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from Nuclear Industries. Vol. I at p. 203.*

IAEA-SM-237/48

THE TOXICITY OF TRITIUM

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Abstract

Among radionuclides of importance in atomic energy, ^3H has relatively low toxicity. There is concern, however, because very large amounts are involved in nuclear fission and fusion, impressive quantities are released to the environment and tritium in its preferred state, water, has free access to living cells and organisms. The main health and environmental worry is the possibility that significant biological effects may follow from protracted exposure to low concentrations in water. To examine this possible hazard and measure toxicity at low tritium concentrations, chronic exposure studies were done on mice and monkeys. During vulnerable developmental periods animals were exposed to ^3HOH and mice were exposed also to ^{60}Co gamma irradiation and energy-related chemical agents. The biological endpoint measured was the irreversible loss of female germ cells. Effects from tritium were observed at surprisingly low concentrations where ^3H was found more damaging than previously thought. Comparisons between tritium and gamma radiation showed the relative biological effectiveness (RBE) to be greater than 1 and to reach approximately 3 at very low exposures. For perspective, other comparisons were made: between radiation and chemical agents, which revealed parallels in action on germ cells; and between pre- and postnatal exposure, which warn of possible special hazard to the foetus from both classes of energy-related byproducts.

2.

Health Physics. Vol 36 (March). pp. 283-287
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**RADIATION EFFECTS OF TRITIATED
SEAWATER ON DEVELOPMENT
OF THE GOOSE BARNACLE,
*POLLICIPES POLYMERUS***

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Abstract -- Barnacle embryos were reared in Millipore cytology monitors containing approximate tritiated water (HTO) concentrations of background plus 0, 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} , 10^{-1} , and 10^0 $\mu\text{Ci/ml}$. After 32 days the cultures were fixed and the numbers of larvae counted. A "molting index," the percentage of larvae that molted at least once, was used to evaluate the effects of HTO on normal development. Effects were observed at concentrations as low as 7×10^{-6} $\mu\text{Ci/ml}$, and were exponentially related to HTO concentration. Factors affecting sensitivity and possible environmental implications are discussed.

**TRITIUM RADIOBIOLOGICAL EFFECTS IN MAMMALS:
REVIEW OF EXPERIMENTS OF THE LAST DECADE IN RUSSIA****M. I. BALONOV**

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Abstract -- This review briefly describes techniques and basic results of experimental investigations in mice and rats on metabolism, dosimetry, and radiobiological effects of tritium oxide and some tritiated biogenic compounds (glucose, amino acids, and nucleosides) during the last 10 to 15 years in Russia. The content of water in tissue cells of mammals is shown to be 15 to 40% less than in whole tissue. The kinetics of tritium incorporation from oxide (HTO) and its retention in DNA of hemopoietic tissues were studied. The contribution of bound tritium to dose strongly depends on the chemical form of tritium and reaches 90% when labeled L-lysine is injected. Specific features of the action of HTO on hemopoietic tissue were investigated in tests of damage and repair of DNA, induction of chromosome aberrations in cells, content of nucleic acids, kinetics of cell populations, immunity parameters, carcinogenesis, decrease of life span, induction of dominant lethal mutations in germ cells in male mice, and reciprocal translocations in mouse spermatogonia. According to these tests, the radiobiological effects of tritium beta radiation in the form of oxide is 2 to 6 times higher than for gamma radiation of ^{137}Cs . The frequency of dominant lethal mutations induced by labeled lysine, thymidine, and deoxycytidine is 3 to 12 times higher than those induced by equal HTO activity. The results of these investigations are used to standardize HTO and the various biogenic compounds of tritium, improve techniques of indirect dosimetry, provide medical aid to personnel, and estimate population risk.

4.

From Intl. Atomic Energy Agency symposium, Vienna, 1979.
Biological Implications of Radionuclides Released from
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TRITIATED URACIL, TRITIATED THYMIDINE, AND BROMODEOXYURIDINE-INDUCED MUTATIONS IN EUCARYOTIC CELLS

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Abstract --- The induction of gene conversion at the ARG-4 locus in strain BZ34 of *Saccharomyces cerevisiae* was examined after the cells incorporated 6-³H uracil under optimum growth conditions for 16 hours, and then received damage at 4⁰ C from tritium decays at very low dose rates of 1.4-27.6 tritium decays per hour. The results were compared to the results of gene conversion induced by ⁶⁰Co. Each decay of tritium under these conditions was equivalent to a dose of 3.67 rads. This value is very similar to the estimated value of 2.6 rads expected when uniform distribution of dose in the cells is taken into consideration. These results are contrasted with results from acute ³H₂O experiments that suggest a relative biological effectiveness (RBE) of 2.8. The induction of resistance to 6TG in Chinese hamster ovary (CHO) cells has been studied after incorporation of ³H-methyl thymidine, 6-³H-thymidine, and bromodeoxyuridine under several experimental conditions. The induction of mutations by incorporated 6-³H-thymidine is about three times as effective as the induction of mutations by tritiated-methyl thymidine. Since these results are obtained for cells frozen in the G₁ stage of the cell cycle, it may be influenced by the loss of indirect effects of tritium radiation and by life cycle effects since G₁ is a sensitive time for mutations induced by ionizing radiation. The induction of mutations by BUdR depends on the portion of the DNA that is replicated during exposure to BUdR: early replicating DNA damage is associated with induction of 6TG resistance. These results suggest that the determination of the RBE for tritium decays in model eucaryotic systems like yeast and cultured Chinese hamster cells will be influenced by the precise experimental conditions employed. In particular, experiments with mammalian cells will be affected by 'hot times' for mutagenesis in the cell cycle and 'hot positions' within the DNA in the nucleus, and also by the position of tritium decay within the DNA-incorporated molecule.

5.

Health Physics. Vol. 36 (February). pp. 135-145
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TESTIS MASS LOSS IN THE MOUSE INDUCED BY TRITIATED THYMIDINE, TRITIATED WATER, AND ^{60}Co GAMMA IRRADIATION

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Abstract -- The reduction of testis mass of the mouse following single injections of tritiated thymidine (1.0-20 μCi tritium/g body mass) or tritiated water (10-40 μCi tritium/g body mass) and ^{60}Co gamma rays (delivered to match the dose-rate vs. time curve in the 40 μCi tritium/g body mass tritiated water group) was investigated at times from 1 hr to 24 weeks after injection. Measurements of the testicular retention of tritium were also made at these times.

There was a progressive loss in mass, up to 30% after 4-5 weeks, followed by an irregular recovery which was more delayed in the case of the tritiated-thymidine-injected animals.

The effectiveness of tritium compared with ^{60}Co gamma rays, calculated using the average absorbed dose to the testis, was 1.43 for tritiated water and 2.07 for tritiated thymidine. A significant effect on the testis mass was seen after the injection of tritiated thymidine at 1.0 μCi tritium/g body mass, which delivered an average absorbed dose to the testis of about 3.5 rad (0.035 Gy) during 16 weeks. Calculations suggest that tritium from tritiated thymidine "fixed" in the testis was about twice as effective as the more labile and uniformly distributed tritium from tritiated water and that in terms of injected amount tritiated thymidine is unlikely to be more than five times as effective as tritiated water even at very low injected amounts.

6.

Health Physics. 65(6): 698-712; 1993

ORGANICALLY BOUND TRITIUM

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Abstract — Tritium released into the environment may be incorporated into organic matter. Organically bound tritium in that case will show retention times in organisms that are considerably longer than those of tritiated water which has significant consequences on dose estimates. This article reviews the most important processes of organically bound tritium production and transport through food networks. Metabolic reactions in plant and animal organisms with tritiated water as a reaction partner are of great importance in this respect. The most important production process, in quantitative terms, is photosynthesis in green plants. The translocation of organically bound tritium from the leaves to edible parts of crop plants should be considered in models of organically bound tritium behavior. Organically bound tritium enters the human body on several pathways, either from the primary producers (vegetable food) or at higher trophic level (animal food). Animal experiments have shown that the dose due to ingestion of organically bound tritium can be up to twice as high as a comparable intake of tritiated water in gaseous or liquid form. In the environment, organically bound tritium in plants and animals is often found to have higher specific tritium concentrations than tissue water. This is not due to some tritium enrichment effects but to the fact that no equilibrium conditions are reached under natural conditions.

7.

Health Physics. Vol. 36 (January), pp. 35-38
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INCORPORATION OF TRITIUM IN GRAIN PLANTS

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Abstract -- Maize and barley plants were grown from seed for a period of 30 days in an enclosure in which the soil water and atmospheric vapour contained equilibrium concentrations of tritiated water. At the end of the experiment the plant water of the maize and the barley contained 95 and 84%, respectively, of the environmental concentration of tritium. The tritium-to-hydrogen ratio in plant dry matter was 60% for maize and 45% for barley of the environmental tritium-to-hydrogen ratio. The results show a significant isotope effect which reduces the tritium content of food grown in a continuously contaminated environment.

8.

Mutation Research, 50 (1978) 101-110
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UNUSUAL DOSE-RESPONSE OF CHROMOSOME ABERRATIONS INDUCED IN HUMAN LYMPHOCYTES BY VERY LOW DOSE EXPOSURES TO TRITIUM

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Abstract

Leukocyte cultures of human peripheral blood were chronically exposed for 48 h to tritiated water and [³H] thymidine using a wide range of tritium doses, and aberrations in lymphocyte chromosomes at the first metaphases were examined. In the experimental conditions, the types of aberrations induced by radiation emitted from both tritiated water and [³H] thymidine were mostly chromatid types, such as chromatid gaps and deletions. The dose-response relations for chromatid breaks per cell exhibited unusual dose-dependency in both cases. It was demonstrated that at higher dose range the yields of chromatid breaks increased linearly with dose, while those at lower dose range were significantly higher than would be expected by a downward extrapolation from the linear relation. Partial-hit or partial-target kinetic events appeared at very low dose exposure.

9.

Health Physics, 1969. Vol. 17, pp. 547-557.
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CYTOLOGICAL DAMAGE PRODUCED IN THE MOUSE TESTES BY TRITIATED THYMIDINE, TRITIATED WATER AND X-RAYS

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Abstract -- Irradiation of the mouse testes was carried out using internally distributed tritium from injected tritiated thymidine and tritiated water and also using external whole body irradiation with 200 kVp X-rays.

The inability of intermediate spermatogonia to divide twice and produce resting primary spermatocytes was used as a criterion of biological damage and the following conclusions were reached:-

(i) Injected amounts of both tritiated thymidine and tritiated water equivalent to less than 2 $\mu\text{Ci/g}$ body mass produced cell death in the testes;

(ii) Tritiated thymidine was four times more effective in killing spermatogonia than equivalent injected amounts of tritiated water;

(iii) For the same total dose to the whole body of 200 kVp X-rays, acute irradiation (dose rate 1.6 rads/min) delivered in less than 30 min, was more effective for cell lethality than irradiation at low dose rates (<0.7 rad/hr) spread over 72 hr.

(iv) 5 μCi $^3\text{HTdR}$ injected/g body mass, 20 μCi tritiated water injected/g body mass and 30 rads to the whole animal from 200 kVp X-rays delivered at an exponentially decreasing dose rate similar to that obtained from the two tritiated compounds, produced equivalent cell death within the testes over 72 hr.

(v) The RBE of tritium as tritiated thymidine or tritiated water relative to 200 kVp X-rays was found to be in the range 1.3-2.4.

CUMULATIVE GENETIC EFFECTS FROM EXPOSURE OF MALE MICE TO TRITIUM FOR TEN GENERATIONS

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Abstract

Three sublines of C57 Black/6M mice were propagated from three sibling pairs from the same litter. All breeders were weaned at 28 ± 2 days of age. At each generation in experimental lines weaned male breeders aged 35 days either received a single injection of tritiated thymidine ($1 \mu\text{Ci/g}$ of body weight) or were exposed for 5 weeks to tritiated drinking water ($10 \mu\text{Ci/ml}$). Control male breeders and all female breeders received tap water. At 10 weeks of age all breeders were sibling-mated. Breeders were allowed to produce one litter. The time of delivery, the litter size and sex ratio was recorded. At each generation the offspring in the three lines were sibling-mated in the same sequence as their parents. At the 6th generation total offspring in the sibling line received tritiated thymidine numbered 108 versus 336 in the sibling line exposed to tritiated water, in contrast with 721 in the control sibling line. At that point it was decided to reduce the subpopulation in all three sibling lines to 20 couples which were assigned to further sibling-matings. The 6th generation couples (20 in either subline) generated at the 9th generation 624 animals in the control subline versus 386 and 476 in the subline exposed to tritium. The data suggest a trend toward reduction in the subpopulation of offspring propagated from male parents exposed to tritiated water or tritiated thymidine. This particular trend repeated itself whenever the various sublines were re-propagated from an equal number of couples. At the 9th generation 50 couples were assigned to a special study on reproductive fitness. The F_1 and F_2 offspring from the 50 couples in each subline were observed to evaluate litter size and infant mortality. The litter size was decreased and infant mortality was increased in experimental sublines (chi-square test, $P < 0.05$). The F_2 offspring was assigned to a separate investigation of the occurrence of dominant lethal mutations. In control, as well as in experimental lines, at 70 days of age males were sibling-mated with their sisters. All animals received tap water and were not exposed at any time to tritiated thymidine or tritiated water. Fifteen days after mating females were sacrificed and the ovaries and uterine content were examined. Preimplantation loss values were significantly increased in experimental versus control sublines (chi-square test, $P < 0.01$). The dose to male sperm over a 35-day period was estimated at 3.7 rads from tritiated water and at 3.9 rads from tritiated thymidine under our experimental conditions.

11.

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OVERVIEW OF TRITIUM: CHARACTERISTICS, SOURCES, AND PROBLEMS

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Abstract -- Tritium has certain characteristics that present unique challenges for dosimetry and health-risk assessment. For example, in the gas form, tritium can diffuse through almost any container, including those made of steel, aluminum, and plastics. In the oxide form, tritium can generally not be detected by commonly used survey instruments. In the environment, tritium can be taken up by all hydrogen-containing molecules, distributing widely on a global scale. Tritium can be incorporated into humans through respiration, ingestion, and diffusion through skin. Its harmful effects are observed only when it is incorporated into the body. Several sources contribute to the inventory of tritium in our environment. These are 1) cosmic ray interaction with atmospheric molecules; 2) nuclear reactions in the earth's crust; 3) nuclear testing in the atmosphere during the 1950s and 1960s; 4) continuous release of tritium from nuclear power plants and tritium production facilities under normal operation; 5) incidental releases from these facilities; and 6) consumer products. An important future source will be nuclear fusion facilities expected to be developed for the purpose of electricity generation. The principal health physics problems associated with tritium are 1) the determination of the parameters for risk estimation with further reduction of their uncertainties (e.g., relative biological effectiveness and dose-rate dependency); 2) risk estimation from complex exposures to tritium in gas form, tritium in oxide form, tritium surface contamination, and other tritium-contaminated forms, with or without other ionizing radiations and/or nonionizing radiations; 3) the dose contributions of elemental tritium in the lung and from its oxidized tritium in the gastrointestinal tract; 4) prevention of tritium (in oxide form) intake and enhancement of tritium (oxide form) excretion from the human body; 5) precise health effects information for low-level tritium exposure; and 6) public acceptance of tritium leakage and waste disposal from reactors and fuel reprocessing plants.

TRITIUM INCORPORATION IN RATS CHRONICALLY EXPOSED TO TRITIATED FOOD OR TRITIATED WATER FOR THREE SUCCESSIVE GENERATIONS**Z. PIETRZAK-FLIS, I. RADWAN, Z. MAJOR and M. KOWALSKA***Central Laboratory for Radiological Protection. Warsaw, Poland***Abstract**

Wistar rats have continuously ingested tritiated food (48.1 kBq/g) or tritiated water (37.0 kBq/ml) from three weeks before mating of P₀ through delivery of the F₃ generation. An analysis of tissues at various ages during treatment shows that: (1) tritium incorporation into tissues of rats life-time exposed to tritiated food was on the average 3.53±0.38 times higher than after similar exposure to tritiated water; (2) the highest organically bound tritium concentrations were found in ovaries and lungs of rats exposed to tritiated food. Tritiated water exposure gave the highest concentrations of this isotope in testes and lungs; (3) exposure of females to tritiated water during 64 days, including pregnancy and lactation, is not sufficient to attain equilibrium concentrations of organically bound tritium in all the studied tissues, while in the tritiated food exposure group such equilibrium concentrations were attained in the majority of tissues; (4) the dose rate estimation on the basis of tritium in body water leads to underestimation of absorbed dose in the tissues of rats, especially after exposure to tritiated food.

RADIOTOXICITY OF TRITIUM-LABELLED MOLECULES

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Abstract

The effect of tritiated organic compounds and ^3H -water on the growth of chloroleukaemia cells was studied in permanent suspension culture. In terms of environmental activity concentration, ^3H -leucine (protein precursor), ^3H -uridine (RNA precursor) and ^3H -thymidine (DNA precursor) were roughly 10, 100, and 1000 times as toxic as ^3HOH to rapidly growing malignant cells. In addition to measurement of the growth rate by cell counting, more subtle changes in the kinetic behaviour of the population were followed by pulse-labelling with ^3H -thymidine. As the technique of labelled mitoses showed, radiation-induced changes in population kinetics are apparent even after a very short, low level exposure of the cells to ^3H -thymidine (30-min pulse at $0.04 \mu\text{Ci}\cdot\text{ml}^{-1}$). These results indicate that in evaluating the harmful effects of tritium it is essential to consider several factors such as the chemical nature of the tritiated substance entering the body and the metabolic status of the cells of the organism.

14.

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TRITIUM RADIOBIOLOGY AND RELATIVE BIOLOGICAL EFFECTIVENESS

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Abstract

During the past decade, a large number of radiobiological studies have become available for tritium -- many of them focusing on the relative biological effectiveness of tritium beta rays. These and previous studies indicate that tritium in body water produces the same spectrum of radiogenic effects (e.g., cancer, genetic effects, developmental abnormalities, and reproductive effects) observed following whole-body exposure to penetrating radiations such as gamma rays and x rays. However, tritium beta rays are of greater biological effectiveness than gamma rays and x rays. For example, tritium in the oxide form is about 2 to 3 times more effective at low doses or low dose rates than gamma rays from ^{137}Cs or ^{60}Co . When tritium is bound to organic molecules, relative biological effectiveness values may be somewhat larger than those for tritium in oxide form. Tritium administered to animals or to cells *in vitro* as tritiated amino acids results in relative biological effectiveness values that appear similar to those obtained for tritium in oxide form; however, if administered as tritiated thymidine, the relative biological effectiveness values appear to be about two-fold higher. It is clear from the wealth of tritium data now available that relative biological effectiveness values for tritium beta rays are higher than the quality factor of unity generally used in radiation protection.

EFFECTS OF A SINGLE INJECTION OF TRITIATED WATER DURING ORGANOGENY ON THE PRENATAL AND POSTNATAL DEVELOPMENT OF MICE

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Abstract --- Pregnant NMRI mice were injected with tritiated water during organogeny. Corresponding to single injections of 0.07 mCi tritium per g body weight of dams on Day 9 post conceptionem (p.c.), the offspring showed a significantly decreased weight of brain and genital tract organs. In offspring aged 4.5 months the number of oocytes was substantially reduced and the seminiferous epithelium was in a state of disintegration. At 2 months, however, these offspring were fertile. 0.135 mCi/g affected fertility of females but not that of males. After injection of 0.27 mCi tritium/g neither sex of the offspring appeared to be fertile at the age of 2 months. At 18 months the ovarian tumour incidence of exposed offspring was increased approximately five-fold over controls (tritium: 67%, controls: 14%). 0.54 mCi/g caused perinatal mortality in 100% of offspring. Injecting 0.54 mCi tritium/g on Days 7, 9 or 11 of pregnancy, the foetuses were stunted, but incidence rates of gross malformations as well as of skeletal anomalies were negligible. Prominent histological findings were common – retardation of the prosencephalon and marked hypoplasia of the gonads. Oocyte nuclei stained with haematoxylin appeared to be pale. In contrast to controls, pachytene stages were extremely rare. In the testicular cords large centrally spaced spermatogonia were absent. The placental weight was reduced. The treatment of dams on Days 7 and 9 led to increased resorption of embryos. After injection of 0.81 mCi tritium/g on day 9 p.c. the rate of resorption was about 50%. Tritium administered at higher activity levels than 1 mCi/g caused maternal mortality. In the uteri of surviving and of dead dams all embryos were in a state of resorption.

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ASSESSMENT OF POTENTIAL RADIATION DOSE TO MAN FROM AN ACUTE TRITIUM RELEASE INTO A FOREST ECOSYSTEM

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Abstract -- On 2 May 1974, 479,000 Ci of tritium gas escaped from a Savannah River Plant exhaust stack. The tritium first reached the ground in a pine forested area and was partially assimilated into the ecosystem. Samples of vegetation collected and measured for a period of 70 days showed an increase in the levels of tritiated water. Cycling of the tritium retained in the forest ecosystem resulted in a higher concentration of tritiated water vapor in the air at breathing height near the forest floor than that calculated by the usual models used for predicting air concentration. In addition, the model for tritium cycling in the forest predicts a diurnal cycle of tritiated water vapor concentration with higher concentrations at night when air movement under the canopy is slower.

The potential dose to the maximum individual because of inhalation and skin absorption of tritium as HTO after the release was calculated in three ways: (1) by using the body water model from the *ICRP Publication 2* and assimilation during the passage of the puff, (2) by using the body water model from the *ICRP Publication 2* and assimilation during an extended exposure period to tritium determined by the experimental measurements and (3) by using a three-compartment dosimetry model with retention half-times of 9, 30 and 450 days with the extended exposure period used in (2). The potential doses were 0.14, 0.80 and 0.89 mrem, respectively. These potential doses show the necessity of considering the interaction of radioactive material with the ecosystem for dose calculation.