10.0 ANIMAL WELFARE CONSIDERATIONS

10.1 Extent to which FETAX Will Reduce, Refine, or Replace Animal Use for Human Developmental Hazard Assessment

In terms of human developmental toxicology, FETAX is proposed as a screen for hazard identification, and thus would not totally eliminate the use of mammals in teratogenicity and developmental toxicity testing. If validated, the use of this *in vitro* assay would, however, reduce reliance on mammalian tests, and thereby reduce the number of mammals used. Each successful FETAX assay would potentially eliminate the use of approximately 190 rats and 112 rabbits in the typical segment 2 mammalian test. FETAX also offers substantial refinement in the way animals are used. Federal guidelines for teratogenicity and developmental toxicity testing recommend the use of 16 to 24 litters of rats for each dose level (U.S. EPA, 1991; U.S. FDA, 1994). In comparison, FETAX not only employs a non-mammalian alternative, but it is stated that fewer organisms are used per dose level (ASTM, 1991; 1998). In addition, embryos and not adult animals are used in FETAX, another refinement in the assay (ASTM, 1991; 1998).

The per-dose group numerical advantage stated in the ASTM FETAX Guideline (1991, 1998) for FETAX disappears when the recommended numbers of dose groups and of replicate experiments are taken into consideration. In a typical segment 2 test for one compound, ten different dose groups are tested for each species, both rodent and lagomorph. Typically, six dose groups of rodents and non-rodents are used in a pilot study, and four dose groups for each species is used in the definitive study. For the rodent segment of the segment 2 test, rats are usually used. Fifteen rats are used for each pilot study dose group, and 25 rats are used for each definitive test dose group (total number of rats = 190). For the lagomorph, rabbits are usually used, with eight rabbits used in each pilot study dose group, and 16 rabbits used in each dose group for the definitive portion of the test (total number of rabbits = 112). FETAX uses 40 to 50 embryos per dose level (80 to 100 for the concurrent control group), with a minimum of seven dose groups tested per range-finder assay, and five dose groups tested in each of three replicate tests, for a minimum of at least 1300 embryos (ASTM, 1991; 1998). Also, it is recommended that each

study be conducted with and without metabolic activation, which would require a minimum of at least 2600 embryos.

FETAX would also reduce animal usage if the assay could be used:

- in the earliest stages of product development, to select for further development those compounds that are the least likely to cause developmental toxicity;

- to compare the developmental toxicity potential of a new chemical that is only a slight modification of an existing chemical that has already been tested *in vivo*; and

- to evaluate compounds for which testing is not routinely performed, usually because the anticipated exposure is very low (Spielmann, 1998).

10.2 Section 10 Conclusions

FETAX is proposed as a screen for human hazard identification, and thus will not totally eliminate the use of mammals in teratogenicity and developmental toxicity testing. However, if accepted as a screen, use of this *in vitro* assay would reduce reliance on mammalian tests, and would thereby reduce the number of mammals used.