



Evaluating Safety of Vaginal Microbicides

Scientists are developing vaginal microbicides as an important new female-initiated HIV prevention tool. Before they can be licensed, candidate microbicides must go through a series of tests to evaluate their safety. This document explores the questions that scientists hope to answer when they conduct safety evaluations of microbicides, including how safety is evaluated and the strengths and limitations of current methods used.*

What is safety?

Product developers tend to think about safety as a person's ability to use a product without negative impact on the body. While communities share this concern, community members sometimes conceptualize "safety" more broadly. When asked about their safety concerns regarding an upcoming microbicide trial, one group of community members, for example, wanted to know if the product would cause relationship problems between spouses or cause people who participated in trials to be stigmatized. Others wanted to know if the microbicide would cause problems if they ingested it during oral sex, or if the drug would negatively affect a woman's fertility.¹

In order to get a drug approved for human use, researchers and drug regulators concentrate on a very specific notion of what it means to be safe. Generally, they focus on whether and under what conditions the product might cause harm. If the potential benefit a new drug provides does not outweigh any harm it may cause, the drug regulators will not approve it for use. While both the biological and social effects of any product are important to understand, this document will focus on how scientists explore the effects of a microbicide on the body.

* In addition to vaginal use, microbicides are also being developed and tested for possible rectal use. While this is an important topic of research, a specific discussion of evaluating the rectal safety of microbicides involves different research criteria and thus is not addressed in this article.

¹ Rhonda R White, "Community Perspectives on Safety Evaluation in Microbicide Trials." Presentation made at the Microbicide Safety Consensus Meeting sponsored by the HIV Prevention Trials Network, March 1 - 3, 2006, Bethesda, Maryland USA. Available at: http://www.hptn.org/network_information/MicrobicideSafety2006.htm

What safety questions are researchers trying to answer?

For the purposes of supporting regulatory approval of a new vaginal microbicide, researchers address a variety of important questions:

- Does the product harm the vaginal lining (epithelium) or create an inflammatory response that may facilitate HIV or other STI transmission?
- Does it affect the natural vaginal environment that helps to keep the vagina healthy?
- Does it cause harm if used rectally?
- Does the product penetrate the **vaginal epithelium***?
- Is it absorbed into the blood stream? If so, to what degree?
- Does it have any negative effects on the penis?
- Does the product cause any negative effects on other body functions?
- Is it safe for pregnant women to use?

How do scientists test safety?

Preclinical evaluation

Any new drug, including microbicides, must be thoroughly researched in laboratory tests before it is tested in people. Non-clinical or **pre-clinical research** is the general term for research that takes place before human testing (which occurs in clinics). It includes “in vitro” testing (“vitro” means “glass”, as in test tubes) and animal testing. To get approval for testing in human beings, a researcher must first generate non-human data demonstrating: (1) that the drug is unlikely to harm people and (2) that it may potentially benefit people. If both these conditions are met, drug regulatory agencies will allow the product to be tested among human volunteers.²

Human safety trials

Microbicide testing in human beings involves a series of carefully staged studies called clinical trials. Typically in Phase I safety trials, the candidate product is used by 20-40 healthy volunteers for a limited period of time. These participants are monitored closely to see if the product causes any adverse reactions. Generally, multiple phase I trials are conducted in increasingly diverse groups of participants (e.g. in more countries, among those who are HIV positive). Sometimes a separate Phase II trial is done among a larger group of women, either alone or as a “run-in” to a larger effectiveness trial.

* Words that are defined in the glossary are bolded the first time they appear in the document.

² For a full discussion of nonclinical evaluations of safety, see: Lard-Whiteford SL, Matecka D, O'Rear JJ, Yuen IS, Litterst C, Reichelderfer P; International Working Group on Microbicides. Recommendations for the nonclinical development of topical microbicides for prevention of HIV Transmission: An update. *Journal of Acquired Immune Deficiency Syndrome*. 36(1):541–552.

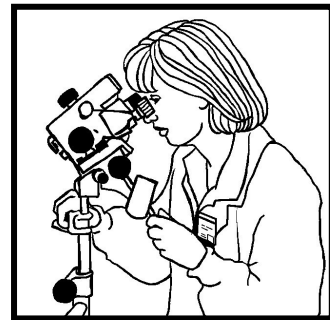
If any evidence of potential harm emerges, research on that candidate is stopped and either the drug is changed in some way to make it safer or it is dropped from consideration as a potential microbicide. If the data generated from the safety trials do not produce any evidence of potential harm, then the candidate can go into an effectiveness trial, known as Phase III, to find out if it really works. Phase III trials involve a significantly greater number of participants, 3,000-10,000, than safety trials. All drugs, including microbicide products, go through similar evaluation steps.

It is important to remember that positive results in Phase I and II clinical trials do not mean that the product has been determined to be completely safe. As noted above, these early phases involve a relatively small number of volunteers. Completion of them means that the product appears to be safe thus far and that it is reasonable to move forward to Phase III trials which provide additional information about product safety as well as efficacy.

How is potential harm evaluated in humans?

Colposcopy is one way of finding out if a product disrupts the cells lining the vagina and cervix (the vaginal epithelium). A colposcope is a tool that provides a magnified view of the vagina and cervix.

During a pelvic exam, the clinician positions the colposcope just outside the vagina and looks through it to check for redness, swelling, or other disruptions to the epithelial surfaces. Any of these conditions might indicate irritation that could make it easier for HIV to get through the vaginal lining and thus increase a woman's risk of becoming infected.



The colposcope is a useful tool, but colposcopy still isn't a perfect way to evaluate safety. One problem with it is that there is often variability between what different healthcare providers observe with colposcopy. In other words, one clinician may interpret and classify what is seen through the colposcope slightly different from the way another clinician would classify it. Colposcopists are trained to use standardized descriptions and classification of what they see, but variability can still exist and these complicate researchers' ability to compare colposcopic findings.

Another important limitation to this method is that although a colposcope can help identify **lesions** or disruptions that are invisible to the naked eye, we still don't know exactly what the presence of these effects means. Do minor disruptions in the vaginal epithelium actually increase the risk of HIV transmission? This is thought to be the case, but until more data are available from large-scale clinical trials, we will not know how much vaginal disruption can occur before a woman is at increased risk. It could be that common minor disruptions actually do not translate into increased risk of HIV.

Furthermore, disruptions in the lining of the vagina could be due to any number of causes other than a microbicide: such as sexual intercourse, tampon use, the use of other vaginal

products or herbs, a microbicide applicator, or other sexually transmitted infections. It can be difficult to determine whether epithelial disruptions observed during a microbicide trial actually occurred because of the candidate microbicide or one of these other causes. One way to address this concern is to compare how often vaginal disruptions are observed among women using the candidate microbicide versus among women who use a comparison product without any active ingredients (called a **placebo** gel). This gives researchers an idea whether epithelial damage is likely to be related to the product rather than some other cause.

Finally, colposcopy may not detect all types of damage that increase a woman's risk of HIV. For example, changes in a cell or inflammatory responses may increase susceptibility to HIV, but without causing any visible changes in appearance, even with the magnification of the colposcope.

Biomarkers

To some extent, because the amount that researchers can learn from colposcopy is limited, scientists are exploring other ways to determine if a microbicide is safe. One way to do this is through the use of **biomarkers**; often also referred to as surrogate markers.

When the body is damaged and its immune system is activated, it produces a number of substances that, when present, may suggest a person's increased susceptibility to HIV infection. In addition to not causing damage, a microbicide should also not disrupt the healthy substances that exist naturally in the vagina. Researchers measure the presence of these substances in the vagina during a trial to see if they have been changed in any way by the test product. Substances that could indicate a problem if increased or decreased (such as healthy vaginal components) are called biomarkers.

Biomarker testing looks for changes in the levels of these substances, but scientists do not yet know exactly which, if any, biomarkers accurately indicate whether a candidate microbicide is safe or not.

Generally, there are two schools of thought in the microbicide community regarding biomarkers: (1) people who believe that biomarkers will strongly correlate with a likelihood of infection and are thus very important to measure and (2) people who are waiting for additional evidence to establish the relevance of biomarkers. The latter group believes that biomarkers are unlikely to play a role in predicting risk and is concerned that national drug regulatory agencies will begin requiring measurements of potential biomarkers, thereby slowing the approval process without really knowing how to interpret the markers.

Scientists who advocate for the use of biomarkers suggest that inflammatory cytokine measurements could be one important safety biomarker. **Cytokines** are natural substances in the body that work by bringing other immune cells (white blood cells) to an area of infection or injury. Normally, this helps the body heal itself. But in the case of HIV, it could actually increase risk because HIV easily infects white blood cells. So

increased cytokine levels in the vagina may be important to note for two reasons. The first is that they may mean that the microbicide is injuring the vagina in some way. The second is that they may mean the woman is at higher risk of HIV if she is exposed because the cytokines are bringing HIV target cells (white blood cells) with them.

Vaginal flora

Scientists want to know that the addition of a microbicide to the vaginal environment does not negatively disrupt the natural protective flora in the vagina. The normal vaginal environment contains a variety of small organisms (micro-organisms) that help keep the vagina healthy. These include an important strain of bacteria called lactobacilli, which produce the protective mild acidity of the vagina and often also produce hydrogen peroxide. Both of these substances may be important in blocking the growth of harmful organisms. Safety evaluations generally include tests in both the laboratory and in women's bodies to see if the product is having a negative effect on the protective micro-organisms that normally live in the vagina.

Pregnancy

Some women may use a microbicide before they realize they are pregnant and others who are pregnant may want to use a microbicide to prevent HIV infection, thus the safety of microbicides among pregnant women must also be evaluated.

Internationally agreed-upon guidelines indicate that all products must go through three preclinical segments of **reproductive toxicology** studies to evaluate whether they might cause harm to a developing fetus. These studies are conducted in animals to answer the following questions:

Segment I: Does the drug affect the animal's ability to get pregnant?

Segment II: What are the effects of the drug on fetal development in animals?

Segment III: Does exposure to the drug during gestation affect the development of a newborn animal during birth or breastfeeding?

Segment I and II are typically done before a product is tested among women. Segment III is usually done while women are enrolled in a Phase III trial of the product.

Currently, only women who do not intend to get pregnant during the period of microbicides trial are allowed to enroll. Trial participants are tested frequently (often monthly) for pregnancy and are required to stop product use if they become pregnant, because the full effects of the product on a pregnancy are not well understood. If a woman becomes pregnant during a microbicide trial, she will no longer receive the test product, but she will continue to receive condoms and safer sex counseling. She will be monitored closely by study staff during the course of her pregnancy.

Every time a woman becomes pregnant during a trial, it has a negative impact on the trial's data. This is because the woman continues to be counted as if she were still using the product even though she isn't. Thus if she acquires HIV, she will be counted as a microbicide "failure", even though she was not using the product at the time she became infected. Studies are designed this way for reasons that are very complicated – but the main point is that the frequent occurrence of pregnancies in microbicide trials has made it difficult to determine if the microbicide actually works.

For example, if many women become HIV infected during a microbicides trial, it could either be because (1) the microbicide doesn't work or (2) because many women became pregnant in the trial and later became HIV positive when they were not using the microbicide. The current design of the trials does not allow us to distinguish between the two possible explanations.

Pregnant women can't be offered the option of continuing to use a candidate microbicide in a trial until the researchers have a better understanding of the effects of microbicides on pregnancy. To achieve this, it would be necessary to complete all three Segments of the reproductive toxicity studies mentioned above before women were enrolled in a Phase III trial of the product. These segments are relatively inexpensive and not time consuming. However, regulatory agencies are likely to also want **carcinogenicity** testing to be completed before allowing pregnant women to remain on product.

Carcinogenicity tests evaluate the potential of a product to cause cancer. These tests take at least two years and cost approximately US\$2 million. Requiring completion of these tests prior to a Phase III clinical trial would significantly delay the onset of phase III trials.

An unanswered question remains. Does it make sense to delay the start of Phase III trials (and, hence, potential access to an effective microbicide) by up to two years in order to do the work that would enable trial participants to continue with product use even if they become pregnant. On the one hand, a microbicide is urgently needed to offer millions of women a means to protect themselves from HIV. On the other hand, once a microbicide is on the market, it is likely to be used by some pregnant women regardless of whether or not the effects on pregnant women are understood. Thus, determining the effects on pregnant women prior to market introduction is necessary and the time and resources to accomplish this should be invested now.

Why are Phase III efficacy trials important when thinking about safety?

Candidate microbicides may potentially have any number of side effects that must be evaluated, but perhaps the most important safety concern is that any future product does not *increase* the risk of HIV or other STIs. Since the intact vaginal epithelium is a very good barrier to infection, damage to that barrier could increase risk of HIV infection to a degree that could override the potential benefit of a microbicide. Therefore, the data

gathered in Phase III trials show both whether an experimental microbicide actually reduces HIV risk and answer a critical safety question: whether the microbicide might inadvertently *facilitate* infection.

Data from today's large-scale effectiveness trials (Phase III) are also extremely important to improve our ability to understand and evaluate the likely safety of microbicide candidates in the future. Until scientists know whether various colposcopic findings and biomarker changes correlate directly to increased incidence of HIV or other STIs in humans, we will not know for sure which of these findings really signify potential harm. Once these factors are better understood, we will have an improved ability to choose the safest and most promising microbicides.

Conclusion

Safety evaluation of candidate microbicides is an evolving science. As data from human effectiveness trials become available, they will improve researchers' ability to predict possible harm because scientists will know which laboratory and animal tests are good predictors of how the product may affect humans.

As advocates, we must press hard to ensure that all candidate microbicides undergo rigorous scientific tests to evaluate both their potential benefit and their potential harm.

But we must also recognize the limits of science. Some possible side effects just don't show up during clinical trials because they take decades to develop (like some cancers). Some are so rare that they only show up in one out of several hundreds of thousands of people are using a product. Scientists attempt to predict the potential of new drugs to have negative consequences by testing the products in animals, but animals are rarely perfect models for human biology.

Finally, we must all learn from the community's notions of safety. Are there other specific elements of safety and potential harm that would be useful to monitor? All research involves risk, but by working together, scientists and the community can maximize benefit and minimize potential harm.

Glossary

Antiretroviral Therapy (ARV)—Drugs that suppress or stop a retrovirus. One such retrovirus is the human immunodeficiency virus (HIV) that causes AIDS.

Biomarker—A substance whose presence or change in level in the body can be used as an indication of another substance or process in the body.

Carcinogenicity—Any substance that helps promote or facilitates the development of cancer due to the disruption or damage of cellular function.

Colposcopy—A procedure in which a tool called a colposcope is used to examine an illuminated, magnified view of the cervix, the tissue of the vagina, and the vulva. This procedure helps a clinician assess the safety of a microbicide product by looking at any changes in the vagina.

Cytokines—Natural substances in the body that increase in number in response to inflammation or infection. They are being considered as a biomarker in microbicide development to help assess safety.

Genotoxic—Substances which are capable of causing genetic mutation.

Lesions—Abnormal tissue in the body.

Mutagenicity—The ability of a substance to change the genetic information in a cell and thus increase the number of mutations above the natural background level. As many mutations cause cancer, mutagens are typically also carcinogens.

Placebo—A medicine that has no inherent activity. It is often used in a study to provide a comparison to a product being tested. This comparison helps to determine if any effects of a candidate product (including both side effects and efficacy) are due to the product itself or if the effects would have happened without the use of the active product.

Preclinical research—This literally means “before the clinic.” It refers to any work done to create a drug and study it before it is tested in humans. This includes laboratory work to create the compound and any testing in animals.

Reproductive toxicology—The study of the effects of chemicals on the adult reproductive systems, the embryo, fetus, and newborn.

Systemic—Affecting the entire body.

Vaginal epithelium—the layer of cells lining the vagina and cervix.