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Standard Guide for Assessing Mammal Health at Chemically Contaminated Terrestrial Sites Using Rodent Sperm Analysis[1]

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INTRODUCTION

Contaminated terrestrial properties provide opportunities for determining whether or not toxicological responses of concern have arisen in ecological receptors contacting them. The guide, employing direct health status assessment of rodents captured at contaminated sites, can furnish information to support such determinations, thereby providing a greater degree of realism in health assessments for resident mammals than that offered in generic desktop assessments. This guide's direct health status assessment design involves only wild-type animals in their natural state, with this arrangement circumventing a commonplace species extrapolation element, one that introduces a considerable degree of uncertainty in ecological risk assessments (ERAs). Of note, the guide is not itself a terrestrial ERA method, but rather an additional tool to inform such efforts. In the area of notating significant effects, the guide's biological thresholds-for-effect replace arbitrarily or negotiated differences, or both in response that are only assumed to be biologically meaningful (for example, a 20 % decrement in a measure), and that typically are not confirmed in field studies (**1**).[2] Due to the availability of the thresholds-for-effect, the guide allows for bright-line determinations to be made, creating an opportunity to supply a high level of conservatism to outcomes (see 9.4). Importantly, Rodent Sperm Analysis (RSA) is distinctly different from others in concept and practice because it aims to make yes/no determinations (that is, that reproductive impact has occurred or that it has not) as opposed to generating estimates of the likelihood of certain toxicological outcomes arising in the future. RSA provides a useful line of evidence for ERA, wherein mammal reproduction is a common endpoint, and it may simplify remedial decision-making for contaminated terrestrial sites.

This guide, notably removed from testing with laboratory-reared, commercially available, and chemically naive organisms that are subjected to brief chemical exposures in controlled environments, is predicated on a fundamental underlying premise consistent with the field condition considered (**2, 3**). It is recognized that sufficient time has elapsed at contaminated sites for toxicological effects of concern to have been elicited. Given that typical sites are minimally 30 years old by the time they submit to ERA (**2-8**), the described standard understands that if critical effects (here, reproductive impacts) have not appeared over such a time course, they are unlikely to ever occur. Addressing mammals, one of only two tetrapod classes evaluated in ERA (Class Aves is the other), the guide has three unique features that set it apart from conventional ecological assessment tasks. First, the procedure directly assesses health by evaluating the actual animals that inhabit contaminated sites. (Of note, common to RSA and the desktop-based risk characterizations of many conventional ERAs, is the defining of overall mammal health through the key biological function of reproduction.) Second, the procedure considers the three chemical uptake routes of ingestion, inhalation, and dermal contact, whereas conventional ERAs only report hazards for the first of these. The procedure's third unique feature is its ability to evaluate the effects of chemical mixtures, whereas the conventional hazard quotient (HQ)-reliant ERA process can only review chemicals singly. The procedure is supported by the existence of established sperm-based barometers of reproductive capability/success for the rodent grouping. In contradistinction, for most other current biological measures that can be collected in the laboratory or field (for example, an enzyme level, size of an internal organ), it is not known how much of a contaminated site-mediated sublethal change signifies health compromise (**3, 9**).

1. Scope

1.1 This guide describes the procedures for obtaining and interpreting data associated with a direct health status assessment for mammalian receptors at chemically contaminated terrestrial sites where ERA work is either scheduled or ongoing, and irrespective of the number and type of chemicals that may be present. Through reviewing sperm features, the RSA method reports on the reproductive health of male rodents in their natural environmental settings, with these animals serving as surrogates for other (and larger) site mammals (**4**).

1.2 These procedures are applicable at any terrestrial property that supports small mammals (for example, mice, voles, rats, squirrels) and has contaminated soil. Importantly, chemicals of concern in site soils need not be spermatoxins. Additionally, the RSA method considers that any combination of chemicals or other site stressors might collectively act to compromise reproduction, held to be a sensitive toxicological endpoint for mammals. The anticipated primary application of the method will be at historically contaminated sites (such as Superfund sites). The procedures describe tasks conducted in the field and in a laboratory. For the latter, tasks may be conducted either in an on-site mobile laboratory, or in a more conventional laboratory setting. For certain tasks, a make-shift work space may be suitable as well (see 7.3).

1.3 Initial determinations of compromised or non-compromised reproduction in resident male small rodents are made through a cautious comparative review of sperm parameters. Briefly, for the rodents of a given species collected at both a contaminated site and a habitat-matched (non-contaminated) reference location, arithmetic means are first computed for each of the three sperm parameters of count, motility, and morphology. If one or more of the parameter means of the contaminated site rodents reflect an unfavorable shift (that is, count or motility is less than that of reference location animals; the percentage of abnormally-shaped sperm is greater relative to reference location animals), the percent decrease or increase in each mean is compared to the relevant established sperm parameter benchmark, each in the form of that degree of shift in an unfavorable direction that signifies lesser reproductive success (**2**) (see 9.3).

1.4 Advanced determinations of compromised or non-compromised reproduction in larger site-contacting mammals, the true focus of the RSA method and this guide, are made through an applied spatial movements-based extrapolation scheme. Where established sperm parameter benchmark exceedances are not observed in contaminated-site rodents, other mammals contacting a site are also assumed to have non-compromised reproduction. This follows from the latter all having notably lesser degrees of site exposure due to home ranges that are vastly larger than those of rodents. By way of example, with respective home ranges of 400+ and 640 acres for the red fox and white-tailed deer (**10-14**), these species would spend minimal amounts of their time (for example, 5 %) at prototypical contaminated sites that cover areas of 25 acres or less (**15, 16**). Where one or more sperm parameter benchmarks are exceeded in contaminated-site rodents (certainly indicating that the rodents are reproductively compromised), other site mammals may also be reproductively compromised. The greater the disparity between the home ranges of the target species (that is, the site rodent) and any of the other mammals known to contact the contaminated site in question, the less likely it will be that the latter are reproductively compromised. The RSA method employs the same toxicological extrapolation principles as that used for mammals in conventional desktop-based ERAs. In those ERAs, stressor-mediated responses of rodents (of a laboratory-based study) assist with the interpretation of health effects for an expanded list of mammals that cannot conveniently be evaluated directly for health status (for example, fox, skunk, raccoon, deer, coyote, etc.).

1.5 This guide is arranged as follows:

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1527 Practice for Environmental Site Assessments: Phase I Environmental Site Assessment Process

E1689 Guide for Developing Conceptual Site Models for Contaminated Sites

E1848 Guide for Selecting and Using Ecological Endpoints for Contaminated Sites

E2081 Guide for Risk-Based Corrective Action

E2205 Guide for Risk-Based Corrective Action for Protection of Ecological Resources

E2616 Guide for Remedy Selection Integrating Risk-Based Corrective Action and Non-Risk Considerations

[1] This guide is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Action and is the direct responsibility of Subcommittee E50.47 on Biological Effects and Environmental Fate.

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[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.