January 2005

Rodents as receptor species at a tritium

Angel Kelsey-Wall
University of Georgia

John C. Seaman
University of Georgia

Charles H. Jegoe
University of Georgia

Cham E. Dallas
University of Georgia

Karen F. Gaines
Eastern Illinois University, kfgaines@eiu.edu

Recommended Citation

Kelsey-Wall, Angel; Seaman, John C.; Jegoe, Charles H.; Dallas, Cham E.; and Gaines, Karen F., "Rodents as receptor species at a tritium" (2005). *Faculty Research & Creative Activity*. 70.
http://thekeep.eiu.edu/bio_fac/70

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Environmental Radioactivity 82 (2005) 95–104
JOURNAL OF ENVIRONMENTAL RADIOACTIVITY
www.elsevier.com/locate/jenvrad

Rodents as receptor species at a tritium disposal site

Angel Kelsey-Wall[a], John C. Seaman[a,*], Charles H. Jagoe[a], Cham E. Dallas[b], Karen F. Gaines[c]

[a] *The University of Georgia, Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29802, USA*
[b] *The University of Georgia, 353 Robert C. Wilson Pharmacy Building, Athens, GA 30602, USA*
[c] *The University of South Dakota, 175 Churchill – Haines, Vermillion, SD 57069, USA*

Received 20 September 2004; received in revised form 29 December 2004; accepted 4 January 2005

Abstract

New methods are being employed on the Department of Energy's Savannah River Site to deal with the disposal of tritium, including the irrigation of a hardwood/pine forest with tritiated water from an intercepted contaminant plume to reduce concentrations of tritium outcropping into Fourmile Branch, a tributary of the Savannah River. The use of this system has proven to be an effective means of tritium disposal. To evaluate the impact of this activity on terrestrial biota, rodent species were captured on the tritium disposal site and a control site during two trapping seasons in order to assess tritium exposure resulting from the forest irrigation. Control site mice had background levels of tritium, 0.02 Bq/mL, with disposal site mice having significantly higher tritium concentrations, mean = 34.86 Bq/mL. Whole body tritium concentrations of the mice captured at the disposal site were positively correlated with tritium application and negatively correlated with precipitation at the site.

Keywords: Tritium; Tritium oxide; Rodent; *Peromyscus gossypinus*; Savannah River Site; Irrigation; Tritium disposal; Precipitation

---

* Corresponding author. Tel.: +1 803 725 0977; fax: +1 803 725 3309.
E-mail addresses: kelsey-wall@srel.edu (A. Kelsey-Wall), seaman@srel.edu (J.C. Seaman), jagoe@srel.edu (C.H. Jagoe), cdallas@mail.rx.uga.edu (C.E. Dallas), kfgaines@usd.edu (K.F. Gaines).

0265-931X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jenvrad.2005.01.007

1. Introduction

The Department of Energy's (DOE) Savannah River Site (SRS), located near Aiken, SC, produced tritium for national defense purposes for nearly 40 years. In addition, SRS is the only tritium recycling facility in the U.S. Throughout its history, SRS has released tritium into the environment during normal operations, as well as during waste disposal activities (Murphy et al., 1991; Arnett, 1997). Nuclear material processing activities on SRS produced a mixture of wastes, including tritium, that are now contained in the Old Radiological Waste Burial Ground (ORWBG) on the SRS. Seepage from the ORWBG containing tritiated water leached into the water-table aquifer and outcropped within a watershed that feeds Fourmile Branch, a tributary to the Savannah River, resulting in tritium levels above background (Arnett, 1997).

An environmental assessment concluded that tritium would continue to migrate into Fourmile Branch for the next 20–25 years, in spite of predictions that tritium concentrations would decrease due to radioactive decay (Arnett, 1997). To reduce the concentration of tritium reaching Fourmile Branch, a dam was constructed at the base of the seepline to intercept the contaminant plume. The resulting pond, with an average tritium concentration around 520 Bq/mL, is used to irrigate a neighboring 8.9-hectare hardwood/pine forest, known as the Mixed Waste Management Facility (MWMF). The initial operation of the irrigation facility has reduced the concentration of tritium being released into Fourmile Branch by 60% (Blake, 2000; Hitchcock et al., 2004).

To evaluate the efficacy of the remediation effort, tritium levels are routinely measured in the irrigation pond, as well as in soils and vegetation in the irrigation site. The irrigation schedule is thoroughly documented and the local precipitation is monitored. Soil monitoring efforts include measurement of tritium in soil cores, in addition to monthly lysimeter and soil vapor tube sampling to survey the tritium concentrations found in porewater at various depths within the soil profile. Vegetation sampling includes determining tritium concentrations in leaf litter samples, and measurement of vegetation height and species composition. Evapotranspiration of tritium by various tree species at multiple canopy heights is also monitored (Blake, 2000; Rebel et al., 2005).

Prior to the current study, there were few data about tritium in wildlife inhabiting the irrigation site. The uptake of radionuclides by biota has been studied at nuclear facilities with a history of radiation contamination, providing useful information regarding bioavailability and trophic transfer (Hoffman et al., 2003). For example, following the Chernobyl accident, the 30 km zone surrounding the reactor has been used to study the response of wildlife to radiological contamination. At Chernobyl, studies have evaluated the effect of radiation on various levels of biota, including terrestrial (i.e., plants, invertebrates, small mammals, avian populations) and aquatic species (i.e., fish, invertebrates, and plants) (Krivolutzki and Pokarzhevski, 1992; Baker and Chesser, 2000).

In this study, an evaluation of the terrestrial biota inhabiting the site is needed to provide essential information regarding the tritium dynamics within the disposal site.

Rodents are a good choice for the receptor species at the tritium disposal site because they have a high reproductive capacity, are sensitive to the contaminant of interest, and have home ranges that are within the study site itself (Golley et al., 1965a; Gentry et al., 1971; Smith et al., 1971). In addition, rodents, especially mice, have been used for decades in ecological research to determine the fate and transport of contaminants (Johnson et al., 1978; Anthony and Kozlowski, 1982; Hunter et al., 1987). Within SRS, the cotton mouse (*Peromyscus gossypinus*) has been studied extensively as a receptor species to determine the bioavailability of various environmental contaminants, such as radionuclides and metals (Golley et al., 1965b; Punshon et al., 2003; Reinhart, 2003). Because the cotton mouse is one of the most common rodent species in the southeast and thrives in forested ecosystems, it has proved to be the most representative receptor species for this site.

The objectives of this study were to compare the whole body tritium concentrations of rodents within the tritium disposal site with those at a control site and to correlate tritium burdens with facility management practices, more specifically tritium application records and local meteorological conditions at the site.

2. Materials and methods

2.1. Field methods

Two sites were chosen for rodent trapping: the tritium irrigation site and a reference location, Boggy Gut, which is located in an area of the SRS without local sources of radionuclides or other wastes. At the tritium disposal site, trapping was performed using baited Sherman rodent-traps. The traps were deployed in four evenly spaced (23 m) trap-lines comprised of 35 Sherman rodent-traps at 6 m spacing placed across plots 13–19, and 23. Two additional trap-lines consisted of 20 Sherman rodent-traps placed in plots 20 and 21, for a total of 180 traps (Fig. 1).

All traps were checked daily and re-baited as necessary. In the fall season, traps were checked from September 30 until November 22, 2002. Traps were initially baited using sunflower seed, with poor trapping success, until October 22, when the bait was switched to a combination of peanut butter and oatmeal. The spring season lasted from March 31 until May 26, 2003. The peanut butter and oatmeal bait was used again for the spring season. Captured animals were euthanized in the field using cervical dislocation before transport to the laboratory for processing.

At Boggy Gut, traps were deployed in four evenly spaced lines approximately 23 m apart, with approximately 9 m between the traps. Three of the lines contained 19 Sherman rodent-traps, with the fourth line only containing 18 traps because of landscape restraints, for a total of 75 traps. All traps were checked daily and re-baited as necessary. Boggy Gut was only trapped for one season, fall 2002, lasting from September 8 until December 3, 2002. Traps were baited with sunflower seeds for the entire season due to high trapping success. Captured animals were euthanized in the field using cervical dislocation before transport to the laboratory for processing.

2.2. Laboratory analysis

Once at the laboratory, the body weight and sex of captured animals were recorded; whole mice were frozen until required for analysis. The whole body of captured rodents was used to extract body fluid for analysis by sublimation (i.e., freeze drying). The extracted body fluid was analyzed for tritium by liquid scintillation with an estimated detection limit of 0.01 Bq/mL and a counting error below 2% for elevated tritium concentrations (Minaxi Tri-Carb 4000, Packard Instrument Co). QA/QC protocols were performed regularly to verify accuracy of laboratory methods. Compositing of animals was not necessary as the tritium analytical method was sufficiently sensitive to detect tritium concentrations in 70% of the samples given the volume of sample obtained from each animal.

2.3. Exposure estimate calculations

Savannah River Site-Forestry Service (SRS-FS) is responsible for managing the tritium disposal site, which includes maintaining weekly records of the volume of tritiated water applied to each plot. This data set was used to calculate daily average applications for each one week period. In addition, SRS maintains daily records of meteorological data around the entire site, including the area in which the tritium disposal site is located, that are used in determining application rates. These precipitation data are multiplied by area to obtain rain volume for individual plots. Irrigation and precipitation data for the capture plot were used to calculate estimated exposure to tritium in water, as shown in Eq. 1:

$$\text{Exposure} = \frac{(\text{tritium concentration, Bq/mL})_{\text{pond}} \times \left(\text{volume}_{\text{applied}},\ \text{mL}\right)}{\left(\text{volume}_{\text{precip}} + \text{volume}_{\text{applied}},\ \text{mL}\right)} \qquad (1)$$

The tritium concentration of the pond used in the above equation was an average of the monthly pond concentrations over the first and second year of operation, and calculated to be approximately 520 Bq/mL.

2.4. Statistical analysis

Statistical analyses were performed using SAS (version 9.0; SAS Institute). Body weights and whole body tritium concentrations were tested for normality using the Shapiro-Wilk statistic (PROC UNIVARIATE). Analysis of variance (ANOVA; PROC GLM), along with post hoc tests (Tukey, Scheffe and Dunnett), were used to examine relationships between whole body tritium concentrations, capture season, body weight, sex, and location. For correlation analyses, regression models (PROC REG) were used to test for significant relationships between whole body tritium concentrations and irrigation levels and precipitation. For all the tested models, the coefficient of determination was used to provide an estimate of the predictive value of the model. All statistical tests were considered significant at the $p \leq 0.05$ level. In these analyses, instrumental detection limits were used for below detection observations,

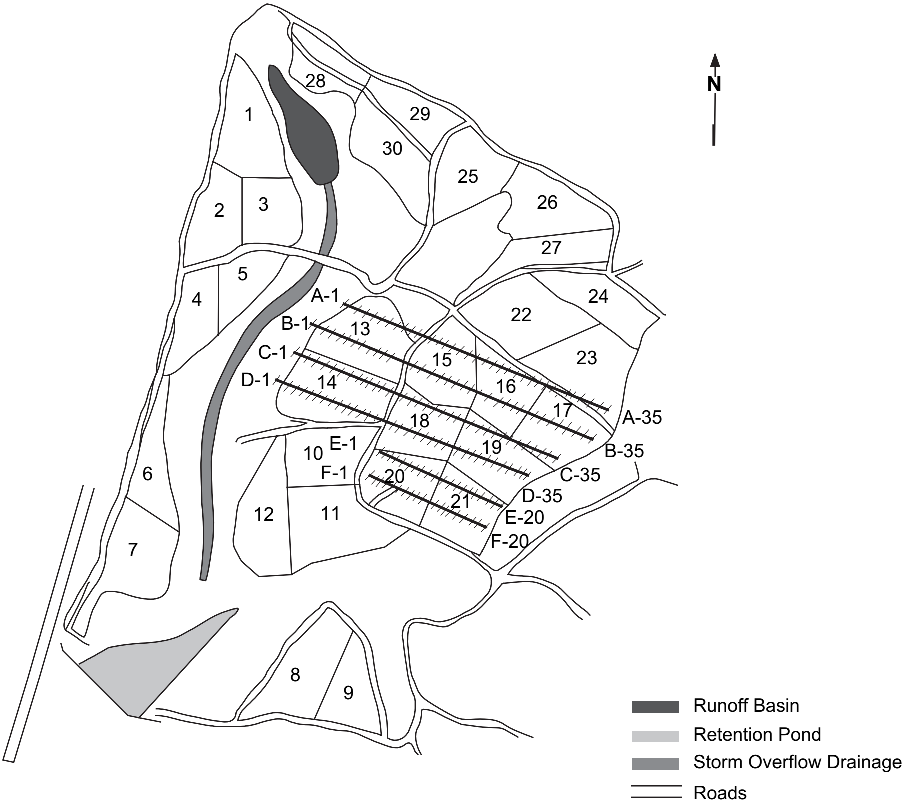

Fig. 1. Tritium disposal site rodent trapping lines. Rodent trapping lines used in the tritium disposal site on the SRS included 4 trapping lines containing 35 Sherman traps and 2 trapping lines containing 20 Sherman traps, for a total of 180 traps distributed among 10 irrigation plots.

which corresponded to approximately 30% of the measured whole body tritium concentrations. Because this type of left-censored data can result in underestimation of the variance and overestimation of the mean, the statistical program Uncensor was used to estimate the mean whole body tritium concentration for Boggy Gut mice. Using the regression order statistics method in Uncensor, a coordinate system was calculated for each ranked, non-censored data point such that the data formed a straight line; the mean and standard deviation of the data set was calculated based on the regression of this non-censored data (Newman et al., 1989).

3. Results and discussion

There were significant differences between whole body tritium concentrations in mice from the tritium disposal site and the reference site, Boggy Gut. Increases in whole body tritium concentrations in mice on the disposal site were related

($r^2 = 0.1820$) to increases in tritium irrigation levels and increases in tritium exposure. Decreases in whole body tritium concentrations in mice on the irrigation site were related ($r^2 = 0.2585$) to increases in precipitation.

Only one species, *P. gossypinus* (cotton mouse), was captured in sufficient numbers to allow statistical analyses. A summary of the capture results can be seen in Table 1. Fourteen cotton mice were captured at the tritium disposal site in fall of 2002, with a maximum whole body tritium concentration of 118.50 Bq/mL, minimum whole body tritium concentration of 10.51 Bq/mL, and mean whole body tritium concentration of 49.99 ± 8.88 Bq/mL. For spring 2003, 11 cotton mice were captured at the tritium disposal site; the whole body tritium concentration ranged from 0.01 Bq/mL to 60.84 Bq/mL, and the mean whole body tritium concentration was 19.75 ± 10.25 Bq/mL. Sixteen cotton mice were captured at Boggy Gut, the reference site, with a maximum whole body tritium concentration of 0.20 Bq/mL, a minimum whole body tritium concentration of 0.01 Bq/mL, and the mean whole body tritium concentration being 0.02 ± 0.08 Bq/mL. Whole body tritium concentrations in mice for the two seasons at the tritium disposal system were not significantly different ($F = 2, 16$; $p = 0.1560$; df = 1, 23), so they were pooled for the remaining statistical analyses. In addition, there were no significant differences between the whole body tritium concentrations of male and female mice ($F = 0.89$; $p = 0.4260$; df = 2, 23) at the irrigation site, so sexes were pooled for analyses.

At the irrigation site, tritium is available to mice by several pathways: direct ingestion as a water source, absorption through the skin, inhalation of the vapor, and ingestion of food and sediment. The high concentrations of tritium in mice from the irrigation site, as compared with locations with only background levels of tritium, such as Boggy Gut ($F = 20.55$; $p < 0.0001$; df = 1), indicate that the organisms in terrestrial environments receiving tritium do take up the radionuclide and may experience quantifiable doses. Previous studies have found that tritium causes harmful effects in rodents at concentrations several orders of magnitude higher than those found at the tritium disposal site (Brooks et al., 1976; Carsten and Commerford, 1976; Gao et al., 2002; Yamamoto et al., 1995). In addition, results from a laboratory study where exposure concentrations were comparable to those of mice inhabiting the tritium disposal site indicated that the tritium burdens of mice at the disposal site are insufficient to induce oxidative stress (Kelsey-Wall et al., 2005).

Table 1
Seasonal cotton mouse field data for locations. Field data for cotton mice captured at both the tritium disposal site and Boggy Gut for fall 2002 and spring 2003 seasons

Season	Location	Total mice	Sex		Maximum tritium concentration (Bq/mL)	Minimum tritium concentration (Bq/mL)	Mean tritium concentration (Bq/mL ± SD)
			M	F			
Fall 2002	Tritium disposal Site	14	5	9	118.5	10.5	50.0 ± 8.8
Fall 2002	Boggy Gut (reference site)	16	10	6	0.2	0.01	0.02 ± 0.08
Spring 2003	Tritium disposal site	11	3	8	60.8	0.01	19.8 ± 10.3

As an isotope of hydrogen, the metabolic pathways for tritiated water in organisms are the same as those for water. It has been theorized that because hydrogen is encountered constantly in the environment and sufficient processes exist to metabolize hydrogen, organisms do not have a capability to bioconcentrate hydrogen, nor therefore, tritium. Indeed, bioaccumulation and biomagnification do not occur with tritium as may be the case with some other contaminants (Murphy, 1993). The data from this study support this observation. Mice captured in the spring season were significantly smaller than those captured in the fall season ($F = 7.00$; $p = 0.0026$; df = 2, 39), suggesting that more juveniles were captured in the spring season. However, as mentioned earlier, whole body tritium concentrations in mice for the two seasons were not significantly different ($F = 2.16$; $p = 0.1560$, df = 1, 23). If bioconcentration occurred in this system, the larger animals, those captured in the fall, which are presumably older and with longer exposure, would be expected to have higher whole body tritium concentrations than those who are younger and smaller. Also, the highest whole body tritium concentration found in the captured mice was only approximately 25% of that in the pond that supplies water to the irrigation system. If tritium was bioconcentrated, the whole body tritium concentration of exposed mice should exceed that of the mean calculated exposure, accounting for irrigation and precipitation. Thus, the data supports the conclusion that tritium does not bioconcentrate (Murphy, 1993; Klaassen 2001).

Because tritium does not bioconcentrate, and because tritium has a relatively short biological half-life (Kelsey-Wall et al., 2005), there should be a relationship between whole body tritium concentrations of exposed mice and the amount of tritium applied to their environment. With an increased availability due to irrigation with tritiated water, whole body tritium concentration of exposed mice should rise. In fact, there is a significant, positive regression, albeit weak, between the volume of tritiated water applied and the mouse whole body tritium concentration (Fig. 2; $F = 4.89$; $p = 0.0376$; df = 1, 23; $r^2 = 0.1820$).

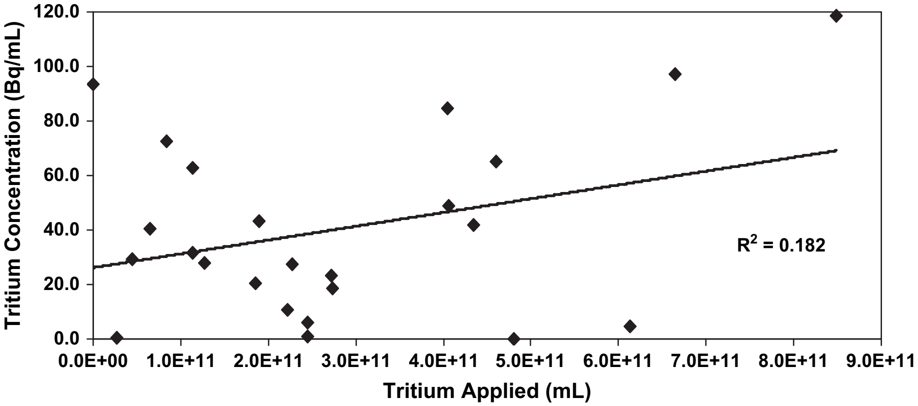

Fig. 2. Tritium disposal site mice whole body tritium concentrations regressed on tritium application. Whole body tritium concentrations of mice captured at the tritium disposal site increased with an increase in the amount of tritium applied ($p = 0.0376$).

Conversely, precipitation should also affect the whole body tritium concentrations of exposed mice. With an increase in precipitation, the whole body tritium concentration of an exposed mouse should decrease, due to the dilution of the tritiated water by rainwater. In this study, a significant, negative regression between the volume of precipitation and the mouse whole body tritium concentration ($F = 7.67$; $p = 0.0112$; $\mathrm{df} = 1, 23$; $r^2 = 0.2585$) was observed.

Taken together, the observed relationships between the whole body tritium concentrations and irrigation volume and precipitation indicate a moderate relationship between tritium dose and whole body concentration.

From the concentration of tritium in the irrigation water, the volume of the tritiated water applied and any dilution of this water with rain water (Eq. 1), an exposure estimate was calculated. As with most other toxicants, the whole body tritium concentration should increase with an increased dose. The data from this field study support this prediction, as there is a significant, positive relationship between the estimated dose and mouse whole body tritium concentration (Fig. 3; $F = 6.97$; $p = 0.0149$; $\mathrm{df} = 1, 23$; $r^2 = 0.2406$).

4. Conclusions

Mice inhabiting the tritium disposal facility have significantly higher whole body tritium concentrations than mice at locations with background levels of tritium. In addition, the whole body tritium concentrations of mice within this system increase with increasing tritium irrigation rates and increasing tritium dose. Also, decreases in whole body tritium concentrations of mice on the disposal site were related to increases in precipitation. The data support the observation that tritium does not

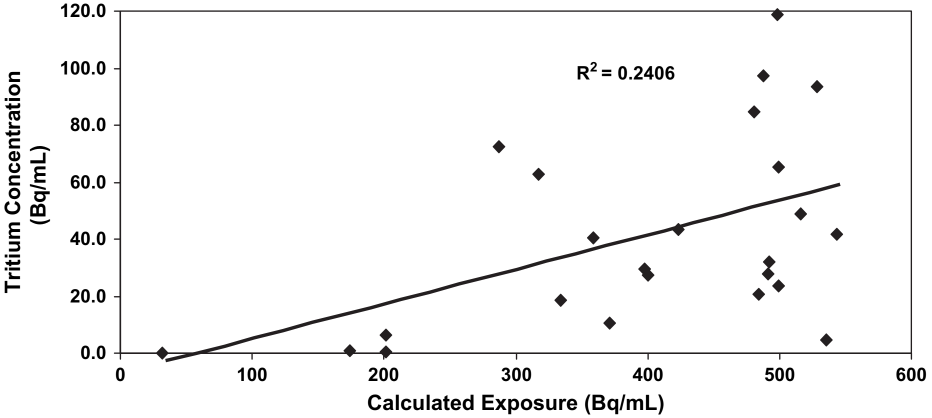

Fig. 3. Tritium disposal site mice whole body tritium concentrations and estimated exposure. Whole body tritium concentrations of mice captured at the tritium disposal site increased with an increase in the estimated tritium dose.

bioconcentrate, and indicate that tritium concentrations in wildlife are directly dependent on exposure concentrations in their immediate environment.

Acknowledgements

The authors would like to thank Mark Komoroski and Brad Reinhart for field assistance, and Jennifer McIntosh and Jane Logan for laboratory assistance. This research was supported by Westinghouse Savannah River Company-Soil & Groundwater Closure Projects and through Financial Assistance Award No. DE-FC09-96SR18546 from the U.S. Department of Energy to the University of Georgia Research Foundation, Inc.

References

Anthony, R.G., Kozlowski, R., 1982. Heavy-metals in tissues of small mammals inhabiting waste-water irrigated habitats. J. Environ. Qual. 11, 20–22.

Arnett, M., 1997. Savannah River Site Environmental Report for 1997. Westinghouse Savannah River Company, Aiken, SC. WSRC-TR-97-00322; WSRC-TR-97-00322.

Baker, R.J., Chesser, R.K., 2000. The Chernobyl nuclear disaster and subsequent creation of a wildlife preserve. Environ. Toxicol. Chem. 19, 1231–1232.

Blake, J.I., 2000. Effectiveness monitoring plan for the tritiated water management facility southwest plume interim measures. Westinghouse Savannah River Company, Aiken, SC. SRI 00-10-R.

Brooks, A.L., Carsten, A.L., Mead, D.K., Retherford, J.C., Crain, C.R., 1976. The effect of continuous intake of tritiated water (HTO) on the liver chromosomes of mice. Radiat. Res. 68, 480–489.

Carsten, A.L., Commerford, S.L., 1976. Dominant lethal mutations in mice resulting from chronic tritiated water (HTO) ingestion. Radiat. Res. 66, 609–614.

Gao, W., Lu, H., Dong, J., Zhang, W., Zhou, X., Jenkins, L.W., et al., 2002. Postnatal growth, neurobehavoiral and neurophysiologic changes of prenatal low dose β-radiation from tritiated water in mice. Neurotoxicol. Teratol. 24, 247–254.

Gentry, J.B., Golley, F.B., Smith, M.H., 1971. Yearly fluctuations in small mammal populations in a southeastern United States hardwood forest. Acta Theriol. 12, 179–190.

Golley, F.B., Gentry, J.B., Caldwell, L., Davenport Jr., L., 1965a. Numbers and variety of small mammals on the AEC Savannah River Plant. J. Mamm. 46, 1–18.

Golley, F.B., Gentry, J.B., Menhinic, E.F., Carmon, J.L., 1965b. Response of wild rodents to acute gamma radiation. Radiat. Res. 24, 350–356.

Hitchcock, D.R., Barton, C.D., Rebel, K.T., Singer, J., Seaman, J.C., Strawbridge, J.D., et al., 2004. A containment and disposition strategy for tritium-contaminated groundwater at the Savannah River Site, South Carolina, United States. Environ. Geosci. 12, 17–28.

Hoffman, D.J., Rattner, B.A., Burton Jr., G.A., Cairns Jr., J., 2003. Handbook of ecotoxicology, second ed. Lewis Publishers, Florida.

Hunter, B.A., Johnson, M.S., Thompson, D.J., 1987. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. 3. Small mammals. J. Appl. Ecol. 24, 601–614.

Johnson, M.S., Roberts, R.D., Hutton, M., Inskip, M.J., 1978. Distribution of lead, zinc and cadmium in small mammals from polluted environments. Oikos 30, 153–159.

Kelsey-Wall, A., Seaman, J.C., Jagoe, C.H., Dallas, C.E., Gaines, K.F., 2005. Biological half-life and oxidative stress effects in mice with low-level, oral exposure to tritium. J. Toxicol. Environ. Health, in press.

Klaassen, C.D., 2001. Cassarett and Doull's toxicology: The basic science of poisons. McGraw-Hill, New York.

Krivolutzki, D.A., Pokarzhevski, A.D., 1992. Effects of radioactive fallout on soil animal populations in the 30 km zone of the Chernobyl atomic power station. Sci. Tot. Environ. 112, 69.

Murphy Jr., C.E., 1993. Tritium transport and cycling in the environment. Health Phys. 65, 683–712.

Murphy Jr., C.E., Bauer, L.R., Hayes, D.W., Marter, W.L., Zeigler, C.C., Stephenson, D.E., et al., 1991. Tritium in the Savannah River Site Environment. Westinghouse Savannah River Company, Aiken, SC. WSRC-RP-90-424-1.

Newman, M.C., Dixon, P.M., Looney, B.B., Pinder III, J.E., 1989. Estimating mean and variance for environmental samples with below detection limit observations. Water Resour. Bull. 25, 905–916.

Punshon, T., Gaines, K.F., Jenkins Jr., R.A., 2003. Bioavailability and trophic transfer of sediment bound Ni and U in a southeastern wetland system. Arch. Environ. Con. Tox. 44, 30–35.

Rebel, K.T., Riha, S.J., Seaman, J.C., Barton, C., 2005. The use of dynamic modeling in optimizing tritium phytoremediation. Environ. Geosci. in press.

Reinhart, B.D., 2003. The use of small mammals as indicators of heavy metal bioavailability in a contaminated riparian zone. M.S. thesis, Athens, GA: University of Georgia.

Smith, M.H., Blessing, R., Chelton, J.G., Gentry, J.B., Golley, F.B., McGinnis, J.T., 1971. Determining density for small mammal populations using a grid and assessment lines. Acta Theriol. 16, 105–125.

Yamamoto, O., Seyama, T., Jo, T., Terato, H., Saito, T., Kinomura, A., 1995. Oral administration of tritiated water (HTO) in mouse. II. Tumour development. Int. J. Radiat. Biol. 68, 47–54.