

# Appendix A: The HPV Animal Test Battery

## Acute Toxicity

Acute toxicity is traditionally evaluated with the Lethal Dose test, or LD50. This Lethal Dose test involves the administration of a substance to a group of animals at increasing doses in order to determine the dose that kills 50 percent of the tested animals within a set time frame. Typically, administration of the test substance is via a tube inserted down the esophagus into the stomach. Other routes of administration include inhalation and applying the substance to the animals' skin (the dermal toxicity test). The test is typically allowed to proceed for 14 days, at which time all the animals who have not died from the test substance are killed. When experimenters are interested in studying dermal toxicity, they shave the fur on the animals' backs and then apply the chemical. The dose is increased until 50 percent of the animals die from the application of the chemical on their skin. All the animals are then killed and dissected.

## Genetic Toxicity

*In vitro* tests are used in which bacteria or cell cultures are treated with the chemical and observed for mutations in the DNA or abnormality in the chromosomes. Animal tests are also used, such as the *in vivo* mammalian erythrocyte Micronucleus Test, in which the chemical is typically injected into the animals' stomachs. The animals are then killed, and their bone marrow is extracted and examined for abnormalities indicative of a structural or numerical chromosomal aberrations.

## Repeat Dose Toxicity

The typical test used is the repeated dose 28-day oral toxicity screening test, in which the chemical is force-fed to young animals through a stomach tube in incremental doses over a period of 28 days. Once a week, the animals are checked for weight loss, changes in eating habits, abnormal behavior, and death. At least 40 animals are used in this test per chemical.

## Reproductive and Developmental Toxicity

Male and female rats and mice are dosed with the chemical and then mated. Problems in reproductive function and fertility are observed. The test provides that 20 pregnant female rats be brought to term. After the animals give birth, they are killed and dissected, with special attention to their reproductive organs. Birth defects in the offspring are noted.

## Acute Toxicity to Fish

Fish are exposed to various concentrations of a chemical for four days. At the end of each 24-hour period, the experimenters count how many fish have died. At least 42 fish are killed for each chemical tested. The goal of the test is to estimate the LC50, or lethal concentration 50, the concentration of chemical that kills half the fish.