contamination once the vial has been opened. It is recommended that any syringe used to remove material from a multi-dose vial is sterilized, particularly if it is intended to remove aliquots over a protracted period of time.

Tritium-labelled compounds in aqueous solution

Aqueous solutions of tritiated compounds should ideally not be stored frozen because concentration of the radioactive compound by slow freezing (molecular clustering) may accelerate the rate of self-radiolysis. This is thought to result from the short penetrating power of the electron from tritium decay, which causes damage in a localized volume. If aqueous solutions must be stored frozen it is advisable to freeze them rapidly (e.g. in a dry ice bath) then store at -20 °C.

General storage practices

The following general practices are intended as a guide but do not supersede the recommendations given in the product specification sheet accompanying each consignment since this contains the most up-to-date and relevant information.

- Store at the lowest molar specific activity required for the use of the compound.
- Disperse solids as much as possible and store them in a dry atmosphere: sealing under vacuum or an inert gas is usually desirable and a number of compounds are supplied sealed in this way.
- Compounds at high specific activities should be stored in ethanol at 1 mCi (37 MBq)/ml at - 20 °C or below, subject to chemical compatibility with ethanol.
- For many compounds of biochemical interest, which are insoluble in aromatic solvents and for which aqueous solutions must be used, 2–10% ethanol should be added to scavenge free radicals. Many of our compounds are supplied in aqueous solutions containing 2 or 3% ethanol as a radical scavenger.
- Keep the solution in the dark and add a bacteriostat or antioxidant where appropriate.
- The recommended storage temperature will be provided in the pack leaflet supplied with the product.
- It is recommended that compounds are re-analyzed immediately before use.
- Where a compound is to be used in aliquots over a period of time it is advisable to subdivide it upon receipt, or to request subdivision before shipment.

IMPORTANT:

- Vials containing radiochemicals must never be stored by immersion in liquid nitrogen because this could lead to trapping of liquid nitrogen in the vial or outer container causing a build up of pressure when the temperature rises.
- Radiochemicals should not be stored or handled in bright sunlight as this may accelerate decomposition.

Applications of radiochemicals

Radiochemicals are widely used in research and assay applications. Certain radionuclides have specific properties, which make them especially suited to particular applications.

Radiotracers

A radiotracer is a labelled compound that is used to follow a particular component through a biochemical transformation or a chemical reaction. The radionuclide used should ideally form part of the compound's molecular skeleton and, unless conversion to the metabolic product(s) is very low, molar specific activity need not be high. Of the elements present in pharmaceutical compounds, only carbon in the form of carbon-14, provides a suitable radioisotope and it has had this status for many years. Early ADME work can be carried out using tritium, but for a full study, this is always followed up using carbon-14.

Radiotracers can be used to investigate the distribution of a compound and its degradation products in animal tissue. Measurement is either by liquid scintillation counting of dissected samples or qualitative and/or quantitative whole-body autoradiography (QWBAR). The latter method allows the levels of radioactivity in the tissue to be estimated independent of the size, localization and structure of the tissue samples analyzed.

Structural studies on macroscopic and microscopic biological materials can be carried out by incorporation of a suitable radiotracer precursor followed by autoradiography (e.g. the localization of receptor sites on cell surfaces by the attachment of radiolabelled steroids). In this application, the energy, and hence the range, of the beta-emission should be low to provide optimal resolution and, because in many cases only small quantities of material are incorporated, specific activities need to be high. Therefore tritium is the preferred nuclide, but iodine-125 is also used. Tritium is sometimes preferred because it is a natural isotope, having virtually the same chemistry and size as hydrogen, whereas iodine is often a foreign element.

Investigation of the structures of biological polymers such as proteins and nucleic acids may be carried out by incorporation of radiochemicals into the growing polymer. For this application the radiochemical should have the highest specific activity possible. Tritium, phosphorus-32, and phosphorus-33 are the isotopes of choice for studies on RNA and DNA: phosphorus-32 is particularly useful when only very small quantities of material are available for study.

Tritiated amino acids of high specific activity are used for protein structure investigations. Proteins that incorporate sulfur-containing amino acids may be studied using very high specific activity L-[35S]Methionine or L-[35S]Cysteine.

Enzymatic studies

The activity of enzymes may be assayed either by following the rate of disappearance of a labelled substrate or the rate of appearance of a labelled product. ³²P- and ³³P-labelled radiochemicals are readily available and are widely used for enzymatic studies. High specific activity is not essential for this type of study, but high radiochemical and biological purity of the substrate are vital (see page 33).

Fig 13. ERK-1, from hamster cell pellet (Biosignal), generated with the recommended protocol for Serine/Threonine Protein Kinase SPA [33P] Enzyme Assay (RPNQ0200).

Assay of biological compounds

Iodine-125 and tritium are the radionuclides most commonly used in these assay procedures. Although iodine does not occur naturally in many biological compounds, it can be bound to suitable substrates (ligands) to give products with similar antibody binding responses to those of the unlabelled compounds to be measured.

The radioimmunoassay is carried out by incubating fixed amounts of tracer ligand and antibody with varying known concentrations of standard unlabelled ligand. The percentage bound is plotted against standard concentration and any unknown samples can be read off from the standard curve that is produced.

The concentration of biological compounds in body fluids, even hormones and vitamins present in extremely small quantities, may be measured using radiochemicals by techniques such as radioimmunoassay (RIA), competitive protein binding (CPB) analysis, receptor-ligand binding, enzyme assays, protein-protein, and protein-DNA interactions. Utilization of scintillation proximity assay (SPA) technology, and radioisotopes such as iodine-125, tritium, and phosphorus-33 enables development of these assays for a wide range of potential applications.

Fig 14. Typical standard curves generated using acetylation and non-acetylation protocols with cAMP - [¹²⁵]] Direct Biotrak SPA (RPA542)

Fig 15. Principle of SPA

Scintillation proximity assay (SPA) technology is a versatile bead-based system which can be used with weakly-emitting beta isotopes, such as iodine-125. tritium and phosphorus-33, for RIA, enzyme assays, receptor-ligand binding, protein-protein, and protein-DNA interactions. The microscopic beads (average diameter 5 μM) contain a scintillant that is stimulated to emit light only when radiolabelled molecules of interest are bound to the surface of the bead. The scintillation signal can be measured on any scintillation counter.

A range of bead coatings allow different biological molecules of interest to be captured on the bead, for example, streptavidin beads capture biotinylated molecules. Radiolabelled molecules that are not bound to the bead are unable to stimulate the scintillant (being too distant from it) and therefore are not detected, so separation of bound and free radioligand is not required. SPA beads replace the need for separation steps involving filter papers or plates.

This reduces both hands-on time and the amount of radioactive waste generated, thus increasing safety and lowering disposal costs. In addition, as a mix and measure method, SPA offers improved assay precision over conventional heterogeneous assay systems and can be used for real-time kinetics studies.

As well as offering a range of generic SPA beads for your own assay development, Amersham Biosciences has a range of fully-configured kits for a number of popular targets.

Glossary of terms

Activity — The number of nuclear transformations that occur in a given quantity of radioactive material in unit time.

Alpha (α**) emission** — Particulate radiation consisting of fast moving helium nuclei (2 protons, 2 neutrons) produced by the disintegration of heavy nuclei of atomic number > 52.

Autoradiography — A method used to determine the position of a radiochemical in a sample by the interaction of its emission with a photographic (X-ray) film.

Becquerel (Bq) — The SI unit of radioactivity. One becquerel is equal to 1 nuclear transformation per second. 1 Bq = 2.70×10^{-11} Ci.

Beta (β**) emission** — An electron ejected from a nucleus during radioactive transformation. Beta particles produced by a given nuclei have a range of initial energies from a maximum, which is characteristic of the nuclide.

Bremsstrahlung — (Literally: braking radiation). Electromagnetic radiation produced when an electrically charged particle, such as an electron, is slowed down by the electric field of an atomic nucleus.

Carrier-free — A preparation of a radioisotope to which no carrier (unlabelled) isotope has been added, and for which precautions have been taken to minimize contamination with other isotopes. Material of high specific activity is often loosely referred to as carrier-free, but more correctly this should be termed material of high isotopic abundance.

Curie (Ci) — The unit of radioactivity. One curie (1 Ci) is 3.7×10^{10} nuclear transformations per second. 1 Ci = 3.7×10^{10} Bq = 37 GBq.

Daughter — Of a given nuclide, any nuclide that originates from it by radioactive decay.

Electron capture — Radioactive transformation in which the nucleus absorbs an electron from an inner orbital. The remaining orbital electrons rearrange to fill the empty electron shell and in so doing energy is released as electromagnetic radiation at X-ray wavelengths and/or as electrons (such electrons are called Auger electrons).

Electron volt (eV) — A unit of energy equal to the kinetic energy acquired by an electron when accelerated through a potential difference of 1 volt $(1 \text{ eV} = 1.6 \times 10^{-19} \text{ J}).$

Enantiomorph — Either of two forms of a substance that are mirror images of each other.

Freeze-drying (lyophilization) — The process of rapidly freezing a solution and removing the solvent by sublimation under vacuum.

Gamma (γ**) emission** — Electromagnetic radiation emitted by atomic nuclei, the wavelength of which is generally in the range 1×10^{-10} to 2×10^{-13} m.

Gray (Gy) — The SI unit of absorbed dose. One gray is equal to joule per kilogram. $1 \text{ Gy} = 100 \text{ rad.}$

Half-life — The time in which the activity of a radioactive nuclide decays to half its initial value.

ICRP — International Commission on Radiological Protection.

Isotope effect — Differences that may be detectable in the chemical or physical behavior of two isotopes of the same element, or of their compounds.

Isotopes — Nuclides having the same atomic number but different mass numbers.

Isotopic abundance — The number of atoms of a particular isotope in a mixture of the isotopes of an element, expressed as a fraction of all the atoms of the element present.

Milliatom — (Milligram atom). The atomic weight of an element in milligrams.

Millimole (mmol) — The molecular weight of a compound in mg.

Nuclide — A species of atom characterized by its mass number, atomic number and nuclear energy state, provided that the mean life in that state is long enough to be observable.

Parent — Of a nuclide, that radioactive nuclide from which it is formed by decay.

Positron — The antiparticle of the electron, having the same mass but an equal and opposite charge. It is produced in certain decay processes.

Radioactive concentration — The radioactivity per unit quantity of any material in which a radionuclide occurs. Normally expressed as radioactivity per unit volume (e.g. Bq(mCi)/ml). It is not the same as specific activity.

Radioactivity — The property of certain nuclides to emit radiation by the spontaneous transformation of their nuclei.

Radiochemical — A compound in which one or more of its atoms is present in a radioactive form.

Radiochemical purity — Of a radioactive material, the proportion of the total radioactivity that is present in the stated chemical form.

Radiolabelled compound — A compound in which one or more of the atoms of a proportion of the molecules is replaced by a detectable radioactive isotope.

Radionuclide (radioisotopic) purity — Of a radioactive isotope, the proportion of the total radioactivity that is present in the stated isotopic form.

Rem — The former unit of dose equivalent. 1 rem = 0.01 Sv.

Sievert (Sv) — The SI unit of dose equivalent. 1 Sv = 1 J/kg.

Specific activity — The radioactivity per unit mass of an element or compound containing a radioactive nuclide. Normally expressed as millicuries per milligram (mCi/mg) (MBq/mg), as millicuries per millimol (mCi/mmol) (MBq/mmol), or as curies per millimole (Ci/mmol) (GBq/mmol).

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