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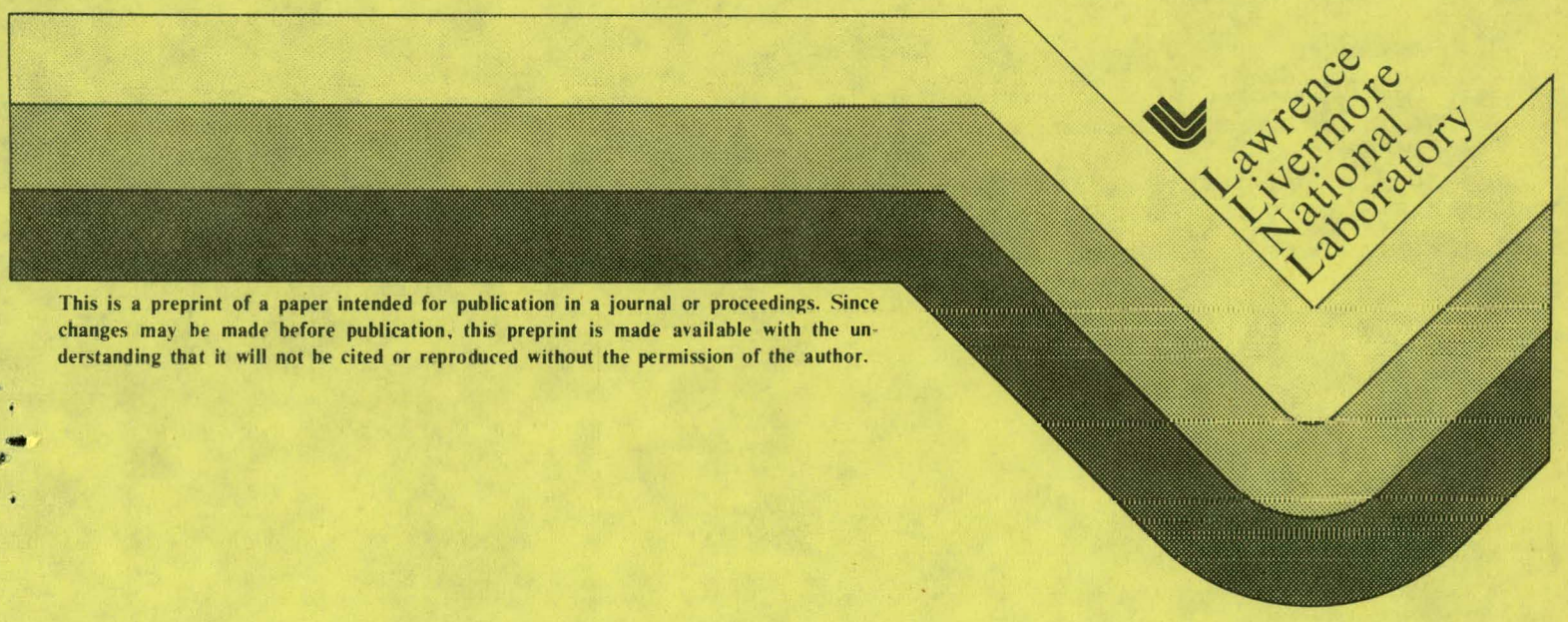

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LOW-EXPOSURE TRITIUM RADIOTOXICITY IN MAMMALS

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INTRODUCTION

Tritium toxicity, when considered in full detail, involves more than radiation effects. It involves isotope effects, transmutation effects, intramolecular position effects, and the biochemistry of ^{3}H. In terms of practical biological importance, however, the ionizing radiation of tritium's beta emission dominates these other features (1). Therefore, attention here is focused on tritium's ionizing radiation (although isotope effects will be considered briefly, for perspective).

Much of the current concern and uncertainty over radiation hazards relates to low exposures. The reasons for this are apparent: the radiobiology of large doses is fairly well understood, while questions of possible dangers from low-level exposure remain matters of interpretation and debate. A recent illustration of difficulties encountered in attempting to deal rigorously with these questions in analyzing risks to man is the so-called "BEIR III controversy" (2-5). The basic problem underlying such difficulties is that available human data are not adequate to establish definitively the shapes of

dose-response curves in the low-dose range---the range of greatest practical interest. Consequently, conclusions (and opinions) about effects of low doses vary with the choice of mathematical model used for fitting the incomplete data. Clearly, what is needed is fuller knowledge of the shapes of dose-response curves for low-level exposure.

This is true for tritium also. The radiobiology and toxicology of large doses have been reasonably well studied (for reviews see references 1,6); and human deaths from high exposure (in the luminous paint industry) have been documented (7). But whether or not significant deleterious biological effects are associated with chronic low-level exposure is a question more difficult to answer satisfactorily.

General agreement exists that tritium has low toxicity compared with other important radionuclides (8,9). The contrast is dramatic, for example, in the case of tritium compared with plutonium: ICRP's long-recommended maximum permissible body burden of ^{3}H (1 mCi, for ^{3}H in the form of tritiated water) is of the order of 10^{5} times larger than that for ^{239}Pu (0.04 μCi) (8). Nevertheless, ^{3}H remains of concern for several reasons. First, it is isotopic to the cell's most abundant and ubiquitous atom (H) and can find its way almost anywhere in the living biochemical machinery. Second, large quantities of tritium are involved in atomic energy operations, both fission and fusion; it is released to the environment in amounts larger than for any other radionuclide (neglecting short-lived atoms) excepting the noble gas ^{85}Kr. And when nuclear fusion becomes successfully harnessed for peaceful purposes, tritium will become even more important. Enormous amounts

will be manufactured and used as fuel. And, finally, tritium's thermodynamically preferred state is water (^{3}HOH), which gives it unimpeded access to cells and organisms.

Now, for perspective in which to view low exposures, let us first briefly consider large doses of ^{3}HOH and the relative importance of tritium's radiation compared with a representative non-radiation effect.

RADIATION EFFECTS AND ISOTOPE EFFECTS COMPARED

Because ^{3}H and ^{1}H differ in atomic weight by a factor of 3 (the largest mass ratio for any isotopic pair in the periodic system), significant isotope effects can be expected. And they do occur, being reflected in both physical and chemical behavior. As shown in Table 1, for example, the physical vapor pressure of ^{3}HOH at 37°C is less than that of ordinary water by the substantial factor of 0.83, corresponding to an approximately 17% difference. Equally large differences are found in chemical bond energies, as shown in Table 2. The energy of the C-^{3}H bond is approximately 17% greater than that of the C-^{1}H bond. The difference, 0.065 eV, can measurably influence chemical reaction rates. This effect can be appreciable when ^{3}H atoms constitute a significant fraction of the H atoms present, but tends to lose importance as tritium concentrations decrease.

Consider now the relation between the number of ^{3}H atoms present and the associated radiation dose delivered. The average tritium beta-ray energy (5.7 keV) is 9.1×10^{-9} erg; therefore 1 μCi of ^{3}H, which disintegrates at 3.2×10^9 dis/day, liberates $(9.1 \times 10^{-9})(3.2 \times 10^9) = 29$ erg/day.

Hence, an ^{3}HOH concentration in H_2O of 1 μCi/ml liberates energy (in the water) at a rate of 29 erg per gram per day, or 0.29 rad/day. And the thousand-fold higher concentration of 1 mCi/ml will irradiate at 290 rad/day. Hence, if this latter concentration is present in body water (cells being taken as 70% H_2O), the dose rate to tissue will be 0.7 x 290 or approximately 200 rad/day.

Now assume acute exposure of a person to ^{3}HOH such that the initial concentration in body water is 1 mCi/ml. Taking the biological half-time of ^{3}HOH in that person as 4 days (the normal value in man is higher, some 7 to 12 days, but can be greatly shortened by increased fluid intake to treat the tritium poisoning), the 1 mCi/ml will deliver to the person approximately 600 rad in the first 4 days, a total-body dose well into the lethal range. (Incidentally, it is interesting to note that this lethal concentration of tritium in body water, 1 mCi/ml, would follow intake by an adult of about 40 Ci of ^{3}HOH; and 40 Ci of pure ^{3}HOH is only 27 mg, or 0.025 ml!)

We wish now to know the relative number of ^{3}H and ^{1}H atoms corresponding to 1 mCi/ml in body water. On the one hand, tritium disintegrates at 0.016% per day; so 1 mCi corresponds to $(3.2 \times 10^{12})/0.016\% = 2 \times 10^{16}$ tritium atoms. On the other hand, 1 ml of H_2O (1 g, or 1/18 mole) corresponds to $(1/18)(6 \times 10^{23}) = 0.33 \times 10^{23}$ H_2O molecules or 0.66×10^{23} H atoms. Hence, for 1 mCi/ml, the ratio of ^{3}H to ^{1}H atoms is $(2 \times 10^{16})/(0.66 \times 10^{23})$, or approximately 1 in 3×10^{6}! In other words, only one ^{3}H atom in about three million ^{1}H atoms (a concentration that could give at most only a minute isotope effect) will deliver a <u>lethal</u> whole-body radiation dose. Clearly, the isotope effect, although very real,

is overwhelmed by radiation effects. We shall therefore for practical purposes neglect isotope effects, along with other non-radiation effects, in considering tritium toxicity.

A special feature of tritium radiation, however, is the very low energy of the beta particles (5.7 keV average, 18 keV maximum). This low energy results in very short particle ranges in tissue (10), ranges that are less than cell dimensions, as illustrated schematically in Fig. 1. Another result of the low energy is that the ionization density along beta-ray tracks (even though the tracks are short) is significantly greater than that associated with secondary electrons from, say, gamma rays. And this must be fully considered in radiotoxicity assessment.

TRITIUM RADIOTOXICITY AT LOW EXPOSURE

To assess the possibility that deleterious effects in mammals may result from chronic exposure to low concentrations of ^{3}H in water (the primary practical problem), it is necessary to examine endpoints that might be especially radiosensitive. Using such endpoints, one must then determine whether biological effects can in fact be detected at interestingly low exposure levels, e.g. in the range of ICRP's maximum permissible concentrations for occupational exposure (0.1 and 0.03 μCi/ml, for 40 and 168 h/week (8)). Then, in order to quantify radiotoxicity, it is necessary to obtain dose-response data for such effects at low exposure levels. It is highly desirable also to have a reliable basis for extrapolation to even lower doses and, if possible, to man.

Effects in Mice

Our most extensive studies aimed at these goals have been on mice (11-14). Animals were exposed chronically to low levels of ^{3}HOH during the vulnerable developmental periods, and the biological endpoint measured was the killing of female germ cells in the ovary (oocytes in the juvenile mouse are known to be unusually radiosensitive (15,16)). Loss of ovarian germ cells, an irreversible injury because oocytes cannot be replaced after birth, was quantified by microscopic counting in serial sections. Comparisons with controls gave cell-survival data and quantitative dose-response relations. Results from these studies have been summarized recently (17) and are reviewed here together with new findings.

Young animals were chronically exposed to tritium in body water (via maternal drinking water) throughout development until two weeks of age, and their ovaries were then studied. Dose-dependent germ-cell destruction was found at all tritium levels examined---in a more than 100-fold range of concentrations from 12 μCi/ml down to 0.085 μCi/ml. Results from several investigations (11,12,14) are schematically shown in the left-hand panel of Fig. 2. It is seen that mouse oocyte survival decreases exponentially with exposure level. There is no evidence of a "safe" threshold below which cells are not killed; and the LD_{50} level is only 2 μCi/ml which, under the these chronic exposure conditions, delivers 0.44 rad/day.

This great effectiveness of tritium, shown by dose-dependent, in-vivo cell destruction measureable at exposure levels even below 0.1 μCi/ml (in a range comparable with ICRP's maximum permissible concentrations for occupational

exposure, and delivering only 0.02 rad/day), exceeds that previously predicted for any detectable mammalian response (18). Furthermore, the threshold-free exponential dose-response curve suggests that extrapolations can be made for predicting effects at even lower exposures.

However, the question arises: Are these mouse data relevant to man? The high radiosensitivity known to characterize mouse oocytes, especially in the juvenile animal, suggested the possiblity that the remarkable response observed might be peculiar to mice. Clearly, low-exposure tritium data were needed for species more closely related to man.

Effects in Monkeys

We approached this question with studies on squirrel monkeys (19). As in the mouse experiments, exposure was continuous by way of ^{3}HOH in the pregnant mothers' drinking water. Body-water levels ranging from 3.1 μCi/ml down to 0.05 μCi/ml were studied. Ovaries of newborn tritium-exposed monkeys displayed tremendous germ-cell loss. Results from oocyte counts are shown in the right-hand panel of Fig. 2.

As in the mouse, oocyte survival in the squirrel monkey decreased exponentially with exposure level (Fig. 2), and there was no evidence of a threshold. The response in this monkey is more dramatic even than that in the rodent (the mouse oocyte was previously considered to be perhaps the most radiosensitive of all mammalian cells). The LD_{50} level for these primate germ cells is only one-fourth of that for mouse oocytes, 0.5 μCi/ml, delivering 0.1 rad/day. (The dashed curve in Fig. 2 is an alternative

interpretation of the data which excludes a single outlier point (see ref. 19); it is not possible yet to rule out this interpretation which, if real, suggests that some 70% of the germ cells are more sensitive than described above, with an LD_{50} level close to 0.07 μCi/ml). The squirrel-monkey data in Fig. 2 indicate that for a dose rate of only 1 rad/day (from 4.5 μCi/ml) the female germ-cell population would be completely (99.9%) destroyed in the fetus before birth!

These startling results in a primate led us to examine chronic tritium exposure in additional species (20). It was important to determine if the unexpectedly high germ-cell radiosensitivity in prenatal squirrel monkeys was a general phenomenon in primates (and might therefore be expected in man). Two species of macaque were studied, rhesus (Macaca mulatta) and bonnet monkey (Macaca radiata), with continuous maternal exposure to ^3HOH in drinking water during the last two trimesters of gestation. Details of the results are still under analysis, but it can now be said that the striking effects seen in squirrel monkeys were not observed in the macaques. While this may be comforting with regard to possible sensitivity of human female germ cells, caution must be exercised. It is conceivable that observed differences between squirrel monkeys and macaques may be related to differences in the exposure durations used (entire gestation for squirrel monkeys, last two trimesters for macaques). This continues under study.

It is clear, however, that of the four mammalian species we have so far studied, two (mouse and squirrel monkey) show marked vulnerability during early development. Female germ-cell destruction occurs from surprisingly low levels of ^3HOH exposure, even from levels in the range of long-accepted permissible concentrations for occupational exposure.

Tritium and Gamma Radiation Compared

Because ^{3}HOH caused such marked effects, it was important to determine whether special attributes of tritium were involved or whether similar effects would be seen following exposure to a more "standard" radiation such as gamma rays. Therefore mice were continuously exposed to low-level ^{60}Co gamma radiation during in-utero and postnatal development in a manner rigorously comparable with the chronic ^{3}HOH exposures. Oocyte survival was determined in offspring 14 days after birth. Results (12,14) are shown by the gamma-ray curve in the upper portion of Fig. 3, where oocyte survival is plotted as a function of the dose rate and of "effective" dose (see ref. 12). In comparison with the straight-line tritium response, also shown in the upper portion of Fig. 3, the gamma-ray curve is both less steep and downward-bending.

Tritium is clearly more effective than gamma rays, and especially so at low exposures. Its RBE compared with gamma radiation, defined as the ratio of doses producing equal effects, is greater than 1 at all exposures examined and increases inversely with dose and dose rate, reaching a value close to 3, as seen in the lower portion of Fig. 3.

The inverse variation of RBE with dose (exposure level) is in agreement with the theory of dual radiation action (21), and the low-dose value of about 3 is in agreement with physical microdosimetric measurements on tritium and ^{60}Co radiation (22), as shown in Fig. 4. It should be noted---and emphasized---that the tritium RBE increases at low exposure not because of any rise in effectiveness of tritium (its effectiveness is constant throughout the exposure range studied, as seen by the straightness of the dose-response

curve); rather, the RBE increases because the effectiveness of gamma rays becomes less at low exposure. Relationships between these findings and radiation protection recommendations, in particular the value of the quality factor (Q) (23), have recently been discussed elsewhere (24).

Further Studies in Mice

Because mouse oocyte radiosensitivity differs with animal's age, it was important to carefully determine effectiveness of chronic ^{3}HOH exposure during the juvenile period when murine oocytes are most vulnerable, 5-18 days after birth. Such an experiment, carried out in collaboration with K. Takimoto, gave results (to be published) which are not significantly different from those shown in Figs. 2 and 3. This validated the use of "effective" dose (derived by adjustment for sensitivity differences before and after birth, see reference 12) for expressing results from prolonged chronic exposure throughout development. And, importantly, it gave additional confirmation to the exponential character of the tritium dose-response curve.

Now, because the tritium dose-response curve is exponential and the curve for gamma rays is downwardly bending, which together suggest absence of oocyte recovery following tritium exposure and presence of recovery (at least some) following gamma irradiation, it was important to determine whether more acute ^{3}HOH exposure would produce a steeper response curve. Such an experiment, carried out with the collaboration of N. Nakamura and using highly sensitive juvenile mice given single injections of ^{3}HOH, yielded results (to be published) identical again to those mentioned above. The curve was not steeper. While further aspects of this experiment are still under study, the

observations give added support to the notion that oocyte recovery does not occur after tritium exposure. On the other hand, preliminary results from split-dose gamma-ray experiments, also done in collaboration with N. Nakamura and which are still in progress (to be published), strongly support the existence of some significant degree of oocyte recovery following gamma irradiation.

These recent investigations, though yet unfinished, are adding to our understanding of the extremely sensitive mouse oocyte system that continues to give such important quantitative data for evaluating tritium radiotoxicity at low exposures.

CONCLUSIONS

The studies of tritium radiotoxicity reviewed here, involving chronic ^{3}HOH exposures in mammals, demonstrate in both mice and monkeys that biological effects can be measured following remarkably low levels of exposure---levels in the range of serious practical interest to radiation protection.

These studies demonstrate also that deleterious effects of ^{3}H beta radiation should not be expected to differ significantly from those of gamma radiation at <u>high</u> exposures. In contrast, however, at <u>low</u> exposures---which are of most practical concern---tritium is significantly more effective than gamma rays, rad for rad, by a factor approaching 3. This is an important feature of tritium radiotoxicity. It is important for hazard evaluation and radiation protection because knowledge concerning biological effects of

chronic low-level radiation exposure has, through the years, come mainly from gamma-ray data; and predictions based on gamma-ray data will underestimate tritium effects---especially at low exposures---unless the RBE is fully taken into account.

ACKNOWLEDGMENT

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Table 1. Ratio of Vapor Pressures (VP) of ^{3}HOH and ^{1}HOH at Various Temperatures

Temperature °C	VP of ^{3}HOH / VP of ^{1}HOH
25.7	0.77
37.0	0.83
75.6	0.89

Table 2. Chemical Bond Energies

Bond	Energy, eV
C - ^{1}H	3.793
C - ^{3}H	3.858

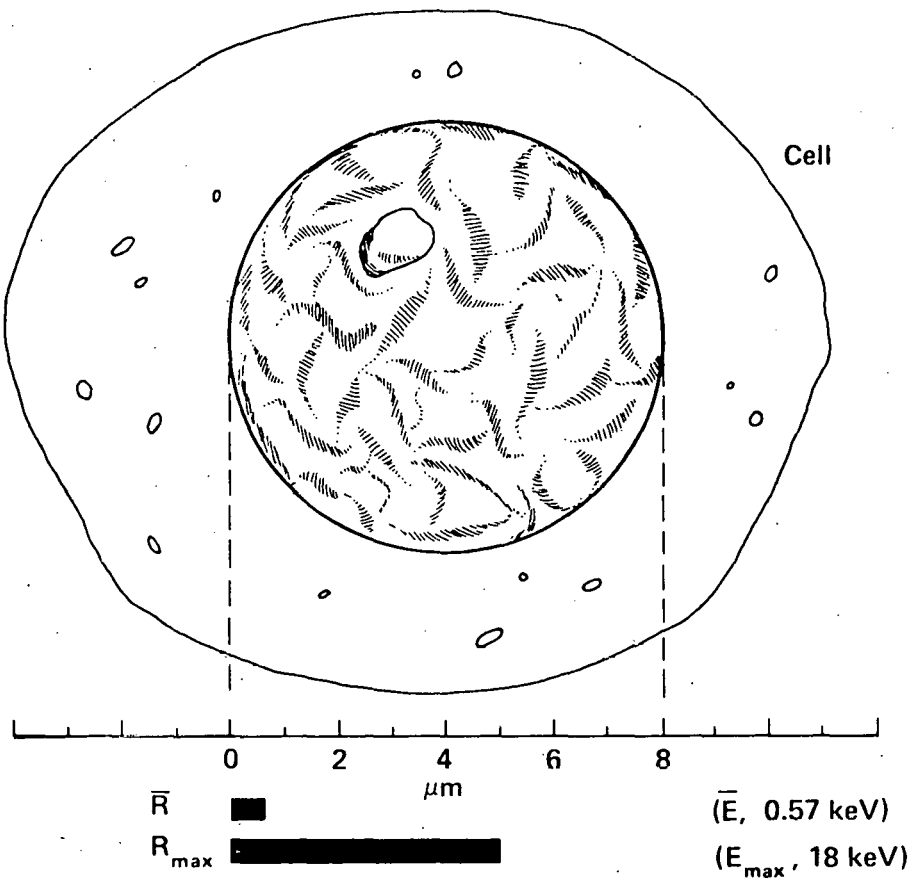
FIGURE LEGENDS

Fig. 1. Average range ($\bar{R}$) and maximum range (R_{max}) in tissue of ^{3}H beta particles, shown in comparison with cellular dimensions.

Fig. 2. Survival curves for oocytes in mice and squirrel monkeys exposed continuously to ^{3}HOH during prenatal development (plus 14 postnatal days for mice). (Modified from reference 17.)

Fig. 3. Mouse oocyte survival curves for chronic ^{3}HOH and ^{60}Co gamma-ray exposures and the RBE of tritium compared with gamma radiation. (From reference 17.)

Fig. 4. Low-Dose RBEs for tritium and 250 kVp x rays, with ^{60}Co gamma rays as reference radiation, estimated from physical measurements and shown as functions of site diameter (see reference 22). (From reference 24.)



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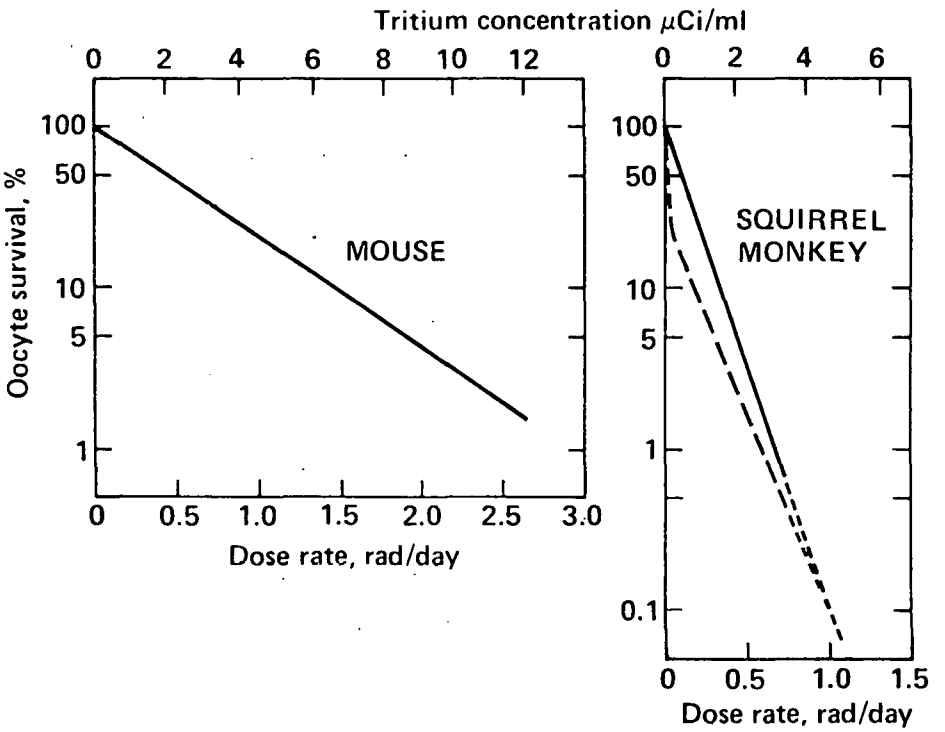

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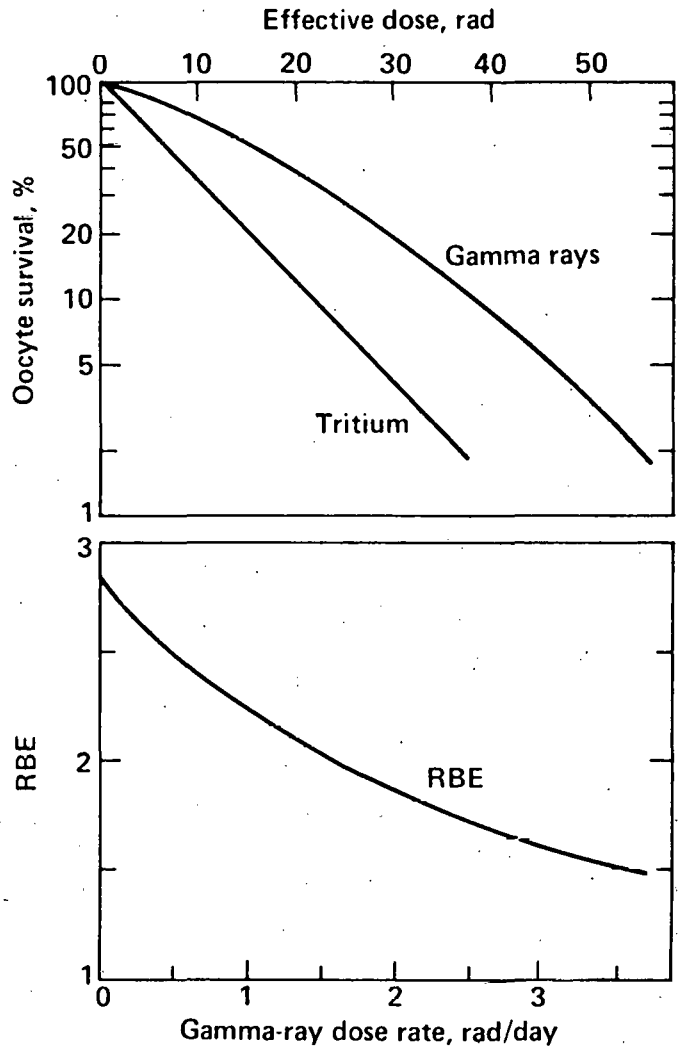

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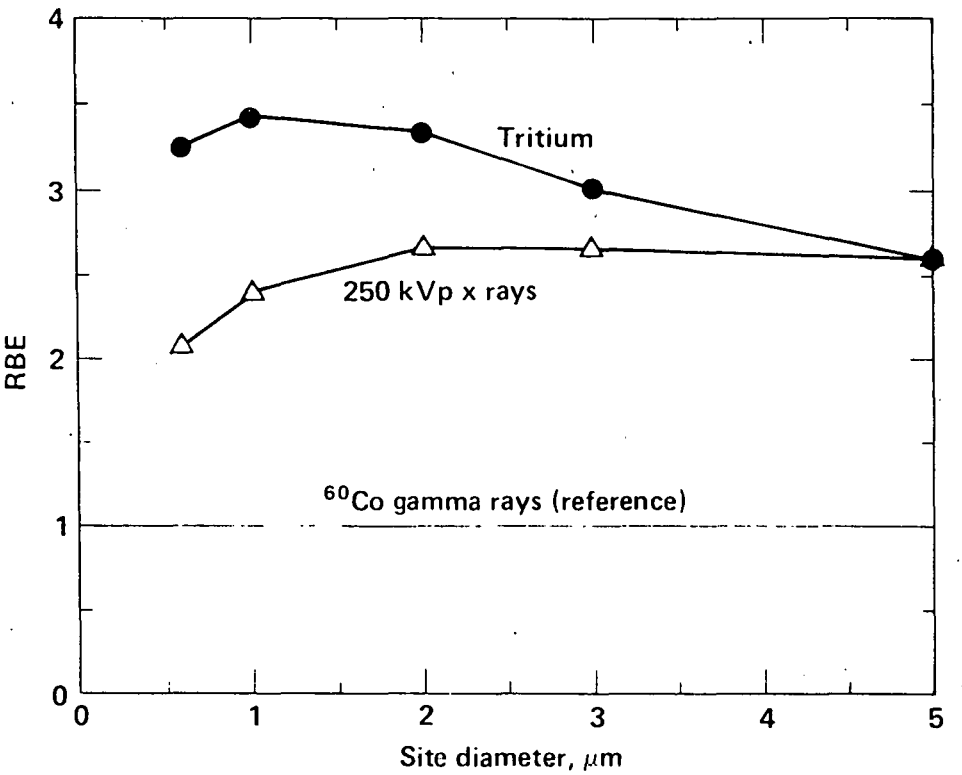

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