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ACCUMULATION OF TRITIUM IN VARIOUS SPECIES OF FISH REARED IN TRITIATED WATER

Environmental Monitoring & Support Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Las Vegas, Nevada 89114

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by

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16. ABSTRACT

The release of tritium into aquatic ecosystems has resulted from nuclear industry operations. Because of the projected expansion of the nuclear power industry and associated fuel reprocessing plants, such releases can be expected to increase and to require further assessment of the environmental impact. Considerable information exists for the behavior and fate of tritiated water in mammals; however, few experimental data are available on the incorporation of tritium from tritiated water into fresh water fishes. Of particular interest are fish that begin and end their lives in a tritiated environment. In the present study trout and channel catfish eggs were hatched and the fish maintained in tritiated water for four months. In addition, two species of minnows native to the southwest United States were maintained in tritiated water and offspring from these fish were reared in tritiated water for five months. Some of these native fish were maintained for two months in large outdoor pools in which part of their food was grown naturally. The tritium concentrations in aquaria water were held constant during the experimental period. The results show that for these species of fish living in tritiated water the concentration factor for organic bound tritium is generally less than unity. The concentration factor is defined as the specific activity of tritium in dried body tissue divided by that in the water in which the fish were reared. Literature concerning behavior of tritium in aquatic food chains after release in nuclear industry effluents is reviewed.

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1. INTRODUCTION

Substantial amounts of tritium have been released into the environment as a result of nuclear industry operations. Most of this tritium becomes incorporated into water molecules to form tritiated water (HTO) and can then be incorporated into the organic constituents of plants and animals which come in contact with the water [1, 2, 3].

Because of the projected expansion of the nuclear power industry and associated fuel reprocessing plants, the environmental releases of tritium can be expected to increase. Current design of fuel reprocessing plants is such that most of the tritium entering the plant in spent nuclear fuel is released to the environment as tritiated water [4, 5]. The capacity of the worldwide environment for diluting such tritium to negligible levels is not unlimited [6]. Furthermore, to the extent that such tritium is not rapidly diluted throughout the water of the world, the tritium concentrations in certain areas would be higher than estimated for rapid dilution in the entire hydrosphere. It has been reported that tritium released to the environment as tritiated water may be concentrated in some organisms through ecological food chains [7]. If these organisms are part of a food chain leading to man, the significance of environmental releases of tritium must be reexamined.

The accumulation of tritium in organisms through ecological food chains is being studied at the U.S. Environmental Protection Agency's National Environmental Research Center at Las Vegas. Tritium accumulation in fish through aquatic food chains is one of the studies in progress.

Considerable information exists for the behavior and fate of tritiated water in mammals [1, 2, 3, 8]; however, little experimental data are available on the incorporation of tritium from tritiated water into fresh water fishes. To determine the significance of tritium in the food chain of fish, tritium incorporation into fish from tritiated water alone must be evaluated. This report presents results for tritium incorporation into fish living in tritiated water and eating non-tritiated food. In an associated experiment, some fish were raised in tritiated water in which a substantial portion of their food was grown.

2. METHODS

2.1 Fish in aquaria

Small fish of two species native to the southwestern United States and suitable for rearing in small aquaria were collected from streams and placed in tritiated water. These species, *Gamusia affinis* (mosquito fish) and *Poeciliopsis occidentalis* (Gila topminnow), both of the family *Poeciliidae*, are live-bearing in that eggs are hatched within the mother and both present live young. Both species have a lifetime of about three years. Adult fish, one to two years old, were used and the species would not interbreed.

Fertilized trout (*Salmo gairdnerii*) eggs, obtained from a hatchery, were hatched in 380-liter (100 U.S. gal.) tanks with cooled and filtered circulating tritiated water. The trout were reared in this same tank to an age of 140 days and then placed in non-tritiated water for 72 days to determine the gross tritium loss rate from the fish.

Fertilized eggs of the channel catfish (*Ictalurus lacustris*), obtained from a catfish farm, were hatched in a tank identical to the trout tank, and fry were reared to adulthood in this tank. Trout and catfish were selected for study because they are used as food by man.

Young produced by mosquito fish and Gila topminnows were separated from the adults at birth and reared in different aquaria. Information was thus obtained on tritium incorporation into fish which start and end their lives in tritiated water. Some of these young fish were placed in non-tritiated water at an age of 200 days and periodic samples were collected to determine the rate of tritium loss from the fish.

The tritium concentration in all aquaria was maintained at a constant level throughout the study. Hatching of eggs and care of fish was performed as recommended in the literature [9, 10, 11]. Trout and catfish were fed a balanced commercial ration. The mosquito fish and Gila topminnows were fed a mixed diet of commercial foods for tropical fish. These commercial foods did not contain tritium incorporated in non-exchangeable positions [3].

2.2 Fish in outdoor pools

Six plastic-lined pools, constructed at the EPA Experimental Farm on the Nevada Test Site, were filled with 3700 liters (980 U.S. gal.) of tritiated water. Inorganic fertilizer, tritium and green algae (Chlorella pyrenoidosa) were introduced and the algae allowed to produce a full bloom. The tritiated water level in the pools was maintained by water level controllers connected to a reservoir of tritiated deionized water.

Windblown dirt, debris, and hay from the farm entered the pools, and a variety of insect life was observed in the pools before fish were introduced. While the algae and biota which spent a significant proportion of their lifetime in the pools would have considerable tritium metabolically incorporated into their tissues, the same would not be true for material which blew into the pools [2, 3]. Mosquito fish placed in the pools on several occasions resulted in complete mortality within a few days. Despite identical treatment, the pools developed individual characteristics after several weeks, and four pools then provided adequate conditions to maintain fish. Adult mosquite fish, one to two years old, were introduced into three pools, and young mosquito fish, four months old, into the fourth pool. The food supply from algae, insects, and windblown debris appeared to be adequate for these fish, and no additional food was provided. The pools were stocked in mid-July, and the fish harvested in mid-October when freezing temperatures threatened survival. Some of the adult fish were returned to tritiated laboratory aquaria and fed commercial fish food to determine the importance of the tritiated food in the environment.

2.3 Sampling and analysis

Water samples were collected from aquaria in the laboratory on a weekly basis, and farm pool water samples were collected about every two weeks on inspection visits. Tritium concentrations in aquaria and pools were maintained at 1 nCi/ml ±10 percent. This concentration was selected to provide easily measurable tritium levels and not yield unacceptably high radiation dose rates to fish. The fish received a dose of about 1 rad/yr [12].

Fish from pools and aquaria were collected periodically for analysis. Freeze-dried tissues of whole fish were oxidized in a Model 300 Packard Tricarb Sample Oxidizer and the water of oxidation analyzed for tritium.

All water samples were analyzed for tritium specific activity (μCi/gH or an equivalent unit) using a Beckman Model 100 Liquid Scintillation Counter. The scintillator solution contained 7g diphenyloxazole (PPO) and 1.5g p-bis-(o-methylstryl)-benzene (bis-MSB) dissolved in a mixture of p-xylene and Triton N-101 with a volume ratio of 2.75:1. Samples were analyzed by liquid scintillation techniques previously described [13, 14]. All samples were counted to at least ±2 percent at the 95 percent confidence interval.

3. RESULTS

3.1 Fraction of specific activity equilibrium

Results are presented to show the ratio between the tritium specific activity in freeze-dried fish tissue and that in water in which the fish were grown. A ratio, or fraction, greater than unity would indicate concentration of tritium within the organic constituents of fish relative to the water environment. No fraction greater than unity was measured in these experiments.

The results obtained for mosquito fish and Gila topminnows grown in tritiated water and fed a commercial diet are shown in Table I. The fraction of equilibrium attained is about 0.5 for both fish born in the tritiated water and fish introduced into tritiated water as adults. The data in Table II for trout and catfish indicate that a somewhat lower fraction of equilibrium is attained in these game fish grown in tritiated water and fed a commercial diet.

The data in Table III show that, for mosquito fish grown in outdoor pools in which a substantial fraction of their food was grown, the fraction of equilibrium attained is about 50 to 90 percent higher than for aquarium fish eating commercial food. The mosquito fish introduced into the pools as one- to two-year-old adults were full grown and did not increase in size during their sojourn in the pools. The fish introduced at 180 days of age, however, increased in body weight by a factor of three during the 94 days in the pool. As mentioned earlier (Section 2.2) the outdoor pools differed in the amount and type of biota present. The pool containing the young fish appeared to have very sparse populations of plant and insect life. Whether this is due to the fish keeping such populations sparse or to other unfavorable environmental conditions in the pool, it appears likely that the small fish ate a greater fraction of their diet from sources outside the pool than did the adult fish. Two of the three pools in which adult fish were kept contained lush growths of biota, and in the third pool populations of biota were intermediate between these two pools and that in which the young fish were raised. The fraction of tritium equilibrium attained in adult fish tissues in these pools (Table III) appears to be in proportion to the observed growth of biota in the pools. This fraction approached but did not exceed unity.

3.2 Tritium loss from fish tissue

After several months of growth in tritiated water while eating commercial fish foods, some of the trout and mosquito fish were transferred to non-tritiated water. Data on the loss of tritium from tissues of these fish are given in Table IV and Figure 1, curves C and D. The data for mosquito fish indicate that about 50 percent of the tritium in tissue is eliminated with a biological half-time of about five days, and the remainder is eliminated at a much slower rate. The two data points for trout (line D in Figure 1) are sufficient only to indicate that not all the tritium in tissue is eliminated rapidly when the trout are placed in non-tritiated water. The two data points are connected by a dashed line to facilitate association of the data and not to represent an estimate of the tritium loss rate.

Adult mosquito fish from two of the farm pools were placed in tritiated water in laboratory aquaria. The aquaria water had the same tritium concentration as the farm pools; however, the fish food was changed from the food grown in the farm pools, plus some outside sources (Sections 2.2 and 3.1), to a commercial diet. The data for these fish are given in Table V and Figure 1, curves A and B. Only two samples were obtained for fish from the pool which had attained the highest fraction of equilibrium, 0.92. After 28 days in the aquarium the tritium concentration in these fish had decreased some 20 percent. These two data points are connected by a dashed line in the figure only to facilitate association of the data. The more interesting data are for the fish from the pool in which the fraction of equilibrium had reached 0.8 after 93 days in the pool. After 41 days in the aquarium the fraction of equilibrium in tissues of these fish had decreased to about that expected for fish grown in tritiated water and fed commercial food (Table I). The tissue concentration remained at this level after an additional 98 days in tritiated water.

4. DISCUSSION

4.1 Tritiated water exposure

The fraction of tritium specific activity equilibrium in tissue of small fish living in tritiated water (Table I) was found to be about 30 percent higher than that reported for tissue of mammals exposed to tritiated drinking water [1, 2, 3]. The reason for this difference is uncertain; however, one explanation is that the small fish eat small biota which grow naturally even in clean aquaria. Intake of such organisms with tritium already incorporated into protein, fat, and carbohydrate molecules would tend to increase the tritium specific activity in tissues of the fish. This explanation is supported by the data for trout and catfish in Table II. The trout were raised in very clean and cold water (11°C) in which little biota grew. In addition, the trout ate large amounts of commercial food and grew rapidly to a size for which small aquatic biota would be expected to be a small portion of their total diet. The highest tritium specific activity in trout tissue was about 20 percent less than that for mosquito fish. The catfish grew very slowly during the first 60 days and possibly ate substantial amounts of biota growing in their warm (24°C) and relatively messy aquarium. By the time the catfish were 133 days old they were 7 to 8 cm in length, ate large quantities of commercial food, and kept their aquarium clean by devouring everything possible. From the very small fish at age 60 days to the much larger ones at age 133 days, the tritium specific activity in tissue decreased by approximately 30 percent to about the same level as for trout.

4.2 Influence of tritiated food

The consumption of food grown in their tritiated environment increases the tritium specific activity in tissue of mosquito fish to levels about 50 to 90 percent higher than in fish grown in tritiated water and fed commercial foods. Algae, which reproduce from carbon dioxide, water and inorganic materials in the water, incorporate tritium from tritiated water into cell components. The algae were introduced into pools to provide food for both fish and other aquatic organisms in the pools. The pools were filled initially with water from an irrigation reservoir which contained populations of unidentified aquatic organisms. Although food produced in this manner could have provided adequate food for the fish, other foods not produced in the tritiated environment (Sections 2.2 and 3.1) were probably eaten. The tritium specific activity in organic components of this material would not be in equilibrium with the aquatic environment and, therefore, would tend to lower the fraction of equilibrium in the fish. An example of this is the insect larvae developed from eggs in which all the nutrients are contained. If the adult insect producing the eggs lives and/or feeds in other than the tritiated pool environment, the larvae when hatched would contain little tritium bound to organic molecules. It should be noted that such introduction of food material from outside the tritiated water environment is a phenomenon which also occurs in natural waters.

4.3 Loss of tritium from fish tissue

Limited data are presently available on the loss of tritium from fish tissue after transfer to a less tritiated environment (Figure 1). It is clear, however, that a portion of tritium in tissue is not excreted rapidly. This finding is of significance in environmental monitoring in that the tritium concentration in fish tissue as a result of previous tritium exposures may be higher than in the ambient water. Furthermore, this elevated concentration may persist for longer than indicated by the data shown in Figure 1 because the fish are eating organisms from an entire food chain which has had a similar tritium exposure history. The organic tritium persistence in the food chain to the organism of interest is the major factor of importance, and limited data in the literature [15] indicate that such tritium persists in ecological cycles for several years.

5. CONCLUSIONS

5.1 Fish living in tritiated water

The experimental results indicate that for fish living in tritiated water and eating foods produced elsewhere, the tritium specific activity in tissue would reach about 50 percent of that in the water.

5.2 Fish living in a tritiated environment

The consumption by fish of foods grown in tritiated water where the fish are living increases the tritium specific activity in fish tissues significantly as compared to tissues of fish eating foods not grown in their tritiated environment. The fraction obtained by dividing the tritium specific activity in fish tissues by that in the water of their environment approached, but did not exceed, unity in the studies described.

5.3 Persistence of tritium in fish tissue

Tritium incorporated into fish tissue from tritiated water or from fish food grown in tritiated water is not excreted rapidly. Approximately 50 percent of this tritium appears to have a half-time of about five days, and the remainder has a much longer half-time in the mosquito fish studied.

Mention of commercial products used in connection with work reported in this article does not constitute an endorsement by the Environmental Protection Agency.

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TABLE I. SMALL FISH GROWN IN AQUARIA AND FED COMMERCIAL FOOD: SPECIFIC ACTIVITY OF TRITIUM IN FREEZE-DRIED FISH TISSUE AS A FRACTION OF THAT IN WATER IN WHICH THE FISH WERE GROWN

SPECIES	AGE[a]	DAYS IN HTO	FRACTION[b]
Mosquito fish	180 d	21	0.42
"	1-2 yr	60	0.37
"	1-2 yr	66	0.31
"	1-2 yr	101	0.44
"	1-2 yr	106	0.47
"	1-2 yr	158	0.73[c]
"	1-2 yr	198	0.52
"	1-2 yr	203	0.52
Gila Topminnow	1-2 yr	79	0.56
Gila Topminnow	---	88	0.49
"	---	185	0.45

a. Age at time of introduction into tritiated water; dash (-) indicates fish were born in tritiated water.

b. Tritium specific activity in dry tissue divided by that in aquaria water.

c. These fish were inadvertently fed for three days with brine shrimp reared in tritiated water for a different experiment.

TABLE II. GAME FISH GROWN IN AQUARIA AND FED COMMERCIAL FOOD: SPECIFIC ACTIVITY OF TRITIUM IN FREEZE-DRIED FISH TISSUE AS A FRACTION OF THAT IN WATER IN WHICH THE FISH WERE GROWN

SPECIES	DAYS IN HTO[a]	FRACTION	COMMENT ON SAMPLE
Trout	1	0.27	Dead eggs and debris
	9[b]	0.34	Fry with yolk sac
	14	0.23	Dead fry and debris
	22[c]	0.22	Dead fry and debris
	32	0.39	Dead fry
	119	0.42	Fish
	140	0.42	Fish
Catfish	3[d]	0.51	Dead eggs
	49	0.43	Small fish
	53	0.45	Small fish
	62	0.58	Small fish
	133	0.41	Small fish

a. Fertilized eggs placed in tritiated water when received
b. All trout eggs hatched by day 9
c. Fry began eating well on day 22
d. All catfish eggs hatched by day 7

TABLE III. MOSQUITO FISH GROWN IN OUTDOOR POOLS:
SPECIFIC ACTIVITY OF TRITIUM IN FREEZE-DRIED FISH TISSUE
AS A FRACTION OF THAT IN WATER IN WHICH THE FISH WERE GROWN

AGE[a]	DAYS IN HTO	FRACTION
180 d	11	0.36
180 d	39	0.56
180 d	94	0.74
1-2 yr	20	0.54
1-2 yr	58	0.63
1-2 yr	93	0.80
1-2 yr	93	0.92
1-2 yr	93	0.88

a. Age at time of introduction into tritiated water.

TABLE IV. LOSS OF TRITIUM FROM TISSUES OF FISH GROWN IN TRITIATED WATER AND THEN TRANSFERRED TO NON-TRITIATED WATER

SPECIES	AGE[a]	DAYS IN H_2O	FRACTION[b]
Mosquito fish born in HTO	200 d	0	0.52
"	200 d	8	0.39
"	200 d	11	0.30
"	200 d	60	0.19
Trout hatched in HTO	119 d	0	0.42
"	119 d	72	0.18

a. Age at time introduced into non-tritiated water
b. Tritium specific activity in dry tissue divided by that in aquaria water during HTO exposure

TABLE V. LOSS OF TRITIUM FROM TISSUES OF FISH GROWN IN TRITIATED OUTDOOR POOLS AND THEN TRANSFERRED TO TRITIATED AQUARIA AND FED COMMERCIAL FOOD

SPECIES	AGE[a]	DAYS IN HTO[b]	FRACTION[c]
Mosquito fish adults	1-2 yr	0	0.92
"	1-2 yr	28	0.74
Mosquito fish adults	1-2 yr	0	0.80
"	1-2 yr	6	0.62
"	1-2 yr	22	0.58
"	1-2 yr	41	0.51
"	1-2 yr	139	0.52

a. Age at time transferred from outdoor pools to aquaria containing HTO
b. Days in aquaria containing same HTO concentration as outdoor pools
c. Tritium specific activity in dry tissue divided by that in aquaria water

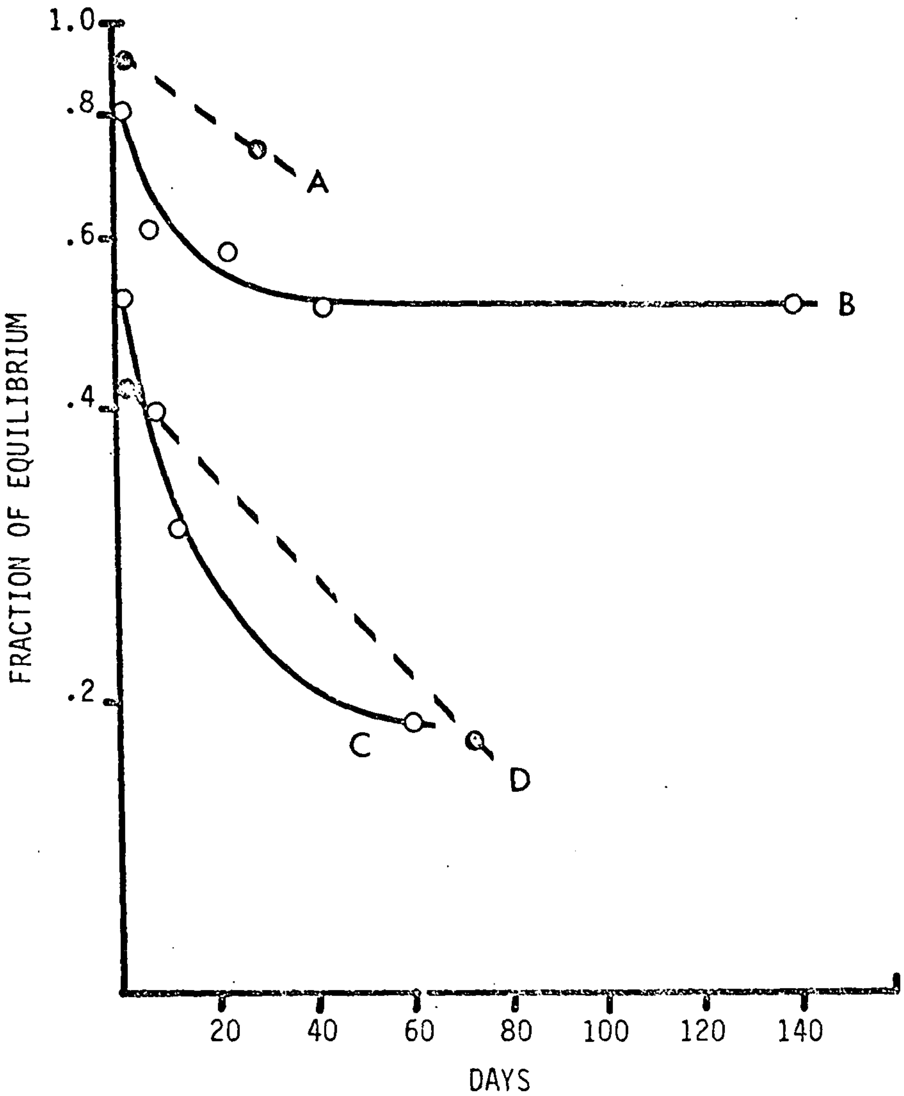

FIGURE 1. LOSS OF TRITIUM FROM FISH AFTER TRANSFER TO A LESS TRITIATED ENVIRONMENT

A/B MOSQUITO FISH FROM OUTDOOR POOLS:TO HTO
C MOSQUITO FISH BORN IN HTO:TO H_2O
D TROUT HATCHED IN HTO:TO H_2O

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16. ABSTRACT

The release of tritium into aquatic ecosystems has resulted from nuclear industry operations. Because of the projected expansion of the nuclear power industry and associated fuel reprocessing plants, such releases can be expected to increase and to require further assessment of the environmental impact. Considerable information exists for the behavior and fate of tritiated water in mammals; however, few experimental data are available on the incorporation of tritium from tritiated water into fresh water fishes. Of particular interest are fish that begin and end their lives in a tritiated environment. In the present study trout and channel catfish eggs were hatched and the fish maintained in tritiated water for four months. In addition, two species of minnows native to the southwest United States were maintained in tritiated water and offspring from these fish were reared in tritiated water for five months. Some of these native fish were maintained for two months in large outdoor pools in which part of their food was grown naturally. The tritium concentrations in aquaria water were held constant during the experimental period. The results show that for these species of fish living in tritiated water the concentration factor for organic bound tritium is generally less than unity. The concentration factor is defined as the specific activity of tritium in dried body tissue divided by that in the water in which the fish were reared. Literature concerning behavior of tritium in aquatic food chains after release in nuclear industry effluents is reviewed.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Water Tritium Fishes Trout Catfishes Minnows	Tritiated water Aquatic environment Aquatic biology Tritium accumulation in fish Tritium concentration factor	0606 0618 1802 1808

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