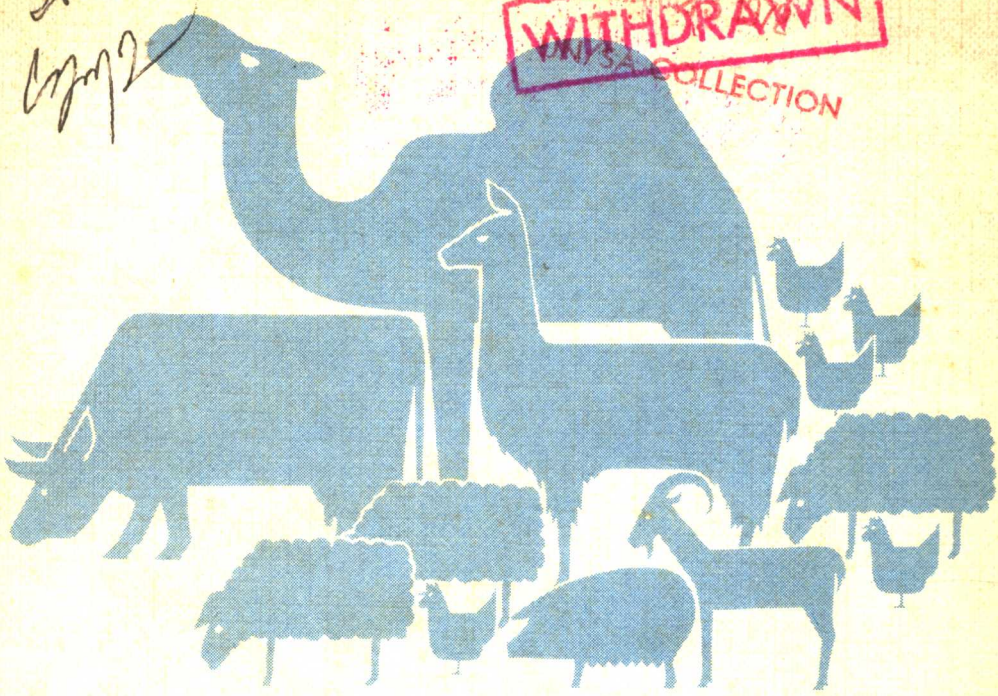

TECHNICAL REPORTS SERIES No. 60

Laboratory Training Manual on the Use of Radionuclides and Radiation in Animal Research

THIRD EDITION

JOINT FAO/IAEA DIVISION OF
ATOMIC ENERGY IN FOOD AND AGRICULTURE

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1972

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LABORATORY TRAINING MANUAL ON THE USE OF RADIONUCLIDES AND RADIATION IN ANIMAL RESEARCH

THIRD EDITION

A JOINT UNDERTAKING BY THE
FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS AND THE
INTERNATIONAL ATOMIC ENERGY AGENCY

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1972

FOREWORD

The use of radionuclides and radiation has proved to be a powerful tool in the agricultural sciences. This manual is designed to give the agricultural researcher the basic terms and principles necessary for understanding ionizing radiation, its detection and measurement, its associated hazards, and some of the more common applications. Basic laboratory experiments to illustrate this purpose are included. Such understanding is necessary if one wishes to gain the most information from the use of radionuclides and to design research experiments for the greatest effectiveness. It is expected that each user of this manual will have had education in basic chemistry, physics, mathematics and statistics as well as his agricultural science speciality.

The further progress of each user will depend on his need and desire to expand his learning by additional reading and training. A list of useful references is included.

The Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA), in co-operation with the Government of the United States of America, Cornell University and Colorado State University, have jointly sponsored several international training courses on the use of radionuclides and radiation in animal research.

Outstanding scientists from various countries have given lectures and devised and conducted the laboratory exercises. Research workers from all over the world have attended these courses in order to apply this experience in their own countries.

The applied part of this present manual, containing a series of detailed laboratory exercises in the use of radionuclides and radiation in animal sciences, represents the efforts of the various instructors who have participated in these training courses.

The first edition of this manual, published in 1966, followed a similar manual (Technical Reports Series No.29) on soil-plant relations research. Since then other manuals (Technical Reports Series No.61, on entomology, and No.114, on food irradiation technology and techniques) have appeared in this series jointly published by FAO and IAEA.

The second edition of the present manual, published in 1968, was enlarged by the addition of several exercises in the basic part.

FAO and IAEA would like to thank all scientists who contributed to the success of the training courses in animal research and in particular Dr. Cyril L. Comar and Dr. Francis A. Kallfelz of Cornell University, who prepared the applied part of the first edition of this manual from lectures given by them and other scientists during the courses. For the additional exercises in the second edition thanks are due to Dr. W. Mulligan of Glasgow University and Dr. F. Lengemann of Cornell University. Dr. James E. Johnson of Colorado State University revised both the basic part and applied part of this third edition.

SOME BASIC SYMBOLS AND UNITS FREQUENTLY USED IN THIS TEXT

Time (in general)	t
Number of radioactive atoms	N
Radioactive decay constant	λ
Radioactive half-life	$T_{\frac{1}{2}}$
Radioactivity or activity	A
Gamma-ray intensity	I
Number of recorded counts	C
Count rate	r
Unit of count rate	cpm (counts per minute)
Count rate of background	r_b
Count rate of sample plus background	r_{s+b}
Approximately equal to	$\simeq$
Tracer radioactivity	R
Amount of stable tracee	S
Specific activity (R/S)	a
Units of specific activity	μCi/g, cpm/g
Biological half-life	$T_{\frac{1}{2}B}$
Biological rate constant	k
Exchange rate	ρ

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1. PROPERTIES OF RADIONUCLIDES AND RADIATIONS

1.1. Atomic model: definitions

Radioactive atoms have unstable nuclei. To understand the reasons for this instability, it is necessary to describe the structure of atoms. An atom is composed of a positively charged nucleus which is surrounded by shells of negatively charged (orbital) electrons. The nucleus contains protons and neutrons (collectively termed nucleons) as its major components of mass. Protons are positively charged and have a mass of 1.007277 atomic mass units. Neutrons have no charge and a mass of 1.008665 atomic mass units. Nuclei have diameters of approximately 10^{-12} cm; the diameters of atoms, which include the orbital electrons, are approximately 10^{-8} cm or 1 Ångström unit.

The number of protons in the nucleus (Z) determines the number of electrons and hence the chemical nature of the element. The atoms of a particular element may, however, not all have the same number of neutrons in the nucleus. Atoms that have the same Z but different neutron numbers are called isotopes, because they occupy the same place in the periodic chart. Isotopes, therefore, have identical chemical properties.

As the protons and neutrons represent the major part of the mass of the atom and each has an atomic weight close to unity, the mass number M, which is the sum of the proton and neutron number, is close to the atomic weight. Nuclides (any species of nuclei) are described symbolically by the designation

$$^{M}_{Z}X$$

where X is the chemical symbol for the element.

Nuclei are held together by attractive forces between the nucleons which balance the coulombic repulsion of protons. However, the attractive forces are of shorter range than the repulsive forces and therefore as the atomic number increases, the number of neutrons must increasingly exceed the number of protons. The neutron to proton ratio in the nucleus is the parameter which primarily describes the stability of the nucleus. As the atomic number increases, a point is eventually reached where addition of neutrons is not sufficient to overcome the repulsive forces and above $Z = 83$ (bismuth) all nuclei are unstable or radioactive. There is also a more or less well-defined optimum neutron to proton ratio for stability of each element. In general, isotopes which have a neutron to proton ratio greater than or less than the stable value for that element are radioactive. All species of radioactive nuclei are called radionuclides.

Radionuclides disintegrate spontaneously, each at a characteristic decay rate. Radioactive nuclei, upon disintegration, may emit alpha (α) or beta (β) particles as well as gamma (γ) rays. These are termed ionizing radiations. Alpha particles are doubly ionized He nuclei ($^{4}_{2}He$), and alpha decay is characteristic of the radioactive heavy elements occurring in nature. Beta particles are electrons of either negative (β^-) or

positive (β^+) charge, while gamma-rays are electromagnetic radiation of very short wavelength. The energy of electromagnetic radiation is concentrated into packets termed photons.

An example of α-decay is given by the following nuclear reaction

$$^{226}_{88}\text{Ra} \longrightarrow {}^{4}_{2}\text{He} + {}^{222}_{86}\text{Rn} + \text{Q (energy)}$$

The total energy Q of a nuclear reaction is calculated from the decrease in atomic mass substituted in the equation of Einstein $E = mc^2$, where m is the mass and c is the velocity of light. Since momentum is conserved, the α-particle in this case receives the majority of the kinetic energy.

An excess of neutrons in a nucleus (generally for the lighter elements) can result in β^- decay. An example is

$$^{24}_{11}\text{Na} \longrightarrow {}^{0}_{-1}\text{e} + {}^{24}_{12}\text{Mg} + \text{Q}$$

In this case, a neutron in the $^{24}_{11}$Na nucleus is transformed into a proton resulting in an increase in Z number.

A deficiency of neutrons in the nucleus may be counteracted by the emission of a positron. An example is

$$^{65}_{30}\text{Zn} \longrightarrow {}^{0}_{1}\text{e} + {}^{65}_{29}\text{Cu} + \text{Q}$$

In this case, a proton in the $^{65}_{30}$Zn nucleus is transformed into a neutron, resulting in a decrease in Z number.

A deficiency of neutrons in the nucleus may alternatively be increased by the capture of an orbital electron. This is termed electron capture (EC) or, commonly, K capture as it describes capture of the K electron. An example is

$$^{85}_{38}\text{Sr} + {}^{0}_{-1}\text{e} \longrightarrow {}^{85}_{37}\text{Rb} + \text{Q}$$

This process is accompanied by the emission of characteristic X-rays since the hole in the K shell must be filled by an electron, etc.

After the ejection of a particle, or K capture, the energy of the resulting nucleus may not be at its ground state. The excess energy of this "excited" nucleus is emitted as one or more γ-rays.

A γ-ray may interact with an orbital electron in the decaying atom whereby the electron is ejected from the atom and the photon ceases to exist. This process results in the combined emission of a fast electron and a characteristic X-ray and is known as internal conversion.

In some instances, two alternate modes of decay of the nucleus may occur. An example is

$$^{40}_{19}\text{K} \longrightarrow {}^{0}_{-1}\text{e} + {}^{40}_{20}\text{Ca} \quad (\beta^- \text{ decay, 82\% of disintegrations})$$

$$^{40}_{19}\text{K} + {}^{0}_{-1}\text{e} \longrightarrow {}^{40}_{18}\text{A} \quad (\text{EC, 11\% of disintegrations})$$

In the case of the EC mode a γ-ray also results.

In nature, a number of radionuclides are known and many more can be produced artificially by nuclear fission, by neutron activation or by particle accelerators.

Summarizing, radionuclides will emit particles and/or electromagnetic photons of the following nature:

α-particle	doubly positively charged particle, containing two neutrons and two protons and originating at high speed from the nucleus;
β^--particle	high-speed electron from the nucleus, negatively charged;
β^+-particle	high-speed positron from the nucleus, positively charged;
γ-ray photon	electromagnetic energy packet coming from the nucleus at the speed of light;
X-ray photon	electromagnetic energy packet coming from an electron shell at the speed of light, following K capture or internal conversion;
I.C. electron	(internal conversion electron) electron emitted as a result of the interaction between a γ-ray and a valence electron;
Neutron	particle with no charge and a mass close to that of a proton.

1.2. Radioactive decay

The decay of radioactive atoms is comprised of individual random events. However, if a sample contains a sufficiently large number of atoms of a radionuclide, their average statistical behaviour can be described by precise laws. The radioactive decay law is developed as follows:

Let N = the number of radioactive atoms of a given radionuclide present at any time t. The rate of decay dN/dt is proportional to the number of atoms present or

$$\frac{dN}{dt} = -\lambda N \qquad (1)$$

where λ is the proportionality constant, termed the decay constant. The sign is negative because the number N decreases with time.

Rearranging Eq. (1) to solve for λ

$$\lambda = -\frac{dN/dt}{N} \quad \text{or} \quad -\frac{dN/N}{dt} \qquad (2)$$

or the decay constant is the fraction of radioactive atoms that decay per unit time.

Equation (1) may be integrated (the steps in this equation are given in Appendix I) to give

$$N = N_0 e^{-\lambda t} \qquad (3)$$

where N_0 is the number present at any starting time and N is the number remaining after a period of time t; e is the base of natural logarithms.

It can be seen from Eq. (3) that the decay of radioactive atoms is exponential with time. Also, it follows that the time for N_0 to be reduced to $\frac{1}{2}$ its initial value is a constant independent of N_0.

Let N_0 be reduced to $\frac{1}{2}N_0$ in time t termed the half-life or $T_{\frac{1}{2}}$. Then from Eq. (3)

$$\tfrac{1}{2}N_0 = N_0 e^{-\lambda T_{\frac{1}{2}}} \tag{4}$$

or

$$\frac{1}{2} = e^{-\lambda T_{\frac{1}{2}}} \tag{5}$$

Inverting and taking the natural logarithm of both sides:

$$\lambda T_{\frac{1}{2}} = -\ln \tfrac{1}{2} = 0.693 \tag{6}$$

Thus, the decay constant and the half-life are constants, and are characteristic of a given radionuclide. The $T_{\frac{1}{2}}$ has units of time and the decay constant that of reciprocal time.

Since the disintegration rate, dN/dt, is termed the radioactivity or, simply, activity (A) of the sample, $A = \lambda N$, one can write from Eq. (3):

$$A = A_0 e^{-\lambda t} \tag{7}$$

The half-life of a radionuclide may be determined graphically by plotting the disintegration rate (as determined by a suitable counting instrument) versus time on log-linear co-ordinate paper. Referring to Eq. (7), if one takes the natural logarithm of both sides the result is

$$\ln A = \ln A_0 - \lambda t$$

converting this to common logarithms this becomes

$$\log A = \log A_0 \frac{-\lambda t}{2.3}$$

Therefore, plot of A on the log co-ordinate versus time on the linear co-ordinate will be a straight line with a slope of $-\lambda/2.3$. This is graphically illustrated in Fig. 1.

The unit of activity is the curie (Ci) defined as equal to 3.7×10^{10} disintegrations per second or 2.22×10^{12} disintegrations per minute.

Thus, 1 curie	(Ci)	$= 2.22 \times 10^{12}$	dis/min
1 millicurie	(mCi)	$= 2.22 \times 10^{9}$	dis/min
1 microcurie	(μCi)	$= 2.22 \times 10^{6}$	dis/min
1 nanocurie	(nCi)	$= 2.22 \times 10^{3}$	dis/min
1 picocurie	(pCi)	$= 2.22$	dis/min

In practice, radionuclides will often be accompanied by variable quantities of stable isotopes of that element. The stable form is called the carrier. Specific activity is the term used to describe the ratio of radioactive atoms to carrier atoms. The specific activity is defined as the total activity of a particular radionuclide per unit mass of its element

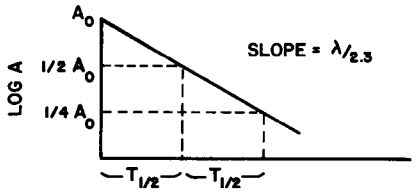

FIG. 1. Decay curve of a single radionuclide.

or compound. Common units are μCi/g or μCi/mmole. The specific activity of "carrier free" activity (that is all the atoms of the element present are the same isotope) can be calculated as follows

$$A\ (\mu Ci) = \lambda N\ (\mu Ci)$$

Divide both sides by N, the number of atoms; the result is specific activity in μCi per atom

$$\frac{A}{N}\ \frac{(\mu Ci)}{(atom)} = \lambda\ (time^{-1})$$

If N^0 is Avogadro's number and M is the molecular or atomic weight, then specific activity may be expressed as μCi per gram

$$\text{Specific activity} = \frac{A(\mu Ci) \times N^0(atoms\ mole^{-1})}{N(atoms) \times M(gram\ \ mole^{-1})} = \frac{\lambda N^0}{A}\quad \frac{(\mu Ci)}{(gram)} \quad (8)$$

An example of the production of carrier free and non-carrier free activity will be given in Section 1.4.4.

1.3. The energy of radiations

The energy unit used with regard to radiation is the electron volt (eV). This is equivalent to the kinetic energy acquired by an electron accelerated through a potential difference of one volt. The most commonly used multiple is the unit MeV or million electron volts (10^6eV). One MeV is equal to 1.6×10^{-6} ergs.

The kinetic energies of the particles and photons emitted by radionuclides have characteristic values. The energies of α-particles and X- or γ-rays are constant or discrete. The energies of β-particles, however, ejected by a given radionuclide vary from zero up to a certain maximum energy (E_{max}) that is available to the β-particles. This is because a variable part of E_{max} is taken away by a neutrino in every β-particle decay. Neutrinos cannot be detected by ordinary methods as they have no charge and essentially no mass. As a consequence, the β-particles show a continuous spectrum of energies from zero to E_{max}. The β-energies given in a table or chart of nuclides are E_{max} values; the average β-particle energy is usually about one-third E_{max}. Internal conversion electrons on the other hand are monoenergetic.

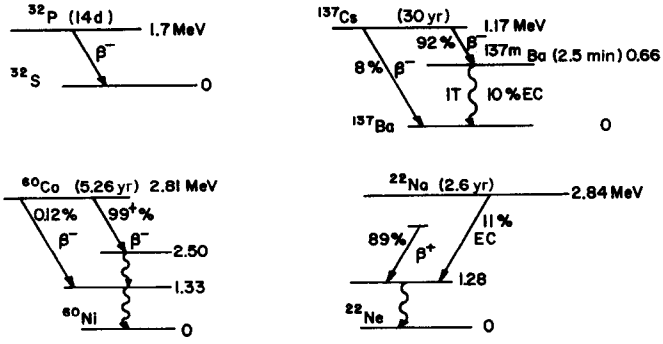

FIG. 2. Decay schemes showing characteristic radiations and energies of four different radionuclides.
IT = Isometric transition. Different types of the same nucleus are called isomers.
EC = Electron capture, K capture.

The characteristic radiations and energies for a given radionuclide are often shown in the form of decay schemes. Examples of the decay schemes of four commonly used radionuclides are shown in Fig. 2.

1.4. Interaction of radiation with matter

1.4.1. Interaction of alpha particles with matter

The α-particles ejected from any particular radionuclide are monoenergetic. Their initial kinetic energies are in the order of several MeV and, since ionization potentials and bond energies are in the range 1-12 eV, the α-particles are capable of causing ionization as well as electronic excitation of the atoms or molecules along their path. Ionization is complete removal of the valence electron and excitation is raising electrons to higher energy levels in their orbits. Since the valence electron participates in any chemical bond of the atom, ionization destroys the integrity of that bond. Alpha particles are doubly charged and of comparatively heavy mass and, therefore, form a dense track of ion pairs (i.e. ejected electrons and positively charged ions) along their path. Therefore, they lose energy relatively rapidly in matter by these processes. As the α-particle dissipates its energy along its path the velocity of the particle decreases and at zero kinetic energy the particle acquires two electrons from its surroundings and becomes a helium atom. The range, i.e. the distance that an α-particle can penetrate into any matter (absorber) depends on the initial energy of the particle and the density of the absorber. The range of an α-particle is relatively small and amounts to several centimetres in air and several microns (10^{-3} mm) in tissue for energies in the order of 1 - 10 MeV.

1.4.2. Interaction of beta particles with matter

Beta particles lose energy in matter by ionization and excitation just as do α-particles. The mass of the β-particle, however, is only 1/7300

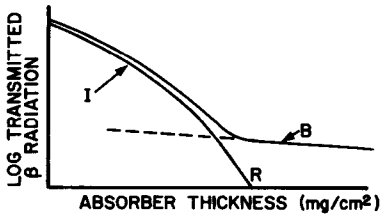

FIG. 3. Curve demonstrating the transmitted β-radiation as a function of absorber thickness.
I = Intensity of transmitted β-radiation.
B = Bremsstrahlung component (and γ-ray component).
R = Approximate range of β-particles in absorber material.

of the mass of the α-particle and β-particles have only unit charge. They will, therefore, scatter more, penetrate further into matter and produce a less dense track of ion pairs than will α-particles. The range of β-particles in matter is also a function of the initial energy of the particle and the density of the absorber, but this range is not so well defined because of the zig-zag path (due to scattering) of the electron. The range of β-particles of 1 MeV initial energy is approximately 300 cm in air and 0.4 cm in tissue.

Because of the fact that β-particles have a continuous spectrum of energies up to E_{max}, their absorption in matter is approximately exponential with absorber thickness. Therefore, when the β-radiation transmitted by an absorber is plotted as a function of absorber thickness on semi-log paper, a fairly straight line is obtained over a portion of the curve (Fig. 3).

The curve becomes reasonably flat at R, approximately the range for β-particles with E_{max}. Although nearly all the β-particles are stopped by this thickness of absorber, one still finds transmission of radiation, because the β-particles interact with the atoms of the absorber giving rise to non-characteristic X-rays, or bremsstrahlung. In addition, any γ-rays will contribute to this component. By subtracting this component from the composite curve, the pure β-transmission curve I is obtained.

Positive β-particles, termed positrons, lose their kinetic energy in matter in exactly the same manner as negative β-particles. However, when the kinetic energy of the positron has been reduced to zero by ionization and excitation, the positron is annihilated together with an electron giving rise to two annihilation photons of 0.51 MeV each. (0.51 MeV is the equivalent energy of the rest mass of an electron.)

Absorption and scattering of β-particles are important in their measurement in samples. Absorption and scattering will occur in the sample cover, the detector window, the walls of the shield, the intervening air and in the sample itself. These effects will all influence the counting rate, self-absorption being the most important. This will be illustrated by Laboratory Experiment 1.4.

1.4.3. Interaction of gamma rays and X-rays with matter

Electromagnetic radiation is considerably more penetrating than particulate radiation of the same energy. This is because the electromagnetic radiation must first undergo an absorbing event to produce a

"secondary" ionizing particle before its energy may be dissipated. Gamma rays will be absorbed in matter as a function of their energy, the Z, and the density of the absorbing material. (γ-rays and X-rays differ only as to their origin and interact identically in matter). Three types of absorbing event may occur:

(1) photoelectric absorption

(2) Compton absorption

(3) pair-production absorption

(1) Photoelectric absorption is predominant for low-energy γ-rays and for absorbers of a high Z material. The γ-ray interacts with a K or L electron of the absorber atom and ejects it from the atom with a kinetic energy equal to the initial γ-ray energy minus the binding energy of that K or L electron. Thus, an electron is produced with kinetic energy to produce ionizations and excitations along its path exactly in the manner of a β-particle.

In Fig. 4, the coefficient for photoelectric absorption is given for water as a function of γ-ray energy. The absorption coefficient is a measure of the probability of absorption.

(2) Compton absorption is the interaction of the γ-ray with an outer electron of the absorber atom. Part of the initial kinetic energy is transferred to this electron and the γ-ray photon is scattered off in a new direction at a lesser energy. As can be seen from Fig. 4, this effect is at a maximum in water for γ-rays of about 0.5 MeV. The effect rises only slightly with increasing Z. The electron ejected will produce ionization and excitation again exactly in the manner of the β-particle.

(3) When the γ-ray has an initial energy of 1.02 MeV or greater it may undergo pair-production absorption. In this process, the γ-ray interacts with the positive field of the nucleus of the absorber atom and is completely annihilated producing a positron-electron pair. Since it requires 1.02 MeV for this pair formation, this is the threshold for this event. Any γ-ray energy above this required 1.02 MeV is imparted as kinetic energy to the positron-electron pair. Both the positron and the β-particle cause ionization and excitation along their respective paths. The two 0.51-MeV photons produced upon annihilation of the positron must be absorbed by Compton or photoelectric events or a combination of the two. In Fig. 4, the absorption coefficient for pair-production is labelled as K.

Considering the above processes, a beam of monoenergetic γ-rays is absorbed exponentially as a function of thickness of the absorbing material. For a beam of intensity I the change in intensity per unit absorber thickness, dI/dx, is proportional to the intensity of the beam.

$$\frac{dI}{dx} = -\mu I \tag{9}$$

This is identical to the well-known Lambert-Beer law for attenuation of monochromatic light. The proportionality constant μ is termed the total linear attenuation coefficient. Exactly analogous to radioactive decay, μ is the fraction of the original intensity removed from the beam per unit thickness. Equation (9) is identical to the radioactive decay law and may be integrated to give