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Editorial

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In Vitro Toxicology Testing: It's Time to Report the Sex of Cells

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In light of evidence taken from numerous fields indicating that males are routinely used more than females as test subjects,[1-3] and the resulting poor outcomes of such practice,[4,5] a conversation on balancing the sex of sample sets is not a new one. In a 2014 meta-analysis of nearly 300 studies, Prendergast and colleagues discovered that females have erroneously been considered more variable owing to their estrous cycle; instead, males were found to be more inconsistent under some experimental conditions.[6] These results were subsequently confirmed by meta-analysis of microarray datasets.[7] If we are to agree that sex of the subject (be it animal or human) is important to disclose, then the lack of representation to this day is troubling – and it is furthermore troubling that many studies fail even to report the sex of tissues or cells, meaning we have no idea as to the relevance of sex to measured outcomes. There have been several calls to action on this point in the biomedical field,[3] yet we as a toxicology community have not yet been fully persuaded of our failure.

In toxicological studies, cultured cells are routinely used to identify molecular mechanisms driving chemical actions in our environment, the results of which are foundational to designing whole-animal experiments (using so-called "tiered testing") and developing toxicological profiles that provide guidance by predicting harm.[8] As part of sound experimental design, researchers often report several specifics of cultured cells, such as species origin and media conditions, but regularly do not report the sex of their cells,[9] and are seemingly not challenged on this point by peer reviewers and publishing friends.

This invisibility cloak on sex reporting exists and extends beyond any semblance of welcome, despite the fact that every somatic cell indeed has a sex, and sex-specific cell behavior can occur without considering gonadal hormone exposure history. As such, sex chromosome complement, especially in cells studied in culture, has tremendous potential to impact gene expression and resulting mechanistic signaling pathways,[10] and therefore stands to have significant implications for risk assessment, disease susceptibility, and harm prevention.

The sex of cells can clearly matter, and matter in a big way, and the brain has been particularly well studied for its sexual dimorphism at this level. For example, isolated dopaminergic neurons display different morphologies, independent of hormonal status, depending on whether they come from males or females,[11] and male hippocampal neurons are more likely than female ones to be killed by the oxidizer peroxynitrite.[12] Still other studies using embryonic neurons from male and female rats have found males to be more sensitive to ischemic environments, while females have been found more sensitive to apoptotic agents.[13] Regarding exposure to potential toxicants, female murine cells have demonstrated greater sensitivity than their male counterparts to ethanol.[14] This partial list will no doubt continue to grow as more researchers seek to validate or eliminate sex as an experimental factor. Indeed, as others have suggested,[15] the identification of cell sex may explain why observed effects for "identical conditions" may result in dissimilar findings. Should we really expect a cell derived from a female to behave exactly the same as one derived from a male?

Sexual dimorphisms of course exist for cells beyond the brain, and dimorphic cell responses can occur through specific metabolic pathways. For example, researchers have found

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evidence that human cells display a wide variety of different metabolites across the sexes, such as the Carbamoyl-phosphate synthase 1 (CPS1) gene, involved in protein and nitrogen metabolism.[16] This is in keeping with other basic research that has repeatedly shown that males and females metabolize drugs differently.[17,18]

Moving forward, we should hold ourselves to a higher standard of awareness. In a 2015 report by Wan and colleagues,[19] researchers used quantitative Polymerase Chain Reaction (qPCR) – a very common molecular method – to newly identify NIH/3T3 (ATCC No.: CRL-1658) murine embryonic fibroblast cells as female; 3T3 cells have long been used in toxicological experiments, most recently to assess developmental toxicity to therapeutic drugs[20] and phototoxic potential,[21] but also to document cell behavior on nanotubes,[22] response to nanoparticles,[23] and antioxidant response.[24]

Importantly, sex determination using qPCR is now added to the list of several other available tools that can be used to determine the sex type of cultured cells, including immunodetection of H-Y antigen,[25] nested PCR,[26] Southern blot,[27] and enzymatic assays.[28] It should also be noted that it is likely that sex of a cell must be determined more than once over prolonged passage in culture – e.g., many "male" cells, such as the T-84 colonic carcinoma epithelial cell line, have been found to have lost the Y chromosome over time.[15]

Especially in this era of genomics, understanding sex and gender in science seems fundamental and relevant to basic and applied sciences. Enhanced reporting efforts that clearly identify the cell source as female or male would not only enrich our understanding of risk assessment and mechanisms of action for chemical toxicity, but might also contribute to building a new framework with which to understand complex chemical interactions. In the end, sex is a biological variable that could affect measured endpoints – and is just as relevant as other experimental features such as culture conditions.

In summary, there seems to exist a pervasive assumption that the sex of cells in culture in not important,[29] even in the face of mounting evidence that sex influences gene expression in cultured cells, tissues, and beyond.[30-32] Potential benefits of identifying cell sex have been raised for biomedical science writ large,[8,33] and here, now, for toxicology more pointedly. Yet, unless it is the focus of their research, most investigators rarely consider whether cells bearing an XY genotype will behave the same as an XX genotype. It is not expected that sex will play a role in every experimental outcome, but at the very least it should be documented as a biological factor and eliminated as having a significant influence on experimental data.

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