

**BCHEM 365****Lecture 12****December 04, 2018****Units of radioactivity**

Table 12.1 presents the units commonly used to describe radioactivity

Table 12.1. Units of radioactivity

Unit	Abbreviation	Definition
Counts per min or second	c.p.m. or c.p.s.	The recorded or counted rate of decay
Disintegrations per min or sec	d.p.m. or d.p.s.	The actual rate of decay
Curie	Ci	The no. of d.p.s equivalent to 1 g of radium ( $3.7 \times 10^{10}$ d.p.s.)
Millicurie	mCi	$\text{Ci} \times 10^{-3}$ or $2.22 \times 10^9$ d.p.m.
Microcurie	$\mu\text{Ci}$	$\text{Ci} \times 10^{-6}$ or $2.22 \times 10^6$ d.p.m.
Becquerel (SI unit)	Bq	1 d.p.s
Terabecquerel (SI unit)	TBq	$10^{12}$ Bq or 27.027 Ci
Gigabecquerel (SI unit)	GBq	$10^9$ Bq or 27.027 mCi
Megabecquerel	MBq	$10^6$ Bq or 27.027 $\mu\text{Ci}$
Electron volt	eV	The energy attained by an electron accelerated Through a potential difference of 1 volt. Equivalent to $1.6 \times 10^{-19}$ J
Roentgen (pronounced (Rentghen)	R	The amount of radiation that produces $1.61 \times 10^{15}$ ion-pairs $\text{kg}^{-1}$
Rad (for energy absorbed)	rad	The dose that gives an energy absorption of $0.01 \text{ Jkg}^{-1}$ radiation
Gray (for energy absorbed)	Gy	The dose that gives an energy absorption of $1 \text{ Jkg}^{-1}$ . Thus $1 \text{ Gy} = 100 \text{ rad}$
Rem (for biological effect)	rem	The amount of radiation that gives a dose in humans equivalent to 1 rad of X-rays
Sievert (pronounced (Sehvert) (for biological effect)	Sv	The amount of radiation that gives a dose in humans equivalent to 1 Gy of X-rays. Thus $1 \text{ Sv} = 100 \text{ rem}$

Use Table 12.2 to convert amount of radioactivity from one unit to the other

Table 12.2 Conversion of units of radioactivity

To convert radioactivity from – to –	Multiply by
Ci to Bq	$3.7 \times 10^{10}$
Bq to Ci	$2.7 \times 10^{-11}$
Ci to dpm	$2.22 \times 10^{12}$
mCi to dpm	$2.22 \times 10^9$
dpm to Ci	$4.50 \times 10^{-13}$
dpm to mCi	$4.50 \times 10^{-10}$
Bq to dpm	60
TBq to dpm	$6.0 \times 10^{13}$
dpm to Bq	$1.67 \times 10^{-2}$
dpm to TBq	$1.67 \times 10^{-14}$

### Specific activity and concentration of radioactivity

Two properties of radioisotopes make it necessary to introduce the concept of specific activity and concentration of radioactivity: 1) Radioactive decay is spontaneous and random, and (2) Toxicity of radiation.

Owing to their toxic nature, radioisotopes are usually embedded in a nonradioactive carrier or base and so that not all the substance supplied by a manufacturer will be radioisotopes. In such a case, it is important to know the proportion of the purchased radioactive reagent that is actual radioisotope, that is, we want to find the activity of the radioisotope present in a substance. We describe this as **specific activity**.

**Specific activity** is the amount of activity per unit gram or amount of sample. The units of specific activity is commonly expressed as Ci mg<sup>-1</sup> or Ci mmol<sup>-1</sup>. Other units include Bq mol<sup>-1</sup>, or d.p.m. μmol<sup>-1</sup>, Bq g<sup>-1</sup>, or d.p.m./μg. Specific activity gives a guide to which method to use for detection of decay events, i.e., whether solid or liquid scintillation counting; and which method to use to obtain optimal assay efficiency.

### Equations on specific activity

We learned earlier that radioactive decay is random, and that the probability of decay for a radionuclide (radioisotope) is fixed and is a characteristic or a physical property of the

radioisotope. Remember the half lives of radioisotopes. There are few exceptions where some isotopes which decay by electron capture undergo changing decay rates, e.g.,  $^7\text{Be}$ . Similarly, the number of decay events that occur within a given time of a **specific number of atoms** of that radioisotope is also a fixed physical quantity (the decay constant). Specific activity is defined as the activity per quantity of atoms of a particular radioisotope.

Radioactivity is therefore expressed as the decay rate of a particular radionuclide with decay constant,  $\lambda$ , and the number of atoms  $N$ :

$$-\frac{dN}{dt} = \lambda N \dots (1) \text{ where } N \text{ is the number of radioisotopes, } \lambda \text{ is the decay constant.}$$

Mass of the radionuclide can be calculated from the number of moles. Number of moles is defined severally:

$$\text{No. of mol} = \frac{\text{mass (g)}}{\text{mol.mass (g/mol)}} ; \text{ or } \text{No. of mol} = \frac{\text{No. of atoms (N)}}{\text{Avogadro No. (N}_A\text{)}} ;$$

Mass = No. of mol  $\times$  Mol. mass. Substitute No. of moles with the Avogadro relation, we get:

$$\text{Mass} = \frac{N}{N_A} \times \text{Mol. mass}$$

$$\text{Specific activity} = \frac{\text{Radioactivity}}{\text{mass}} = \lambda N \div \frac{N \times \text{Mol. mass}}{N_A}$$

$$\text{Specific activity} = \frac{\lambda N \times N_A}{N \times \text{Mol. mass}} ; \text{ But } \lambda = \frac{\ln 2}{T_{1/2}}$$

$$\text{Specific activity} = \frac{\ln 2 \times N \times N_A}{T_{1/2} \times N \times \text{Mol. mass}} ; \text{ so that } \text{Specific activity} = \frac{\ln 2 \times N_A}{T_{1/2} \times \text{Mol. mass}}$$

Units of specific activity is shown below:

$$\text{Specific activity} = \frac{\ln 2 \times N_A (\text{mol}^{-1})}{T_{1/2} (\text{sec}) \times \text{Mol. mass (g mol}^{-1}\text{)}}$$

When half life is given in years, it may be converted to seconds as: No. of years  $\times 365 \times 24 \times 60 \times 60$  sec)

#### Purchasing a radiolabel for biochemical assay

Suppliers of radiolabeled compounds sell in units of total activity. Assuming Perkin-Elmer sells [ $^3\text{H}$ ]leucine in a vial labeled as 500  $\mu\text{Ci}$ . The total activity is 500  $\mu\text{Ci}$  (hot radioisotope). This radioactivity is contained in a certain volume (hot plus cold radioisotope) to give a radioactive concentration, of say, 1 to 5  $\text{mCi/mL}$ . Not all molecules in the solution will have  $^3\text{H}$  (the non-radioactive component is the cold carrier). The "specific activity" gives the radioactivity concentration and depends on the half-life of the radioisotope. If every molecule were labeled, the maximum theoretical specific activity obtained for  $^3\text{H}$  will be say, 5000  $\text{Ci/mmol}$ . Due to pre-calibration and efficiency issues with the counters, this number is never seen on a label, rather, suppliers offer a range of specific activities for their products, such as 400, 1,000 and 3,000  $\text{Ci/mmol}$  for  $^3\text{H}$ , the highest often being the most expensive. Using the number on the label, one can calculate the total chemical concentration and the ratio of hot to cold radioisotope.

#### **Concentration of radioactivity and handling**

A radioactive sample bottle has information on concentration, dates, and calibration on it. The purpose of this information is to guide clients on the sample with time as decay events takes place all the time.

#### **Concentration**

It is the amount of material (both hot (labeled) and cold (unlabeled)) dissolved in a known volume of liquid. The concentration of a radioactive sample is expressed in two ways:

1. Radioactive concentration or radiochemical concentration: it is the total amount of radioactivity per unit volume, i.e.,  $\text{mCi/ml}$  or  $\mu\text{Ci/ml}$ . Also known as activity per ml.
2. Molar concentration - the total concentration (in moles) of both labeled and unlabeled product per unit volume as ( $\mu\text{M}$ ) or  $\mu\text{Ci/mmol}$ . it is also known as activity per mole. A linear relationship exists between radiochemical concentration and the molar concentration

#### **Dates**

1. Fresh lot date: the date to manufacture a radiochemical
2. Stock date: the date the radiochemical was manufactured

3. Ship date - the date on which a particular product was dispensed and shipped from the source to the recipient. The actual dispensed volume is adjusted so that the vial will contain the specified amount of radioactivity on the calibration date.
4. Calibration date - date on which the concentration of the radioactive material is exactly the concentration described on the technical data sheet accompanying the shipping. This date accommodates for shipping period during which decay events will be occurring. The manufacturer specifies a date for calibration and states on the container, the radioactive concentration on the calibration date. If a product is shipped before the calibration date, it will arrive with a higher radioactive concentration than that described on the technical data sheet. If the product is shipped after the calibration date, the radioactive concentration will be lower. If product delays on shipping and is calibrated at a later date than that specified by the manufacturer, the radioactive concentration will be lower
5. Pre-calibration date: is when the activity is calibrated for a future date to compensate for the decay events during shipping.

Advantages of using very high specific activity are as follows:

1. Products of a reaction involving the labeled precursor can be produced at high specific activity, e.g., DNA probes for southern or northern blotting. The higher the specific activity, the more accurate is the count
2. With high specific activity, small quantities of radiolabeled compound can be added such that the equilibrium of the metabolic concentrations is not distorted by dilution

### Choice of a radioisotope

The choice of a radioisotope depends on the precise requirements of the experiment. Table 12.3 gives a guide.

Table 12.3. Relative merits of commonly used  $\beta$ -emitters

Isotope	Advantages	Disadvantages
$^3\text{H}$	<ul style="list-style-type: none"> <li>-Safety</li> <li>-High specific activity possible</li> <li>-Wide choice of labeling positions in inorganic compounds</li> <li>-Very high resolution in</li> </ul>	<ul style="list-style-type: none"> <li>-Low efficiency of detection</li> <li>-Isotope exchange with environment</li> <li>-Isotope effect</li> </ul>

	autoradiography	
$^{14}\text{C}$	Safety -Wide choice of labeling positions in inorganic compounds. -Good resolution in autoradiography	Low specific activity
$^{35}\text{S}$	High specific activity Good resolution in autoradiography	Short half-life Relatively long biological half-life
$^{33}\text{P}$	High specific activity Good resolution in autoradiography Less hazardous than $^{32}\text{P}$	Lower specific activity than $^{32}\text{P}$ Less sensitive than $^{32}\text{P}$ High cost
$^{32}\text{P}$	High specific activity Ease of detection Short half-life simplifies disposal	Short half-life affects costs and experimental design External radiation hazard Good resolution in autoradiography

Source: J. Slater (2002).

### Inherent advantages and restrictions of radiotracer experiments

Radiotracer experiments offer the most sensitive detection limits among other chemical and physical techniques, such as spectrophotometry, gravimetry, titrimetric assay, electrophoresis, etc. For example, sample can be diluted many times without interfering with the detection of  $^3\text{H}$  labeling. It is thus possible to detect the occurrence of metabolic substances that are normally present in tissues at low concentrations that other chemical methods could not. Radiotracer techniques allow *in vivo* studies to be carried out to a far greater degree than can any other technique.

### RESTRICTIONS

Different isotopes exhibit different rates of reactions in mixtures, primarily arising from the differences in mass number between the isotopes. The largest difference in rates of reaction is recorded in  $^1\text{H}$  and  $^3\text{H}$ , the effect being small for  $^{12}\text{C}$  and  $^{14}\text{C}$  and almost insignificant for  $^{33}\text{P}$  and  $^{32}\text{P}$ . Because this phenomenon affects the rate of reaction or the equilibrium constant, the effect is described as **kinetic isotope effect (KIE)** or **thermodynamic isotope effect (TIE)**, defined as a change in the rate of chemical reaction when one or more of the atoms in the reactants is(are) replaced by its isotope. Molecules that differ only in their isotopic composition due to difference in number of neutrons are isotopologues. For example, water may exist from protium ( $^1\text{H}_2\text{O}$ , light),  $^2\text{H}^1\text{HO}$ , semi-light water; from deuterium  $^2\text{H}_2\text{O}$  =

$^2\text{D}_2\text{O}$ , heavy water; or from tritium ( $^3\text{H}_2\text{O}$ ,  $^3\text{H}^1\text{HO}$ , super-heavy water). Mathematically, the KIE is computed as ratio of the rate of reaction of the light reaction ( $K_L$ = equilibrium constant of the light reaction) to rate of reaction of the heavy reaction ( $K_H$ = equilibrium constant of the heavy reaction)

$$KIE = \frac{K_L}{K_H}$$

The heavy isotopes more often possess lower vibrational frequencies and require larger energy to reach the transition state or dissociation, hence slower rate of reaction. KIE is therefore predominantly positive. When the bond dissociation energy between the heavy molecules is lower than that for the light molecules, the KIE becomes  $<1$ . Isotope effect is employed in determination of reaction mechanism, and in pharmacokinetics where bonds that are vulnerable to dissociation are protected by substitution with their isotopes.

### Safety aspects

The greatest disadvantage of using radioisotopes is their toxicity from the ionizing radiation they produce. When they are absorbed, radiation causes ionization and free radicals are formed that interact with the cell's macromolecules, causing mutation of DNA and hydrolysis of proteins. Toxicity of radioisotopes depends on the following:

1. Amount of radiation absorbed by the body
2. The energy,  $E$ , of the absorbed radiation
3. The biological effect of the radiation

Originally, radiation hazard was measured in terms of **exposure**. This is the quantity expressing the amount of ionization in air. The unit of exposure is roentgen (R) (pronounced rentghen). 1 R is the amount of radiation that produces  $1.61 \times 10^{15}$  ion-pairs  $(\text{kg air})^{-1}$  or  $2.58 \times 10^{-4}$  coulombs  $(\text{kg air})^{-1}$ .

The amount of energy required to produce one ion-pair in air is  $5.4 \times 10^{-18}$  joules and so the amount of energy required to produce  $1.61 \times 10^{15}$  ion-pairs  $(\text{kg air})^{-1}$  =

$$1.61 \times 10^{15} \times 5.4 \times 10^{-18} = 0.00869 \text{ J (kg air)}^{-1}$$

This amount of energy is approximately 0.01 J kg<sup>-1</sup> air. This is the amount of energy associated with one unit of radioactivity exposure or 1 R.

The unit Roentgen is now considered inadequate because:

- It is defined with reference to X-rays or  $\gamma$ -rays only.
- The energy associated with the radiation is not considered. The amount of ionization in materials other than air is likely to be different.

A new concept, **radiation absorbed dose** (Rad) is used. Rad is defined as the dose of radiation that gives an energy absorption of 0.01 J (kg absorber)<sup>-1</sup>. This is now changed to Gray, the SI unit. One Gray is defined as the dose of radiation that gives an energy of absorption of 1 J kg<sup>-1</sup>, that is, 100 Rads. The unit Gray is also limited by no indication of biological hazard associated with the absorption of the energy of radiation. Because different types of radiation are associated with different degrees of biological hazard, the use of Gray unit becomes inadequate in describing the hazard to living organisms.

It is therefore necessary to introduce a correction factor, known as the **weighting factor** (*W*) which considers the biological effect of any radiation compared to that of X-rays. So, a combination of energy of radiation absorbed plus the biological effect on the object produces a new unit called Sievert (Sv) (pronounced *sehvert*) and is known as the **equivalent dose**.

$$\text{Equivalent dose (Sv)} = \text{Gy} \times W$$

Sievert measures the probability that a specific dose of a particular energy of radiation will cause a biological effect. One Sv is equal to 1 J kg<sup>-1</sup>

Majority of isotopes used in biological research decay by beta emission. This is considered to have a biological effect that is very similar to X-rays and has a weighting factor of 1. So, for a  $\beta$ -radiation of 1 Gray, the magnitude of energy absorbed plus biological effect becomes:



$$Sv = 1 \text{ Gy} \times W = 1$$

where  $W = 1$

for alpha particles with stronger ionizing power and a weighting factor,  $W = 20$ , and having energy absorbed equal to say, 1 Gray, the toxic effect becomes:

$$\text{Therefore, for } \alpha\text{-radiation, Toxic effect (Sv)} = 1 \text{ Gy} \times 20 = 20 \text{ Sv}$$

Absorbed dose from known sources can be calculated from knowledge of the following:

1. The rate of decay of the source
2. The energy of radiation
3. The penetrating power of the radiation
4. The distance between the source and the researcher.

As radiation is emitted from a source in all directions, the level of irradiation will be related to the area of a sphere,  $4\pi r^2$ . Thus the absorbed dose is inversely related to the square of the distance from the source ( $r$ ), because, the shorter the distance, the higher the absorbed dose. That is, if distance is doubled, the dose is quartered.

A useful formula is

$$\text{dose}_1 \times \text{distance}_1^2 = \text{dose}_2 \times \text{distance}_2^2 \dots (1)$$

The relationship between radioactive source and absorbed dose is illustrated in Fig. 12.1. The rate at which dose is delivered is referred to as dose rate and is expressed in  $Sv \text{ h}^{-1}$ . It can be used to calculate the total dose upon exposure. For example, a source may be delivering  $10 \mu\text{Sv h}^{-1}$ . If you worked with the source for 6 hours, your total dose would be  $60 \mu\text{Sv}$ .

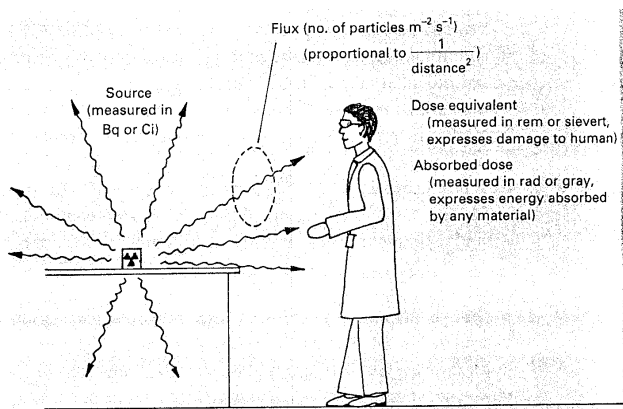


Fig. 12.1. Relationship between radioactive source and absorbed dose.

Currently, international guidelines for dose limit for workers exposed to radiation is 15 mSv in a year for whole body, but this is rarely ever reached by biologists because levels of radiation used are so low. Limits are set for individual organs. The most important of these are hands ( $500 \text{ mSv year}^{-1}$ ); lens of the eye ( $150 \text{ mSv year}^{-1}$ ). Work that may cause an individual to exceed one-tenth of the dose limit must be carried out in a controlled area. Work in biosciences rarely involves a worker receiving a measurable dose. Controlled areas are required in only certain circumstances, such as for isotope stores or radioiodination work. A major problem however in biosciences is the internal radiation hazard. This is caused by radiation entering the body by inhalation, ingestion, absorption or puncture. Such hazard is encountered in work involving open sources, i.e., liquids and gases. Most work in biology involves manipulations of radioactive liquids. Control of contamination is achieved by the following:

1. Comply with local rules, specified by the employer
2. Conscientious personal conduct in the lab
3. Regular monitoring
4. Carry out work in some kind of containment

In some human experiments, radioactivity is ingested by volunteers. In this case, guidelines of a National Radiological Protection Board must be followed. The Annual Limit on Intake (ALI) involves a person receiving a dose limit to the whole body or a particular organ. Table 12.4 provides the ALI for some commonly used isotopes

Table 12.4. Annual Limit on Intake (ALI) for some commonly used isotopes.

Radioisotope	ALI (MBq)
$^3\text{H}$	480
$^{14}\text{C}$	34
$^{32}\text{P}$	6.3
$^{125}\text{I}$	1.3

Most countries have a board for management of radiation protection. In the U.S., there is Code for Federal Regulations on Radiation; in U.K., there is Radioactive Substances Act of 1993 and the Ionizing Radiations Regulation of 1999. In Ghana, the Environmental Protection Agency issues out certification to companies and institutions complying with radiation rules.

The following precautions pertain to handling radioisotopes.

1. Maximize the distance between yourself and the source
2. Minimize the time of exposure
3. Maintain shielding at all times

### **Applications of radioisotopes in biological sciences**

#### **1. INVESTIGATING ASPECTS OF METABOLISM**

##### **Metabolic pathways**

Radioisotopes are frequently used for tracing metabolic pathways. This usually involves adding a radioactive substrate, taking samples of the experimental material at various times, extracting and separating the products by chromatography, electrophoresis, etc. Radioactivity detectors can be attached to gas-liquid chromatography or HPLC columns to monitor radioactivity coming from the column during separation.

Radioactivity can also be located on paper or thin layer chromatography either with a Geiger-Müller scanner or by autoradiography. Radioisotopes can be used to confirm the metabolic pathway of a compound. For example, it is possible to predict the fate of individual carbon atoms of [ $^{14}\text{C}$ ]acetate through the Krebs cycle. Methods have been developed whereby intermediates of

the cycle can be isolated and the distribution of carbon within each intermediate can be ascertained. This method is called the [specific labeling pattern](#).

For example, there are numerous ways by which glucose can be oxidized, the most important ones being by glycolysis, followed by the Krebs cycle, together with the pentose phosphate pathway. Tissues possess the necessary enzymes for both pathways and it may interest researcher to establish the relative contribution of each to glucose oxidation. Both pathways involve the complete oxidation to CO<sub>2</sub> but the origin of the CO<sub>2</sub> in terms of the six carbon atoms of glucose is different. Thus, it is possible to trap the CO<sub>2</sub> evolved during the respiration of specifically labeled glucose (e.g. [6-<sup>14</sup>C]glucose in which only the C-6 atom is radioactive or [1-<sup>14</sup>C]glucose and obtain the contribution of each pathway to glucose oxidation.

### **Metabolic turnover times**

Radioisotopes offer a convenient method of ascertaining turnover times for particular compounds. Using the example of turnover of proteins in rats, a group of rats are injected with radioactive amino acid and left for 24 hours. During this time, most of the amino acid is assimilated into proteins. The rats are then killed at suitable time intervals and radioactivity in organs or tissues of interest determined. Using this method, it has been shown that liver protein is turned over in 7 – 14 days; skin and muscle protein is turned over every 8 – 12 weeks; collagen is turned over at a rate of less than 10% per annum.

### **Studies of absorption, accumulation and translocation**

Radioisotopes have been used in the study of the mechanisms and rates of absorption and translocation of inorganic and organic compounds by both plants and animals. Such experiments yield evidence on the route of translocation and sites of accumulation of molecules of biological interest.

### **Pharmacological studies**

Radioisotopes are widely used in the development of new drugs. The site of drug accumulation, the rate of metabolism and the metabolic products of a new drug must all be determined. Radiotracers are almost indispensable in each of these areas of study. For instance,

autoradiography on whole sections of experimental animals yields information on sites and rate of accumulation, while techniques used in metabolic studies can be used to follow the rate and products of metabolism.

## ANALYTICAL APPLICATIONS

### Enzyme and ligand binding studies

Any enzyme can be assayed using radiotracer methods. Radioisotopes have been used in the study of the mechanism of enzyme action and in studies of ligand binding to membrane receptors.

### Isotope dilution analysis

Many compounds present in living organisms are at such low concentrations and in mixtures of similar compounds so that they cannot be accurately assayed. Isotope dilution analysis offers a convenient and accurate way of determining the quantity of chemical substances that are in very concentrations, and offers the advantage of no need to isolate the substance before quantitation. Isotope dilution analysis works on the basis that the specific activity of the substance exists in a constant ratio, in other words, constant signal ratio. For example, one can use  $^{59}\text{Fe}$  to determine the amount of iron in blood. Iron exist in seven isotopes, of which four are stable, and three are radioactive.

$^{54}\text{Fe}$ : protons= 26 no. of neutrons=28 – stable

$^{55}\text{Fe}$ : protons= 26 no. of neutrons=29 – radioactive,  $\beta$ - emitter,  $t_{1/2}$ = 2.73 years

$^{56}\text{Fe}$ : protons= 26 no. of neutrons=30 – stable, forms 91.7% of iron in the earth

$^{57}\text{Fe}$ : protons= 26 no. of neutrons=31 - stable

$^{58}\text{Fe}$ : protons= 26 no. of neutrons=32 - stable

$^{59}\text{Fe}$ : protons= 26 no. of neutrons=33 - radioactive,  $\beta$ - emitter,  $t_{1/2}$ = 44.6 days

$^{60}\text{Fe}$ : protons= 26 no. of neutrons=34 - radioactive,  $\beta$ - emitter,  $t_{1/2}$ = 2.6 million years

Isotope dilution involves the following steps:

1. Add a known amount of a labeled substance (the standard, i.e., the isotope form of the analyte) to the analyte which contains the normal substance to be assayed and mix

thoroughly. For example,  $^{56}\text{Fe}$  in blood may be assayed using  $^{59}\text{Fe}$  in isotope dilution method.

2. Mixing of the standard with the analyte dilutes the concentration of the standard. This is the basis for the isotope dilution method.
3. Method is based on the mark-and-recapture method used in ecology to determine population size.
4. This method is based on the premise that, the proportion of marked individuals in a population is equal to the proportion of marked individuals in a sample drawn from the entire population.
5. This method can be used to determine population size.

$^{59}\text{Fe}$  is mixed with the protein which is known to contain iron, say Ferritin, but the amount of iron in the ferritin is not known, and that is what is being measured. Following mixing, a sample of the mixed iron (radioactive + nonradioactive) is subsequently isolated, assayed for total iron and the radioactivity determined.

If the original specific activity of the protein- $^{59}\text{Fe}$  mixture was 10,000 d.p.m.  $(10\text{ mg})^{-1}$  and the specific activity of the isolated mixed iron (radioactive + nonradioactive) was 9,000 d.p.m.  $(10\text{mg})^{-1}$ , then the difference in specific activity is due to the unlabeled iron in the protein ( $x$ ) which has diluted the original iron in the labeled form.

That is:

$$\frac{9,000}{10} = \frac{10,000}{10 + x} \dots (1)$$

Therefore  $x = 1.1\text{ mg}$ . This is the amount of unlabeled iron in the ferritin.

### **Isotope dilution calculation**

### **Question**

To determine the nutritional quality of protein in soybean, the content of lysine was determined by isotope dilution analysis. To an acid hydrolysate of the protein (1 mg) a 0.5  $\mu\text{mole}$  of  $[^3\text{H}]\text{lysine}$  ( $1 \text{ Ci mol}^{-1}$ ) was added. A sample of lysine was isolated from the hydrolysate by chromatography and the specific activity determined by scintillation counting at 25 % efficiency. The value obtained was 2,071 c.p.m.  $\mu\text{g}^{-1}$ . What is the % (w/w) lysine content of soybean protein?

$$1\text{Ci} = 22.2 \times 10^{11} \text{ d.p.m.}; M_r \text{ lysine} = 148.$$

### Solution

To solve this problem we apply equation (1) above. The original specific activity in the acid hydrolysate was:  $1 \text{ Ci mol}^{-1}$ , that is,

$$1 \text{ mole} = 1 \text{ Ci}$$

Therefore 0.5  $\mu\text{mole}$  will contain

$$0.5 \times 10^{-6} \text{ mole} \times 1 \text{ Ci/1 mole}.$$

Convert all units including Ci to d.p.m in a  $\mu\text{g}$

We have original specific activity to be  $0.5 \times 10^{-6} \text{ mole} \times 22.2 \times 10^{11} \text{ d.p.m/ mole}$

This gives  $11.1 \times 10^5 \text{ d.p.m.}$

Convert no. of moles of lysine to mass by multiplying by the  $M_r$  (the relative molecular mass).

$$0.5 \mu\text{mole of lysine} \times 148 = 74 \mu\text{g}.$$

Since the counting efficiency was only 25%, it is necessary to multiply the observed count by 4 to correct the counts to 100% efficiency.

$$25\% \text{ gives } 2,071 \text{ c.p.m.}\mu\text{g}^{-1}$$

$$\text{Therefore } 100 \% \text{ will give } 2071 \times 4 = 8,284 \text{ d.p.m. } \mu\text{g}^{-1}$$

Using equation (1),

$$\frac{8284 \text{ d.p.m.}}{1\mu\text{g}} = \frac{11.1 \times 10^5 \text{ d.p.m.}}{(74 + x)\mu\text{g}}$$

where  $x$  is the lysine content of the sample.

From this equation  $x = 60 \mu\text{g}$  or 6% of the 1 mg protein sample.

### **Radiodating**

Radioisotopes are used in determining the age of rocks, fossils and sediments. In this technique, it is assumed that the proportion of an element that is naturally radioactive has been the same throughout time. From the time of fossilization or deposition, the radioactive isotope will decay. By determining the amount of radioisotope remaining and from a knowledge of the half-life, it is possible to date the sample. For example, if the radioisotope normally comprises 1% of the element and it is found that the sample actually contains 0.25% radioactivity, then it can be assumed that two half-lives have elapsed since deposition. If the half-life is 1 million years then the sample can be dated as being two million years old.

## **OTHER APPLICATIONS**

### **Molecular biology techniques**

Radioisotopes are used in DNA and RNA sequencing, DNA replication, transcription, synthesis of complementary DNA, recombinant DNA technology and many similar studies

### **Clinical diagnosis**

Radioisotopes are used in medicine for diagnostic tests. Lung function tests use  $^{133}\text{Xe}$ . Kidney function test uses [ $^{131}\text{I}$ ]iodohippuric acid. In haematology, blood cell lifetimes, blood volumes and circulation times are determined with radioisotopes.

### **Ecological studies**

The migratory patterns and behavior patterns of many animals can be monitored using radiotracers. Examination of food chains where the primary producers may be made



radioactive and the path of radioactivity followed throughout the resulting chain can be performed.

### **Sterilization of food and equipment**

Very strong  $\gamma$ -emitters are now widely used in the food industry for sterilization of prepacked foods such as milk and meats. Usually  $^{60}\text{Co}$  or  $^{137}\text{Ce}$  is used, but care must be taken to ensure that the food product is not affected in any way. So, doses have to be used such that sterilization is not complete, nevertheless spoilage can be reduced.  $^{60}\text{Co}$  and  $^{137}\text{Ce}$  are also used to sterilize Petri dishes and syringes.

### **Mutagens**

Radioisotopes may cause mutations particularly in microorganisms. In various microbiological studies, mutants are desirable. For example, development of new strains of a microorganism that produce higher yields of a desired microbial product frequently involve mutagenesis by radioisotopes.