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**Ecological Effects of Contaminants in the Upper Three Runs Integrator
Operable Unit**

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ABSTRACT

For risk assessment purposes, the 780 km² Savannah River Site (SRS) has been partitioned spatially into Integrator Operable Units (IOUs) that correspond to the watersheds of the Savannah River tributaries that drain the SRS. The streams within each watershed are “integrators” because they receive contaminants transported by surface or subsurface flow from all sources within their watersheds. Ecological receptors within the streams are exposed to these contaminants, and their health reflects the severity of contamination within the watershed. The Upper Three Runs (UTR) drainage is one of the six IOUs on the SRS. The UTR IOU includes five IOU subunits that correspond to the upper (UTR-upper), middle (UTR-middle), and lower (UTR-lower) reaches of UTR; Tinker Creek; and Tims Branch. Also included in the UTR IOU are three relatively small tributary streams: Mill Creek, McQueen Branch, and Crouch Branch. Tinker Creek, Mill Creek, and UTR-upper are largely undisturbed by SRS operations. UTR-middle, UTR-lower, Tims Branch, Crouch Branch, and McQueen Branch are potentially impacted by SRS waste sites or industrial operations.

Assessment of the ecological effects of contaminants in the SRS IOUs involves 1) the measurement of contaminant levels in sediment, fish, crayfish, and surface water; 2) the use of contaminant exposure models that estimate potential contaminant doses to ecological receptors; and 3) field bioassessments of the fish and invertebrate assemblages inhabiting SRS streams. The data generated by these studies provide a broad basis for a weight-of-evidence characterization of the extent and severity of contaminant related ecological impacts on SRS aquatic ecosystems.

There was little evidence that metal concentrations were unusually elevated in UTR-middle and UTR-lower, both located downstream from SRS waste sites and industrial areas. Concentrations of metals in sediments and fish in these subunits were comparable to those in reference subunits that were unaffected by SRS operations. Contaminant exposure models showed that most metals in UTR-middle and UTR-lower were not present at levels high enough to harm mammals and birds, with the possible exceptions of Hg and Al in UTR-lower. However, these two metals exceeded toxicity benchmarks in reference subunits as well as UTR lower. Elevated levels of Hg in UTR-lower probably resulted from the aerial deposition of Hg from non-SRS sources (as shown for other streams in the Savannah River basin) coupled with the presence of wetlands in the vicinity of UTR-lower that facilitated Hg methylation. Al exceedances may have been related to naturally high Al levels in SRS soils. Bioassessments based on fish and macroinvertebrate assemblage structure confirmed the conclusion that UTR was unimpaired by metals released by SRS operations. Two sampling methods – a multiple habitat sampling protocol and Hester-Dendy artificial substrates – showed that macroinvertebrate community structure in potentially impacted reaches of UTR was comparable or superior to community structure at most reference sites. IBI values based on electrofishing samples showed that fish community structure in potentially impacted reaches of UTR was comparable or superior to that in UTR-upper, which was unaffected by SRS operations. Examination of the health and condition of individual fish provide

further evidence that contamination in UTR was not adversely affecting fish from this stream.

In addition to UTR, the UTR IOU includes Tinker Creek, Mill Creek, Crouch Branch, McQueen Branch, and Tims Branch. Tinker Creek and Mill Creek were reference streams characterized by low levels of metals in sediments, fish, and water and diverse macroinvertebrate and fish communities indicative of high ecological integrity. Macroinvertebrate and fish community data indicated that the upper reaches of McQueen Branch were ecologically degraded, but that ecological integrity improved in the lower reaches. The upper reaches were scoured by runoff, deeply channelized, and had poor habitat quality. Similarly, macroinvertebrate samples indicated ecological degradation in the upper reaches of Crouch Branch but improvement further downstream. Biological degradation in the upper reaches may have been related to elevated copper concentrations in discharge from the H-02 outfall. Reductions in copper levels in the H-02 discharge contributed to improvements in the Crouch Branch macroinvertebrate community over time, but it remained inferior to reference site communities as a likely result of erosion related reductions in habitat quality. The concentrations of several metals were elevated in the sediments of Tims Branch, and the contaminant exposure models for this stream showed possible risks to mammals and birds as a result of elevated Hg levels in fish. In addition, both fish and macroinvertebrate bioassessments indicated that ecological integrity was low in Tims Branch. Contributing factors may include the periodic release of anoxic water from beaver ponds located upstream of the Tims Branch sample site as well as poor physical habitat. However, elevated levels of metals in sediments and, to a lesser extent, fish make it impossible to completely eliminate the possibility of metal related toxicity in this stream.

INTRODUCTION

The Department of Energy (DOE) has conducted industrial operations at the Savannah River Site (SRS) near Aiken, SC since the early 1950s resulting in the release of a variety of contaminants into some SRS streams. In an effort to understand the effects of these contaminants, the 780 km² SRS has been partitioned spatially into Integrator Operable Units (IOUs) that correspond to the watersheds of the Savannah River tributaries that drain the SRS. The streams within each watershed are “integrators” because they have the potential to receive contaminants transported by surface or subsurface flow from all sources within the watershed including Operable Units (OUs; i.e., waste units). Ecological receptors feeding within stream-based food chains are exposed to these contaminants, and their health can be considered an integrative indicator of the severity of contamination within the watershed. Because of their large size, the SRS IOUs have been subdivided into IOU subunits to facilitate the assessment process and identify areas of possible contamination with more precision.

The SRS has a comprehensive process for assessing the ecological effects of contaminants in the IOUs and IOU subunits. It involves 1) the collection of contaminant data from a variety of environmental media with an emphasis on sediment, fish, crayfish, and surface water; 2) the use of contaminant exposure models that estimate potential contaminant doses to ecological receptors (EPA 1993, ERD 1999a, ERD 1999b); and 3) field bioassessments of the fish and invertebrate assemblages inhabiting SRS streams. The data generated by these studies provide a broad and integrative basis for a weight-of-evidence characterization of the extent and severity of contaminant related ecological impacts on SRS aquatic ecosystems.

The SRS has been divided into six IOUs, five of which are stream drainages and one of which is the Savannah River and associated floodplain swamp contiguous with the SRS. These IOUs are subdivided into multiple IOU subunits. Several of the IOU subunits have no waste sites or industrial facilities within their boundaries and are considered relatively undisturbed reference subunits. Upper Three Runs (hereafter referred to as UTR) is one of the six IOUs on the SRS. It includes five IOU subunits that correspond to the upper, middle, and lower portions of UTR, Tinker Creek, and Tims Branch (Figure 1). The latter two subunits are tributaries of UTR. The UTR IOU also includes three additional tributary streams: Mill Creek, McQueen Branch, and Crouch Branch. The UTR IOU is the subject of this report, although data from several reference subunits are also included for comparison with the UTR subunits. The objective of this report is to assess the ecological integrity of the UTR IOU and to identify data gaps that need to be filled.

MATERIALS AND METHODS

Study area

The UTR IOU encompasses a large area that includes portions of Aiken and Barnwell counties located outside of the SRS (Figure 1, Table 1). Approximately

250 km² of the UTR watershed is within the SRS. Tributaries of UTR located within the SRS include Tinker Creek, Tims Branch, Crouch Branch, and McQueen Branch. Mill Creek is also located within the UTR IOU, but this stream discharges into UTR's main tributary, Tinker Creek, rather than directly into UTR. There are a number of SRS operational facilities and waste areas within the UTR IOU (WSRC 1998a); however, much of UTR is located upstream from SRS industrial areas. Similarly, Tinker Creek and Mill Creek are in portions of the SRS that are comparatively undisturbed by SRS operations. Several chemical constituents of potential concern from human health or ecological perspectives have been found in water and/or sediments within portions of UTR (WSRC 1998a).

Contaminant data

The contaminants covered in this report included metals in soil, surface sediments, surface water, and biota (primarily fish and crayfish). Soils and sediments were combined because they are often difficult to distinguish in wetland environments and are collectively referred to as sediment. Fifteen metals were evaluated: Al, Sb, As, Ba, Be, Cd, Cr, Cu, Pb, Mn, Hg, Ni, Se, V, and Zn. Potential sources of these metals include industrial and waste disposal processes on the SRS, land use practices existing before the establishment of the SRS, and natural geological factors.

Much of the contaminant data were collected for risk assessment purposes by the SRS Soil and Groundwater Remediation and Support Group, but substantial data were collected for a variety of other purposes by the SRS Environmental Monitoring Department, Savannah River National Laboratory, Savannah River Ecology Laboratory (SREL), and others. All data were collected and organized by the IOU project team in a geographic information system (GIS) format that provides information on data provenance, sampling times, sampling locations, and data quality. Additional sources of data used in this study included information on metal levels in fish and crayfish collected by SREL, most of which is included in Bryan et al. (2004 and 2005). Data were intensively scrutinized for analytical problems and other quality control issues before entry into the data base (EGIS 2007). The samples represented in the data set were collected between 1990 and 2005. Although data have been collected from all SRS subunits, only data from the UTR IOU and the SRS reference subunits (including subunits both within and outside the UTR IOU) are included in this report. The reference subunits included UTR-upper, Tinker Creek, Fourmile Branch-upper, Pen Branch-upper, Pen Branch-middle, and Meyers Branch (more information on the reference subunits can be found in Paller et al. [2008]). More information about the contaminant data is presented in Paller et al. (2008).

Fish and crayfish concentrations were usually derived from whole fish, sediment concentrations from bulk sediment, and water concentrations from unfiltered water. Surface water measurements were often below detection limits (i.e., censored), and multiple detection limits occurred in some cases. Censoring occurred less frequently with sediment and biotic data. Differences in metal speciation that could affect metal toxicity were not evaluated because supporting environmental information needed to assess metal chemistry was usually lacking. Constituents were considered to be present

in the most toxic state likely to occur (e.g., all mercury in fish and crayfish was conservatively assumed to be methylmercury because methylmercury rather than inorganic mercury predominates in the bodies of fish).

Contaminant exposure models

Contaminant exposure models are used to calculate exposure doses for metals that pose potential risks to ecological receptors through ingestion of contaminated media. These exposure doses are compared with chronic toxicity reference values (TRVs) to identify potentially hazardous constituents. Exposure point concentrations (EPCs) were calculated to represent the exposure doses for each metal in each medium in each subunit. Three types of EPCs were used based on the sample size and amount of data censoring.

They are listed below in decreasing order of preference and frequency of usage:

- 1) The upper 95% confidence limit (UCL) of the mean was used as a conservative estimate of the average exposure scenario when there was sufficient uncensored data for accurate computations. UCLs were computed with ProUCL 4.0 software (Singh and Singh 2007), which identifies an appropriate UCL based on the data distribution and prevalence of censoring.
- 2) The maximum concentration was used when at least some data exceeded detection limits but the number of detects were insufficient to compute UCLs.
- 3) One half of the maximum detection limit was used when all data were below the detection limit(s).

The EPCs for each medium in each subunit were used in contaminant exposure models developed for the river otter *Lontra Canadensis*, and the belted kingfisher *Ceryle alcyon*. Both are representative of the environments under study, locally common, and vulnerable to contaminants because they feed largely on aquatic organisms and are near the apex of the aquatic food chain. The duration of exposure was assumed to be long-term and the receptors were assumed to spend all of their time in the evaluation areas (i.e., subunits) because of the relatively large size of the subunits. The primary exposure pathways were assumed to be ingestion of food, surface water, and soil. Dermal and inhalation pathways were not considered because they are generally insignificant compared with ingestion pathways and insufficiently understood to properly evaluate.

The diet of the river otter consists largely of fish but includes invertebrates, amphibians and reptiles (collectively termed herptiles), birds, and mammals. Estimated percent composition was 65% fish, 15% invertebrates, 10% herptiles, 5% birds, and 5% mammals (EPA 1993). The belted kingfisher has a more restricted diet consisting of 70% fish, 15% amphibians and reptiles, and 15% invertebrates (EPA 1993). Invertebrates consumed by the otter and belted kingfisher were assumed to be crayfish represented by specimens of *Cambarus latimanus* and *Procambarus* spp collected from the IOU subunits. No assumptions were made regarding the species and size of fish consumed; however, the fish represented in the SRS data bases were generally among the more common and larger species and larger individuals found in the subunits. Metal concentrations in herptiles were unmeasured but assumed to be the same as in fish because both types of organisms are ectothermic and feed mainly on aquatic/ riparian invertebrates and small vertebrates. Bird and mammal data were largely unavailable so

contaminant levels in these food sources were estimated using tissue to sediment concentration ratios (CRs):

$CR = C_{\text{animal}}/C_{\text{soil or sediment}}$ where:

CR = the tissue to soil concentration ratio for a particular metal

C_{animal} = average metal concentration in animal whole body (wet weight) from site i

$C_{\text{soil or sediment}}$ = average metal concentration in sediment (dry weight) from site i .

The CRs were based on metal concentrations in cotton rats *Sigmodon hispidus* and sediment collected from five sites exposed during the extended drawdown of a contaminated reservoir on the SRS (Paller and Wike 1996). The CRs computed for mammals were also used for birds.

Data were available for crayfish from only six IOU subunits including the Tims Branch subunit in the UTR IOU. Metal concentrations in crayfish were estimated for other subunits based on the relationship between metal concentrations in crayfish tissue and fish tissue from the subunits with both types of data:

$CR = C_{\text{crayfish}}/C_{\text{fish}}$ where:

CR = the tissue to tissue concentration ratio for a particular metal

C_{crayfish} = average metal concentration in crayfish whole body (wet weight) from subunit i

C_{fish} = average metal concentration in fish (whole body wet weight) from subunit i .

Crayfish to water and crayfish to sediment CRs were also developed but not used because of their high variability among IOUs compared with crayfish to fish CRs (see Paller et al. 2008 for more information).

The contaminant exposure models followed EPA (1993), Sample et al. (1996), and ERD (1999b). Ingestion rates were based on dietary composition, gross energy content, the assimilation efficiency for each food, and the metabolic rates of the receptors. Allometric models (EPA 1993) were used to compute metabolic rates and water ingestion rates. The soil ingestion rate for the otter was assumed to be 2.8%, which is the soil consumption rate of the red fox (EPA1993), another mammalian carnivore of approximately comparable size. The soil consumption rate of the kingfisher, a species for which soil consumption data for comparable species were lacking, was assumed to be 2%. Ingestion rates for all pathways were summed:

$$ED_{\text{total}} = \sum_{i=1}^n ED_{\text{food } i} + ED_{\text{water}} + ED_{\text{soil}}, \text{ where:}$$

ED_{total} = total exposure dose from all sources (mg/kg/d)

$ED_{\text{food } i}$ = exposure dose from ingestion of food source i

ED_{water} = exposure dose from ingestion of water

ED_{soil} = exposure dose from ingestion of soil.

The exposure dose resulting from each pathway was represented as a daily intake normalized to body weight (mg/kg/d). Total daily exposure (ED_{total}) was compared with lowest observed adverse effect levels (LOAELs) that represented the lowest metal concentrations that cause adverse chronic effects. LOAELs were taken from ERD (1999b) and from Sample et al. (1996) (LOAEL values are shown in Paller et al. 2008). LOAELs rather than more conservative NOAELs were used to provide a more realistic estimate of probable risks. A hazard quotient (HQ) was calculated for each IOU subunit

by dividing the total exposure dose by the LOAEL. The contaminant exposure models were used to estimate ecological risks for each UTR and reference subunit based on the EPCs for each contaminant source. More detailed explanations of the models and their derivation is presented in Paller et al. (2008) and Paller and Dyer (2010a and 2010b).

Bioassessment using fish assemblage data

Bioassessment is the use of information about the organisms within a stream to assess environmental quality. The Index of Biotic Integrity (IBI) is a bioassessment method that uses fish assemblage data to assess the biotic integrity of streams (Karr et al. 1986). Biotic integrity is the ability of a stream to support a self-sustaining biological community and ecological processes typical of undisturbed, natural conditions (Angermeier and Karr 1994). The IBI is a multimetric index composed of a number of community, population, and organism level variables (or metrics) that are ecologically important and sensitive to environmental disturbances of various types.

The IBI used in this study was adjusted for the composition of the ichthyofauna of the upper South Carolina coastal plain. It included 10 metrics, each scored one, three, or five which summed to a maximum total index value of 50 (Paller et al. 1996). It also adjusted for differences in sample area (i.e., species/area effect), stream size, and sampling effort making it possible to directly compare IBI values from streams of different size, sample areas of different size, and stream reaches sampled with different numbers of electrofishing passes. The IBI was calculated for each of the three 50 m segments at each location. The statistical significance of differences between IBIs at sites potentially influenced by SRS waste sites (i.e., sites located downstream from SRS waste sites) and reference sites was assessed with the Kruskal-Wallis test ($P \leq 0.05$) because the IBI data were non-normally distributed and with heterogeneous variances.

Fish assemblage data for computation of the IBI were collected from three locations in UTR, two locations in Crouch Branch, two locations in McQueen Branch, one location in Tims Branch, two locations in Mill Creek, and one location in Tinker Creek at various times between 1997 and 2007 (Table 1). These locations included both reference and potentially impacted sites. The former were located in the upper reaches of UTR (Tyler Bridge Road and Road 8-1), Mill Creek, and Tinker Creek. Additional reference sites representing conditions unaffected by SRS operations were located in Pen Branch, and Meyers Branch (more information on reference sites can be found in Paller and Dyer 2004).

Fish collections were made by electrofishing three 50 m stream segments. Small stream sites were sampled by a single crew using a single generator or battery powered Smith-Root backpack electrofisher, and medium-size sites (i.e., Tinker Creek) were sampled by two crews using two backpack electrofishers. Because of their exceptional depth and width, sites on UTR were sampled using a boat mounted electrofisher. A single pass was made through each 50 m segment while moving upstream at all sites. All microhabitats were sampled in an effort to obtain as many species and individuals as possible. Fish were collected with dip nets after they had been stunned by DC current, identified to species, and released.

The IBI was supplemented with the Fish Health Assessment Index (HAI, Adams et al. 1993) and the condition (K) factor. The HAI is a method for assessing the health and condition of individual fish based on dissection and internal examination. The HAI helped to determine whether contaminant concentrations were high enough to adversely affect fish health. For comparability among sites, only redbreast sunfish were collected for determination of the HAI. These fish were returned to the laboratory where they were sacrificed, weighed, measured (total length), and examined using the methods described in Klemm et al. (1993). Sixteen variables were recorded for each fish including:

- blood parameters (hematocrit, leukocrit, plasma protein);
- length and weight,
- appearance of the eyes, gills, pseudobranchs, spleen, kidneys, liver, hindgut; and
- damage to the skin, fins, and thymus.

The HAI was computed by assigning each variable (e.g., appearance of the liver) from each fish a numerical score based on evidence of pathology, with higher scores indicating greater pathology. HAI values were calculated for 10 fish collected by electrofishing from each of nine sample sites (including two reference sites) during 1996 and 1997. Sample sites representing the UTR IOU were located in UTR-lower and Mill Creek (Figure 1, Table 1). The statistical significance of differences between HAI values at potentially disturbed sites and reference sites was assessed with the Kruskal-Wallis test ($P \leq 0.05$).

The K factor, which expresses the relationship between a fish's weight and its length, was calculated using the following formula:

$$K = (W \times 10^5) / L^3,$$

where W equals weight in g and L equals total length in mm. Like the HAI, condition factors are an indicator of fish health, with low condition factors suggesting poor health. Condition was calculated for each redbreast sunfish that was necropsied for determination of the HAI.

Bioassessment using macroinvertebrate assemblage data

Biological surveys can involve fish, benthic macroinvertebrates, or periphyton (Barbour et al. 1997). Although bioassessments are often based on only a single assemblage, a more complete and accurate assessment can be produced by the use of more than one taxonomic assemblage because different taxonomic groups may respond differently to the same stressors (Mount et al. 1984, Yoder and Mount 1995, Paller 2001).

Macroinvertebrates samples were collected from UTR using artificial substrates (Hester-Dendy multiplate samplers) during 1997/1998, 2000, and 2007. A different method similar to SCDHEC's Timed-Qualitative Multiple Habitat Sampling Protocol (MHSP) (SCDHEC, 1998) was used to collect macroinvertebrates from most sites in 2003 and 2007, and from Crouch Branch in 2007. Each Hester-Dendy sampler consisted of fourteen 7.6 cm plates separated by approximately 0.3-1.0 cm (total surface area of 0.179 m²). Five samplers were deployed at each site. They were hung from a line stretched across the stream so that they did not contact the bottom and retrieved after a 28

day colonization period. Organisms were removed from the samplers, preserved in 70% ethanol, and taken to the laboratory for microscopic identification (usually to the genus level).

The MHSP involved the collection of macroinvertebrates from natural substrates using a timed, multiple habitat sampling approach consisting of three man-hours of sampling effort at each sampling location. All available natural habitats were sampled with a D-frame dip net, kick net, hand sieve, white plastic pan and fine mesh sampler with the objective of collecting as many different macroinvertebrate taxa as possible during the allotted time. Macroinvertebrates were placed in ethanol and returned to the laboratory for microscopic identification. The MHSP protocol is designed to ensure that all the habitats at a site are thoroughly sampled to obtain a good representation of the macroinvertebrate community. Details of the sampling protocol are provided in SCDEC (1998). The MHSP protocol was used to collect macroinvertebrates from all sites in the UTR IOU (Figure 1, Table 1).

Data collected with the MHSP was analyzed to produce a bioclassification rating based on two metrics: the number of EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa and a biotic index (BI) that reflects the relative pollution tolerances of individual taxa (Lenat, 1993). Each metric is usually assigned a score of 1 to 5 for each site, and the two scores were averaged to produce a combined bioclassification score. However, preliminary work on the SRS showed that the MHSP sometimes produced inaccurate scores leading to the development of a modified version of the MHSP for SRS streams. The modification consisted of adjusting the EPT metric for stream size and adjusting the overall scoring system by calibrating it against SRS streams known to be minimally disturbed. Because larger streams typically support greater macroinvertebrate diversity than smaller headwater streams (Vannote et al., 1980), larger streams would be expected to have more EPT taxa than smaller streams. The EPT metric was adjusted by drawing a “maximum species richness line” (Fausch et al. 1984) through the highest points on a plot of number of EPT taxa versus stream size and dividing the area below the line into five areas of equal size corresponding to the five bioclassification categories (see Paller and Dyer 2004 for more details). In general, these adjustments increased the scores for streams of high and moderate quality, but did not affect the scores of low-quality streams.

Habitat quality was assessed in conjunction with the MHSP using a methodology developed by SCDHEC (1998). Variables included epifaunal substrate, pool substrate, pool variability, sediment deposition, channel flow status, channel alteration, channel sinuosity, bank stability, vegetative protection, and riparian vegetation. These variables were scored, and the scores were summed to produce an overall rating of habitat quality.

The statistical significance of differences between macroinvertebrate metrics at sites potentially influenced by SRS waste sites (i.e., sites located downstream from SRS waste sites) and reference sites was assessed with the Kruskal-Wallis test ($P \leq 0.05$), followed by a Dunn’s test for comparisons between the pooled reference sites and individual potentially impacted sites ($P \leq 0.05$).

RESULTS AND DISCUSSION

Metal concentrations in the Upper Three Runs IOU

EPCs for contaminants in sediment indicated that UTR-middle and UTR-lower (both potentially impacted) differed little from UTR-upper, Tinker Creek, and the other reference subunits (Table 2). In contrast, the concentrations of some metals (Cr, Cu, Pb, Mn, Ni, and Zn) were elevated in Tims Branch compared with the reference subunits. EPCs for contaminants in fish tissues indicated that UTR-middle and UTR-upper differed little from the other reference subunits (Table 3). An exception was Hg, which was somewhat elevated in fish from UTR-lower as a likely consequence of greater rates of mercury methylation in the wetlands connected with this portion of the stream (Paller et al. 2003). Al, Sb, Ba, Be, Se, and V were unmeasured in fish from Tims Branch. Concentrations of the other metals in fish from Tims Branch were unexceptional compared with the reference sites, although nickel was somewhat elevated. Metal concentrations in crayfish were measured only in the Tims Branch subunit and in several subunits outside of the UTR IOU. EPCs for metals in Tims Branch crayfish were generally lower than in these other subunits (see Paller et al. 2008 and Paller and Dyer 2010a for more information on metals in crayfish). Metal concentrations in water were lower and more variable than in sediment and fish. EPCs for contaminants in water indicated that a few metals (Al, As, Ba, and Cd) were somewhat elevated in UTR-middle and UTR-lower compared with Tinker Creek and the other reference subunits (Table 4), although none of these were associated with exceedances of toxicity reference values in the otter and kingfisher contaminant exposure models (see next section). More detailed data on metal levels in individual subunits are available in Paller et al. (2008).

Contaminant exposure models

Contaminant exposure model results were generated for all five UTR subunits, although a lack of fish tissue metal data resulted in underestimates of the true exposure doses for a few metals in Tims Branch (Table 3). The otter contaminant exposure model showed that the exposure doses for most metals did not exceed the TRV (i.e., HQ < 1) indicating a low risk to mammals (Table 5). Exceptions included Al in UTR-lower and Hg in UTR-lower and Tims Branch. Hazard quotients were 1.3 for Al in UTR-lower, 2.6 for Hg in UTR-lower, and 1.4 for Hg in Tims Branch. Comparable HQs for these metals were observed in two of the reference subunits (2.1 for Hg in PB-middle and 2.0 for Al in FMB-upper). Like the otter model, the kingfisher model showed exceedances of the TRV for Hg in UTR-lower (HQ = 2.1) and Tims Branch (HQ = 1.4) (Table 6). A comparable exceedance (HQ = 1.8) for Hg occurred in PB-middle, a reference subunit (Table 6).

Both models indicated that Hg posed a risk to mammals and birds in two potentially impacted UTR subunits (UTR-lower and Tims Branch) but also in Pen Branch (PB-middle), a reference subunit. Sensitivity analysis indicated that the primary Hg exposure pathway for both the otter and kingfisher was fish consumption (Paller et al. 2008). Hg occurs in relatively high levels in fish throughout the Savannah River basin as a result of atmospheric deposition from non-SRS sources (EPA 2000), and it is likely that

this factor contributed to the observed exceedances for Hg. In addition, the lower reaches of UTR (i.e., UTR-lower) are associated with wetland habitats, as are the lower reaches of many Savannah River tributaries. These wetlands support conditions that promote Hg methylation and the accumulation of Hg in aquatic biota (Paller et al. 2003). These factors, combined with the comparable Hg related risk observed in at least one reference subunit (PB-middle), suggest that the Hg exceedance observed in UTR-lower was unrelated to SRS operations. In the case of Tims Branch, it is possible that Hg in water discharged from a ground-water treatment system contributed to the Hg exceedances observed in this stream. Additional treatment was added to remove mercury from this discharge after fish were collected from Tims Branch (2006), and it is possible that mercury levels in fish have since declined, although this cannot be verified without additional sampling.

Reasons for the TRV exceedance observed for Al in the otter model are uncertain. However, an even greater Al exceedance occurred in FMB-upper, a reference subunit (Table 5), suggesting that the Al exceedance in UTR-lower may have been unrelated to SRS operations. Al exceedances in the SRS subunits may be related to high Al levels in SRS soils. Kaolinite [$\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$] is mined in the region and is a component of the SRS geological strata. Furthermore, Al speciation can significantly affect toxicity (Klöppel et al. 1997), and Al toxicity to aquatic organisms is strongly influenced by water hardness (Gensemer 2009). These facts raise the possibility that total Al, as used in this exposure assessment, may not be the appropriate form of Al to compare with the relatively low Al TRV for mammals (19.3 mg/kg/d). More information concerning the contaminant exposure model results, including a discussion of uncertainties associated with the models, can be found in Paller et al. 2008.

IBI

Fish assemblage samples for computation of the IBI were collected from UTR-upper, UTR-lower, Tinker Creek, Crouch Branch, McQueen Branch, Tims Branch, and Mill Creek (Table 1, Figure 1). UTR-lower, Crouch Branch, McQueen Branch, and Tims Branch were potentially impacted (i.e., downstream from industrial waste sites and/or NPDES outfalls). Mill Creek and Tinker Creek were reference streams (i.e., largely undisturbed by SRS activities). All locations were sampled in 1997-1998 (hereafter referred to as 1997), 2000, 2003, and 2007 with the exception of Tinker Creek, which was sampled only in 2003 and 2007. Sample site locations in some streams changed over time (Table 1, Figure 1), mostly because of flooding by beaver impoundments. Fish assemblages were also sampled at four additional reference sites outside of the UTR IOU; the sites were in Meyers Branch and Pen Branch (Paller and Dyer 2004).

IBI values at sample sites in the UTR IOU did not exhibit consistent temporal trends between 1997 and 2007, except at Tims Branch where the IBI appeared to decrease with time (Figure 2). The average IBI over 1997 – 2007 at UTR-lower, which was downstream from important waste sites in F and H Areas, was 40.8. This value was lower than the average IBI at Tinker Creek (43.7) and Mill Creek (46.3), two of the reference locations in the UTR IOU, but considerably higher than the average IBI at UTR-upper (32.0). UTR-upper, the third reference location in the UTR IOU, was

upstream of SRS disposal sites and industrial facilities. Low IBI values at UTR-upper, and to a lesser extent UTR lower, are at least partly related to low sampling efficiency. UTR is a deep, swiftly flowing stream with extensive overhanging brush (an average of 43% of the stream surface area at UTR-upper, Paller and Dyer 2007) that is difficult to effectively electrofish. The low conductivity of UTR water (Halverson et al. 1997) likely also reduced electrofishing success. These factors could result in an apparent, rather than real, reduction in biotic integrity. From the standpoint of evaluating SRS impacts, the most important point is that IBI values at UTR-lower, a potentially impacted area downstream from SRS waste sites, were higher than at UTR-upper, the reference area upstream from the waste sites.

IBI values at the potentially impacted tributary streams in the UTR IOU, Crouch Branch, McQueen Branch, and Tims Branch, were variable but, on the average, lower (38.7, 39.7, and 34.3, respectively) than at Mill Creek (46.3) and Tinker Creek (43.7), the two reference tributaries in the UTR IOU (Figure 2). A Kruskal-Wallis test of differences among all potentially impacted UTR sites (UTR-lower, Crouch Branch, McQueen Branch, and Tims Branch) and all reference sites pooled (i.e., all sites in Tinker Creek, Mill Creek, Meyers Branch, and Pen Branch) was significant ($P \leq 0.05$). Dunn's tests of individual differences between each potentially impacted site and the pooled reference sites were significant for Tims Branch and McQueens Branch but not UTR-lower or Crouch Branch. It is likely that the relatively low IBI values at McQueens Branch, represented by a sample site near Road F, were largely the result of physical habitat degradation. The stream channel near Road F is deeply incised as a likely result of storm run-off from the Defense Waste Processing facility upstream from the sample site. The cause of the depressed IBI in Tims Branch is more difficult to definitively identify. Habitat is poor in this stream, and large amounts of iron floc on the stream bed of Tims Branch suggest the release of anoxic water from beaver ponds located upstream of the sample site. However, this stream is also characterized by relatively high metal concentrations in sediments suggesting the possibility of toxicity related effects.

HAI and K

The average HAI for the 10 redbreast sunfish collected during 1997 from Mill Creek, an undisturbed tributary of Tinker Creek, was 23. This value was considerably lower than the average HAI (72) from Meyers Branch, the other undisturbed reference site at which HAI and K values were calculated (low HAIs indicate good health). This difference between reference sites indicates substantial variability in the frequency of pathologies among fish from undisturbed streams. The average value for UTR-lower (near Road A.2) was 67. The HAI values for fish from UTR-lower were not significantly different (Kruskal-Wallis test) from the HAI values for fish from the reference sites ($P < 0.05$) indicating no increase in pathology in fish from the portion of UTR potentially subject to waste site discharge.

An analysis of condition factors (K) also failed to show any indication of poor health among redbreast sunfish from UTR. The average K for redbreast sunfish from UTR-lower was 1.90, which compares favorably with the average condition factors in the reference streams, Meyers Branch (1.50) and Mill Creek (1.62) (high condition factors

suggest good health). A Kruskal-Wallis test indicated no significant difference between condition factors for fish from UTR-lower and fish from the reference streams.

HDMI and MHSP

The MHSP protocol was used to sample macroinvertebrates at most sites within the UTR IOU in 2003 and 2007. The MHSP was also used to sample macroinvertebrates at Crouch Branch in 2006. Habitat quality for benthic macroinvertebrates was assessed in conjunction with the MHSP (see Methods and Materials). Macroinvertebrates were sampled primarily or solely with Hester-Dendy multiplate samplers during 1997/1998, 2000, and 2007. Results from the two methods are not directly comparable because of differences in field methods, metrics used for analysis, and scoring scales; however, both are indicators of stream ecological health.

In 2003 MHSP sampling sites were located in UTR-upper, UTR-middle, Tims Branch, Crouch Branch, Tinker Creek, Mill Creek, and McQueen Branch. There was more than one sample site in many of these streams (Table 1, Figure 1). Tims Branch near Road 2 and Crouch Branch near Road 4 received MHSP bioclassification scores of Poor (Table 7). The Tims Branch location was characterized by an incised channel and large amounts of iron floc (iron hydroxide) on the stream bottom. Crouch Branch at Road 4 is just downstream from the H-02 NPDES outfall, which discharges noncontact cooling water with elevated Cu levels. Steps have been taken to reduce Cu concentrations at the outfall, but the stream may be impacted by this or other factors as indicated by its low MHSP bioclassification score. Both Tims Branch and Crouch Branch at Road 4 received low Habitat Scores (81-95) reflecting relatively poor habitat for macroinvertebrates at both sites (Table 5).

With the exception of Tims Branch and Crouch Branch at Road 4, all of the sampling sites in the Upper Three Runs drainage were rated fair or higher using the MHSP scoring system (Table 7). However, none, including the reference sites at Tyler Bridge Road, Tinker Creek, and Mill Creek, received more than a “Good” score. Based on studies conducted by Dr. John Morse at Clemson University and others, UTR is an outstanding resource. Ratings of Fair to Good- for this stream and other undisturbed streams in the UTR watershed suggest that the SCDHEC bioclassification rating system may underestimate benthic community quality in some cases. Deep water in UTR and Tinker Creek at Tyler Bridge Road may have reduced sampling efficiency, thus lowering the scores for these streams, although scores were also relatively low for other undisturbed sites that were easier to sample.

The MHSP was used to sample macroinvertebrates from Crouch Branch in 2006 to determine if environmental conditions were improving in this stream (ETT 2006). There were four sample sites that spanned the length of the stream from its headwaters near Road 4 to its confluence with UTR (sites 5, 5B, 5C, and 5A, Table 1, Figure 1). Adjusted (see Materials and Methods) MHSP scores from the headwaters to the confluence were 1.3, 1.5, 2.3, and 2.5, respectively, indicating an increase in environmental quality with distance from the headwaters but only “Fair+” status at even the most downstream site. These scores were comparable to the MHSP scores obtained

in 2003 (Table 7) indicating little change over time. ETT (2006) attributed the relatively low MHSP scores in Crouch Branch to a combination of poor water quality in the headwaters and poor physical habitat caused by erosion.

The MHSP was again used to sample most sites in the UTR IOU during 2007 (Table 7). Moderate improvements compared with 2003 were observed at most sites in UTR and Tinker Creek, and MHSP scores in Crouch Branch improved substantially. In contrast, MHSP scores decreased at the Z Area sample site in McQueen Branch and at the Mill Creek reference sites. These declines were the result of beaver impoundments that converted formerly lotic into largely lentic habitats that did not support typical populations of stream invertebrates. MHSP scores also declined in another reference site, Pen Branch (Road C) because of low water levels.

Hester-Dendy artificial substrates were the primary method of collecting macroinvertebrate data on the SRS in 1997/1998, 2000, and 2007. These data were used to calculate the Hester-Dendy multimetric index (HDMI, Paller and Specht 1997) with data collected in 1997/1998 and 2000. The original HDMI included eight macroinvertebrate metrics, each scored one, three, or five resulting in a minimum possible score of eight and maximum possible score of 40, with high scores indicating high biotic integrity and low scores indicating biotic impairment. However, one of the eight metrics, percentage Tanytarsini, was not used when analyzing the 1997/1998 data because Tanytarsini were rare at all sample sites during those years. This reduced the minimum and maximum scores to seven and 35, respectively, for the 1997/1998 data. Biocriteria thresholds for rating the ecological health of sites (as good, fair, poor, etc.) were not developed for the HDMI. Instead, HDMI values at potentially impacted sites were compared statistically with values at reference sites, with the occurrence of significantly lower scores at potentially impacted sites indicating ecological impairment. The reference sites used in this comparison were Pen Branch near Road C, Pen Branch near Road B, Meyers Branch, Mill Creek, and UTR near Road 8-1. All of these sites were in undisturbed stream reaches upstream of SRS industrial areas and outfalls. Additional data concerning the reference sites, statistical methods, and HDMI computations can be found in Paller and Dyer (2004).

In 1997/1998, the HDMI was calculated for UTR-upper (Tyler Bridge Road, UTR-middle (Road C), Crouch Branch (Road 4), McQueen Branch (Z Area), Tims Branch (Road 2), and Mill Creek (Telephone Cable Road). The site in UTR-upper was a reference site, and the other sites were potentially impacted. The average HDMI was 27.4 at UTR near Road C and 22.2 at McQueen Branch. Neither value differed significantly from the average HDMI at all control sites pooled (30.4, Table 8), although the HDMI at McQueen Branch was marginal. Average HDIMs at Crouch Branch (11.8) and Tims Branch (18.2) were significantly lower than at the control sites.

The HDMI was again calculated for the UTR IOU during 2000. The average HDMI values for UTR-upper and Mill Creek, both of which were reference sites, were 34.8 and 38.0, respectively. These values were comparable to the HDMI values at the other reference sites sampled during 2000 (Table 8). The average HDMI at UTR-middle

(near Road C), which was located in a potentially disturbed area, was 32.8. This value was also relatively high and did not differ significantly from the control site values. However, average HDMI values at Crouch Branch (18.0), Tims Branch (22.5), and McQueen Branch (15.2), were significantly lower the average for the pooled control sites.

The HDMI results were similar to the MHSP results. Both methods indicated acceptable environmental quality in UTR but environmental degradation in Crouch Branch and Tims Branch. As previously described, biological degradation in Crouch Branch may have been related to elevated Cu concentrations in discharge from the H-02 outfall located upstream from Road 4. Reductions in copper concentrations in the H-02 discharge (Specht 1999) likely contributed to improvements in Crouch Branch HDMI values from 11.8 in 1997/1998 to 18.0 in 2000 (Table 7). However, the relatively low HDMI observed in 2000 indicated that either recovery was incomplete or that the macroinvertebrate community was influenced by the relatively poor habitat quality in this stream (Table 6)

Unlike Crouch Branch, the HDMI in Tims Branch exhibited relatively little change between 1997/1998 and 2000 (Table 7). The periodic release of anoxic water from upstream beaver ponds may have contributed to the depressed HDMI in Tims Branch. These beaver ponds were in the old bed of Steeds Pond just upstream of the sample area. Water flowing out of the old pond bed appeared to contain an iron hydroxide floc, which suggests that the beaver ponds were probably producing anoxic conditions that resulted in the release of ferrous iron from the pond sediments. A water sample collected just downstream from the old Steeds Pond dam indicated that total iron and aluminum were high (3620 and 1620 $\mu\text{g/l}$, respectively), but that none of the metals that were measured, including dissolved iron, exceeded EPA water quality criteria (Paller and Specht 2001).

The HBMI from McQueen Branch decreased from 1997/1998 (18.2) to 2000 (15.2) (Table 7). This change was probably related to the relocation of the sampling site from Z Area to Road F because of flooding by beaver dams near Z Area. The Road F sampling location was scoured by runoff during construction of the Defense Waste Processing Facility (DWPF) in the 1980's, was deeply channelized, and had poorer habitat than the sampling location near Z Area. It is also possible that discharges from NPDES outfalls upstream from the sampling area contributed to the low HDMI in McQueen Branch near Road F.

Hester-Dendy artificial substrates were also used to collect macroinvertebrates from the UTR IOU in 2007 (Table 1, Figure 1). Reference sites included UTR-upper, Mill Creek, and Tinker Creek as well as sites in Pen Branch, Meyers Branch, and upper Fourmile Branch. Metrics used to analyze the 2007 Hester-Dendy data included total number of taxa, number of Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT), and a biotic index (BI) that reflects the relative pollution tolerances of individual taxa. All three metrics are known to be effective indicators of environmental quality and the latter two are also used in the MHSP (SCDHEC 1998).

Numbers of EPT taxa were higher at UTR-middle and UTR-lower, both potentially impacted, than at any of the reference sites (Figure 3). Similarly, the total number of macroinvertebrate taxa was higher at UTR-middle than at any reference site and higher at UTR-lower than at all reference sites except UTR-upper (Figure 4). BI scores were lower at UTR-middle and UTR-lower than at most of the reference sites (low BI scores indicate the presence of more pollution sensitive taxa, Figure 5). These results indicate good water quality and habitat in the lower and middle portions of UTR. In contrast, number of EPT taxa, total number of taxa, and the BI in the headwaters of Crouch Branch (Road 4) and Tims Branch compared unfavorably with the reference sites. All three metrics improved in the lower reaches of Crouch Branch (near the UTR confluence). These results are similar to those of the preceding analyses which indicate good ecological health in UTR but less favorable conditions in Tims Branch and the upper reaches of Crouch Branch.

Summary for the Upper Three Runs IOU

There was little evidence that metal concentrations were unusually elevated in UTR-middle and UTR-lower, both located downstream from SRS waste sites and industrial areas. Concentrations of metals in sediments and fish in these subunits were comparable to those in reference subunits that were unaffected by SRS operations. Contaminant exposure models showed that most metals in UTR-middle and UTR-lower were not present at levels high enough to harm mammals and birds, with the possible exceptions of Hg and Al in UTR-lower. However, these two metals exceeded toxicity benchmarks in reference subunits as well as UTR-lower. Elevated levels of Hg in UTR-lower probably resulted from the aerial deposition of Hg from non-SRS sources (as shown for other streams in the Savannah River basin, EPA 1999a), coupled with the presence of wetlands in the vicinity of UTR-lower that facilitated Hg methylation. Al exceedances may have been related to naturally high Al levels in SRS soils. Bioassessments based on fish and macroinvertebrate assemblage structure confirmed the conclusion that UTR was unimpaired by metals released by SRS operations. Two sampling methods – a multiple habitat sampling protocol and Hester-Dendy artificial substrates – showed that macroinvertebrate community structure in potentially impacted reaches of UTR was comparable or superior to community structure at most reference sites. IBI values based on electrofishing samples showed that fish community structure in potentially impacted reaches of UTR was comparable or superior to that in UTR-upper, which was unaffected by SRS operations. HAI and K data provide further evidence that contamination in UTR was not adversely affecting fish from this stream.

In addition to UTR, the UTR IOU includes Tinker Creek, Mill Creek, Crouch Branch, McQueen Branch, and Tims Branch. Tinker Creek and Mill Creek were reference streams characterized by low levels of metals in sediments, fish, and water and diverse macroinvertebrate communities indicative of high ecological integrity. Macroinvertebrate and fish community data indicated that the upper reaches of McQueen Branch were ecologically degraded, but that ecological integrity improved in the lower reaches. The upper reaches were scoured by runoff, deeply channelized, and had poor habitat quality. Similarly, macroinvertebrate samples indicated ecological degradation in the upper reaches of Crouch Branch but improvement further downstream. Biological

degradation may have been related to elevated copper concentrations in discharge from the H-02 outfall. Reductions in copper levels in the H-02 discharge contributed to improvements in the Crouch Branch macroinvertebrate community over time, but it remained inferior to reference site communities as a likely result of erosion related reductions in habitat quality. The concentrations of several metals were elevated in the sediments of Tims Branch, and the contaminant exposure models for this stream showed possible risks to mammals and birds as a result of elevated Hg levels in fish. In addition, both fish and macroinvertebrate bioassessments indicated that ecological integrity was low in Tims Branch. Contributing factors may include the periodic release of anoxic water from beaver ponds located upstream of the Tims Branch sample site as well as poor physical habitat. However, elevated levels of metals in sediments and, to a lesser extent, fish make it impossible to completely eliminate the possibility of metal related toxicity in this stream.

REFERENCES

- Adams, S.M., A.M. Brown, and R.W. Goede. 1993. A quantitative health assessment index for rapid evaluation of fish condition in the field. *Transactions of the American Fisheries Society* 122: 63-73.
- Angermeier, P.L., and J.R. Karr. 1994. Biological integrity versus biological diversity as policy directives. *Bioscience* 44:690-697.
- Barbour, M.T., J. Gerritsen, B.D. Snyder, J.B. Stribling. 1997. Revision to rapid bioassessment protocols for use in streams and rivers: periphyton, benthic macroinvertebrates, and fish. U.S. Environmental Protection Agency, EPA 841-D-97-002. Washington, D.C.
- EGIS (Environmental & Geographic Information Systems). 2007. Data management plan for the integrator operable (IOU) program. Westinghouse Savannah River Company, Aiken, SC. U-DMP-G-00001.
- EPA (United States Environmental Protection Agency). 1993. Wildlife exposure factors handbook, volume I. U.S. Environmental Protection Agency, Washington, DC. EPA/600/R-93/187.
- EPA (United States Environmental Protection Agency). 2000. Total maximum daily load (TMDL) development for total fish & fish consumption guidelines in the middle and lower Savannah River watershed. ERA Region 4, Atlanta, GA.
- ERD (Environmental Restoration Division). 1999a. Ecological constituents of potential concern selection process. Savannah River Site, Aiken, SC. ERD-AG-003, P.7.2, Revision 0.

ERD (Environmental Restoration Division). 1999b. Terrestrial toxicity reference values (TRVs) (including exposure dose (ED) and hazard quotient (HQ) calculations). Savannah River Site, Aiken, SC. ERD-AG-003, P.7.3, Revision 0.

ETT (ETT Environmental, Inc.). 2006. Macroinvertebrate stream assessment of Crouch Branch. ETT Environmental, Inc., Craftsman Court, Greer, SC.

Fausch, K. D., J. R. Karr, and P. R. Yant. 1984. Regional application of an index of biotic integrity based on stream fish communities. *Transactions of the American Fisheries Society*. 113: 39-55.

Halverson, N.V. and 20 others. 1997. SRS ecology environmental information document. WSRC-TR-97-0223. Westinghouse Savannah River Company, Aiken, SC.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, I.J. Schlosser. 1986. Assessing biological integrity in running waters: a method and its rationale. Special Publication 5 of the Illinois Natural History Survey.

Klemm, D.J., Q.J. Stober, J.M. Lazorchak. 1993. Fish field and laboratory methods for evaluating the biological integrity of surface waters. U.S. Environmental Protection Agency, EPA/600/R-92/111. Cincinnati, Ohio.

Lenat, D.R. 1993. A biotic index for the southeastern United States: Derivation and list of tolerance values, with criteria for assigning water-quality ratings. *Journal of the North American Benthological Society* 12:279-290.

Mount, D.I., N. Thomas, M. Barbour, T. Norberg, T. Roush, R. Brandes. 1984. Effluent and ambient toxicity testing and in-stream community response on the Ottawa River, Lima, Ohio. Permits Division, Washington, D.C., and Office of Research and Development, Duluth, Minnesota. EPA 600/3-84-080.

Paller, M.H., Wike, L.D. 1996. Potential ecological effects of contaminants in the exposed PAR Pond sediments. Savannah River Site, Aiken, SC. WSRC-TR-96-0292.

Paller, M.H. 2001. Comparison of fish and macroinvertebrate bioassessments from South Carolina coastal plain streams. *Aquatic Ecosystem Health & Management* 4:175-186.

Paller MH, Jagoe CH, Bennett H, Brant HA, Bowers JA. 2003. Influence of methylmercury from tributary streams on mercury levels in Savannah River asiatic clams. *Science of the Total Environ* 325:209–19.

Paller, M.H. and S.A. Dyer. 2004. Biotic integrity of streams in the Savannah River Site integrator operable units. Savannah River National Laboratory, Aiken, SC. WSRC-TR-2004-00562, Revision 0.

Paller, M.H. and J.W. Littrell. 2007. Long-term changes in mercury concentrations in fish from the middle Savannah River. *Science of the Total Environment* 382:375–382.

Paller, M.H. and S.A. Dyer. 2010a. Ecological effects of metals in streams on a defense materials processing site in South Carolina, USA. *Human and Ecological Risk Assessment* 16: 1095-1114.

Paller, M.H. and S.A. Dyer. 2010b. Ecological Risk Assessment of Metals in Savannah River Tributaries on the Savannah River Site. Proceedings of the 2010 South Carolina Water Resources Conference, held October 13-14, 2010, at the Columbia Metropolitan Convention Center, Columbia, SC..

Paller, M.H., S.A. Dyer, and D.P. Coughlin. 2008. Exposure of ecological receptors to metals in the Savannah River Site Integrator Operable Units, analysis of refined data from multiple environmental media. Savannah River National Laboratory, Aiken, SC. WSRC-STI-2008-00365.

Sample, B.E., D.M. Opresko, and G.W. Suter, II. 1996. Toxicological benchmarks for wildlife. Oak Ridge National Laboratory, Oak Ridge, TN. ES/ER/TM-86/R3.

SCDHEC (South Carolina Department of Health and Environmental Control). 1998. Standard operating and quality control procedures for macroinvertebrate sampling. Technical Report No. 004-98. Bureau of Water, Division of Water Monitoring, Assessment and Protection, Aquatic Biology Section.

Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 130-137.

Yoder, C.O. and E.T. Rankin. 1995. Biological response signatures and the area of degradation value: new tools for interpreting multimetric data. Pages 263-286 in W.S. Davis and T.P. Simon (editors). *Biological assessment and criteria: tools for water resource planning and decision making*. Lewis Publishers, Boca Raton, Florida.

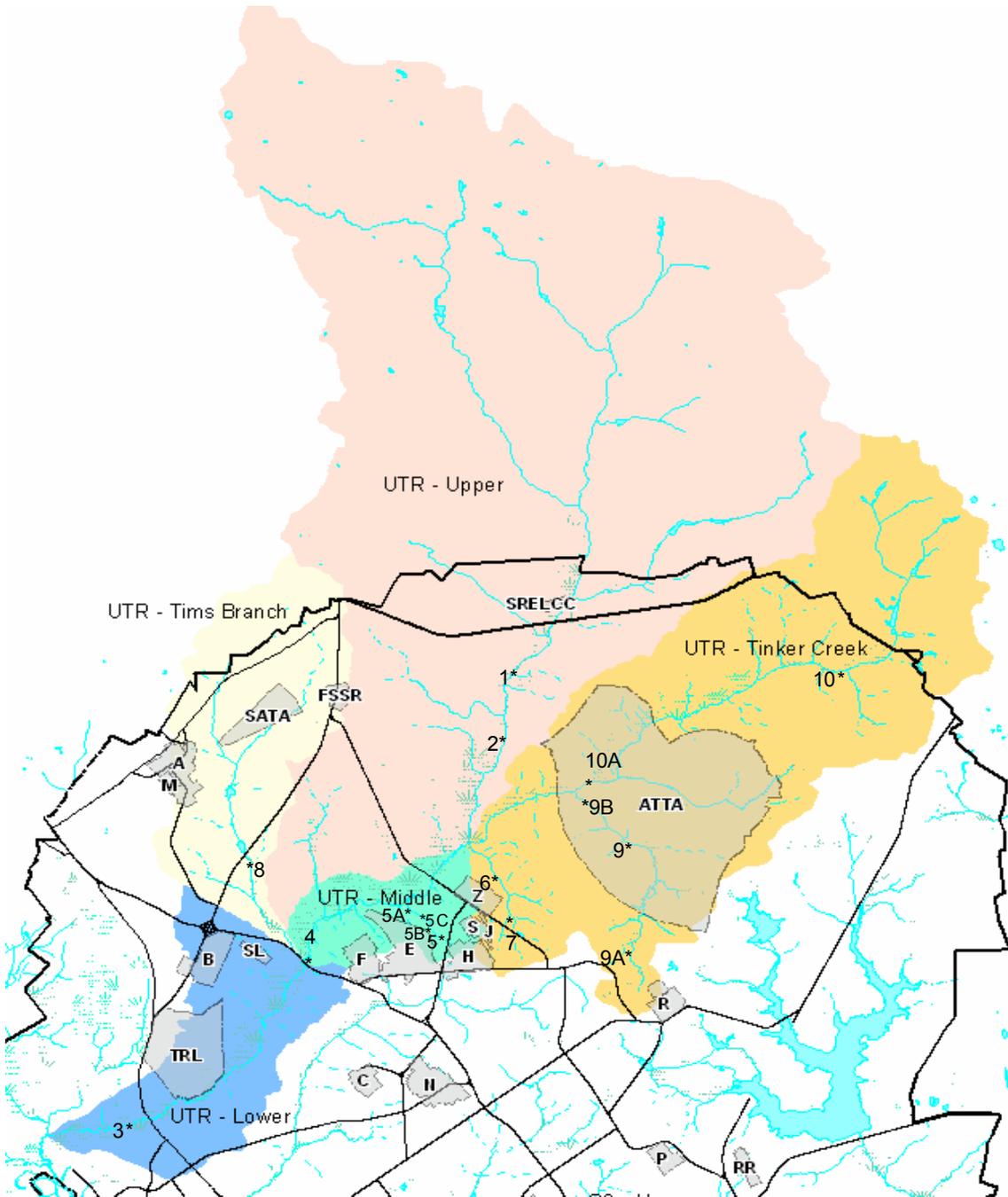


Figure 1. Map of the Upper Three Runs Integrator Operable (IOU) unit and subunits showing approximate locations of sample sites (indicated by asterisks and accompanying numbers – corresponding site names can be found in Table 1).

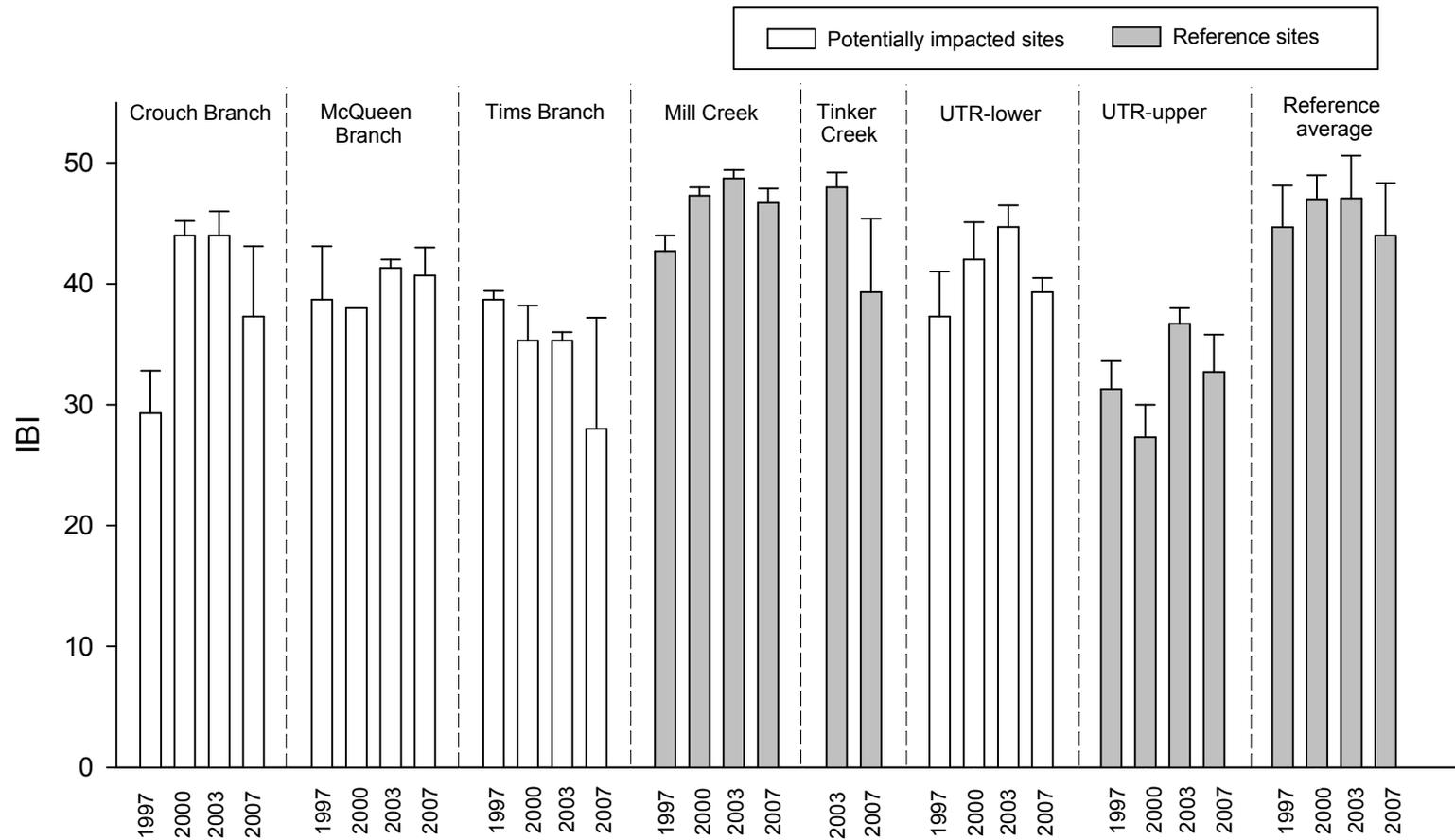


Figure 2. Average IBIs (standard deviation) for sample sites in the UTR IOU (see Table 1 and Figure 1 for more information on locations). Average IBIs at six undisturbed reference sites are included for comparison. The six reference sites included in the reference site average were located in Meyers Branch, Tinker Creek, Mill Creek, and Pen Branch. Error bars represent standard deviations.

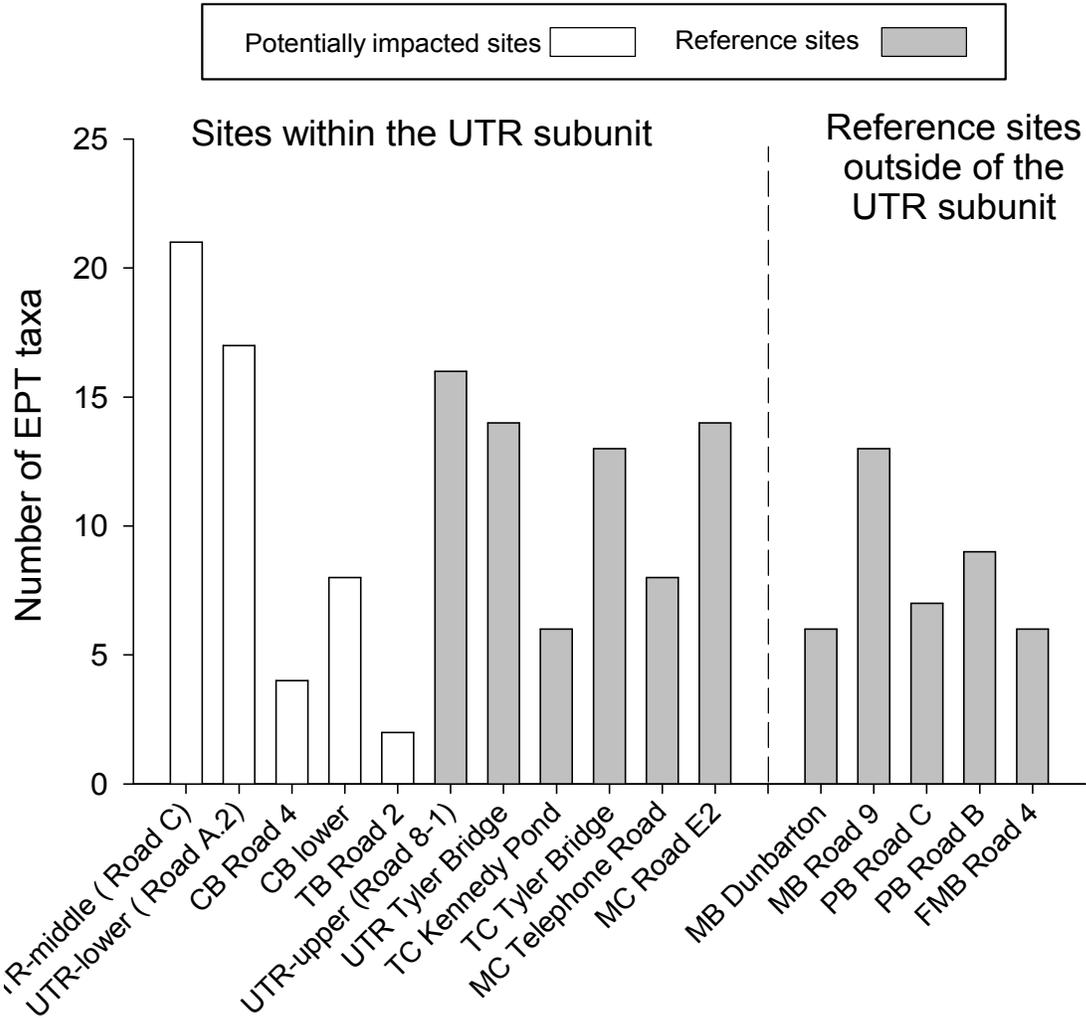


Figure 3. Number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa at sample sites in the UTR IOU during 2007. Undisturbed reference streams located outside the UTR IOU are also shown. Streams include UTR (Upper Three Runs), CB (Crouch Branch), TC (Tinker Creek), MC (Mill Creek), TB (Tims Branch), MB (Meyers Branch), PB (Pen Branch), and FMB (Fourmile Branch).

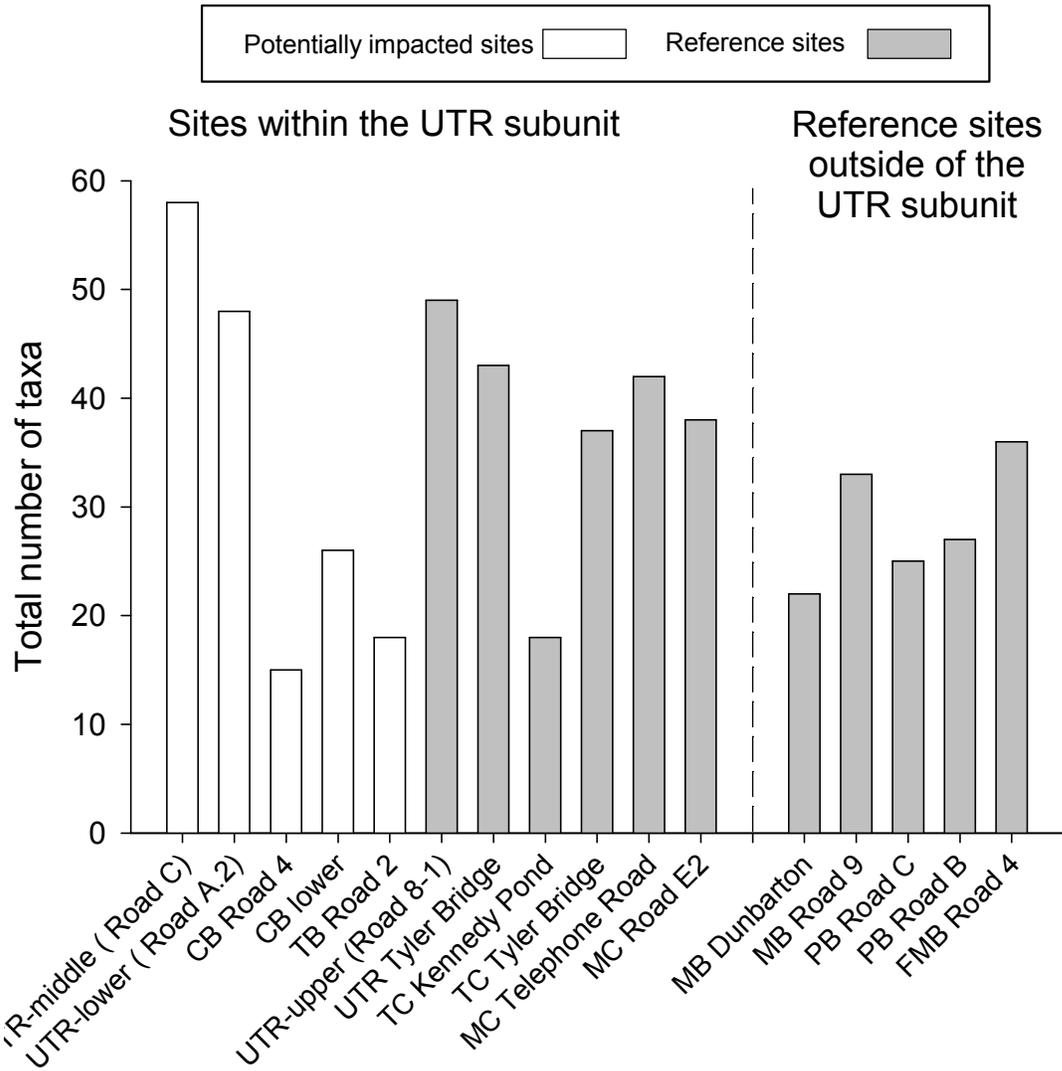


Figure 4. Total number of taxa at sample sites in the UTR IOU during 2007. Undisturbed reference streams located outside the UTR IOU are also shown. Streams include UTR (Upper Three Runs), CB (Crouch Branch), TC (Tinker Creek), MC (Mill Creek), TB (Tims Branch), MB (Meyers Branch), PB (Pen Branch), and FMB (Fourmile Branch).

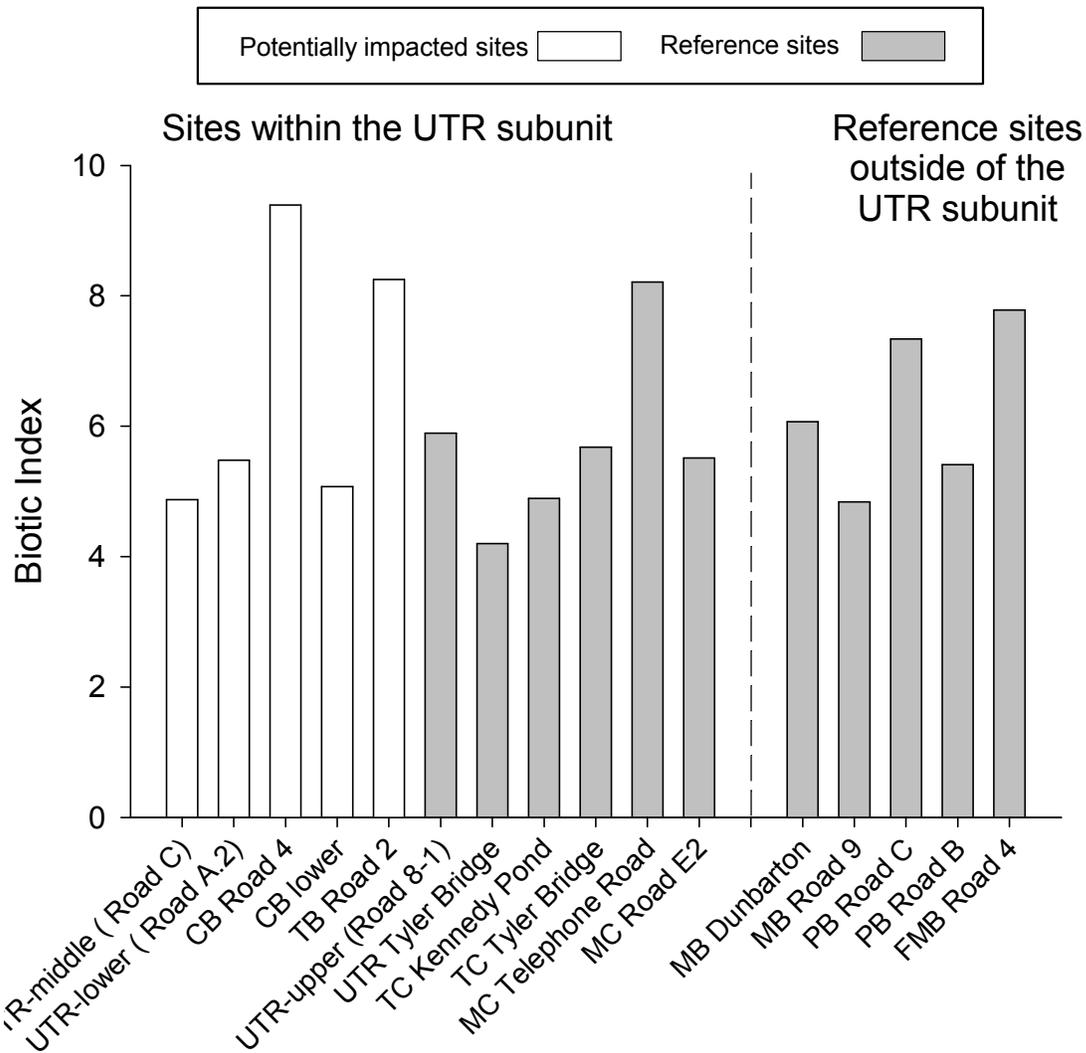


Figure 5. . Biotic index values for macroinvertebrates at sample sites in the UTR IOU during 2007. Undisturbed reference streams located outside the UTR IOU are also shown. Streams include UTR (Upper Three Runs), CB (Crouch Branch), TC (Tinker Creek), MC (Mill Creek), TB (Tims Branch), MB (Meyers Branch), PB (Pen Branch), and FMB (Fourmile Branch).

Table 1. Sample sites in the Upper Three Runs (UTR) IOU. Also indicated are the station type (reference [R] or possibly impacted by SRS waste sites [PI]), and the type of data collected at each sample station: Index of Biotic Integrity (IBI), habitat (H), tissue contaminant levels in whole fish (T), tissue contaminant levels in fish fillets (TF), fish Health Assessment Index (HAI), fish condition factors (K), Hester-Dendy macroinvertebrate index (HDMI) or Multiple Habitat Sampling Protocol (MHSP). Approximate locations of the sample sites are shown in Figure 1.

IOU subunit	Stream	Location	Type	Data collected	Year	Station number	Longitude	Latitude
UTR-upper	Upper Three Runs	Tyler Bridge Rd	R	IBI, H, T, HDMI	1998	1	-81.631	33.353
				IBI, H, HDMI	2007			
UTR-upper	Upper Three Runs	Rd 8-1	R	IBI, HDMI, H	2000	2		
				IBI, MHSP	2003			
				HDMI, H	2007			
UTR-lower	Upper Three Runs	Rd A.2	PI	IBI, H, HAI, K, T	1998	3	-81.695	33.286
				IBI, H	2000			
				IBI, H	2003			
				IBI, H, HDMI	2007			
UTR-middle	Upper Three Runs	Rd C	PI	T, HDMI	1998	4	-81.756	33.236
				HDMI	2000			
				MHSP	2003			
				HDMI, H	2007			
UTR-middle	Crouch Branch	Rd 4	PI	IBI, H, HDMI	1997	5	-81.649	33.291
				HDMI	2000			
				MHSP	2003			
				MHSP	2006			
				HDMI, H	2007			
UTR-middle	Crouch Branch	Downstream from Rd 4		MHSP	2006	5B	-81.653	33.293
UTR-middle	Crouch Branch	Above (0.5) km UTR confluence	PI	MHSP	2006	5C	-81.657	33.298

Table 1 continued.

IOU subunit	Stream	Location	Type	Data collected	Year	Station number	Longitude	Latitude
UTR-middle	Crouch Branch	Near UTR confluence	PI	IBI, H	2000	5A	-81.661	33.301
				IBI, H, MHSP	2003			
				MHSP	2006			
				BI, H, HDMI	2007			
UTR-Tinker Creek	McQueen Branch	Z Area	PI	IBI, H, HDMI	1997	6	-81.634	33.310
				MHSP	2003			
				HDMI, H	2007			
UTR-Tinker Creek	Tinker Creek	Kennedys Pond Rd	R	T	1997	10	-81.530	33.372
				IBI, H	2003			
				IBI, H, HDMI,	2007			
UTR-Tinker Creek	Tinker Creek	Tyler Bridge Rd	R	MHSP	2003	10A	-81.713	33.337
				HDMI, H	2007			
UTR-Tinker Creek	McQueen Branch	Rd F	PI	IBI, HDMI, H	2000	7		
				IBI, H	2003			
				IBI, H, HDMI	2007			
UTR-Tinker Creek	Mill Creek	Telephone cable Rd	R	IBI, H, HAI, K, HDMI	1997	9	-81.568	33.331
				IBI, HDMI, H	2000			
				IBI, MHSP	2003			
				HDMI, H	2007			
UTR-Tinker Creek	Mill Creek	Monroe Owens Rd	R	MHSP	2003	9A	-81.591	33.288
UTR-Tinker Creek	Mill Creek	Rd E-2		IBI, H, HDMI	2007	9B	-81.607	33.333
UTR-Tims Branch	Tims Branch	Rd 2	PI	IBI, H, HDMI	1997	8	-81.714	33.315
				IBI, HDMI, H	2000			
				IBI, H, MHSP	2003			
				IBI, H, HDMI	2007			

Table 2. Exposure point concentrations^a (EPCs, ppm) and sample sizes for metals in sediment from the Upper Three Runs subunits. The exposure point concentrations for six reference subunits^b are also shown for comparison.

Metal	Reference ^b		UTR-upper		UTR-middle		UTR-lower		Tinker Creek		Tims Branch	
	EPC	n	EPC	n	EPC	n	EPC	n	EPC	n	EPC	n
Al	7314	268	4060	86	6882	41	15264	10	5976	24	14074	17
Sb	2.089	246	6.09	84	0.281	31	0.321	2	16.00	21	6.186	12
As	1.119	261	0.372	87	2.292	50	5.79	8	1.814	22	2.963	16
Ba	55.2	271	22.74	86	48.66	52	84.82	10	31.12	23	83.46	16
Be	0.332	231	0.571	85	1.219	29	1.12	2	0.318	20	0.403	9
Cd	0.213	233	0.548	85	0.875	31	1.00	6	0.912	21	5.549	28
Cr	7.77	303	7.369	88	11.04	70	11.09	12	6.792	32	80.93	47
Cu	16.4	271	2.697	88	4.383	48	5.498	13	4.438	24	42.75	25
Pb	8.20	283	4.926	89	8.083	54	17.67	12	6.722	24	49.3	41
Mn	142.9	249	10.97	86	175.6	48	444.7	10	66.85	15	775.6	9
Hg	0.042	236	0.129	89	0.0402	41	0.126	11	0.0202	22	0.169	21
Ni	2.1	266	2.122	86	4.302	49	6.364	12	2.938	23	980.2	32
Se	0.681	232	1.069	87	1.35	30	0.906	3	0.615	20	0.37	8
V	17.70	244	7.534	85	32.26	43	42.45	8	22.98	24	43.38	16
Zn	20.7	257	6.032	88	111.4	28	99.08	13	69.11	24	1633	27

^aUpper 95% confidence limits (UCL) of the mean or maxima if sample size was insufficient to compute a UCL

^bReference subunits include Upper Three Runs-upper, Tinker Creek, Fourmile Branch-upper, Pen Branch-upper, Pen Branch-middle, and Meyers Branch. Data from all six were combined to produce the reference site EPCs.

Table 3. Exposure point concentrations^a (EPCs, ppm) and sample sizes for metals in fish from the Upper Three Runs subunits. The exposure point concentrations for five reference subunits^b are also shown for comparison.

Metal	Reference ^b		UTR-upper		UTR-middle		UTR-lower		Tinker Creek		Tims Branch	
	EPC	n	EPC	n	EPC	n	EPC	n	EPC	n	EPC	n
Al	49.1	8	5.49	3	16.3	3	22.5	6	**	**	**	**
Sb	0.9	10	1.2	3	0.85	3	0.974	6	0.235	2	**	**
As	1.3	13	0.22	3	0.245	3	0.53	6	0.115	2	0.24	585
Ba	25.6	13	19.9	3	2.71	3	4.125	6	4.25	2	**	**
Be	nd	2	** _c	**	**	**	**	**	**	**	**	**
Cd	0.2	13	0.03	3	0.43	3	0.25	6	0.06	2	0.012	585
Cr	0.5	13	0.28	3	0.49	3	0.225	6	0.21	2	**	**
Cu	1.0	10	0.94	3	2.3	3	0.88	6	0.74	2	0.80	585
Pb	6.4	13	0.215	3	0.245	3	0.25	6	0.115	2	0.10	585
Mn	41.6	10	6.02	3	8.97	3	8.441	6	29.2	2	36.6	585
Hg	0.2	22	0.16	15	0.08	40	0.49	28	0.03	2	0.28	585
Ni	nd	10	0.22	3	0.245	3	0.25	6	0.115	2	0.63	585
Se	11.6	13	1	3	0.81	3	0.98	6	0.51	2	**	**
V	nd	2	**	**	**	**	**	**	**	**	**	**
Zn	33.8	10	44.1	3	13.8	3	35.22	6	30.5	2	29.7	585

^aUpper 95% confidence limits (UCL) of the mean or maxima if sample size was insufficient to compute a UCL

^bReference subunits include Upper Three Runs-upper, Tinker Creek, Fourmile Branch-upper, Pen Branch-middle, and Meyers Branch. Data from all six were combined to produce the reference site EPCs.

^c“***” indicates no data available

Table 4. Exposure point concentrations^a (EPCs, ppm) and sample sizes for metals in water from the Upper Three Runs subunits. The exposure point concentrations for six reference subunits^b are also shown for comparison.

Metal	Reference ^b		UTR-upper		UTR-middle		UTR-lower		Tinker Creek		Tims Branch	
	EPC	n	EPC	n	EPC	n	EPC	n	EPC	n	EPC	n
Al	2.003	232	0.849	104	14.32	10	0.312	120	0.38	89	1.301	133
Sb	0.007	53	0.030	9	0.03	44	0.0019	8	0.003	6	0.0041	21
As	0.012	66	0.0158	17	0.003	37	0.0869	8	0.0025	6	0.0039	21
Ba	0.061	76	0.0231	25	0.216	12	0.048	8	0.0198	7	0.0581	21
Be	0.000	94	0.0002	20	0.0016	29	0.0003	28	0.0006	35	0.0006	40
Cd	0.002	233	0.0031	115	0.0012	40	0.512	148	0.004	75	0.0028	169
Cr	0.005	265	0.0032	115	0.0115	24	0.0029	161	0.0014	89	0.0066	189
Cu	0.023	281	0.0117	115	0.0111	26	0.0076	164	0.0132	122	0.01	178
Pb	0.019	253	0.0136	104	0.0231	30	0.0152	161	0.0024	106	0.0097	175
Mn	0.190	219	0.104	112	0.241	6	0.062	157	0.0713	66	0.0704	157
Hg	0.009	301	8E-05	158	4E-05	39	3E-05	204	2E-05	82	0.0002	210
Ni	0.007	255	0.0068	123	0.0071	30	0.0075	164	0.0042	88	0.0159	184
Se	0.002	74	0.0025	25	0.0131	43	0.0018	8	0.0025	6	0.006	21
V	0.005	36	0.0025	9	0.0127	22	0.0118	8	0.025	6	0.0207	21
Zn	0.505	274	0.0515	112	0.0708	16	0.0317	156	0.0946	123	0.0512	171

^aUpper 95% confidence limits (UCL) of the mean or maxima if sample size was insufficient to compute a UCL

^bReference subunits include Upper Three Runs-upper, Tinker Creek, Fourmile Branch-upper, Pen Branch-upper, Pen Branch-middle, and Meyers Branch. Data from all six were combined to produce the reference site EPCs.

Table 5. River otter contaminant exposure model results for the Upper Three Runs IOU subunits. Fifteen metals were evaluated, but only metals that exceeded the TRV (toxicity reference value) for at least one subunit are shown. Reference (R) subunits in other IOUs are also shown for comparison with potentially impacted (PI) sites. Hazard quotients (total exposure dose/LOAEL) are shown for metal exposures that exceeded the TRV.

IOU	IOU subunit	Type ^a	Al	Ba	Hg
Upper Three Runs (UTR)	UTR-middle	PI			
	UTR-lower	PI	1.3		2.6
	UTR-upper	R			
	Tims Branch ^b	PI			1.4
	Tinker Creek	R			
Fourmile Branch (FMB)	FMB-upper	R	2.0	1.1	
Pen Branch (PB)	PB-upper ^b	R			
	PB-middle	R			2.1
Savannah River Floodplain swamp (SRFS)	SRFS-upstream	R			1.4

^a R=reference, PI=potentially impacted

^b The total exposure dose was underestimated in these IOU subunits for some metals due to lack of information for at least one exposure pathway

Table 6. Belted kingfisher contaminant exposure model results for the Upper Three Runs IOU subunits. Fifteen metals were evaluated, but only metals that exceeded the TRV (toxicity reference value) for at least one subunit are shown. Reference (R) subunits in other IOUs are also shown for comparison with potentially impacted (PI) sites. Hazard quotients (total exposure dose/LOAEL) are shown for metal exposures that exceeded the TRV.

IOU	IOU subunit	Type ^a	Ba	Hg
Upper Three Runs (UTR)	UTR-middle	PI		
	UTR-lower	PI		2.1
	UTR-upper	R		
	Tims Branch ^b	PI		1.2
	Tinker Creek	R		
Fourmile Branch (FMB)	FMB-upper	R	1.1	
Pen Branch (PB)	PB-upper ^b	R		
	PB-middle	R		1.8
Savannah River Floodplain swamp (SRFS)	SRFS-upstream	R		1.2

^a R=reference, PI=potentially impacted

^b The total exposure dose was underestimated in these IOU subunits for some metals due to lack of information for at least one exposure pathway

Table 7. MHSP water quality rating scores and habitat scores for streams in the Upper Three Runs (UTR) IOU during 2003 and 2007. Reference (R) sample sites in the Fourmile Branch (FMB), Pen Branch (PB), and Steel Creek (SC) IOUs are included for comparison with potentially impacted (PI) sites.

IOU	Stream	Location	Type	Original Score*	Rating	Adjusted Score*	Adjusted Rating	Habitat Score
UTR	UTR-upper	Tyler Bridge Rd	R	3.2	Good-Fair	4.0	Good	168
	UTR-middle	Rd C	PI	4.0	Good	4.5	Good+	136
	Tims Branch	Rd 2	PI	1.0	Poor	1	Poor	95
	Crouch Branch	Rd 4	PI	1.2	Poor	1.2	Poor	81
	Crouch Branch	Near UTR	PI	2.0	Fair	2.5	Fair+	108
	Tinker Creek	Kennedy Pond Rd	R	2.7	Good-Fair	3.5	Good-Fair+	150
	Tinker Creek	Tyler Bridge Rd	R	3.5	Good -	4.5	Good+	146
	Mill Creek	Monroe Owens Rd	R	2.5	Fair +	3.0	Good-Fair	171
	Mill Creek	Telephone Cable Rd	R	3.2	Good-Fair	4.5	Good+	168
	McQueen Branch	Z Area	PI	3.5	Good -	5.0	Excellent	149
FMB	Fourmile Branch	Rd 4	R	1.5	Fair -	2.0	Fair	126
PB	Pen Branch	Rd C	R	3.5	Good -	4.8	Excellent	155
	Pen Branch	Rd B	R	2.5	Fair +	3.0	Good-Fair	160
SC	Meyers Branch	Old Dunbarton Rd	R	1.7	Fair	2.1	Fair	140

Table 7. continued.

IOU	Stream	Location	Type	Original Score*	Rating	Adjusted Score*	Adjusted Rating	Habitat Score
2007								
UTR	UTR-upper	Tyler Bridge Rd	R	3.5	Good-Fair	4.5	Good	175
	UTR-upper	Road 8-1	R	3.5	Good-Fair	4.5	Good	175
	UTR-middle	Rd C	PI	4.2	Good	5.0	Good+	159
	UTR-lower	Rd A2	PI	4.8	Good	5.0	Good+	167
	Crouch Branch	Rd 4	PI	2.0	Poor	3.0	Poor	147
	Crouch Branch	Near UTR	PI	3.5	Fair	4.5	Fair+	103
	Tinker Creek	Kennedy Pond Rd	R	2.5	Good-Fair	3.0	Good-Fair+	160
	Tinker Creek	Tyler Bridge Rd	R	4.5	Good -	5.0	Good+	175
	Mill Creek	Road E2	R	1.7	Fair +	2.5	Good-Fair	147
	Mill Creek	Telephone Cable Rd	R	1.2	Good-Fair	1.7	Good+	94
	McQueen Branch	Z Area	PI	1.3	Good -	2.0	Excellent	139
	McQueen Branch	F Area	PI	3.0	Good -	4.0	Excellent	117
PB	Pen Branch	Rd C	R	1.5	Good -	1.5	Excellent	129
	Pen Branch	Rd B	R	3.1	Fair +	4.3	Good-Fair	141
SC	Meyers Branch	Road 9	R	4.1	Fair	4.8	Fair	154
	Meyers Branch	Old Dunbarton Rd	R	3.0	Fair	4.5	Fair	165

*The original score is based on criteria established by SCDHEC (1998). The adjusted score incorporates changes to the EPT metric that reflect the effects of stream size and local conditions (see Methods and Materials for more information).

Table 8. HDMI values for sites in the Upper Three Runs (UTR) IOU. Reference (R)sample sites in the Fourmile Branch (FMB), Pen Branch (PB), and Steel Creek (SC) IOUs are included for comparison with potentially impacted (PI) sites. The highest possible HDMI scores were 35 in 1997/1998 and 40 in 2000. The column “Avg. as % of max.” represents the average HDMI as a percentage of the maximum possible HDMI for each time period.

IOU	Stream	Location	Type	Avg.	Avg. as % max.	St. err.	Max.	Min.
1997 and 1998								
UTR	UTR-upper	Tyler Bridge Rd	R	27.4	78	3.1	35	17
	UTR-middle	Rd C	PI	27.4	78	2.3	35	21
	Crouch Branch	Rd 4	PI	11.8	34	0.8	15	11
	McQueen Branch	Z Area	PI	22.2	63	3.6	29	9
	Tims Branch	Rd 2	PI	18.2	52	0.5	19	17
	Mill Creek	Telephone cable	R	32.6	93	1	35	31
FMB	Fourmile Branch	Rd F	R	12.6	36	1.6	19	11
PB	Pen Branch	Rd C	R	31	89	1.9	35	25
	Pen Branch	Rd B	R	31.4	90	1.8	35	27
SC	Meyers Branch	Old Dunbarton Rd	R	29.8	85	1.7	35	25
2000								
UTR	UTR-upper	Rd 8-1	R	34.8	87	2.1	40	26
	UTR-middle	Rd C	PI	32.8	82	1.9	38	28
	Crouch Branch	Rd 4	PI	18.0	45	2.0	24	12
	McQueen Branch	Rd F	PI	15.2	38	1.7	22	12
	Tims Branch	Rd 2	PI	22.5	56	3.4	28	14
	Mill Creek	Telephone cable	R	38.0	95	1.1	40	34
FMB	Fourmile Branch	Rd F	R	17.2	43	1.6	22	14
PB	Pen Branch	Rd C	R	35.2	88	2.1	40	28
SC	Meyers Branch	Old Dunbarton Rd	R	33.2	83	1.4	38	30