

# The Growing Importance of Mitochondrial Toxicity Assessment in Drug Discovery and Development

## "*Primum non nocere.*" First, do no harm.

It's one of the guiding principles underlying the ethical practice of healthcare. More than that, it's a cautionary tale – one that resonates throughout the world of drug discovery and development. History has shown it's one thing to develop a drug that makes a positive impact on the medical condition of interest, it's quite another to ensure that a drug isn't also causing unintended health issues elsewhere.

Mitochondrial toxicity is a case in point. Around the turn of the millennium, several drugs were brought to the market only to be quickly pulled again, in what turned out to be very high-profile failures for the companies involved. The reason the drugs were withdrawn? Liver failure, which was eventually determined to be associated with metabolic/mitochondrial toxicity. Among these drugs was a promising antidiabetic medication called troglitazone, which itself was eventually linked to more than 400 cases of acute hepatic failure, resulting in hundreds of millions of dollars in legal costs for its developer.

The withdrawal of troglitazone and others brought heightened scrutiny to the importance of mitochondrial toxicity as a core criterion of drug safety. It was clear that traditional *in vitro* cytotoxicity tests were inadequate; these assays relied on mammalian cells, which have the ability to circumvent certain toxicities by accelerating glycolysis and inhibiting mitochondrial function through a mechanism known as the Crabtree effect. The net result was a disconnect between the *in vitro* and *in vivo* situations where potential mitochondrial toxins could be missed under certain assay conditions.

## Mitochondrial toxicity in the spotlight

At the time, the only available method for directly measuring mitochondrial respiration relied on a device known as the Clark electrode. A breakthrough at the time of its invention in the early 1960s, the Clark electrode was nonetheless a single-chamber technology that was unsuitable for the throughput needs of the pharma industry.

The Glu/Gal assay, developed by Luxcel Bioscience (now a part of Agilent) in collaboration with Pfizer, was a milestone in the assessment of mitochondrial toxicity. This assay uses glucose- and galactose-conditioned media in combination with the redox-sensitive dye MTT. Although it is reasonably effective and relatively inexpensive to perform, the Glu/Gal assay has shortcomings that limit its use as an indicator of mitochondrial safety. One issue is that it has relatively poor sensitivity; more problematic is the inability of the assay to differentiate between drugs that inhibit mitochondrial function solely by uncoupling the electron transport chain and drugs that exert toxicity through multiple cellular mechanisms. Thus, there is potentially important mechanistic information that this assay is simply not designed to deliver.

A number of companies, including Seahorse Bioscience (now a part of Agilent), recognized the potential to assess mitochondrial function using fluorescent probes that were sensitive to oxygen and pH. This led to the introduction of real-time assays of mitochondrial function that report oxygen consumption and glycolytic flux with a high degree of sensitivity, in a convenient 96-well microtiter plate format that is scalable for practical use in drug development labs.

## Tightening the net

The pharmaceutical industry has expressed a need for more stringent approaches to mitochondrial toxicity screening beyond what available assays have been able to provide, in hopes of avoiding potentially huge development costs (or worse). The introduction of the Agilent Seahorse XF assay platform, accompanied by scientifically controlled and validated real-time metabolic assays kit formulations, has opened new avenues for examining mitochondrial health in a timely and effective manner.

In their efforts to prevent potentially toxic compounds from slipping through the net early in the drug discovery process, a group of scientists at Genentech led by Dr. Tomomi Kiyota and Dr. William Proctor have compared the performance of Glu/Gal assays with results from Agilent Seahorse XF-based methods, particularly the XF Mito Stress Test (MST) assay. This single assay analyzes multiple parameters relevant to mitochondrial health, including basal respiration, ATP-linked respiration, maximal and reserve capacities, and non-mitochondrial respiration.

The results from [Kiyota and Proctor](#) demonstrated that the XF assay has the potential to identify mitochondrial toxins that could not be identified using Glu/Gal methodology (Figure 1). In fact, the Glu/Gal assay failed to identify troglitazone as an inhibitor of mitochondrial function; in contrast, the MST assay sensitively and clearly demonstrated dose-dependent toxicity for this well-known mitochondrial poison.

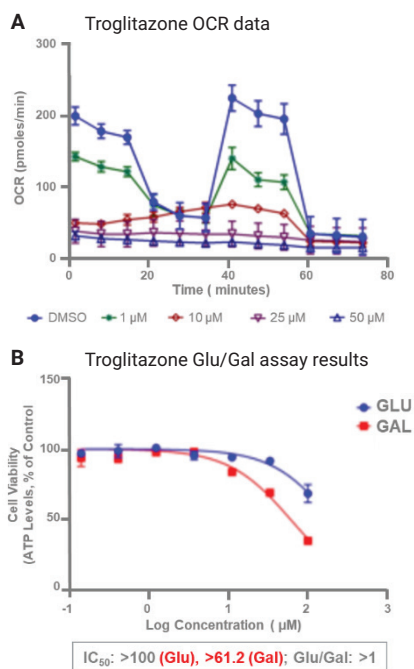
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**Figure 1.** Comparison of Agilent Seahorse OCR-based XF assay and Glu/Gal total ATP assay. (A) The XF assay showed clear mitochondrial toxicity for troglitazone. (B) Mitochondrial toxicity was not detected for troglitazone using the Glu/Gal method.

The MST assay provides essential insights into mitochondrial dysfunction and allows users to investigate functional differences among cell types, drug candidates, and genetic or biochemical interventions. Building on the principle of the MST assay, Agilent has developed a streamlined Seahorse XF Mito Tox assay solution that can easily be integrated into a drug discovery workflow with improved throughput and data interpretability. In light of its combination of sensitivity, specificity, and throughput, the XF Mito Tox assay is deemed an ideal approach for confident identification of mitochondrial liability, thus helping to de-risk the drug discovery pipeline.