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Comparison of the Bioavailability of Elemental Waste Laden Soils Using "In Vivo" and "In Vitro" Analytical Methodology and Refinement of Exposure/Dose Models

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Research Objective

Our hypotheses are: 1) the more closely the synthetic, in vitro, extractant mimics the extraction properties of the human digestive bio-fluids, the more accurate will be the estimate of an internal dose; 2) performance can be evaluated by in vivo studies with a rat model and quantitative examination of a mass balance, calculation and dose estimates from model simulations for the in vitro and in vivo system; and 3) the concentration of the elements Pb, Cd, Cr and selected Radionuclides present in the bioavailable fraction obtained with a synthetic extraction system will be a better indicator of contaminant ingestion from a contaminated soil because it represents the portion of the mass which can yield exposure, uptake and then the internal dose to an individual.

Research Progress and Implications

As of April 15, 1998, we have made significant progress in the development of a unified approach to the examination of bioavailability and bioaccessibility of elemental contamination of soils for the ingestion route of exposure. This includes the initial characterization of the soil, “in vitro” measurements of bioaccessibility, and “in vivo” measurements of bioavailability. We have identified the basic chemical and microbiological characteristics of waste laden soils. These have been used to prioritize the soils for potential mobility of the trace elements present in the soil. Subsequently we have employed a mass balance technique, which for the first time tracked the movement and distribution of elements through an “in vitro” or “in vivo” experimental protocol to define the bioaccessible and the bioavailable fractions of digested soil. The basic mass balance equation for the “in vitro” system is:

$$MT = MSGJ + MIJ + MR.$$

where MT is the total mass extractable by a specific method, MSGJ, is the mass extracted by the saliva and the gastric juices, MIJ is the mass extracted by the intestinal fluid, and MR is the unextractable portion of the initial mass. The above is based upon the use of a synthetic digestive bio-fluids model that includes the saliva, gastric juices, and intestinal fluids. The system has been devised to sequentially extract elements from soil by starting with an extraction by the saliva and carrying the entire mixture to the subsequent bio-fluids for further extraction. The residence time of the soil in each extractant and the liquid to mass ratio in the gastric juices are based upon typical values known for the human digestive system. Experiments were conducted to examine the sensitivity of the extractions to changes in these major variables. The results indicated the lack of significant extraction after 2 h of residence in gastric fluid. The range of variation of the liquid to mass ratio was element dependent over the interval 100:1 and 5000:1. The final values used for the extraction protocol were: 2 h residence time , and a ratio of 1000:1. Details of the chemical composition of the extraction protocol are found in Hamel, 1998.

The mass balance technique was tested in the “in vitro” system for a number of soils. One of major interest was a residential soil from Bunker Hill, ID which was impacted by mining wastes. It was used because of experimental evidence of human bioavailability of lead in the soil based on an adult feeding study conducted at Columbia University. We examined the particle size fraction of <125 μm diameter soil which contained a total Pb concentration of 2900 μg/g. The results of our experiments on soil found the total bioaccessible fraction for lead in the synthetic digestive fuilds to be 70%+/-11%. The human study found the bioavailable fraction, as indicated only by the blood lead levels, to be at least 26%+/-8.1%.

A non-radioactive soil from Savannah River have been subjected to the “in vitro” ; mass balance using the same protocol as above. The Pb level was the only one with values of any interest, but was less that 50 μg/g. In any case, the bioaccessibility of the Pb in the soil was approximately 40%. In this case the analyses were completed on the size fractions <75 μm and < 250 μm in diameter, and the bioaccessibility was the same for both fractions. Based upon other studies, the results are consistent with an un-contaminated background soil.

Another major aspect of the project is the “in vivo”; studies being conducted on a Sprague-Dawley rat animal model with some of the same soils used for the “in vitro” studies. In this case we feed 15 rats contaminated soil, and sacrificed them at 3-4 days post feeding with the soil in their food. The internal organs, blood and feces were subsequently subjected to extraction and analysis for the elements examined in the “in vitro” system. The approach was again designed to provide a mass balance of the elements as they distributed throughout the body, and then provide an estimate of the bioavailable fraction of the element in the soil.

The results for our first experiment using a soil contaminated with As, Cr, Pb collected in Jersey City, NJ. The relationship between what is known as the bioaccessible and the bioavailable fractions was dependent upon the element. The As, Cr, and Pb were 65%, 7.1% and 62% bioaccessible, respectively. The amount of bioavailable As, Cr, and Pb was 44%, 0.2%, and 2%, respectively. Even with the large variations between elements the results were physiologically plausible because all of the material that is released into the digestive (bioaccessible) will not be transported across the stomach or intestinal lining into the blood and other tissues (bioavailable).

Thus far part of our research has focused on how to best develop an “in vitro”; system which minimizes the number of steps used to obtain data on bioaccessibility. For that reason we are now optimizing the system for the analysis of only the bioinaccessible portion of the mass. This will allow us to add more of the minor components of the various biological fluids to the extraction protocols while reducing the number and complexity of the analyses. Initial results suggest that the bioaccessiblity moves closer the bioavailable fraction with the addition of some key organic compounds.

Planned Activities

We have recently developed 1) a self-contained sieving system for the fractionation of soil that contains radionuclides by particle size, and 2) protocols for the extraction of the Radionuclides with the “in vitro” system. These will now be applied to a radionuclide laden soil from Savannah River. Parallel tests will be completed with the “in vivo” system. We are in the process of analyzing the data obtained for an “in vivo” and “in vitro” mass balance of a standard NIST soil. The results will be valuable for future inter-comparisons of our system with other approaches to bioavailability. Future activities will include continued “in vitro” studies in association with Consortium for Risk Evaluation with Stakeholder Participation (CRESP).

Other Access To Information

S. Hamel, Buckley, B., and Lioy, P.J., Bioaccessibility of Metals in Soils for Different Liquid to Solid Ratios in Synthetic Gastric Fluid, Environmental Science and Tech. 32, 358-363, 1998.

S. Hamel, The Estimation of Bioaccessibility of Heavy Metals in Soils Using Artificial Biofluids. **www.cresp.org/, http://eohsi.rutgers.edu/.**