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**Ecological Effects of Metals in Streams on a Defense Materials
Processing Site in South Carolina, USA**

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ABSTRACT

The Savannah River Site (SRS) is a 780 km² U.S. Department of Energy facility near Aiken SC established in 1950 to produce nuclear materials. SRS streams are “integrators” that potentially receive water transportable contaminants from all sources within their watersheds necessitating a GIS-based watershed approach to organize contaminant distribution data and accurately characterize the effects of multiple contaminant sources on aquatic organisms. Concentrations of metals in sediments, fish, and water were elevated in streams affected by SRS operations, but contaminant exposure models for *Lontra Canadensis* and *Ceryle alcyon* indicated that toxicological reference values were exceeded only by Hg and Al. Macroinvertebrate community structure was unrelated to sediment metal concentrations. This study indicated that 1) modeling studies and field bioassessments provide a complementary basis for addressing the individual and cumulative effects of contaminants, 2) habitat effects must be controlled when assessing contaminant impacts, 3) sensitivity analyses of contaminant exposure models are helpful in apportioning sampling effort, and 4) contaminants released during fifty years of industrial operations have not resulted in demonstrable harm to aquatic organisms in SRS streams.

Keywords: ecological risk; metals; streams; nuclear site; watersheds; exposure models

INTRODUCTION

The Savannah River Site (SRS), located near Aiken SC, was established in 1950 to produce nuclear materials for national defense. Industrial operations at this 780 km² Department of Energy facility have resulted in the release of various contaminants into the SRS environment. Many of the industrial areas, waste disposal sites, and other discrete sources of contamination on the SRS have been evaluated and are being remediated. However, there are several streams on the SRS that have received and may still receive contaminants through surface and subsurface transport mechanisms, some of which are not fully evaluated or controlled. Site-specific approaches for assessing risks posed by contaminants in these streams are inadequate because the areas are very large, multiple drainages are involved, levels and types of contamination vary, and there are multiple contaminant sources in each drainage. To deal with these issues, SRS streams have been partitioned into Integrator Operable Units (IOUs) and IOU subunits that, respectively, correspond to entire streams and portions of streams. The streams are “integrators” because they have the potential to receive contaminants that can be transported by water from any source within their watershed. Organisms feeding within stream-based food chains are exposed to these contaminants, and their health is potentially affected by the extent of contamination.

The possibility of multiple types of impacts to SRS streams necessitated a broad assessment approach that included contaminant exposure models representing higher trophic levels and field studies of biota representing other ecosystem components. Probabilistic exposure models estimated potential contaminant doses and effects on ecological receptors (EPA 1993, ERD 1999a, 1999b) based on the pattern and severity of

contamination in the streams on the SRS. Bioassessments of aquatic macroinvertebrate and fish assemblages were conducted to determine the severity and extent of chemical and physical impacts and provide empirical data for comparison to the contaminant exposure model results. Both fish and macroinvertebrate assemblages were sampled because different taxonomic groups may respond differently to the same stressors (Mount et al. 1984, Paller 2001). A GIS-based watershed approach was used to organize the contaminant distribution data, accommodate the size and complexity of the SRS, and accurately characterize the effects of multiple potential contaminant sources acting within the same watershed.

The objectives of this paper are to describe the application of probabilistic contaminant exposure models on a watershed scale, compare the results of these models to bioassessments of lower trophic level organisms, and provide a perspective on the legacy of contamination resulting from more than 50 years of industrial operations at a major U.S. installation involved in the national defense industry.

MATERIALS AND METHODS

Study Area

The SRS is located in the Sand Hills ecoregion on the upper coastal plain of South Carolina. Land cover is primarily pine forest, followed by upland hardwood forest, and lowland hardwood forest. Only a small percentage of land supports industrial facilities. SRS streams are low gradient, acidic to neutral in pH, and have sandy substrates often covered with woody debris (Paller and Dyer 2003). There are five major stream drainages on the SRS: Upper Three Runs (UTR), Fourmile Branch (FMB), Pen Branch (PB), Steel Creek (SC), and Lower Three Runs (LTR) (Figure 1). Each drainage

constitutes an IOU, and each IOU is subdivided into subunits representing ecologically distinct stream reaches (16 in total) for risk assessment (Table 1). Six subunits had no waste sites or industries in their drainages and were considered reference (R) subunits that represented ambient conditions. The remaining subunits were considered potentially impacted (PI) because they received various types of discharges and/or had waste sites within their drainage boundaries.

Contaminant Exposure Models

Analyses were based on metal concentrations in surface (0 –15 cm) wetland sediments, surface water, fish and crayfish. Al, Sb, As, Ba, Be, Cd, Cr, Cu, Pb, Mn, Hg, Ni, V, and Zn were evaluated because they were involved in SRS operations and identified as possible problems in preliminary screening studies. Data on the environmental concentrations of these metals were collected by numerous organizations for various purposes and organized in a geographic information system data base that provided information on data provenance, sampling and analysis dates, sampling locations, data quality, and other factors (EGIS 2007). Data were intensively scrutinized for analytical problems and other quality control issues before entry into the data base (EGIS 2007). Samples reported in this paper were collected between 1990 and 2005. Preliminary analyses indicated an absence of temporal trends during this time period that would necessitate partitioning by time for accurate analyses of potential environmental effects.

Water and sediment data were available for 16 subunits, fish data for 14 subunits, and crayfish data for five subunits. Sample sizes (i.e., number of measurements) varied among media and IOU subunits. Sample sizes for surface water and sediment were

usually large (often over 100). Sample sizes for fish were low (e.g., 2-6) in subunits where fish were composited (often >10 fish per sample) but sometimes exceeded 100 in subunits where fish were analyzed individually. Crayfish were analyzed individually, and sample sizes ranged from 16-35 per subunit. The IOU subunits described herein were represented by nearly 49,000 individual metal measurements.

Fish and crayfish concentrations were usually derived from whole organisms, sediment concentrations from bulk sediment, and water concentrations from unfiltered water. Elemental concentrations were usually determined by ICP-MS, ICP-AES (or cold vapor methods for Hg). Analytical methods generally followed EPA protocols (e.g. EPA 1995). Surface water measurements were often below detection limits, and multiple detection limits sometimes occurred. Differences in chemical or physical state that could affect metal toxicity were not evaluated because 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 was assumed to be methylmercury).

Exposure point concentrations (EPCs) were calculated to represent the metal concentrations in each medium from each subunit. Three types of EPCs were used based on sample size and amount of 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).

Multivariate ordination methods were used to compare EPCs between the PI and R subunits. Principal components analysis (PCA) is an “unconstrained” method that objectively identifies the strongest patterns in the data, regardless of their source. Redundancy analysis (RDA) is a “constrained” ordination technique that identifies patterns associated with specific explanatory variables (Jongman et al. 1995). PCA was used as an exploratory tool to identify the predominant patterns of metal contamination among subunits regardless of their cause followed by RDA to specifically investigate patterns related to differences between the PI and R subunits. Monte Carlo permutation tests were used to determine whether differences between the PI and R subunits were significant ($P < 0.05$) Input data for the ordinations consisted of the EPCs for each metal in each medium in each subunit.

Differences in metal concentrations between PI and R subunits were also compared with univariate Mann-Whitney Wilcoxin or Gehan tests (ProUCL, Sing and Sing 2007). The Gehan test was used more frequently because it is better for data with multiple detection limits. However, the Mann-Whitney Wilcoxin test was used when the number of metal measurements was less than 10 because the Gehan test requires comparatively large samples (EPA 2001). The total number of tests was 45 (15 metals

in three media: sediment, fish, and water), but an experiment-wise error rate was not computed because the tests were intended to identify potentially problematic constituents (i.e., type II error would be more serious than type I).

The preceding analyses identified metals that were present in elevated concentrations and might cause ecological risks. The risks were evaluated with contaminant exposure models developed for the river otter *Lontra Canadensis* and the belted kingfisher *Ceryle alcyon*. Both are locally common, 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 subunits, which were relatively large. 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 relatively insignificant and insufficiently understood to properly evaluate.

Estimated composition of the otter diet was 65% fish, 15% invertebrates, 10% herptiles, 5% birds, and 5% mammals (EPA 1993). Estimated composition of the kingfisher diet was 70% fish, 15% amphibians and reptiles, and 15% invertebrates (EPA 1993). Invertebrates consumed by the otter and kingfisher were assumed to be crayfish represented by *Cambarus latimanus* and *Procambarus* spp collected from the IOU subunits. No assumptions were made regarding the species and size of fish consumed, but the fish collected for tissue analysis were generally among the larger species and individuals within 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):

$$CR = C_{\text{animal}}/C_{\text{soil or sediment}} \text{ where:} \quad 1$$

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 SRS reservoir (Paller and Wike 1996). The CRs computed for mammals were also used for birds.

Data were available for crayfish from only five IOU subunits. Metal concentrations in crayfish from other subunits were estimated from the relationship between metal concentrations in crayfish and fish from the five subunits with both types of data:

$$CR = C_{\text{crayfish}}/C_{\text{fish}} \text{ where:} \quad 2$$

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 .

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 similar 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:

$$ED_{total} = \sum_{i=1}^n ED_{food\ i} + ED_{water} + ED_{soil}, \text{ where:} \quad 3$$

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

$ED_{food\ i}$ = 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). ED_{total} was compared with lowest observed adverse effect levels (LOAELs) representing the lowest metal concentrations that caused adverse chronic effects (ERD 1999b, Sample et al. 1996). LOAELs rather than more conservative NOAELs were used to more realistically estimate risks. Model details are presented in Paller et al. (2008).

The contaminant exposure models were used to compute probabilistic and deterministic risk estimates. Probabilistic estimates were derived using Monte Carlo methods (Decisioneering, Inc. 2001) in which metal concentrations and CRs were represented as probability distributions. The metal distributions were modeled after histograms of the concentration data (with values below the detection limit set equal to half the detection limit), and the probability distributions for the CRs were assumed to be normal (but truncated at the minimum and maximum CRs observed in the field). Where

significant correlations occurred among different media, the simulation was constrained so that values were selected based on the correlations among variables. Other model parameters (ingestion rates, LOAELs, and receptor body weights) were represented as point estimates because their probability distributions were unknown. Sensitivity analyses were conducted by calculating the rank correlation between input variable values and model output values. All PI stream subunits were combined and all R stream subunits were combined for the probabilistic evaluations. Deterministic evaluations were conducted for individual PI and R subunits. Lastly, hazard quotients (HQ: $ED_{\text{total}}/\text{LOAEL}$) for each metal in each subunit were ordinated using PCA to depict cumulative risks among subunits.

Macroinvertebrate and Fish Assemblages

Macroinvertebrates were collected using artificial substrates (Hester-Dendy multiplate samplers) and a timed natural substrate sampling protocol. Each Hester-Dendy artificial substrate consisted of fourteen 7.6 cm plates separated by about 0.3-1.0 cm (total surface area of 0.179 m²). The samplers were suspended above the bottom and retrieved after a 28 day colonization period during the summer and fall of 2007. Organisms were usually identified to genus. Hester-Dendy samplers were deployed at one to four sites in each subunit (including 10 reference sites), and five samplers were deployed per site. Analyses of the Hester-Dendy data were based on five metrics previously shown to be informative of environmental quality: total taxa richness; number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa; a biotic index (BI) based on the pollution tolerances of individual taxa (Lenat, 1993), total density of all organisms, and total biomass (i.e., ash-free dry weight) of all organisms (Paller et al. 1997).

A natural substrate sampling protocol similar to that described in SCDHEC (1998) was used to collect macroinvertebrates during the summer and fall of 1997, 2000, and 2003. Organisms were collected from natural substrates during three man-hours of effort at each site using a D-frame dip net, kick net, hand sieve, white plastic pan and fine mesh sampler. The objective was to collect as many taxa as possible and accurately represent species composition. Analysis of the natural substrate sampling data was based on five metrics that indicate environmental quality: total taxa richness, number of EPT taxa, percent clingers (a behavioral group sensitive to sedimentation), percent ephemeroptera, and total number of organisms (Maxted et al. 2000). Habitat quality was assessed in conjunction with natural substrate sampling using methods similar to SCDHEC (1998). Variables included stream width and depth, epifaunal substrate, pool substrate, pool variability, sediment deposition, channel flow status, channel alteration, channel sinuosity, bank stability, vegetative protection, and riparian vegetation. These variables (excluding width and depth) were combined to produce a summary score (HABSCOR) indicative of overall habitat quality (SCDHEC 1998).

Fish assemblage data were collected from one to three locations in each subunit including four reference locations. Three 50 m stream segments (sufficient to adequately represent species diversity and composition, Paller 1995) were electrofished at each location during the summer and fall of 2003 and 2007. Smaller streams were electrofished with a generator or battery powered DC electrofisher, and larger streams were electrofished with a boat-mounted DC electrofisher. A single pass was made through each 50 m segment, and all microhabitats were sampled. Fish were usually identified to species. Analysis of the fish assemblage data was based on nine metrics

known to be reflective of stream biotic integrity: total species richness; numbers of Cyprinidae, benthic insectivore, and piscivore species; percentages of tolerant, Cyprinidae, Centrarchidae, and generalized insectivore species; and fish density (Paller et al. 1996).

Multivariate differences in biotic metrics among sample sites were analyzed with PCA and RDA, with the former used for exploratory analyses and the latter for statistically testing relationships among biotic metrics, habitat, and metal concentrations in sediment (indicated by EPCs). Sediment metal data were used because they constituted the most complete contaminant data set and were likely most representative of differences in metal contamination among the IOU subunits. Partialling techniques with habitat quality and stream size as covariables were used to assess the independent effects of sediment metal concentrations on biotic metrics. The significance of these effects was assessed using montecarlo permutation tests (Jongman et al. 1995).

RESULTS

Metal Concentrations

Metal concentrations varied among media but were usually highest in sediment and lowest in water (Table 2). Concentrations in fish were usually lower than in sediments, except for Hg, which is highly bioaccumulative. Concentrations of Al, As, Ba, Cu, and Mn were higher in crayfish than fish (Table 3), probably because of physiological or ecological differences between these organisms; e.g., copper is a component of the abundant respiratory protein, hemocyanin, in crayfish. In contrast, Hg concentrations were generally lower in crayfish than fish, possibly because many fish feed at higher trophic levels and reach older ages than crayfish resulting in greater Hg

bioaccumulation (EPA 1997). Metal concentrations in water were typically low compared with the other media.

Univariate tests of the sediment data showed that concentrations of most metals were significantly ($P < 0.05$) higher in the PI subunits than the R subunits (Table 2). Tests of the fish tissue data showed that Al, Cr, Cu, Mn, Hg, Ni, and Zn were significantly higher in the PI subunits than the R subunits, and tests of the water data showed that Al, Cd, Cr, and Zn were significantly higher in the PI subunits than the R subunits.

The multivariate technique of PCA sometimes elucidates different patterns than RDA, because RDA may miss variability that is unrelated to constraining environmental variables. However, both methods indicated that the strongest patterns in the data were associated with differences between the PI and R subunits, so only the more informative RDAs are presented. The RDA for the sediment data indicated that metal concentrations in the PI subunits were significantly ($P = 0.006$) higher than in the R subunits (FMu, PBm, PBu, TNK, MB, and UTu). This difference accounted for 23% of the variability in the metal data (x-axis in Figure 2). Metals that contributed the most to the difference between PI and R subunits (i.e., at least 23% of their variability was related to this distinction) were As, Cd, Cr, Hg, Mn, Ni, and Zn. Additional variability in the data (y-axis) was related to differences among the PI subunits with LTI, LTm, TMS, FMu, and SCu having higher metal levels than UTI, SCl, IGB, FMI, and UTm.

The fish tissue RDA indicated that metal concentrations were significantly ($P = 0.026$) higher in the PI subunits than the R subunits, paralleling the results of the sediment RDA. The difference between the R and PI subunits accounted for 24% of the variability in the metal data. Metals most responsible for the difference among groups

(i.e., at least 24% of their variability was related to the group distinction) were Ni, Cr, Cu, Hg, Pb, and Zn. UTu and UTm separated from the other PI subunits, as also observed in the sediment RDA, indicating that they were less contaminated than the other PI subunits. UTu and UTm are part of the Upper Three Runs drainage, which is large, relatively undeveloped, and receives (or received) contaminants indirectly from tributary streams rather than directly. Two R subunits (PBU and MB) were excluded from the fish tissue RDA because they lacked data for some metals.

The RDA based on water data did not show a significant difference between the PI and R subunits. The prevalence of censoring in the water data likely contributed to this result.

Contaminant Exposure Models

The exposure doses for most metals were higher in the PI subunits than in the R subunits, as shown by the probabilistic otter model (Figure 3). However, with the exceptions of Hg and Al, few doses (0 to 1%) exceeded the LOAEL indicating a lack of significant risk in the PI subunits for most metals. In the otter model 88% of the estimated doses for Hg exceeded the LOAEL in the PI subunits and 19% exceeded the LOAEL in the R subunits. The probabilistic kingfisher model produced similar results for Hg (79.2% exceedance in the PI subunits and 12.8% in the R subunits). Deterministic analyses based on EPCs for individual subunits were used to assess the spatial pattern of Hg doses. Hg exposure doses estimated by the otter model exceeded the LOAEL in one R subunit and seven PI subunits. The highest doses were in the Steel Creek and Lower Three Runs subunits where they were 4.4 – 6.7 times the LOAEL compared with 1.2- 3.2 times the LOAEL in the other subunits. The kingfisher model indicated that Hg

exceeded the LOAEL in one R subunit and seven PI subunits. Exposure doses were 3.7 – 5.5 times the LOAEL in the Steel Creek and Lower Three Runs subunits compared with 1.2 – 2.6 times the LOAEL in the other subunits.

Al exhibited fewer LOAEL exceedances than Hg: 48.5% in the PI sites and 14.6% in the R sites, as shown by the otter model. There were no Al exceedances in the kingfisher model because the avian TRV for Al was relatively high (1100 compared with 19.3 for mammals). The Al exposure doses estimated by deterministic otter models exceeded the LOAEL in one R subunit and seven PI subunits. Hazard quotients for Al (maximum 3.1) were generally lower than for Hg (maximum 6.7) and did not show a pattern among drainages.

Sensitivity analyses of the contaminant exposure models showed that the most important source of mercury exposure was fish consumption (78% of the total dose for both species). Important sources for Al exposure were crayfish (34.9%), sediment (31.4%), and fish (28.3%) consumption. Crayfish were also important for Cu and Mn, and fish were also important for Zn. Contaminant levels in sediment had the strongest influence on the model results for the remaining metals (Figure 4). The influence of contaminant levels in sediment was mediated by the consumption of birds and mammals rather than sediments (with the exception of Al which occurred in high levels in the sediments). Generally, media with high and varying contaminant concentrations relative to other media determined the output of the exposure models.

Cumulative effects constitute a significant consideration when multiple potential stressors co-occur. A simplifying and conservative assumption that can be used to assess the cumulative effects of chemicals is that toxicity is additive (EPA 1998), although even

metals present in toxicologically insignificant concentrations can produce an additive HQ in excess of one if the number of metals is large. The additive HQ was greatest in the Lower Three Runs subunits (LTl and LTm) and least in the Tinker Creek (TC) and Upper Three Runs (UTu, UTm, and UTl) subunits (Figure 5). The additive HQ for most subunits exceeded one especially in the more contaminated PI subunits, where it reached 10.4. It is unlikely that these additive HQs accurately measured total risk, although they are useful in comparing relative risks among locations.

Macroinvertebrate and Fish Bioassessments

PCA of five metrics that represented macroinvertebrates collected from natural substrates indicated that all except % Ephemeroptera were strongly correlated with PCA Axis 1 which accounted for the majority (66.4%) of the variance in the metric data. The Pearson correlation with Axis 1 was 0.83, 0.93, 0.95, 0.35, 0.94, and -0.73 for total number, taxa number, EPT, % Ephemeroptera, % clinger and BI, respectively (Figure 6). Axis 1 was also correlated with stream width and HABSCOR ($r = 0.70$ and 0.72 , respectively), and less strongly with sediment metal concentrations (represented by UCIs, $r = -0.07$ to -0.44), suggesting that habitat had a greater influence than metal concentrations on the metric values. Relationships among the macroinvertebrate metrics, habitat, and sediment metal levels were investigated further with RA employing width and HABSCOR as covariables, thereby permitting a test of the independent effects of sediment metal concentrations. The results showed that the amount of variation in the macroinvertebrate metrics explained by sediment metal concentrations was 24.8% compared with 45.0% by stream width and HABSCOR. A Monte Carlo permutation test showed that sediment metal concentrations did not have a statistically significant effect

(at $P < 0.05$) on the macroinvertebrate metrics after the effects of stream width and HABSCOR were removed.

Analyses similar to those described above were conducted on the five macroinvertebrate metrics representing the Hester-Dendy artificial substrate data. PCA Axis 1, which accounted for 59.5% of the variance in the metric data, was strongly correlated with HABSCOR ($r = -0.63$) but not stream width ($r = -0.02$). Correlations between Axis 1 and sediment metal concentrations ranged from -0.26 to 0.47. RA showed that the amount of variation in the Hester-Dendy metrics explained by sediment metal concentrations was higher (39.4 %) than with the natural substrate metrics, but not statistically significant (at $P < 0.05$) after the effects of HABSCOR were removed.

A third set of analyses showed that stream width and HABSCOR accounted for a small proportion of the variance in the fish assemblage metrics (11.8%), and that the variance explained by sediment metal concentrations (59.2%) was significant ($P < 0.05$). This was the result of comparatively high metal concentrations in two subunits, SCu and TB, both of which suffered from environmental degradation (not represented in the variable HABSCOR) that could also affect fish community structure. SCu was located just upstream from a 4.0 km² reservoir, which may have reduced the number of fish species (because of isolation from downstream source pools), and TB was affected by the release of anoxic water from upstream beaver ponds (Paller and Dyer 2003).

DISCUSSION

There were various sources of uncertainty in the contaminant exposure models including the accuracy and appropriateness of the concentration ratios, contaminant ingestion rates, and TRVs; interactions among metals; and environmental factors that

could affect metal speciation and toxicity. Where possible, uncertainties were addressed by assuming the worst case (e.g., all metals were bioavailable and in their most toxic form). A study such as this that relies on data collection by multiple sources for multiple purposes rather than on purpose-driven data collection also has a relatively high level of uncertainty regarding data quality. Possible problems include instrument error, variable detection limits, method error, recording error, questions about the representativeness of samples, and insufficient data for some locations and media. These issues, except for the last, were addressed by the iterative application of rigorous data quality control procedures prior to and during data analysis.

Despite possible quality control problems, comprehensive environmental data sets, when available, offer advantages, especially when risks must be evaluated for a large area with multiple known and possibly undiscovered sources of contamination. First, they leverage existing resources thereby eliminating or reducing much of the cost associated with sample collection and analysis. Second, the impartiality that results from the inclusion of all known data produces credibility in the regulatory and public venues that adjudicate environmental issues. Last, the use of data from multiple sampling programs with different and often overlapping spatial coverage decreases the possibility of missing impacts resulting from unknown contamination sources, which could occur with more limited data collection efforts that focused on known contaminant sources.

The contaminant exposure models showed that most metals in SRS streams were not present in high enough concentrations to individually pose significant threats to mammals and birds. The most important exception was Hg, which occurs in relatively high levels in fish throughout the Savannah River basin as a result of atmospheric

deposition (EPA 2000). Although atmospheric deposition undoubtedly contributed to the Hg exceedances in the SRS subunits, the most and highest exceedances occurred in SC and LTR. Savannah River water contaminated with mercury from industries located upstream of the SRS (Paller and Littrell 2007) was formerly pumped through these streams for reactor cooling purposes. SRS streams that received reactor cooling water from the Savannah River typically have elevated levels of Hg in biota compared with streams that were not used for reactor cooling (Newman and Messier 1994). Thus, while elevated Hg levels are not likely a direct result of SRS industrial processes, they may be partly related to the former use of contaminated Savannah River water by the SRS.

Al was the only other metal that resulted in significant TRV exceedances (for the otter only). However, the second highest HQ for Al (2.0) occurred in a reference subunit suggesting that the Al exceedances may have been unrelated to SRS operations. The lack of significant differences in sediment Al concentrations between the PI and R subunits supports the same conclusion (Table 2). Al speciation can significantly affect toxicity (Klöppel et al. 1997) raising 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).

A weakness of the contaminant exposure models was their inability to assess cumulative effects because potential mechanisms of cumulative metal toxicity are poorly understood. Nor was it possible to empirically assess these effects (e.g., by conducting physiological studies on otters) for practical reasons. Therefore, the surrogate variables of macroinvertebrate and fish community structure were used to assess possible cumulative effects. Although effects on fish and macroinvertebrates cannot be directly

extrapolated to the otter and kingfisher, both taxonomic groups are sensitive to metals and practical to sample.

Assessment of metal effects on macroinvertebrates and fish was complicated by habitat degradation, which was more prominent in some of the more contaminated streams. These streams were formerly subjected to extreme flow perturbations and other habitat modifications as well as metal contamination, which raised the possibility of conflating habitat and metal related impacts. It was also important to consider the effects of stream size, which is an important natural determinant of biotic structure (Minshall et al. 1985) that could obscure the detection of contaminant related effects.

Macroinvertebrate metrics were uncorrelated with sediment metal concentrations after these variables were accounted for. In contrast, fish community metrics were related to sediment metal concentrations, although these relationships were confounded by co-occurring habitat factors in SCu and TB that could also have depressed fish community structure. Discrimination between habitat and metal related effects on fishes in these streams will require more studies.

The SRS IOU studies support several conclusions:

- 1) Modeling studies and empirical bioassessments are complimentary and provide a basis for addressing the individual and cumulative effects of contaminants at multiple trophic levels.
- 2) It is important to control for the effects of habitat alterations when assessing contaminant impacts because contaminated ecosystems often suffer from physical habitat degradation.

- 3) Sensitivity analyses of contaminant exposure models can be helpful in efficiently apportioning data collection efforts. Sediment and fish contaminant data were far more informative than water data in this study, and crayfish contaminant data provided important model input for selected metals.
- 4) Over 50 years of operations at the SRS have resulted in increased metal concentrations in some streams, but these increases have not caused large-scale degradation and are unlikely to significantly affect populations of organisms that utilize stream ecosystems. Hg may be an exception for higher trophic level organisms, although elevated Hg levels were more likely the result of atmospheric deposition and pumping contaminated Savannah River water into SRS streams than a direct result of mercury releases by SRS operations.

Cumulative effects of multiple stressors including metals, radionuclides, and habitat alterations are still under evaluation on the SRS using multiple lines of evidence; and efforts are continuing to refine the IOU database and fill data gaps that contribute to uncertainty in the contaminant exposure models. The results of these studies will be presented in future reports.

REFERENCES

- Decisioneering, Inc. 2001. Crystal Ball 2000.2. Denver, Colorado, USA
- EGIS (Environmental & Geographic Information Systems). 2007. Data management plan for the integrator operable (IOU) program. U-DMP-G-00001. Westinghouse Savannah River Company, Aiken, SC, USA
- EPA (US Environmental Protection Agency). 1993. Wildlife exposure factors handbook, volume I. EPA/600/R-93/187. US Environmental Protection Agency, Washington, DC, USA
- EPA (US Environmental Protection Agency). 1995. Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods (SW-846), 3rd edition, US Environmental Protection Agency, Washington DC, USA
- EPA (US Environmental Protection Agency). 1997. Volume III: Fate and transport of mercury in the environment. EPA-452/R-97-005. US Environmental Protection Agency, Washington DC, USA
- EPA (US Environmental Protection Agency). 1998. Guidelines for Ecological Risk Assessment. EPA/630/R-95/002F. US Environmental Protection Agency, Washington DC, USA
- EPA (US Environmental Protection Agency). 2000. Total maximum daily load (TMDL) development for total fish & fish consumption guidelines in the middle and lower Savannah River watershed. US Environmental Protection Agency (EPA), Region IV, Atlanta, GA, USA
- EPA (US Environmental Protection Agency).. 2001. Guidance for characterizing background chemicals in soil at superfund sites. EPA 540-R-01-003. Office of

Emergency and Remedial Response, US Environmental Protection Agency,
Washington DC, USA

ERD (Environmental Restoration Division). 1999a. Ecological constituents of potential concern selection process. ERD-AG-003, P.7.2, Revision 0. Savannah River Site, Aiken, SC, USA

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

Jongman RHG, Ter Braak CJF, and van Tongeren OFR. (eds). 1995. Data analysis in community and landscape ecology. Cambridge University Press, Cambridge, USA

Klöppel H, Fließner A, Kordel W. 1997. Behaviour and ecotoxicology of aluminum in soil and water - review of the scientific literature. *Chemosphere* 35:353-363

Maxted JR, Barbour MT, Gerritsen J, Poretti V, Primrose N, Silvia A, Penrose D, and Renfrow R. 2000. Assessment framework for mid-Atlantic coastal plain streams using benthic macroinvertebrates. *J N Am Benthol Soc* 19:128-144

Minshall GW, Petersen RC Jr. and Nimz CF. 1985. Species richness in streams of different size from the same drainage basin. *Am Nat* 125: 16-38

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

- Newman MC, Messier A. 1994. Mercury bioaccumulation in mosquitofish (*Gambusia holbrooki*) sampled on and around the Savannah River Site. SREL-48 UC-66e.
Savannah River Ecology Laboratory, Aiken, SC, USA
- Paller MH. 1995. Relationship among number of fish species, reach length, and sampling effort in South Carolina coastal plain streams. *N Am J Fish Manage* 15:110-120
- Paller MH, Reichert MJM, Dean JM.. 1996. Use of fish communities to assess environmental impacts in South Carolina coastal plain streams. *T Am Fish Soc* 125:633-644
- Paller MH, Wike LD. 1996. Potential ecological effects of contaminants in the exposed PAR Pond sediments. WSRC-TR-96-0292. Savannah River Site, Aiken, SC, USA
- Paller MH, and Specht WL. 1997. A multimetric index using macroinvertebrate data collected with artificial substrates. *J Freshwater Ecol* 12:367-378
- Paller MH. 2001. Comparison of fish and macroinvertebrate bioassessments from South Carolina coastal plain streams. *Aquat Ecosyst Health* 4:175-186.
- Paller MH, and Dyer SA.. 2003. Biotic integrity of streams in the Savannah River Site integrator operable units, 1996 to 2001. WSRC-TR-2003-00149, Savannah River National Laboratory, Aiken, SC
- Paller MH, Littrell JW. 2007. Long-term changes in mercury concentrations in fish from the middle Savannah River. *Sci. Total Environ.* 382:375–382
- Paller, MH, Dyer SA, Coughlin DP. 2008. Exposure of Ecological Receptors to Metals in the Savannah River Site Integrator Operable Units. WSRC-STI-2008-00365.
Savannah River National Laboratory, Aiken, SC, USA

Sample, BE, Opresko DM, Suter, II GW. 1996. Toxicological benchmarks for wildlife.

ES/ER/TM-86/R3. Oak Ridge National Laboratory, Oak Ridge, TN

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

Sing A, Sing AK. 2007. ProUCL Version 4.0 Technical Guide. EPA/600/R-07/041.

US Environmental Protection Agency Office of Research and Development,

Washington, DC, USA

Table 1. Savannah River Site IOUs and IOU subunits. “R” refers to reference subunits and “PI” to subunits that were potentially impacted by SRS waste sites or industrial discharges.

Stream drainage (IOU)	Stream reach or tributary (IOU subunit)	Abbreviation	Type	Watershed Surface area (km ²)	Stream Length (km)
Upper Three Runs	upper	UTu	R	178.3	12.1
	middle	UTm	PI	110.2	20.6
	lower	UTl	PI	32.1	10.1
	Tinker Creek	TC	R	133.4	24.2
	Tims Branch	TMS	PI	43.0	10.7
Fourmile Branch	upper	FMu	R	11.6	4.7
	middle	FMm	PI	30.7	8.7
	lower	FMI	PI	15.5	6.7
Pen Branch	upper	PBu	R	16.6	4.4
	middle	PBm	R	12.9	6.4
	lower	PBl	PI	16.0	5.6
	Indian Grave Branch	IGB	PI	12.4	4.6
Steel Creek	upper	SCu	PI	8.5	3.3
	lower	SCl	PI	12.9	5.9
	Meyers Branch	MB	R	54.9	12.6
Lower Three Runs	middle	LTm	PI	96.6	11.2
	lower	LTl	PI	279.2	17.7

Table 2. Exposure point concentrations (ppm) for metals in sediment, fish, and water in potentially impacted (PI) and reference (R) subunits on the Savannah River Site. Asterisks indicate metals with significantly ($P \leq 0.05$) higher concentrations in the PI subunits based on Gehan ($n > 10$ for PI and R) or Mann-Whitney Wilcoxin ($n < 10$ for PI or R) tests.

Metal	Sediment		Fish		Water	
	PI	R	PI	R	PI	R
Al	9434.0	7314.0	89.7	49.1*	3.141	2.003*
Sb	11.5	2.1	0.6	0.9	0.004	0.007
As	3.9	1.1*	0.3	1.3	0.005	0.012
Ba	134.1	55.2*	19.5	25.6	0.060	0.061
Be	0.7	0.3*	<0.1	0.25	0.001	0.000
Cd	1.6	0.2*	0.1	0.2	0.063	0.002*
Cr	52.1	7.8	1.7	0.5*	0.007	0.005*
Cu	70.0	16.4	2.5	1.0*	0.019	0.023
Pb	418.5	8.2*	1.4	6.4	0.014	0.019
Mn	633.9	142.9*	94.3	41.6*	0.163	0.190
Hg	0.5	<0.1*	0.9	0.2*	0.001	0.009
Ni	104.1	2.1*	1.7	0.22*	0.008	0.007
V	26.9	17.7*	0.5	0.28	0.002	0.002
Zn	171.4	20.7*	140.5	33.8*	0.034	0.005*

Table 3. Comparison of metal concentrations (ppm) in fish and crayfish collected from five potentially impacted subunits on the Savannah River Site.

Metal	Crayfish		Fish	
	Average	Range	Average	Range
Al	448.4	(251.4-647.7)	88.7	(63.3-114.2)
As	1.1	(0.8-1.3)	0.3	(0.2-0.3)
Ba	249.4	(217.5-302.9)	23.3	(20.0-31.4)
Be	0.0	(0.0-0.0)	0.0	(0.0-0.0)
Cd	0.4	(0.1-0.8)	0.1	(0.0-0.2)
Cr	2.7	(1.0-7.5)	1.9	(1.4-2.2)
Cu	38.7	(27.0-44.1)	3.4	(2.6-4.5)
Hg	0.2	(0.1-0.5)	0.8	(0.3-1.3)
Mn	846.7	(453.9-1362.0)	105.3	(76.4-136.8)
Ni	2.2	(1.1-4.7)	2.8	(0.9-6.6)
Pb	2.7	(2.4-2.9)	1.4	(0.7-3.9)
V	1.1	(0.5-1.4)	0.5	(0.4-0.6)
Zn	119.1	(100.8-171.4)	153.1	(135.8-173.6)

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Figure 1. Map of the Savannah River Site (SRS) showing IOU watershed boundaries.

Figure 2. Redundancy analysis of IOU subunits based on metal levels in sediments. The triplot depicts IOU subunits (see Table 1 for definitions), sediment metal levels, and the distinction between potentially impacted (PI) and reference (R) subunits. Arrows indicate directions of increase.

Figure 3. Frequency distributions of estimated exposure doses for selected metals in potentially impacted (PI) and reference (R) IOU subunits. Also shown is the LOAEL (dotted line) and the percent exceedance of the LOAEL for the PI and R subunits.

Figure 4. Pearson correlations between exposure point concentrations in four media (fish, crayfish, sediment, and water) and exposure doses predicted by contaminant exposure models for the otter. Higher correlations indicate greater influence on the model results.

Figure 5. Principle components analysis of IOU subunits based on the hazard quotients for individual metals predicted by the deterministic otter model. Circle diameter is proportional to the cumulative (additive) hazard quotient for all metals (cumulative HQs = 1.2 – 10.4).

Figure 6. Principle components analysis of IOU subunits based on six macroinvertebrate metrics (% Ephemeroptera; % clingers; a biotic index (BI); number of Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT); total number of taxa; and total number of individuals). Arrows indicate the direction and strength of the correlation (indicated by length) of each principle component axis with the

macroinvertebrate metrics, stream habitat quality, stream width, and sediment metal concentrations (the latter are unlabeled).











