

## CELLULAR RESPONSE OF CHO CELLS FOLLOWING EXPOSURE TO EXTRACTABLES AND LEACHABLES FROM SINGLE-USE BIOREACTORS

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The implementation of single-use disposable bioreactors as alternatives to the dominant stainless steel predecessor used in the manufacturing of recombinant therapeutic proteins introduces a variety of new materials into the bioprocessing pipeline. These plastics, when used under normal culture conditions, can degrade and leach breakdown products into the culture media such as bis(2,4-di-tert-butylphenyl) phosphate (bDtBPP).<sup>1,2</sup>

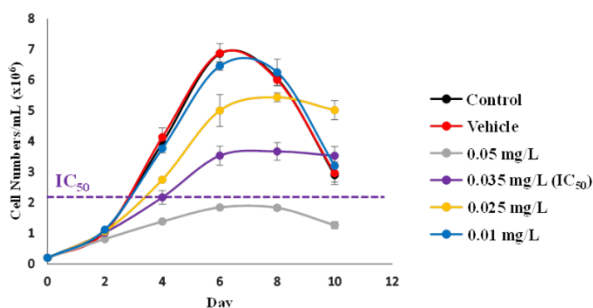


Figure 1 – Impact of various concentrations of the leachable bDtBPP on the bioprocess relevant phenotypes on CHO-K1 cell lines. The concentration of bDtBPP that inhibited cell growth by 50% (IC<sub>50</sub>) after 4 days in culture is a purple broken line.

In the present work the effects of bDtBPP on CHO cell growth was evaluated, using both a parental cell line (CHO-K1) and a mAb1 producing cell line (CHO-DP12). Results showed the effect of bDtBPP at concentrations commonly leached from single-use bioreactors. In particular, the growth curve of the two systems revealed a decrease around 50% of viable cell density, while viability seemed not to be affected, in agreement with previously published observations by Amgen.<sup>1,2</sup> Critical quality attributes (CQAs) for mAb1 produced by CHO-DP12 cell line were analysed, including N-glycan analysis, peptide mapping, aggregation profile, charge and oxidized variants analysis. These analyses showed no differences between produced IgG1 when cells are spiked with bDtBPP.<sup>3</sup>

In order to understand the molecular mechanisms responsible for bDtBPP effects, a multi-omic study

(including glycomic, proteomic and metabolomics) is under investigation.

Following these initial experiments, incubation of media in single-use bioreactors to obtain a preconditioned media containing leachables extracted under normal operational conditions (37°C for 7 days in rocking incubator) was performed. The media was used to evaluate the growth curve of CHO-K1 and CHO-DP12 cell lines; preliminary results showed a detrimental effect on cells growth.

Future experiments will focus on the comparison of media incubated in single-use bioreactors from several brands and models, followed by multi-omic analysis (glycomic, proteomic, metabolomic) of cells cultured with these preconditioned media. This will lead to a better understanding of the molecular mechanisms involving not only bDtBPP but the whole panel of leachables present in single-use bioreactors plastic films.

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