To whom it may concern

Biomedical research relies more and more on the use of vertebrate *in vivo* models to address questions of biological and medical relevance. The mouse is in this respect certainly the most relevant organism, but in the last 20 years the small, tropical zebrafish (*Danio rerio*) has gained enormous popularity as an alternative vertebrate system. World-wide, zebrafish often enable researchers to address specific questions quicker and cheaper than possible in the mouse. Currently, there are more than 700 laboratories using zebrafish, and particular in Europe there is a strong scientific zebrafish community in excess of 300 laboratories. Part of the success of the zebrafish certainly stems from following the '3Rs' principle: research using zebrafish reduces the number of experimental mammalian animals (in particular mice), and derivation of embryos is non-invasive in contrast to other vertebrate model species. Also, it has become clear by now that many disease mechanisms relevant to humans can be quickly resolved in zebrafish, and zebrafish research has both helped to identify human disease genes, as well as generating clinically relevant information.

As so many laboratories use zebrafish, it appears a timely endeavor to standardize husbandry conditions not only on the national, but also on the European level. In this letter we are asking for your support in discussing and finalizing conditions for zebrafish husbandry in European and national laboratories. Since there is a long-standing history in the area of husbandry and maintenance conditions, there is consequently deep expertise from many laboratories, which now needs to be communicated in a concerted manner to the directive authorities. You, as a national representative, are hopefully willing to support this effort which (1) will greatly aid biomedical research, (2) will help European laboratories to maintain scientific excellence, and, most importantly (3) will harmonize husbandry conditions across Europe in an ethically appropriate manner.

Members of the European zebrafish community (http://itgmv1.fzk.de/eufishbiomed/eufish_main.php) have worked together with members of FELASA, the Federation of Laboratory Animal Science Assiciations, to formulate recommended guidelines for zebrafish husbandry conditions (http://www.felasa.eu/working-groups/working-groups-present/zebrafish-housing-husbandry-and-health-monitoring-recommendations/), and a first draft of these guidelines (containing some of the most critical parameters) are attached to this document. A more extensive set of guidelines will follow in due course.

For now, we sincerely hope you will support us in this important endeavor, and help us in discussing husbandry conditions with the directive authorities.

Please do not hesitate to contact us if there should be need for more information.

Light cycle	14h light / 10h dark		
Water provision	Recirculating system with filter, biofilter, UV unit	Daily water exchange of 5-20% is customary	
Water quality	Parameters to be checked include pH and conductivity, nitrite, nitrate.	pH range between 6.5 and 8.0; conductivity between 250 and 600 µS, Nitrate (< 2.5 mg/L), nitrite (≤ 0.025 mg/L)	Parameters to be checked frequently in newly setup system, less frequently in established system.
Stocking density	5 adult fish per liter tank water are recommended but actual number depend on housing and filtering conditions.	See Matthew (2002) and Vargesaan (2007)	Maintaining fish at densities of lower than 1fish/liter will usually cause problems due to over-feeding. Furthermore, it shifts the sex ration dramatically (female bias), which is taken as an indication for a stress situation.
Environmental enrichment	Permanent enrichment devices (plants etc) are not recommended.	There is no evidence at present that adult fish notice, or benefit from, such permanent enrichment.	Tank enrichment with plants, plastic grass or similar tends to cause hygienic problems; it also induces daily and uncontrolled spawning, the effects of which on the fish are unclear at present. Feeding life artemia, however, can be considered as tank enrichment, as it stimulates natural predatory behavior.
Feeding	Mixture of dry food and freshly hatched artemia. Paramecia might be used for fry.		Artemia feeding is considered as tank enrichment.
Temperature	24.5 -29°C for all stages (fry and adults)		Zebrafish can clearly tolerate a much wider range of temperatures, but around 26°C seems to be a preferred temperature.
Single housing	acceptable		Zebrafish are social animals, and maintaining single fish, or ≤5 fish in less than 3 liters should be kept to a minimum. It can be

			necessary, though, as there is no methodology to mark single fish.
Spawning	Separation prior the day of requested spawning. The use of spawning trays, artificial plant material or lowering of the water level can support spawning and increase egg numbers.		
Light cycle	12:12 to 8:16 dark:light		