New England Aquarium

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March 23, 1984

Mr. Thomas Thompson
Division of Fuel Cycle and Material Safety
Office of Nuclear Material Safety and Safeguards
U.S. Nuclear Regulatory Commission
Washington, DC 20555

Dear Mr. Thompson:

The following information is supplied in clarification of New England Aquarium's application for Byproduct Material License renewal request (License No. 20-12743-01).

1. Delete all reference to ^{131}iodine listed under Item 13A, paragraph 5. We do not request authorization to use ^{131}iodine, but do request authorization to use ^{125}iodine. Item 8 presents the inclusive list of elements that we require authorization to utilize.

2. Experiments on iron status and blood volumes of the bottlenose dolphin, Tursiops truncatus, will be conducted only on animals that have been maintained in captivity for a minimum of 1 year and that will not be released back into the marine environment. Three dolphins will be utilized. If in the highly unlikely event that one of the animals died soon after the proposed exepriments in which it was injected with ^{125}iodine, ^{59}iron, or ^{51}chromium, the animal would be stored frozen in a 20' by 20' walk-in freezer located at the New England Aquarium until radioactivity decayed to background levels (approximately 18 months for ^{125}iodine if 15 microcuries are injected). The animal would then be disposed of by incineration.

3. Under Item 8, we requested authorization for the possession of 1 millicurie of ^{125}iodine. As noted under Item 13A, experiments with this isotope will only entail the injection of less than 15 microcuries per animal (a detailed research plan has been drawn up by Dr. Joseph R. Geraci and is enclosed with this letter). We therefore do not anticipate handling 1 microcurie of ^{125}iodine for any one experiment, but requested authorization for this amount in the unlikely event we would need to store this quantity prior to the experiments or awaiting proper disposal.

4. Item 8 requested the authorization for possession of 10 millicuries of ^{32}phosphorus. We do not anticipate handling such a high dosage of ^{32}phosphorus at any one time, but requested authorization for this amount in the event we would need to store this quantity either prior to experimental use or awaiting proper disposal. In the event that 1 millicurie or more of ^{32}phosphorus is handled at any one time, "finger ring" dosimeters and personal film badges will be worn by the appropriate personnel. In the highly unlikely event that 10 millicuries of ^{32}phosphorus is handled at one time, eye protection will be worn in addition to the above mentioned dosimeter monitors.

I hope the enclosed information is sufficient. Don't hesitate to contact me if you have any further questions.

Sincerely,

William E. Robinson
Radiation Protection Officer

WER:CMC
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Iron Status and Blood Volume in Tursiops truncatus

Aim

The aim of this research project is to determine the following in the bottlenose dolphin Tursiops truncatus: $T^1/_2$ of serum iron disappearance, per cent erythron iron uptake, red cell and plasma volume.

Procedure

Three clinically normal adult female animals of approximately 150 kg are to be used. The serum iron disappearance uses radiolabelled iron in the form of ^{59}Fe citrate (Cook et al., 1970). As with red cell volume and plasma volume studies, a 20 to 30 ml sample of blood is withdrawn, the plasma separated by centrifugation and the appropriate portion incubated with the radioactive label in vitro. In lieu of on-site incubation, labelled albumin is commercially available and can be used for plasma volume determination. The resultant incubated mixture is reinfused. The recommended dose in microcuries is 0.3 to 0.5 UCi/kg body weight of ^{59} Fe with a specific activity of 11-15 milli Ci/mg (Cook et al., 1970). Hence, the total dose per animal is approximately 60 UCi. This does not take into account the high fat to body weight ratios of these animals. The ^{59}Fe is incubated with plasma and reinfused via a venous catheter to insure intravascular injection. The animals are required to be out of the water for six to eight hours during which time six to nine 10 ml blood samples are drawn following the reinfusion. This period out of the water is also sufficient to establish red cell volume and plasma volume. ^{59}Fe activity in plasma is measured and $T^1/_2$ serum iron disappearance calculated. On entry into the plasma, radioactive iron is incorporated primarily in the erythron. The biological half-life in normal man is 640 to 6,520 days and the rate of

loss is from 0.011 per cent to 0.11 per cent. Hence, the rate of loss from the body is very slow and must be minimal even if it is not possible to quantitate exactly (McKee et al., 1973). The dose levels of ^{59}Fe used are as used for diagnostic purposes in man and present no hazard to the animals (Cook et al., 1970).

Blood volume studies previously used in porpoises have been single-label methods (Ridgway and Johnston 1966). Double label methods are now recommended by the International Committee for Standardization in Hematology (I.C.S.H.) (Anonymous 1973) (St. Aubin, et al.,1978). The procedure is similar but not identical to that used for T½ initial serum iron disappearance. The animal is bled, a radioactive label incubated with the blood component required and the resultant mixture reinjected via a venous catheter. This requires the animals to be out of the water for approximately two hours during which time four to six samples are needed of 10 ml each. Counting radioactivity in the post infusion samples enables red cell and plasma volume to be calculated. The radioactive dose in microcuries is 50 UCi per animal. [^{51}Cr] - chromate incubated in vitro with red cells and I^{125} labelled human or bovine albumin are used for the red cell and plasma volume determinations respectively (Anonymous 1973). In vitro incubation enables the dose for red cell volume determination to be kept to a minimum. Less than 0.2 UCi/kg bodyweight is recommended by the ICSH (Anonymous 1973). Therefore, the injected dose per animal of ^{51}Cr is approximately 40 UCi.

The ICSH (Anonymous 1973) considers 0.05 UCi/kg body weight is an appropriate dose for the I^{125} labelled albumin. Dose per animal is, therefore, less than 10 UCi per animal for this part of the project.

Using results of the foregoing section in addition to measuring various hematologic and chemistry constituents on the collected samples enables per cent erythron iron uptake to be calculated (Cook et al., 1970). Samples must be taken every other day for two weeks.

Safety Aspects

Dose rates equivalent to or greater than those proposed have been used for diagnostic purposes in man (Cook et al., 1970, Anonymous 1973). The roentgens (R) produced per millicurie per hour at one centimetre for Fe^{59}, Cr^{51} and I^{125} are 6.4, 0.15 and 1.25 while half lives for each are 45 days, 27.8 days and 60 days respectively (Quinby et al., 1970). The yearly rate of loss of iron is 10 per cent of total body iron per year (Hillman and Finch 1979). Hence, the loss to the environment in one year is approximately 1.0 UCi of Fe^{59}. In terrestrial mammals Cr^{51} labelled red cells have an apparent half-life of 20 days (McSherry et al., 1966) T effective of I^{125} and Cr^{51} are considered to be 17 days and 27 days respectively (Quinby et al., 1970). Using I^{131} as an albumin label, Mattheeuw et al. (1966) found albumin in horses to have a $T^1/_2$ of 19.4 days. Globin to which Cr^{51} binds is not reused after red cell breakdown. Hence, it can be seen the loss to the environment of Fe^{59} is extremely low while the low doses, low activity and short half lives of Cr^{51} and I^{125} prevent significant output from the animal.

Normal technical care will prevent on-site contamination. Rubber gloves will be worn while sampling and a gamma counter used to monitor contamination in the unlikely event of a spillage during sampling or infusion.

Blood volume of Tursiops truncatus is 70 ml/kg body weight, which is 10.5 litres for a 150 kg animal. With blood samples of 10 ml, the maximum amount of Fe^{59}, Cr^{51} and I^{125} per sample is 0.045 UCi, 0.05 UCi and 0.01 UCi

respectively. With Fe^{59}, extrapolating from other species, this will be reduced 80 to 90% within six hours to a level of approximately 0.01 UCi per sample.

I have experience using parenteral Cr^{51} for diagnostic purposes in the horse and shall carry out a full pilot study using rabbits before undertaking this project.

Three animals will suffice. Various authors have commented upon the need to elucidate iron status in odontocetes (Ridgway 1972, Medway 1968). This project has the potential to make a significant contribution to the understanding of the unique hematologic picture of marine mammals.

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