**Assigned AAPS Poster Number**

# W4365

# BACKGROUND

## RATIONAL:

**Every new drug application is expected to include some form of extractables profiling and leachables evaluation for the container closure or manufacturing components at highest risk and in closest contact with the drug.**

## EXTRACTABLES & LEACHABLES:

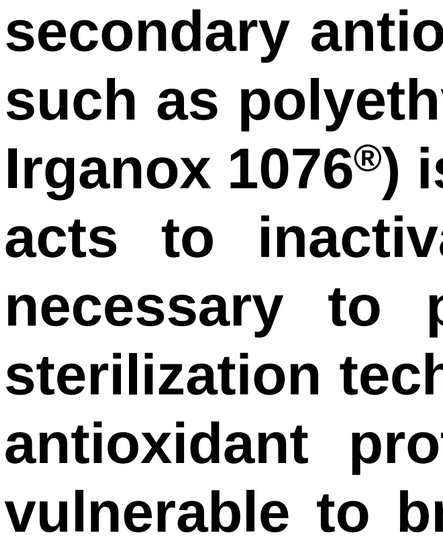
- **Extractables - compounds that can be extracted from a component under *exaggerated* conditions such as in the presence of harsh solvents and/or at elevated temperatures.**
- **Leachables - compounds that leach into the drug product formulation or in-process matrices from the component as a result of direct contact with the formulation under *normal* conditions or accelerated storage conditions.**

## WHY WORRY ABOUT EXTRACTABLES AND LEACHABLES:

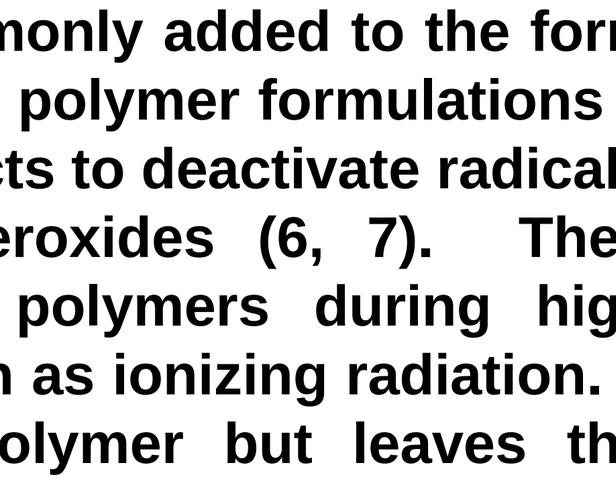
- **The leachable may interfere with the manufacturing process**
- **The leachable may react with one or more of the drug product components**
- **The leachable may be toxic**

## Bis(2,4-di-tert-Butylphenyl)Phosphate:

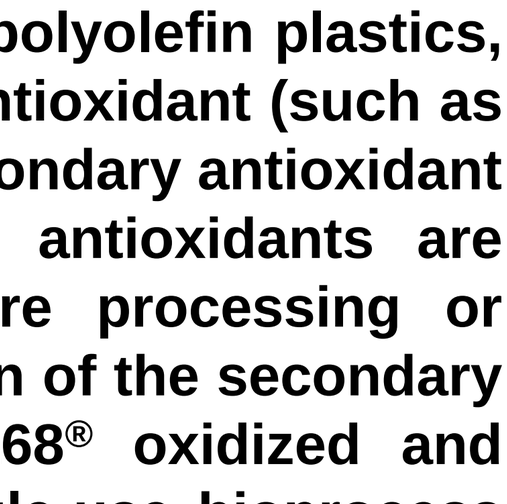
**A prominant extractable from single-use bioprocess containers has been identified as being highly detrimental to cell growth (4). The compound, bis(2,4-di-tert-butylphenyl)phosphate (bDtBPP), is derived from the breakdown of tris(2,4-di-tert-butylphenyl)phosphite (trade name Irgafos 168®). Irgafos 168® is a secondary antioxidant commonly added to the formulation of polyolefin plastics, such as polyethylene (5). In polymer formulations a primary antioxidant (such as Irganox 1076®) is one that acts to deactivate radicals, and a secondary antioxidant acts to inactivate hydroperoxides (6, 7). The secondary antioxidants are necessary to protect the polymers during high-temperature processing or sterilization techniques such as ionizing radiation. The reaction of the secondary antioxidant protects the polymer but leaves the Irgafos 168® oxidized and vulnerable to breaking down. In extractables studies of single-use bioprocess containers, Irgafos 168® and its breakdown products are commonly observed. The most commonly observed compounds are bDtBPP and Irgafos 168® phosphate (tris(2,4-di-tert-butylphenyl)phosphate).**



Irgafos 168®



Bis(2,4-di-tert-butylphenyl)Phosphate



ε-Caprolactam

# The Leachables Profile from Single Use Bioprocess Containers and their Effects on Cell Growth

## CASE STUDY

## Introduction:

**The screening of the final packaging components of human drug products for extractables and leachables has become commonplace in the 15 years since the FDA released their *Container Closure Systems for Packaging Human Drugs and Biologics*. With the increase in prevalence of single-use systems for biomanufacturing, these components require the same scrutiny. The current study examines the leachables profile of three common bioprocess containers and the effects on cell growth of the most prevalent leachable compounds.**

## Methods:

**Three types of single use bioprocess containers were filled with either 60% IPA or water and incubated at 40° C /Ambient Relative Humidity for 20 days. Each bag and corresponding control had 1.0 mL removed at 12, 24, 48 and 72 hours, and 5, 10, 15 and 20 days. The resulting extracts were tested by gradient HPLC using an Agilent 6200 series LC/MS-Time of Flight (TOF) equipped with a multimode source (electrospray and atmospheric pressure chemical ionization) using positive ionization (MMP) and negative ionization (MMN). Table 1 lists the compounds observed in the extractables profiles for each of the bags used in this study with a breakdown by solvent type. For the cytotoxicity assays, CHO K1 cells were plated (5 x $10^4$ per well) in a 96-well plate containing F12K media (with and without FBS). Serial dilutions were made of ε-caprolactin and bDtBPP and were run in triplicate. Four conditions were tested: 1. Media with FBS and no drug; 2. Media without FBS and no drug; 3. Media with FBS and drug; 4. Media without FBS and drug. All plates were incubated at 37° C for 24 hours. Following the incubation, chemiluminescence was measured using CellTiter-Glo (Promega) according to the manufacturer's instructions.**

**Table 1.** Total extractables from the three bag types.

| Bag ID | Water | IPA |
|---|---|---|
| Bag 1 | ε-Caprolactam<br>Bis(2,4-di-tert-Butylphenyl)Phosphate | 3,5-di-tert-Butyl-4-Hydroxybenzaldehyde<br>Bis(2,4-di-tert-Butylphenyl)Phosphate<br>Palmitamide<br>Steamide<br>Erucamide<br>Ethylene Bis Palmitamide<br>Ethylene Bis Heptadecanamide<br>Ethylene Bis Stearamide<br>Irgafos 168 Phosphate |
| Bag 2 | ε-Caprolactam<br>Bis(2,4-di-tert-Butylphenyl)Phosphate | 3,5-di-tert-Butyl-4-Hydroxybenzaldehyde<br>Bis(2,4-di-tert-Butylphenyl)Phosphate<br>Ethylene Bis Heptadecanamide<br>Irgafos 168 Phosphate |
| Bag 3 | Bis(2,4-di-tert-Butylphenyl)Phosphate | 3,5-di-tert-Butyl-4-Hydroxybenzaldehyde<br>Bis(2,4-di-tert-Butylphenyl)Phosphate<br>Palmitamide<br>Ethylene Bis Palmitamide<br>Ethylene Bis Heptadecanamide<br>Irgafos 168 Phosphate |

A B

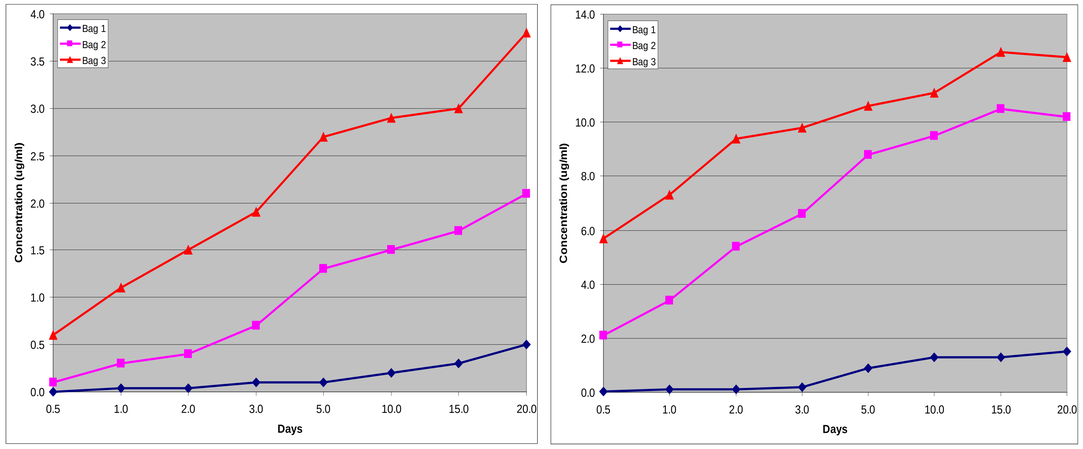



**Figure 1.** Figure 1 A and B show the time dependent extraction of bDtBPP from the three bioprocess bags in water and IPA respectively. This time dependence appears to be nonlinear. Although the data was not fit using a nonlinear algorithm this observation is consistent with what has been previously published for this compound (4).

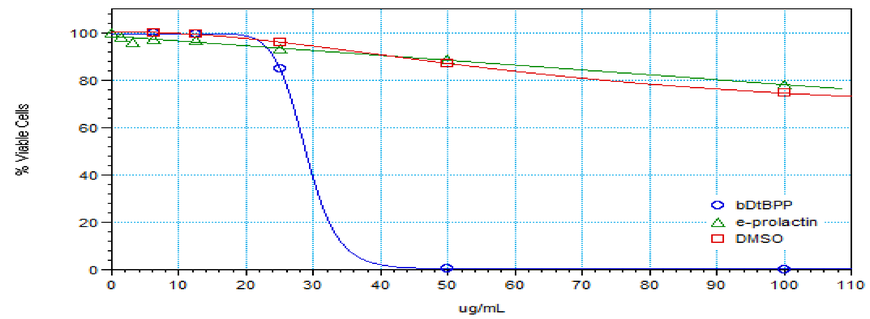



**Figure 2.** Dose-response curves from bDtBPP and ε-Caprolactam spiking cell growth experiments. Each curve represents the average of three independent experiments. The percent viable cell values have been normalized to Control (untreated) cells. The data was fit to the Hill equation.

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## RESULTS

1. **In water, the major extractables were bDtBPP and ε-Caprolactam.**

2. **In the 60% IPA extracts, the bags contained: three Irgafos 168® breakdown products: 3,5-di-tert-butyl-4-hydroxybenzaldehyde, bDtBPP and Irgafos 168 phosphate; and a number of slip agents including: ethylene-bis-palmitamide, ethylene-bis-heptadecanamide, erucamide, and ethylene-bis-steamide along with their breakdown products palmitamide and steamide.**

3. **Extracted bDtBPP concentration initially increased rapidly, but the extraction slowed down significantly after 5-10 days. Maximal levels of water-extracted bDtBPP from the three bags were Bag 1 = 0.5 μg/mL, Bag 2 = 2.1 μg/mL, and Bag 3 = 3.8 μg/mL (Figure 1A). Maximal levels of IPA-extracted bDtBPP from the three bags were Bag 1 = 1.5 μg/mL, Bag 2 = 10.5 μg/mL, and Bag 3 = 12.6 μg/mL (Figure 1B).**

4. **Published $EC_{50}$ values for bDtBBP range from 0.12 to 0.73 μg/mL (4). Two of the three bags exceeded the upper limit by 3- to 5-fold with the third falling just shy of the upper limit with water extraction whereas all three bags exceeded the upper limit when extracted with IPA.**

5. **The $EC_{50}$ value of 29 μg/mL determined in this study was 30 to 300-fold higher than the published values for cell lines derived from the CHO K1 line.**

6. **ε-Caprolactam is relatively nontoxic up to 100 μg/mL.**

## CONCLUSIONS

1. **Single use components have the potential to contribute leachables that can affect both the manufacturing process and the final drug product.**

2. **bDtBBP has a widely variable toxicity profile depending on the cell line exposed to the compound. For a number of published CHO cell lines the observed levels of bDtBPP could negatively impact the manufacturing of biologics as the $EC_{50}$ is 3 to 5-fold lower than the levels of bDtBPP observed. However, the CHO K1 cell line tested in this study would be less affected as the $EC_{50}$ is 2 to 3-fold higher than the levels of bDtBPP observed.**

3. **All single use components need to be evaluated on a case-by-case basis to ensure that bDtBPP or other leachables will not have an adverse impact on the overall manufacturing process.**

## REFERENCE


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