

10.0 Animal Welfare Considerations (Refinement, Reduction, and Replacement)

10.1 How the Five *In Vitro* Test Methods 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 alternatives. 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 non-animal procedures (e.g., *in vitro* technologies), where possible (Russell and Burch 1959)

In 2002, a total of 243,838 rabbits were used in the U.S. for all research and testing purposes, of which 6,324 rabbits were reported as experiencing more than slight or momentary pain and/or distress where anesthetics, analgesics, or tranquilizers could not be administered for scientific reasons (USDA 2002). Eight of these cases were specifically attributed to pyrogenicity testing, presumably based on induction of a fever response (USDA 2002). Thus, although the potential for more than slight or momentary pain and/or distress exists for pyrogenicity testing when a fever response is induced, it does not appear that a fever response is common. In 2006, a total of 239,720 rabbits were used in the U.S. for all research and testing purposes (USDA 2006). No data related to pyrogenicity testing were reported.

In Canada, a total of 18,152 rabbits were used for all scientific purposes in 2006, 3,485 of which were used for regulatory studies and the development of products (Canadian Council on Animal Care [CCAC] 2007). Although no specific data for the number of animals used for pyrogenicity testing were reported, it is likely that the number of rabbits used for this purpose is less than the total of 3,485 used for both regulatory studies and product development.

In the EU, approximately 313,000 total rabbits were used for all scientific purposes in 2005 (Commission of the European Communities [CEC] 2007). Of these, approximately 276,000 rabbits were used for pharmaceutical products and medical device testing (i.e., either research and development, production and quality control, or toxicological and other safety evaluations). Although the number of rabbits specifically used for pyrogenicity testing was not reported, it is likely that this number is significantly less than the total of 276,000.

In the U.K., a total of 21,736 procedures (which used 14,712 total rabbits due to reuse of some test animals) were performed using rabbits for all scientific purposes in 2004 (Home Office 2005). Of these procedures, 8,488 were specifically attributed to pyrogenicity testing in rabbits. Although the total number of rabbits used for these procedures were not provided, it is likely less than 8,488 rabbits based on the assumption that some animals were reused. In 2006, a total of 20,378 procedures (which included 13,397 total rabbits) were performed in the U.K. for all scientific purposes (Home Office 2007). No specific data for pyrogenicity testing were reported in 2006.

The currently accepted pyrogen test methods require the use of rabbits or horseshoe crab hemolymph. The proposed *in vitro* pyrogen test methods address each aspect of animal welfare outlined above. These assays use monocytoid cells of human origin, obtained either from WB donations or from an immortalized cell line. The capability of these five *in vitro* assays to detect Gram-negative endotoxin suggests that they may reduce or eventually replace the use of rabbits and/or horseshoe crab hemolymph for pyrogen testing. However, at the present time, the RPT detects classes of pyrogens that have neither been examined nor validated with the *in vitro* pyrogen test methods and thus, the RPT will still be required for most test substances.

10.2 Requirement for the Use of Animals

10.2.1 Rationale for the Use of Animals

Human blood donations are required for four of the five *in vitro* test methods (WB/IL-1 β , WB/IL-6, Cryo WB/IL-1 β , and PBMC/IL-6) proposed as replacements for the RPT, and as such, humans are the animals used for these assays. While the collection of human blood is a common medical procedure, the many aspects of human blood collection must be considered to ensure that human donors are appropriately treated.