



What is understood by “animal-free research”?

Stina Oredsson^{a,*}, Sandra Coecke^b, Jan van der Valk^c, Mathieu Vinken^{d,e}



^a Department of Biology, Lund University, Lund, Sweden

^b European Commission, Joint Research Centre, Ispra, Italy

^c 3Rs-Centre Utrecht Life Sciences, Utrecht University, Utrecht, the Netherlands

^d European Society of Toxicology In Vitro, the Netherlands

^e Department of In Vitro Toxicology and Dermato-Cosmetology, Vrije Universiteit Brussel, Brussels, Belgium

As scientists, we cannot walk into the future backwards, that is, ignore facts and continue as if they do not exist. It is a fact that the book by Russell and Burch, published in 1959, put forward the three principles (3Rs) of humane animal experimental techniques, calling for Refinement, Reduction and Replacement of animal experiments (Russell and Burch, 1959). The latter R ideally implies full replacement of animal use. In this respect, the number of registered animals used in research during the 60 years following the introduction of the 3Rs principles has decreased substantially in Europe (Törnqvist et al., 2014). At the same time, researchers in various disciplines have adopted cell and tissue culturing techniques for a diversity of purposes, including the reduction and, in some research areas, replacement of animal experiments. However, it is equally a fact that several animal-derived products have been used and are still being used in cell and tissue culturing. Nevertheless, many researchers, regulators, funding organisations, politicians and the general public are unaware of this. It cannot be denied that many of these animal-derived products were of utmost importance in the development of cell culturing techniques. Among such products is fetal bovine serum (FBS), which is typically used to supplement the basal cell culture medium in order to promote cell growth, proliferation and maintenance, as it appeared to be beneficial to most cell types. In addition, it facilitates attachment of cells to culture dishes, and provides, among other factors, essential hormones, minerals, trace elements and lipids. FBS has boosted the development of cell culturing methodologies by assisting in the establishment of cancer cell lines of every tumour origin as well as cell lines developed from primary humans and animal cells. FBS is still extensively used today as a suggested supplement for most cell lines obtained through cell banks. FBS is also used to some extent in the induced pluripotent stem cell (iPSC) area as a supplement in the medium for cell lines to be induced as well as for freezing iPSC cell lines. iPSC cells are indeed successfully used in basic research of diseases, such as cancer and neurological disorders, as well as in the search for improved drugs and in toxicity testing. However, it is a fact that the use and production of FBS raises many scientific and ethical concerns (Jochems et al., 2002). Careful investigation into the worldwide use of FBS has estimated that the

global production per year of raw FBS is around 500,000 l harvested from approximately 1.5 million fetuses (Jochems et al., 2002). Serum is a complex mixture, introducing undefined components into the cell culture medium. Several of these substances have not yet been identified, and in many cases, the effects on cultured cells are as yet unclear (OECD, 2018). Other animal-derived products, such as Matrigel™ from Engelbreth-Holm-Swarm mouse sarcoma, collagen from various animal sources, and antibodies produced in mice, also give rise to considerable animal welfare concerns, which are at odds with the original 3Rs principle. All animal-derived products are batch-collected from different animal groups and do result in confirmed batch-to-batch variability which may contribute to reliability and integrity issues of generated *in vitro* data (OECD, 2018; van der Valk et al., 2017). A collective effort to substitute animal-derived materials and reagents will improve reproducibility and will contribute to the 3Rs. This will take time and in the meanwhile we need incentives for researchers to willingly contribute to the 3Rs by systematically removing animal-derived products. As one such incentive in relation to “animal-free research” in funding and publications, we suggest that there should be a clear distinction in the animal-free research field between research that partly relies on the use of animal-derived products and, thus, only partly contributes to the 3Rs, and research completely devoid of animal-derived products, which are mostly chemically-defined. We also suggest that agencies funding “animal-free research” clearly distinguish which kind of research they support and make this clear to monetary donors. Obviously, the way forward in the on-going high technology research where we can study biological processes with increasing accuracy is to strive towards fully defined systems with known composition. This strive clearly excludes the use of non-defined products such as those derived from animals, but also those from humans and plants. This will necessitate interdisciplinary efforts and increasing awareness at the political level, at the level of funding agencies, research as well as that of journals. It will be an effort ensuring reproducible, credible, and trustworthy scientific data generation in the 3Rs research and development arena.

* Corresponding author at: Department of Biology, Lund University, Sölvegatan 35, 223 62 Lund, Sweden.
E-mail address: stina.oredsson@biol.lu.se (S. Oredsson).

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