THE TOXICOLOGY-METABOLISM LINK

# Cell Metabolism Assays for Toxicology Research

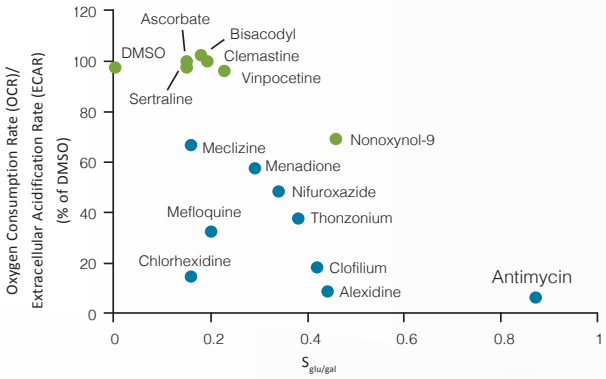
Seahorse Bioscience

A part of **Agilent Technologies**

# SCREENING FOR TOXIC & ADVERSE PHENOTYPES USING FUNCTIONAL METABOLISM ASSAYS

## SCREENING

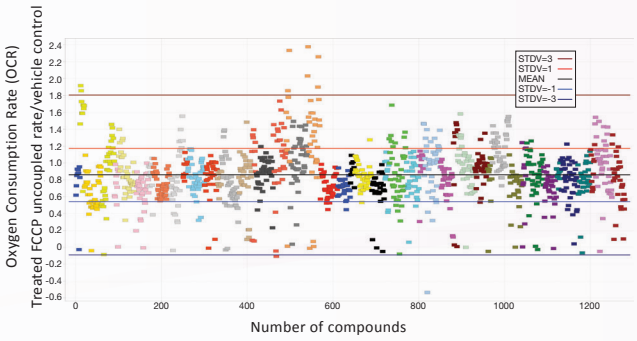
The drug discovery process is evolving to demonstrate increased therapeutic agent efficacy using optimized methodologies, including correlating *in vitro* toxicity and *in vivo* outcomes. Researchers are leveraging Seahorse XF technology to identify potential adverse effects early in the drug discovery process. The Seahorse XF Cell Mito Stress Test measures the key parameters of respiration: basal respiration, proton leak, ATP-linked respiration, maximal respiration, and spare respiratory capacity. The Seahorse XF Glycolysis Stress Test measures the key parameters of glycolytic activity: glycolysis, glycolytic capacity, and glycolytic reserve. These XF assays are used to measure desired and adverse drug affects.




Human fibroblast cells

(Gohil VM *et al.*, 2010. Nat Biotechnol.)

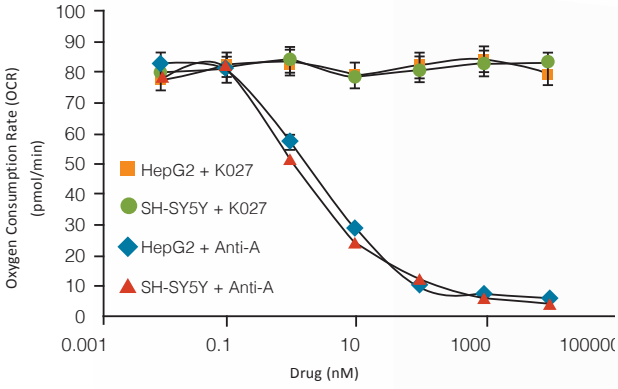
**Seahorse XF assays identifies chemicals that significantly affect metabolism when plotted against cellular growth in galactose -relative to glucose-containing media.**




Rabbit renal proximal tubular cells

(Beeson CC *et al.*, 2010. Anal Biochem.)

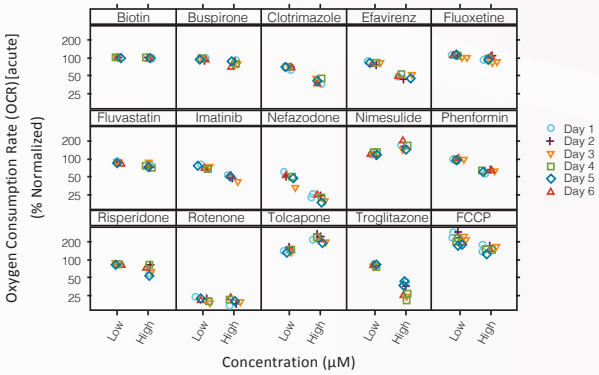
**Seahorse XF assay predicts nephrotoxicity in kidney cells during a primary screening of the Library of Pharmacologically Active Compounds (LOPAC).**




Human liver carcinoma cells (HepG2); Human neuroblastoma cells (SH-SY5Y)

(Prado A *et al.*, 2015. J Appl Toxicol.)

**Seahorse XF assay reveals drug toxicity in liver and neuronal cells.**




Human liver carcinoma cells

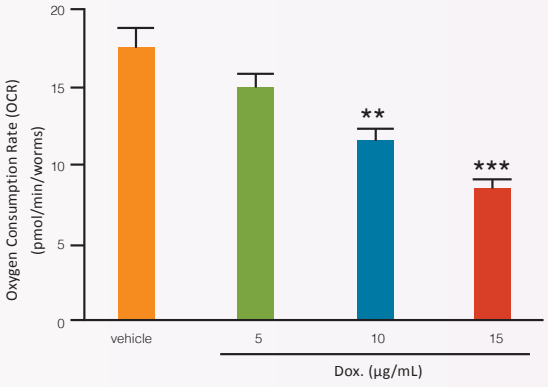
(Wang R *et al.*, 2015. J Biomol Screen.)

**Seahorse XF assay is consistent and reproducible for evaluating the effect of multiple compounds on HepG2 cells.**

# THE WORLD'S MOST ADVANCED METABOLIC ANALYZERS FOR TOXICOLOGY RESEARCH

## MODELS OF TOXICITY

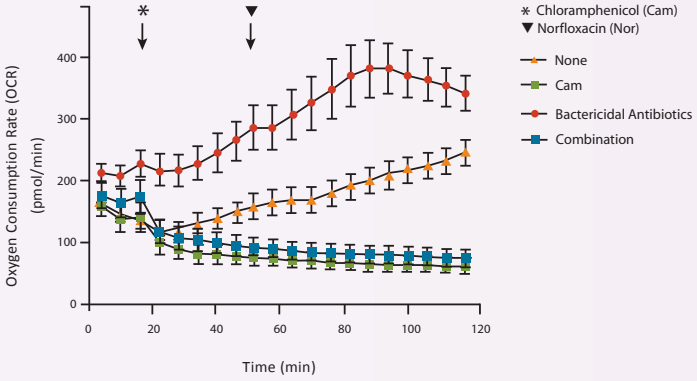
Determining a relevant *in vitro* model that can accurately recapitulate *in vivo* toxicity is crucial to preventing late-stage trial loss. By using Seahorse XF technology to assess functional metabolism, greater comprehensive knowledge about the drug effect can be gained, which is essential to a successful drug discovery program.




*C. elegans*

(Moullan N *et al.*, 2015. Cell Rep.)

**Seahorse XF assay reveals doxycycline (Dox)-mediated mitochondrial dysfunction in *C. elegans*.**



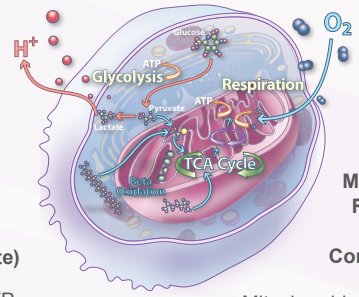

*E. coli*

(Lobritz MA *et al.*, 2015. PNAS.)

**Seahorse XF assay reveals connection between decreased respiration and killing arrest, in *E. coli*, confirming metabolomics data.**

## XF DATA IN PUBLICATIONS

There are over 2,000 references utilizing Seahorse XF technology published in leading journals such as Nature and Cell. Scientists are embracing Seahorse XF technology to identify metabolic phenotypes and reprogramming to target metabolic pathways for therapeutic purposes.

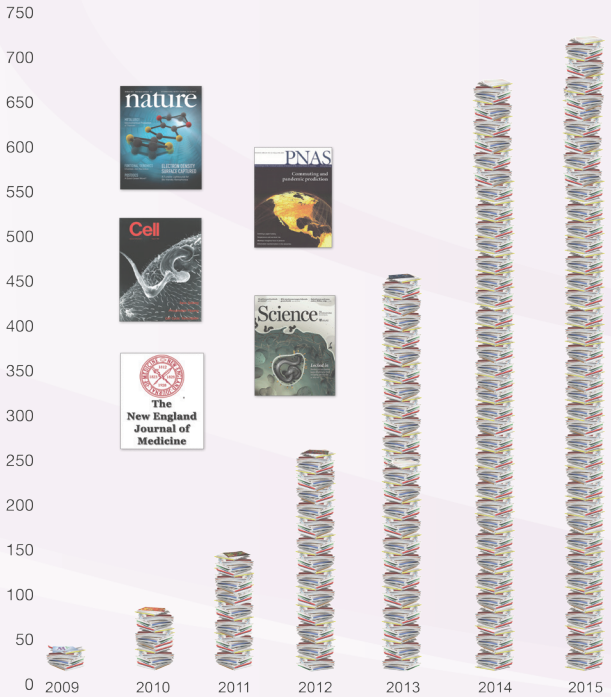



**GLYCOLYSIS — ECAR (Extracellular Acidification Rate)**

Cells generate ATP via glycolysis independent of oxygen, producing lactic acid and protons.Seahorse XF Analyzers measure glycolysis by measuring the extracellular acidification rate (ECAR) of cells.

**MITOCHONDRIAL RESPIRATION — OCR (Oxygen Consumption Rate)**

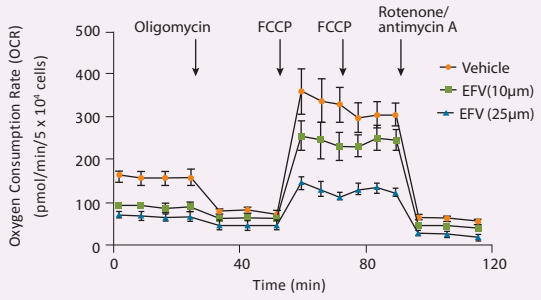
Mitochondria consume oxygen when oxidizing fatty acids or other substrates to generate ATP. Seahorse XF Analyzers measure mitochondrial respiration by measuring the oxygen consumption rate (OCR) of cells.



# MEASURING THE KEY PARAMETERS OF FUNCTIONAL CELL METABOLISM

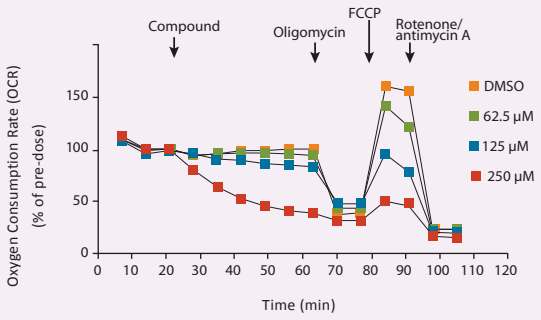
## DRUG TOXICITY

While some therapeutic candidates are widely employed, there can be adverse effects linked to these drugs. Researchers are using Seahorse XF technology to understand the mechanisms associated with adverse effects in clinical situations.



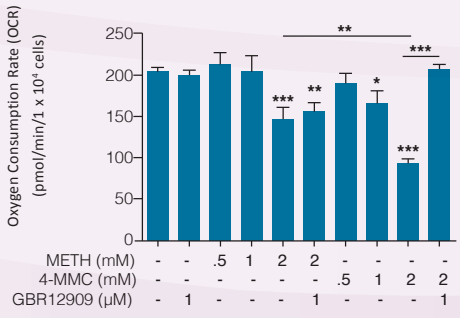

Human glioblastoma cells
(Funes HA *et al.*, 2015. J Antimicrob Chemother.)

**Seahorse XF Cell Mito Stress Test reveals an Efavirenz (EFV) -mediated dose response decline in mitochondrial respiration in human glioblastoma cells.**




Human liver-derived cells
(Kenna JG *et al.*, 2015. J Pharmacol Exp Ther.)

**Seahorse XF Cell Mito Stress Test reveals a dose-dependent reduction in mitochondrial respiration in human liver-derived cells (Huh-7) following treatment with sitaxentan, an endothelial receptor agonist.**

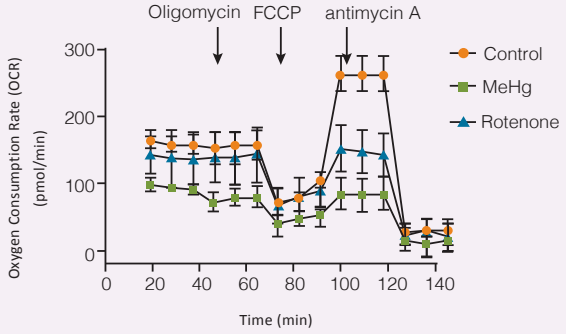



Human neuroblastoma cells
(den Hollander B *et al.*, 2014. Toxicol Sci.)

**Seahorse XF Cell Mito Stress Test reveals a potential rescue of the mephedione-mediated reduction in neuronal cell maximal respiration.**

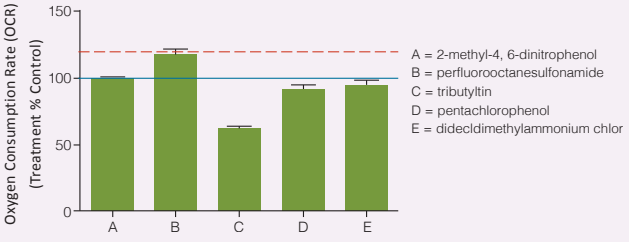
## ENVIRONMENTAL TOXICITY

Exposure to environmental contaminants is a primary health concern. Mitochondria are a key component of cellular response to environmental pollutants. Seahorse XF technology is providing the tools to explore the toxicity of new chemicals.




Rat neuronal cells
(Tiernan CT *et al.*, 2015. Toxicol Sci.)

**Seahorse XF assay reveals mitochondrial dysfunction induced by methylmercury (MeHg) exposure in neuronal cells.**




Rabbit renal proximal cells
(Wills LP *et al.*, 2015. Toxicol Sci.)

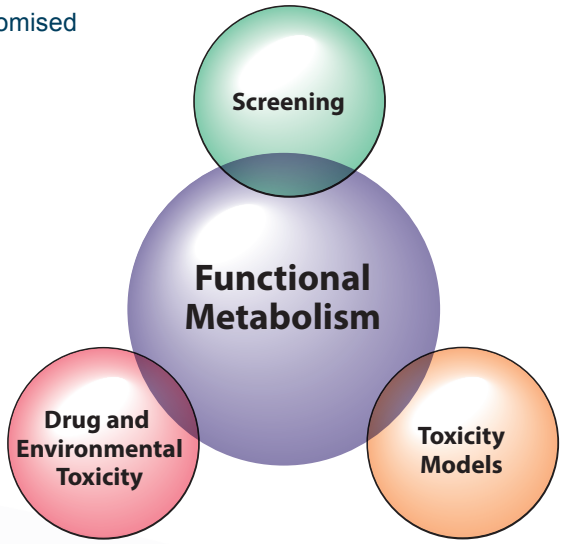
**Seahorse XF assay identifies perfluorooctanesulfonamide, a pesticide and disinfectant, as a mitochondrial uncoupler.**

# FUNCTIONAL METABOLIC ASSAYS

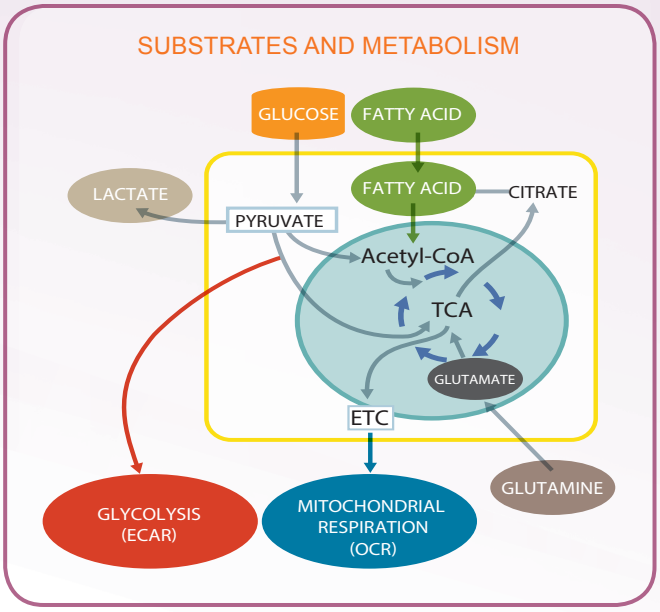
## THE INEVITABLE TOXICOLOGY-METABOLISM LINK

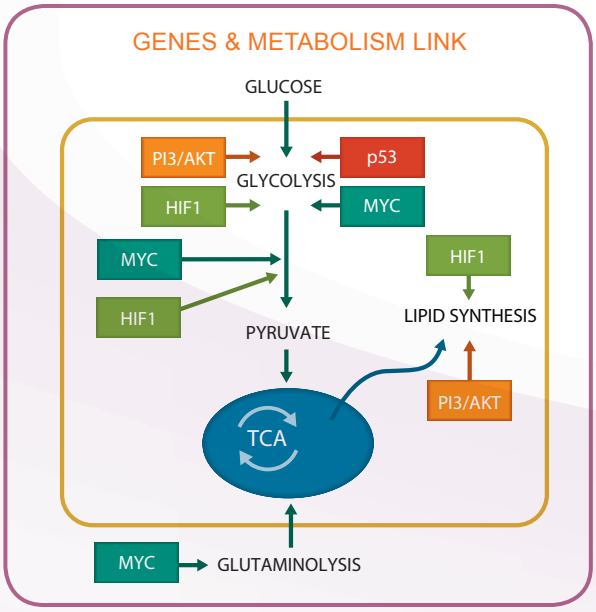
The optimal paradigm for drug discovery includes eliminating ineffective drug candidates early in the process, rather than during the most costly and time-consuming phases of pre-clinical and clinical trials. Furthermore, the elimination of drug candidates goes beyond efficacy to identifying adverse effects, reliably predicting toxicity, and using relevant models that closely mimic the target host, is the ideal strategy.

Mitochondria are at the nexus of cellular energy generation, intracellular signaling, and cell death and survival regulation. Therefore, compromised mitochondria, which strains the expansive and intricate cellular metabolic network, has been linked to various disease pathologies and etiologies. This valuable organelle presents a sensitive system to test for acute and toxic effects.




With traditional cellular toxicology approaches representing only an acute study of drug toxicity, researchers are expanding their focus to include functional metabolism to predict toxicity prior to *in vivo* and patient studies. Seahorse XF technology is providing researchers the necessary tools to measure the functional, metabolic impact of their compound of interest faster and easier, using live cells.



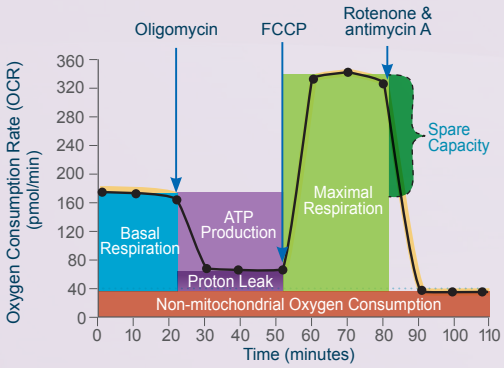



# GOLD STANDARD METABOLIC ASSAYS

## MEASURING THE KEY PARAMETERS OF CELL METABOLISM

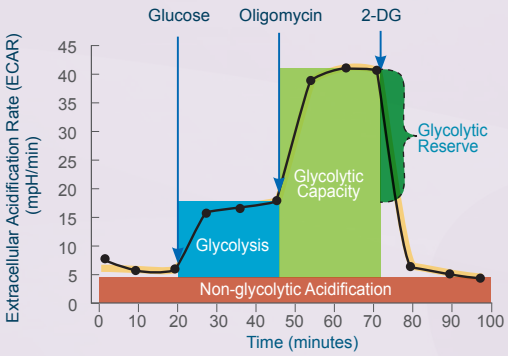
### Seahorse XF Cell Mito Stress Test Profile

Mitochondrial Respiration



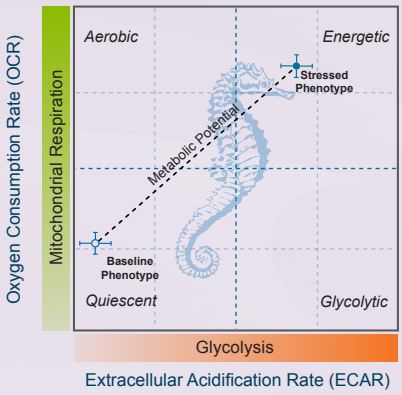

### Seahorse XF Glycolysis Stress Test Profile

Glycolytic Function



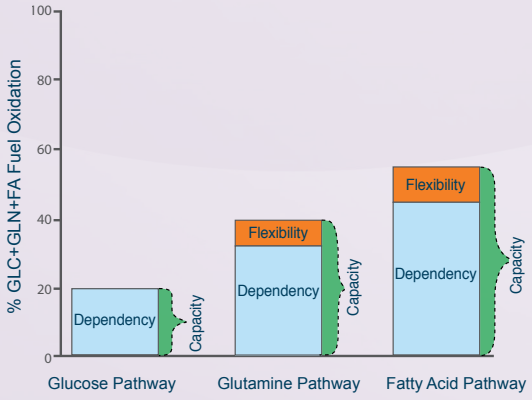

### Seahorse XF Cell Energy Phenotype Test

Metabolic Phenotype & Potential




### Seahorse XF Mito Fuel Flex Test Profile

Mitochondrial Function




Seahorse *Bioscience*

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**Corporate Headquarters**

Seahorse Bioscience Inc.
16 Esquire Road
North Billerica, MA 01862 US
Phone: +1.978.671.1600
www.seahorsebio.com

**European Headquarters**

Seahorse Bioscience Europe
Fruebjergvej 3
2100 Copenhagen DK
Phone: +45 31 36 98 78

**Asia-Pacific Headquarters**

Seahorse Bioscience Asia
199 Guo Shou Jing Rd, Suite 207
Pudong, Shanghai 201203 CN
Phone: 0086 21 33901768


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