

## **10.0 ANIMAL WELFARE CONSIDERATIONS (REFINEMENT, REDUCTION, AND REPLACEMENT)**

### **10.1 How the HET-CAM Test Method Will Refine, Reduce, or Replace Animal Use**

ICCVAM promotes the scientific validation and regulatory acceptance of new methods that refine, reduce, or replace animal use where scientifically feasible. Refinement, Reduction, and Replacement are known as the “Three Rs” of animal protection. These principles of humane treatment of laboratory animals are described as:

- refining experimental procedures such that animal suffering is minimized
- reducing animal use through improved science and experimental design
- replacing animal models with nonanimal procedures (e.g., *in vitro* technologies), where possible (Russell and Burch 1992)

The HET-CAM test method has the potential to refine and reduce animal use in eye irritation testing. The HET-CAM test method refines animal use by the *in vitro* identification of ocular corrosives and severe irritants, when used in the tiered testing scheme. According to the GHS tiered testing scheme, substances are to be initially tested in a valid *in vitro* ocular test method, if available, that assesses severe eye damage (see **Figure 1-2**). If the substance is identified as a corrosive or severe irritant, it may be classified as such and no additional testing is required. If a negative result is obtained from this test, then the substance is to be tested first in a valid *in vitro* test method, if available, for eye irritation. If the substance is identified as an eye irritant, it may be classified as such and no additional testing is required. The next step in the testing scheme is to assess dermal corrosion potential (either by *in vitro* or *in vivo*) methods. If the substance is classified as noncorrosive, it is then evaluated in a single rabbit. The HET-CAM test method, used in the GHS tiered testing strategy, would be used prior to evaluation in any animals (to assess severe eye damage). This test method, therefore, will reduce the numbers of animals subjected to testing and reduce the pain and suffering of rabbits by their exclusion from the testing of corrosives and severe irritants.

### **10.2 Requirement for the Use of Animals**

The HET-CAM test method has been designed so as not to require the use of animals. International regulations have provisions for the protection of animals used for experimental or other scientific purposes. Some provisions indicate the time in which a test method using an animal embryo or fetus is considered an animal, and therefore protected by the regulations. According to some of these regulations, a bird is considered a protected animal (and therefore the test is considered an *in vivo* and not *in vitro* test) when greater than half of the gestation or incubation period has elapsed (day 10.5 of the 21 day incubation period for a chicken embryo) (Animals [Scientific Procedures] Act 1986; EU 1986). The Public Health Service Policy, with which all National Institutes of Health (NIH)-funded research projects must comply, applies to all live vertebrate species. The NIH Office of Laboratory Animal Welfare has provided written guidance in this area, interpreting “live vertebrate animal” to apply to avians (e.g., chick embryos) only after hatching (Kulpa-Eddy J, personal communication; NIH 2000).

It has been proposed that at incubation day nine, the embryonic differentiation of the chicken central nervous system is sufficiently incomplete that suffering from pain perception is unlikely to occur (MSPCA 2005; Liebsch M, personal communication). Evaluations suggest that there are few sensory fibers present at day nine in the avian embryo and that there is significant development of the sensory nerve ending between incubation days 11 and 14 (Romanoff 1960). Studies also have suggested that the extraembryonal vascular systems (e.g., yolk sac, CAM) are not sensitive to pain (Rosenbruch 1997; Spielmann H, personal communication). Combined, these studies suggest that at incubation day nine there is little to no pain perceived by the developing embryo during the conduct of the HET-CAM test method.