Principles of Toxicology Modules

Toxicology is defined as investigation of any adverse effects that physical, chemical, or biological agents may have on living organisms and the environment. Toxicity can be acute or chronic; mild or severe. There are a myriad of interconnected issues that researchers and designers face when determining whether a chemical is toxic or not. Many have to do with the fate of the chemical and then how it impacts living systems and presents itself as toxic. If chemists can change any of the physiochemical properties of the chemicals they design, the chemical bioavailability can be tailored to the acceptable physiochemical level, where it can still carry out its functions while posing less threat to its biological host. When estimating potential toxicity of a chemical, toxicological concepts such as ADME (Absorption, Distribution, Metabolism and Excretion) and bioavailability should be considered, among many others. Physicochemical properties tie into these too as each chemical has a unique set of physical and chemical parameters which play an important role in the toxicity assessment. For example, a chemical can be characterized by its molecular weight, surface area, partition coefficient (LogP), and pKa. These parameters can evaluate if the compound is bioavailable (if it will absorb through the skin, lungs or GI tract) and how fast will it be metabolized and excreted. For a tutorial and background information about toxicology in general, please visit ToxLearn. ToxLearn is a joint project from the U.S. National Library of Medicine's Toxicology and Environmental Health Information Project (TEHIP) and the U.S. Society of Toxicology (SOT). Together, these agencies developed a two module learning tool, helping to highlight key toxicology features and background into further study in toxicology.

Module 3: ADME and Toxicology

Introduction: Chemical impact on health is usually investigated via the concept of ADME. This is how a chemical is **A**bsorbed, **D**istributed, **M**etabolized, or **E**liminated in living systems. Not all chemicals are impactful in the same ways, sometimes metabolism, for example, may not be an issue because of the way our liver metabolizes the compound and converts it into an inert substance. Some chemicals have means to be excreted, while others may not. Considering all aspects of how chemicals get into the body, how they move within the body, and how they get out of the body can help us assess the toxicity of a chemical.

Learning Outcomes: By the end of this module, the student will be able to:

- Define the four key components of ADME
- Relate physicochemical properties of chemicals to the impact they have on ADME
- Predict which physicochemical properties of chemicals have an impact on ADME

Background and Information: ADME (standing for Absorption, Distribution, Metabolism, and Elimination) is an important concept that describes potential impact a chemical or drug may have on a living system within the context of cellular biology and biochemistry. This is because movement and metabolism of molecules is determined by physicochemical properties of the molecule as well as the host system. The movement of molecules is called “kinetics” or “pharmacokinetics” and chemical properties such as polarity, molecule weight, molecular size, chirality, HOMO/LUMO, and many more all have an impact on the ADME potential of a molecule/toxin. ADME is generally used to describe the impact or a drug or pharmaceutical compound. However, the concept of ADME is applicable to non-pharmaceutical compounds, including those from toxic exposure. Drugs are specifically designed using ADME principles; however, chemicals for commercial use are not designed with any guidelines targeting ADME.

Absorption

There are four main routes of exposure:

- Inhalation through the respiratory system: a chemical in the form of a gas, vapor or particulate that is inhaled and can be excreted or deposited in the respiratory system.
- Dermal through skin or eye contact.
- Ingestion through the gastrointestinal system: Absorption through the digestive tract. Ingestion can occur through eating or smoking with contaminated hands or in contaminated work areas.
- Injection: Introducing the material directly into the bloodstream. Injection may occur through mechanical injury from "sharps."

To be absorbed, a substance must cross one of the layers of cells that keeps “us” “in” and the rest of the world “out”: skin (including mucus membranes), lung, and the gastrointestinal (GI) tract. Most substances are absorbed by passive diffusion through membranes. A small number of biologically important atoms and molecules are actively taken up by cells. Examples include sodium, potassium, and calcium ions, amino acids, small sugars (mono- and di-saccarides). If your substance is very similar to one of these, there is an increased chance of cellular uptake. Solubility into membranes is the primary factor affecting absorption.

Distribution

The compound next needs to be able to move from the site of absorption to other areas of the living system if it is to be distributed. Not all compounds move easily. Most often movement is via the bloodstream but other compounds may move cell-to-cell as well. In general, there are four main ways by which small molecules cross biological lipid membranes: (Links to an external site.)

- Passive diffusion. Diffusion occurs through the lipid membrane from a high to low concentration (aka concentration gradient).
- Filtration. Diffusion occurs through aqueous pores, still from high to low concentration as a driving mechanism.
- Special transport. Transport is aided by a carrier molecule. Can move against the concentration gradient (low to high).
- Endocytosis. Transport takes the form of pinocytosis for liquids and phagocytosis for solids.

Many times the mechanism of transport for a certain chemical is unknown, and so we must judge its potential toxicity using other variables (such as molecular weight, ionization (pKa), and octanol/water partition coefficient (logP)).

Metabolism

Compounds begin to break down in the body by a family of enzymes in the liver called the Cytochrome P450 system. These enzymes can convert chemicals to reactive oxygen species (ROS), reactive intermediates, free radicals, and others. For example, redox reactions and potential, with a transfer of electrons, influence the toxicity of a chemical at the intracellular level. Scientific advances in toxicology and chemistry are starting to allow scientists to better understand these kinds of interactions, and they are able to map out more specific pathways, called Adverse Outcome pathways (AOPs). It is through understanding these pathways that a new generation of chemicals will be safely designed by chemists and others.

Excretion

Most excretion occurs through the kidneys as urine or as feces. Excretion is dependent on the process of kidney filtration at the glomerulous, and is largely based on molecular size and charge. Some molecules can be excreted through the skin as sweat and still some may be excreted through the lungs via gas exchange. If excretion is not a complete process, the molecule or metabolic by-product can bioaccumulate and impact living systems adversely. If a compound is lipid-soluble, it will bioaccumulate more quickly in adipose tissue. Bioaccumulation of lipid-soluble compounds such as DDT has been shown to be correlated with adverse health effects such as diabetes, heart disease, obesity, etc.

Several companies have developed tools for predicting toxicity related to ADME, such as ADMET Predictor by Simulations Plus, Inc., ADME/Tox by Sigma-Aldrich, LLC., PhysChem and ADME-Tox Prediction by ACD/Labs, PK/PD Database for Pharmacokinetic Properties by the Laboratory of Computational and Medicinal Chemistry, etc. These tools allow researchers (pharmaceutical and non-pharmaceutical) to predict potential

toxicity in regard to ADME and physicochemical properties. **NOTE:** When investigating ADME in regard to toxicology, most information is centered on drug development. When searching for additional information in regard to toxicity modelling, using key search terms such as “chemical disposition” and “toxicokinetics” will yield additional supporting information for this topic.

Assignment:

Match the following physicochemical properties most likely to have an impact on each component of ADME:

Physicochemical Property	**ADME Component**
Molecular Size	Absorption
REDOX Potential	Distribution
Solubility	Metabolism
LogP/pKA	Excretion

Assignment answer found HERE: ADME and Toxicology Answer Key

Resources:

- Brenner, G., & Stevens, C. (2012). Pharmacology. 4th ed. Pharmacokinetics, Chapter 2. Retrieved from https://www.us.elsevierhealth.com/media/us/samplechapters/9781416066279/Chapter%2002.pdf
- University of Idaho. (2015). eTox, Principles of environmental toxicology lecture series. Retrieved from http://www.webpages.uidaho.edu/etox/lectures.htm
- National Toxicology Program. (2015). Chemical disposition and toxicokinetics. Retrieved from http://ntp.niehs.nih.gov/testing/types/chemdisp/index.html
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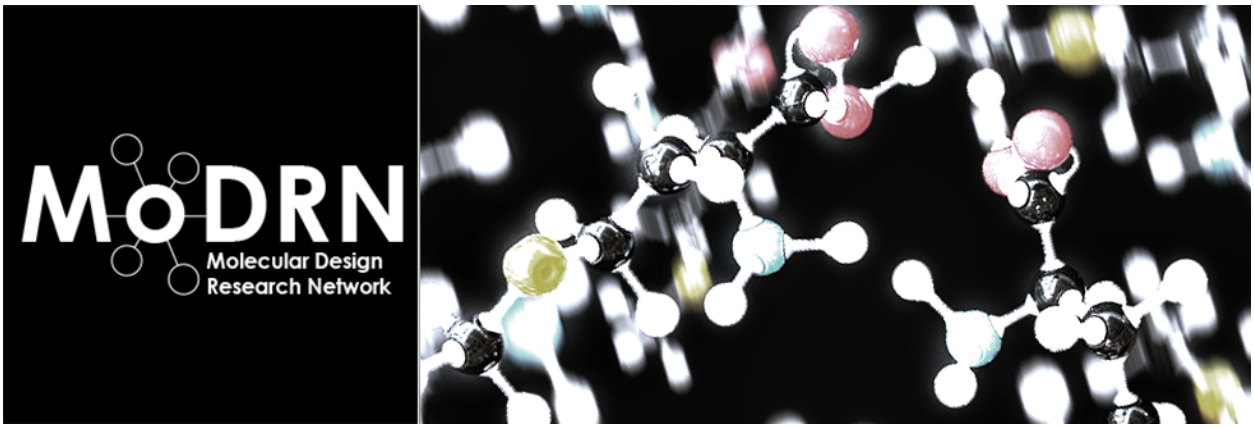
Module 4: Oxidative Stress

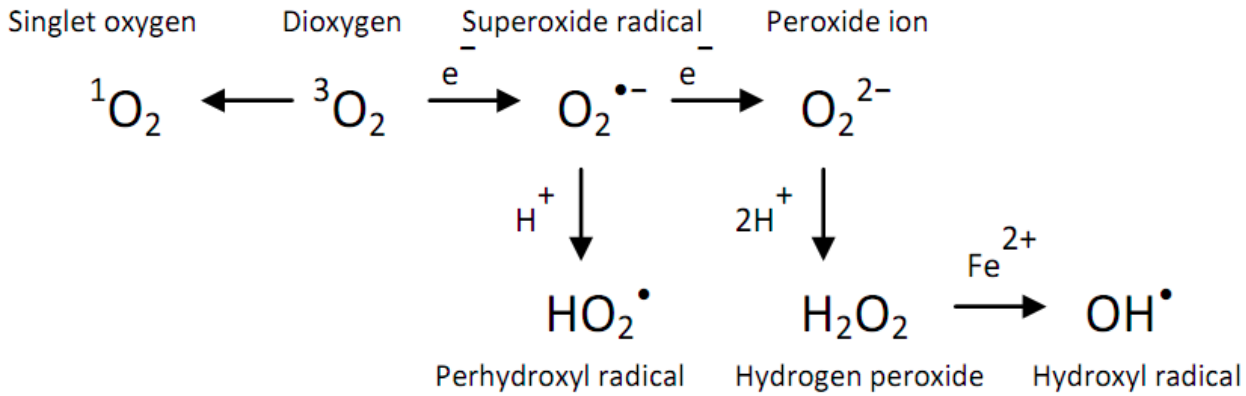


Introduction: Oxidative stress is a condition where elevated level of reactive oxygen species (ROS) exceeds the cellular counteracting antioxidant capacity and thereby causes damages to biological molecules such as lipid, proteins, DNA, and etc. Prolonged oxidative stress is linked to a number of physiological and pathological conditions, such as neurodegradation, immunodepression, and cancer.

Learning Outcomes: By the end of this module, the student will be able to:

- Define oxidative stress
- Define reactive oxygen species and recognize its role in oxidative stress
- Investigate safe molecular design decisions on its potential to produce ROS
- Determine the role the of mitochondria in oxidative stress

Background and Information: The disturbing effects of oxidative stress are mainly delivered by reactive oxygen species (ROS). ROS is a group of highly reactive, oxygen containing chemicals that are derived from molecular oxygen. Commonly encountered reactive oxygen species are summarized in the scheme below.



Chemical and radiation exposures are two main routes for the stimulated generation of ROS. From the safer molecular design perspective, chemicals with a high propensity to induce ROS production should be avoided. Since ROS generation is a redox event, redox properties of chemicals are highly relevant to their likelihood in facilitating ROS production. One possible mechanism for chemicals to induce ROS generation is through disrupting the electron transfer process in mitochondria.

During the respiratory process, electrons that are leaked from the electron transfer chain in the mitochondria can reduce molecular oxygen in order to create peroxide ion and further transforms into other types of ROS. So, a chemical with an appropriate redox property to intervene the electron transfer process in mitochondria can increase the ROS production. Alternatively, certain chemicals can be biologically transformed into reactive harmful radicals. For example, carbon tetrachloride can be activated by cytochrome P450 to produce the trichloromethlyl radical. The trichloromethlyl radical may react with cell and organelle membrane lipids and cause damage. Therefore, the redox activity of a chemical and their biotransformation products should be assessed when designing chemicals with reduced potency to cause oxidative stress.

Another way in which chemicals can cause cellular impact via oxidative stress is by depleting stores of antioxidants. Antioxidants are molecules that "negate" free radicals (aka ROS) by removing the negative oxygen charge by bonding. When antioxidant levels are low, the ROS molecules are able to cause damage to cellular membranes, organelles, and DNA. These ROS are also implicated in numerous diseases such as cardiovascular disease, cancer, hearing impairment, and chronic inflammation, among other negative roles. There is also evidence that ROS may impact cells genetically via the regulation of gene transcription, essentially turning "on" and "off" genes.

Assignments:

1. Summarize the toxicity mechanism of carbon tetrachloride and identify another chemical that produce radicals after biotransformation.

2. Label the reduction potential for all the ROS provided in the top of this page and rationalize the viability between their transformation.

3. Find one research article that details the role of ROS in cellular degradation or human disease. Summarize the impact in 1-2 paragraphs. Include a brief summary of cellular oxidative stress in your review.

Assignment answer found HERE: Oxidative Stress Answer Key

Resources:

- Colton, C., & Gilbert, D. (Eds.). (2007). *Reactive Oxygen Species in Biological Systems: An Interdisciplinary Approach*. Springer Science & Business Media. Retrieved from http://link.springer.com/book/10.1007%2Fb113066
- Noori, S. (2012). An overview of oxidative stress and antioxidant defense system. *Open Access Scientific Reports, 1*(8), 1-9. Retrieved from http://omicsonline.org/scientific-reports/2167-0390-SR-413.pdf
- Villamena, F. A. (2013). Molecular Basis of Oxidative Stress: Chemistry, Mechanisms, and Disease Pathogenesis. John Wiley & Sons Publisher.

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Module 5: Glutathione as a Tool for Testing Gene Function

Introduction: Scientists use many novel molecular techniques with genes since they can provide a more complete view on the development of diseases. Methods such as gene silencing, gene knockout, and gene overexpression allow researchers to study different detoxification pathways at the cellular level. These techniques are used to understand important metabolic pathways, such as glutathione metabolism, which has been identified as one of the body's first line defenses against chemical-induced oxidative stress. A deeper understanding of these mechanisms allows scientists to develop new generations of chemicals with reduced hazard.

Learning Outcomes: By the end of this module, the student will be able to:

- Describe the difference between gene silencing and gene knockout
- Define gene silencing and gene overexpression
- Describe the antioxidant glutathione and how it protects cells against the reactive oxygen species

Background and Information: Each of our 23,000 genes has a defined and very specific role to perform in the human body. Some genes are responsible for cell growth, others assist with cell division and differentiation, while others help to fight off infection or deal with stress. The roles of genes are complex but necessary for normal function and overall well-being. For many years scientists struggled to understand the complexities of gene regulation. Knowing which genes are responsible for cancer, or which genes can prevent it are key questions that many scientists are trying to answer. To determine the role of a particular gene, scientists use extensive molecular studies and advances in genetic engineering to systematically understand how genes operate. One way to uncover gene function is to decrease or increase the expression of the gene's product (for example, gene specific messenger RNA) in an *in vitro* (cell culture) or *in vivo* (whole organism) experimental system. If gene expression is modified, it may affect the system in a positive or negative way, depending on the cellular biochemical pathways involved. These changes can be monitored and quantified. Gene silencing – Reducing gene levels in the cell by interfering with gene transcription or translation machinery. When genes are silenced, their mRNA expression is reduced by 70% or more, which is sufficient to impact the change in the *in vitro* or *in vivo* system. Gene silencing is a relatively straightforward procedure and it is frequently used when low levels of gene expression are required for an organism to survive. This is in contrast to a gene knockout, where genes are completely removed from the organism's genome—a procedure which is permanent, time consuming and it cannot be performed on all genes (e.g. genes critical to

an organism's survival). Bearing in mind that common products of normal gene expression are mRNAs, which are translated into proteins unique to each gene, overexpression refers to increasing gene levels by 1, 2 or more fold, depending on the procedure. Increased gene expression frequently contributes to enhancing or improving a certain feature or process in cells. However, in many cases, higher gene expression doesn't translate into a beneficial outcome. Take cancer for example- overexpression of certain genes, for instance oncogenes, can lead to tumors.

Gene overexpression has been tried with a naturally occurring molecule called glutathione (GSH) made up of the three amino acids: glutamate-cysteine-glycine.

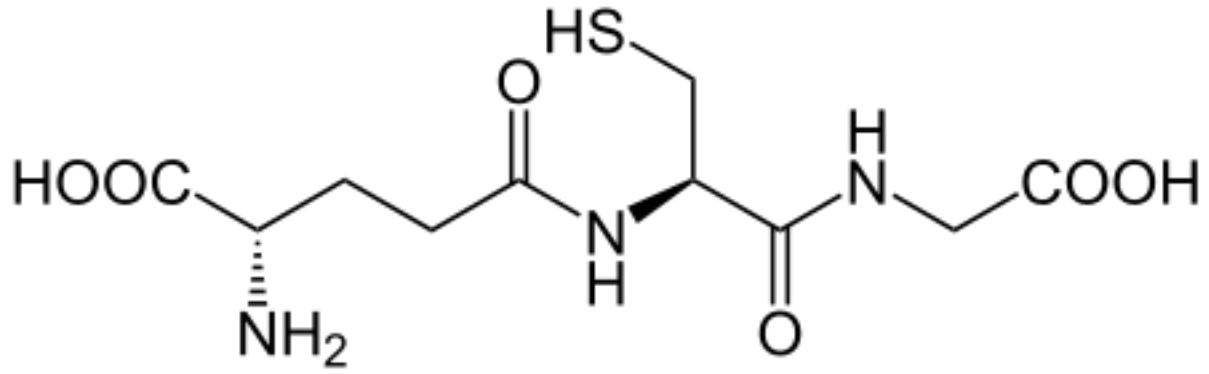


Figure 1. Glutathione - GSH

A small set of enzymes are responsible for the synthesis of GSH which are encoded in the genome of many organisms. GSH is important because it can act as a direct antioxidant in preventing the oxidation of other molecules by giving up electrons. In the process of electron transfer GSH is converted to its oxidized form glutathione disulfide (GSSG).

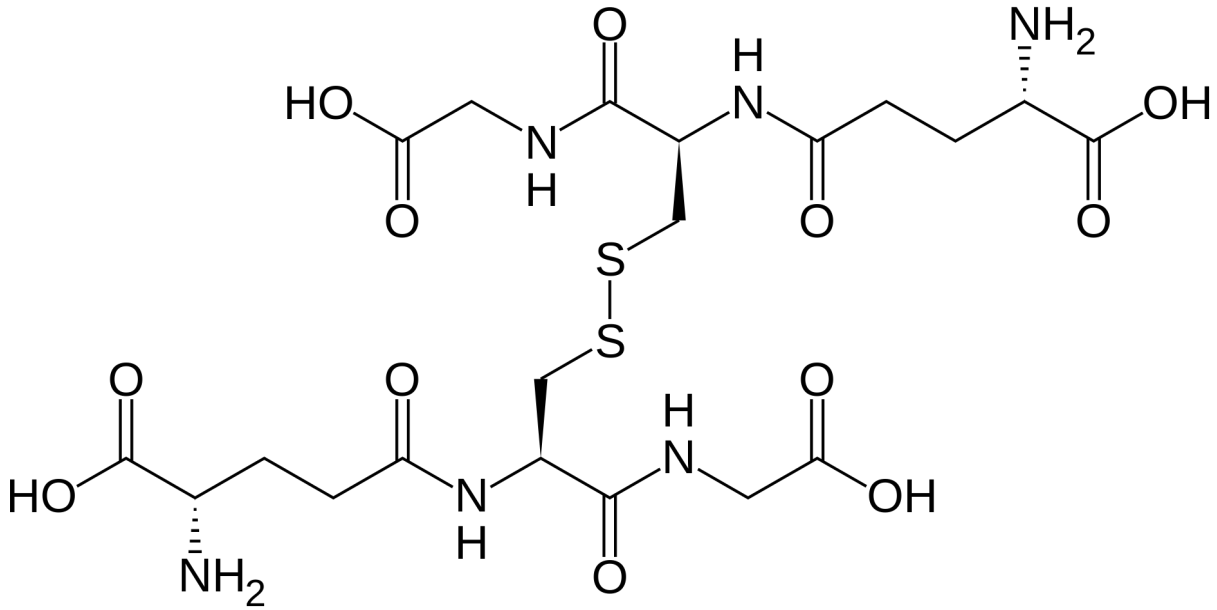


Figure 2. Oxidized Glutathione - GSSG

The really interesting part in the biological chemistry pertaining to GSH levels in cells is the existence of enzymes that can change oxidized GSSG back to the reduced form GSH. The electrons used to reduce GSSG back to GSH are provided by molecules that efficiently act as electron carriers in cells. This is a reliable and efficient way that cells will recycle and maintain a steady level of GSH and cellular antioxidant defense.

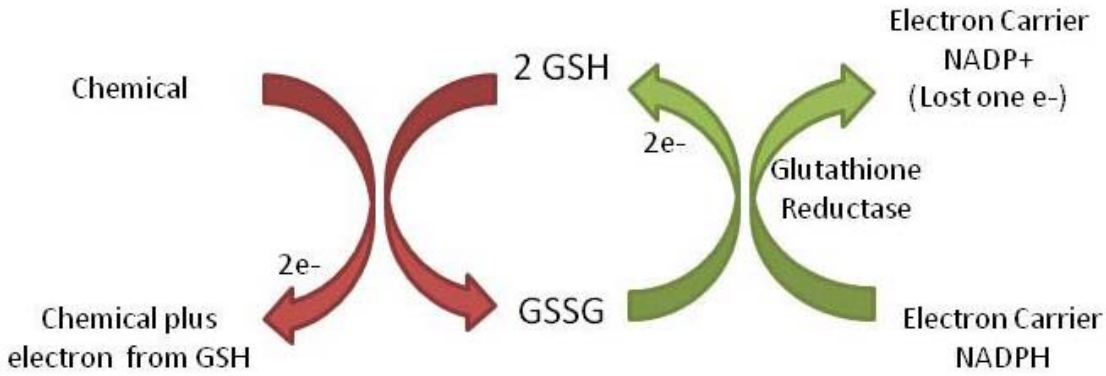


Figure 3. Glutathione Recycling

Glutathione also belongs to a similar group of antioxidant compounds like vitamin C, vitamin A, and vitamin E as well as antioxidant enzymes such as catalase and peroxidases both which help reduce the levels of damage caused by oxidants and chemicals that produce such oxidants. Insufficient levels of antioxidants, or inhibition of antioxidant enzymes can cause oxidative stress and may damage or kill cells. Conversely, an excess (or overexpression) of GSH has been shown to increase cellular resistance to oxidative stress. In other words if cells which overexpress glutathione are exposed to chemicals that cause oxidative stress, these cells are more likely to be resistant to these chemicals. These GSH-enhanced cell lines are frequently used in large toxicology studies, where researchers compare the response of cells to many industrial chemicals. If GSH-overexpressing cells are less sensitive to chemical treatment than healthy cells, chemical toxicity is likely mediated through an oxidative stress related pathway. These tests are designed to determine if chemicals are strong oxidants, what is their toxic potential and to what extent they can compromise cell growth or cause cell death via oxidative stress.

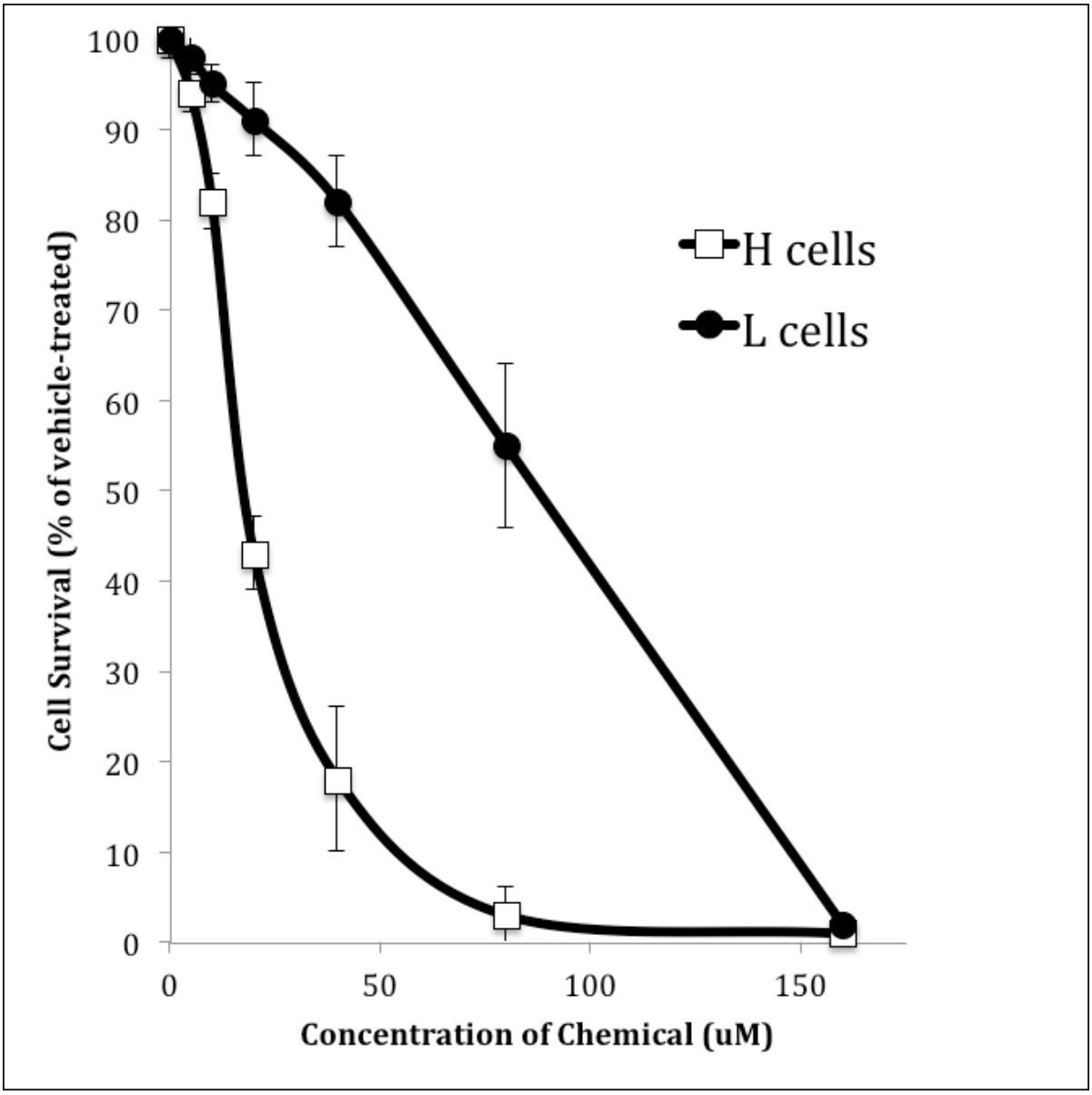
An additional function of GSH is for the detoxification of chemicals. It is conjugated (attached) to many chemicals during a process referred to as phase II metabolism. When toxic chemicals are joined with GSH they become more water soluble and larger in molecular weight (size). These two features are very important in determining the rate at which drugs & toxic compounds are removed from organisms. Phase II metabolism will be covered more in depth in the toxicology module. Using glutathione and other antioxidants are currently an active area of research as you can see by a quick search in the online scientific article database Pubmed (http://www.ncbi.nlm.nih.gov/pubmed). Goto Pubmed.gov and put the term "GSH" in the search query area. How many “hits” did you get?

Assignments:

Assignment #1

Below you will find experimental results for a survival assay conducted on two cell lines [normal and glutathione overexpressing] which were treated with increasing concentrations of a chemical known to cause oxidative stress. As the dose of the chemical increases, cell survival decreases.

A.Which cell line probably overexpresses glutathione?
B.Why does the survival rate decrease for both cell lines, as the dose of the chemical increases?



Assignment #2

Consider the chemical and physical properties of GSH discussed above. Briefly in 2 to 5 sentences, discuss some strategies you may consider using in trying to design a safer chemical.

Assignment Answer found HERE: Glutathione as a Tool for Testing Gene Function Answer Key

Resources

- Awasthi, Y. C. (Ed.). (2006). *Toxicology of glutathione transferases*. CRC Press.

- Botta, D., White, C. C., Vliet-Gregg, P., Mohar, I., Shi, S., McGrath, M. B., ... & Kavanagh, T. J. (2008). Modulating GSH synthesis using glutamate cysteine ligase transgenic and gene-targeted mice. *Drug metabolism reviews*, *40*(3), 465-477.
- Education Development Center, Inc. (2009). Creating transgenic organisms. Retrieved from https://science.education.nih.gov/supplements/nih9/bioethics/guide/teacher/Mod6_transgenics.pdf
- Sheehan, D., MEADE, G., & FOLEY, V. M. (2001). Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. *Biochemical Journal*, *360*(1), 1-16. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1222196/pdf/11695986.pdf
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- Wu, G., Fang, Y. Z., Yang, S., Lupton, J. R., & Turner, N. D. (2004). Glutathione metabolism and its implications for health. *The Journal of nutrition*, *134*(3), 489-492.

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