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Leachables: Minimizing the Influence of Plastic Consumables on the Laboratory Workflows

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Chemical substances leaching out of plastic consumables (leachables) are still frequently underestimated in the majority of life science applications. However, increasing scientific evidence shows that this heterogeneous group of chemicals may significantly affect experiments and pose a likely source of error in various assay systems.

This document provides an overview of the classes of leachables which are known to be critical, and how they can influence various experimental systems. Practical recommendations on how to estimate and significantly reduce the risk of leachables affecting experiments are also summarized.

Introduction

The workflow of laboratory experiments may be influenced by various factors. Routinely, only the assay-specific factors are recognized as relevant and pursued to be controlled. These typically include sample material (quality, integrity, amount), reagent systems (quality, performance) and laboratory equipment (features, performance, quality). On the other hand, more general experimental factors are often unconsidered or not well understood and commonly remain uncontrolled in the laboratory routine. In particular, the role of bioactive contaminants leaching out of consumables – also known as leachables – is generally underestimated as an essential factor likely influencing experimental performance. While possible effects of leachables are routinely taken into account in pharmaceutical or food research, this topic is still underrepresented in the majority of life science applications. Recent scientific evidence indicates, however, that leachables may have various specific as well as general biological effects and pose a likely source of error in many assay systems [1, 2, 3].

Leachables: chemical analysis

In principle, all leachables are chemical substances, which can be released from a given consumable into the sample under certain experimental conditions. These primarily include experiment-specific temperature, time and solvent type. The leaching process may vary greatly according to these experimental conditions, and different chemicals may be present in the sample in different amounts, thus exerting a spectrum of effects on the assay [3]. These effects are particularly relevant for plate-based assays, where a high variability in leachable levels has been observed in different wells, indicating position-dependent influences on the assay (internal data). In addition, cumulative effects have been observed with increasing numbers of pipetting steps or sample handling steps, indicating that even short contact times with the sample may lead to the release of relevant amounts of bioactive contaminants [3, 4].

The majority of laboratory consumables consist of polymers manufactured from petrochemical-based monomers: most common are polypropylene, polyethylene and polystyrene. Contrary to common belief, laboratory plastics contain various polymerization by-products beside the pure polymer: aliphatic hydrocarbons, oligomers, olefin-clusters and products of oxidation (alcohols, aldehydes, ketones). More importantly, however, various chemicals are often added during the manufacturing process to facilitate polymerization, alter consumable properties, or very often simply to increase production speed or reduce production costs. Some additives are indispensable for the production process: metal ions, stabilizers, antioxidants, and so far assessed to be potentially uncritical for biological assays (see Table 1). The majority however, can be largely avoided and has been shown to interfere with various assay systems with critical classes of additives including biocides, plasticizers and slip agents (Table 1).

> **Heavy metals** to catalyze polymerization > **Stabilizers and antioxidants** to stabilize the product > **Pigments** in colored consumables	**Cannot be avoided during production. Most likely non-critical**
> **Clarifiers** to increase transparency > **Antistatic agents** to reduce electric charge > **Surfactants/detergents** to disperse pigments	**Can be avoided in production. Potentially critical**
> **Slip agents** for easier & faster removal from mold > **Biocides** to prevent microorganism growth on plastic > **Plasticizers** to alter mechanical properties	**Can be avoided in production. Affect various assays and are critical**

Additives in the production process - significance in laboratory

Leachables: influences on the workflow in the laboratory
Recent scientific evidence provides numerous examples of critical classes of additives interfering with a broad range of biological assays. These include enzymatic [1, 2, 3] and receptor binding [1, 4], as well as commonly used photometric assays [5]. Leachables have also been shown to cause alterations and growth reduction in various cell culture systems [3, 5]. Examples of recent scientific publications on leachables and their bioactive effects are summarized in table 2.

In recent years, increasing evidence of consumables hampering experimental results has changed the perception of the scientific community, with high quality consumables and comprehensive information on critical additives provided by the manufacturer becoming increasingly important [2, 6, 7, 8].

Minimizing the effects of leachables: practical recommendations
There are several steps researchers can take to minimize the risk of leachables affecting their experiments [2, 3]. Some preliminary basic steps may involve taking inventory of consumables used in the laboratory to gain an overview of the situation and make it easier to react when problems occur. Standardization may also prove a good way to minimize effects of leachables: consistently using consumables from one manufacturer and avoiding changing or mixing them within series of experiments. Furthermore, using consumables from a manufacturer that provides/guarantees a high consistency across consecutive production lots is essential when attempting to minimize technical variances. This is especially important for assays that require the use of consumables from different lots, e.g. long-term routine experiments or high throughput assays. For assays known to be sensitive to leachables, each new production lot purchased should be ordered early enough and tested for any differences against the current batch within the same experimental setup.

Finally, it is important to choose consumables from manufacturers who comply with high quality production criteria (ISO conform, clean room conditions, high degree of automation) and provide consumables made of virgin polymers and strictly free of the critical additives described earlier: slip agents, biocides and plasticizers [3]. Furthermore, strict testing of each individual production lot, as well as external examination and certification of every lot comprising an advanced purity grade, are essential steps in the process of monitoring consistently high product quality. Eppendorf, as a premium consumables manufacturer, is in compliance with all these high production and quality standards. A comprehensive certification of quality standards, which includes the absence of avoidable additives, is provided for all consumables as well as lot-specific certification for advanced purity grades (www.eppendorf.com/certificates).

In summary, an awareness of consumables and leachables as potential sources of interference with the laboratory workflow, coupled with appropriate laboratory routines and strict avoidance of consumables not certified to be free of the critical additives mentioned above provides a best practice guideline for minimizing the risk of leachables affecting the experiments.

Summary

Contrary to common belief, consumables play a vital role in the laboratory workflow. Various additives often used during the production process may leach into the samples and pose a likely source of error in many assay systems. Three classes of additives are particularly critical and were shown in the literature to affect experiments: slip agents, plasticizers and biocides. Choosing high quality consumables, which are certified free of these critical additives, can significantly reduce the risk of negative influences on experiments.

Publication	Consumables	Leachable	Type	Affected Assay
McDonald G.R. et al., Science 322, 917 (2008)	Tubes	DiHEMDA	Biocide	Altered hMAO-B (human monomine oxidase-B) activity
		Oleamide	Slip agent	
		unknown	Surfactant	Inhibition of $GABA_A$ (γ-aminobutyric acid type A) binding
Reid G. et al., GIT Laboratory Journal 9-10, 2-4 (2009)	Tubes	unknown	Surfactant	Inhibition of binding to GABA-A receptors
	Tips	unknown	Unknown	Inhibition of hMAO-B (human monoamine oxidase-B) activity
	Tubes	Oleamide	Slip agent	
Watson J. et al., J Biomol Screen 14(5), 566–572 (2009)	Tips	Erucamide	Slip agent	Alteration in G-protein-coupled receptor assay
Lewis, L. K. et al., BioTechniques 48, 297-302 (2010)	Tubes PCR tubes	Mixture of small molecules (200 - 1400Da)	Unknown	Increased photometric readings at 220 nm and 260 nm affecting DNA/RNA and protein quantification
Hammond M. et al. Biotechnol Prog. Apr: 332-7 (2014)	Single-use bioprocess equipment	bDtBPP Butylphenyl-phosphate	Antistatic stabilizer	Reduced growth of cell cultures
Birch NP. et al. J Neurochem. Apr; 133(1) (2015)	Syringes, tubes	stearic acid, palmitic acid, 1,2-ethanediol, unknown	Plasticizers	Alterations and growth reduction of cultured neurons

Table 2: Bioactive effects of leachables - literature review

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