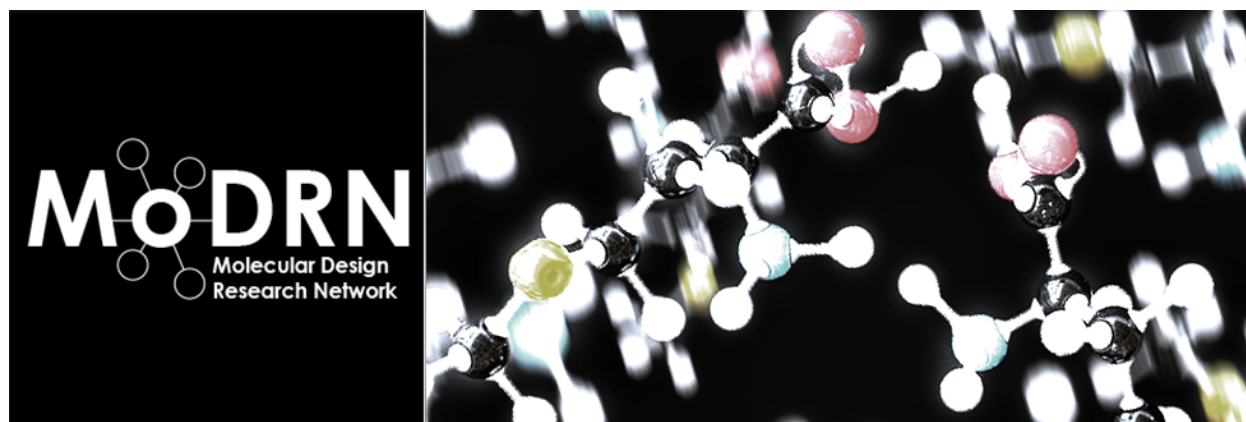


Physicochemical Properties and Toxicology in Chemical Design Modules

Over 700 consumer products are introduced into the US market every year, but only approximately 85% of which are approved for manufacturing in spite of insufficient safety data. This is mainly due to the high cost associated with animal testing to generate toxicity data, and the necessity in developing a specialized set of protocol to evaluate the chemical comprehensively. As a result of this practice, consumers are left to bear the unknown, potentially harmful, factors from these new products until their hazards are realized. Sadly, many of these effects are not immediate, and thus the consequences are often unnoticed until a large quantity of the products have been released to the public and the environment. For this reason, there is an urgent need for a low-cost high throughput reliable screening approach to predict the toxicity of the chemicals during the early designing phase of the product – the *in-silico* approach.

With the rise in cost and increasing restrictions on animal testing in some countries, the *in-silico* approach make use of known chemical data to predict chemical properties and allow chemist to design a chemical that are much more likely to be toxic free for its purposes. Understanding parameters and how they influence ADME, for example, allow chemists to conduct the *in-silico* approach chemical design more efficiently. A chemist designing plasticizers for baby bottles may be able to prescreen candidate molecules and reject ones that are highly bioavailable. There are many chemical descriptors that can assist us in this work.

Module 6: Crossroads of Computational Chemistry and Toxicology



Introduction: There are thousands of chemicals and only a fraction have been evaluated for specific classes of toxicity (carcinogenicity, genotoxicity, developmental toxicity, etc). It is not possible to test all the chemicals with *in vitro* and *in vivo* assays. Computational methods, such as the *in silico* method of testing (and predicting toxicology before development), is the newest way by which to ensure that toxicology is considered in molecular and product design and that potential toxicological impacts are known.

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

- Identify ways in which chemicals are tested for adverse health effects.
- Describe new methods that are helping scientists predict toxicity.
- Investigate the benefits of computational toxicology.

Background and Information: One of the principle goals of the Green Chemistry movement is to use modern methods of chemical synthesis and production to avoid the legacy problems associated with past practices that have caused harm to human health and the environment. This involves consideration of the toxicity of the chemicals being synthesized as well as the toxicity of intermediates used, or waste products generated during that synthesis. However, because of the little amount of toxicity data available for many chemical entities, this remains a big challenge to practitioners of Green Chemistry. One approach being pursued is to use chemical structure activity relationships to predict the toxicity of chemicals using *in silico* and *in vitro* toxicity methods in order to predict *in vivo* toxicity. By applying these methods we hope to be able to design safety into new chemical structures so as to avoid harmful outcomes.

What is *in vitro* Testing? *In vitro* testing is any test that occurs within cells that are not in an organism. In other words, these cells are in a biological medium or culture outside of the organism from which they came. These tests are very helpful in identifying aspects of how a chemical reacts at the cellular level - specifically for absorption, distribution, metabolism and excretion (ADME) processes without doing the testing on the actual organism itself. One disadvantage is that the results may be difficult to apply to a whole organism, as cells in culture may mitigate or offset toxicants differently.

What is *in vivo* Testing? *In vivo* testing is that which is done on the whole organism to study adverse health effects (or other parameters). These may be on microbes, or *Daphnia*, or zebrafish, mice, rabbits, etc. An advantage is that it allows the researcher to take into consideration the toxicological impact on the organism as a whole. A disadvantage is that each species may react differently to a toxicant, so results cannot be fully extrapolated to every species and results must be interpreted carefully. Researchers use many animal models, and it is important to know that one animal model for all chemicals does not exist to predict toxicity to humans or the environment. Also, there may be ethical issues when testing larger organisms such as fish or animals.

What is *in silico*? The United States Environmental Protection Agency (US EPA) defines *in silico* testing as the “*integration of modern computing and information technology with molecular biology to improve agency prioritization of data requirements and risk assessment of chemicals.*” A variety of databases are now available that contain toxicology testing information that can identify chemical induced biological activity mechanisms. *In silico* is the way to test toxicological and chemical properties using a computer and without the need to use *in vitro* or *in vivo* testing methods. Non-testing methods for predictive toxicology is receiving more attention as one way to reduce animal testing. Databases, Structure Activity Relationships (SAR) methods, pharmacophores, homology models, and other approaches such as data mining, network analysis tools, machine learning, etc are all aspects of this methodology. *In silico* results are increasing in use for making policy and regulatory decisions. *In silico* methods do not require animals for testing, like *in vivo* models, and the results can be applied beyond cellular impact in a more complex manner than *in vitro* testing. We refer to this as physiologically-based pharmacokinetic (PBPK) modeling. PBPK is a mathematical modeling process that predicts the absorption, distribution, metabolism and excretion (ADME) of chemical substances (synthetic or natural) in humans and other species.

Video: Dr. Jakub Kostal, Associate Research Scientist, The George Washington University and CSO of Sustainability A to Z

Assignment: Discuss the pros and cons of using *in-silico* testing for chemical toxicity research. Describe some the databases that are used for this type of study. When it is said that *in-silico* testing is “animal-free” is that true for the data that *in-silico* testing uses? Explain.

Resources:

- Raunio, H. (2011). *In Silico Toxicology – Non-Testing Methods*. *Frontiers in Pharmacology*, 2, 33. doi:10.3389/fphar.2011.00033. Retrieved from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3129017/>
- Environmental Protection Agency. (2015). Computational toxicology research. Retrieved from http://epa.gov/comptox/communities_of_practice.html
- Environmental Protection Agency. (2015). Estimating toxicity-related biological pathway altering doses for high-throughput chemical risk assessment. Retrieved from [http://www.epa.gov/ncct/download_files/factsheets/High-Throughput%20Chemical%20Risk%20Assessment Fact Sheet 2-13-2011.pdf](http://www.epa.gov/ncct/download_files/factsheets/High-Throughput%20Chemical%20Risk%20Assessment%20Fact%20Sheet%202-13-2011.pdf)
- National Institute of Health. (2015). ToxLearn: Introduction to toxicology and dose response. Retrieved from <http://toxlearn.nlm.nih.gov/Module1.htm>

This material is based upon work supported by the NSF Division of Chemistry and the Environmental Protection Agency under Grant No. 1339637.

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Module 7: Using ProTox



Introduction: The advent of computational and/or predictive modeling has given scientists an important tool for researching potentially harmful chemicals. This has allowed scientists ranging from drug designers to material chemists to perform molecular pre-screening of thousands of chemicals to determine potential compatibilities to chemicals with already known structures. In addition to finding compounds that are close fits structurally, toxicologists have used these same techniques to assess a chemical's predictive toxicity. Toxicity assessments of chemicals and the endpoints used for evaluation are very important to understand and consider especially as one begins to use new tools for predicting toxicity.

Learning Objectives:

- Identify two new tools that provide toxicity data of a chemical from a variety of sources
- List three toxicity endpoints found in ProTox and Chemspider
- Determine predictive toxicity results for a chemical

Background and Information:

[PROTOX](#) is a web server for the predictive toxicities of small molecules in rodents. It is designed for multiple input formats for chemicals either by generic name, structure, or chemical formula and classifies the chemical based on the programs reference database. This particular model is assessing the predictive "oral" toxicity in rodents and will produce multiple potential results.

PROTOX - Prediction of Rodent Oral Toxicity

PROTOX

Welcome to PROTOX, a webserver for the prediction of oral toxicities of small molecules in rodents! The prediction of compound toxicities is an important part of the drug design development process. Computational toxicity estimations are not only faster than the determination of toxic doses in animals, but can also help to reduce the amount of animal experiments. To read more about reducing animal testing, go to [Animal Ethics 30](#).

Our webserver is based on chemical similarities between compounds with known toxic effects and the presence of toxic fragments. To predict the oral toxicity of your compounds, please click [here](#). For a description of the server and tutorials, go to [FAQ](#). To see statistics about our training set as well as the cross-validation results, please go to [Statistics](#). Should you have further questions, do not hesitate to [contact Us!](#)

Citations

Draai M.N., Banerjee P., Dunkel M., Wetig M.R., Preisner R.: [Protox: a web server for the in silico prediction of rodent oral toxicity](#). *Nucleic Acids Res* (Web server issue 2014); [N45](#).

Schmidt U., Struck S., Gruening B., Hossbach J., Jaeger L.S., Paroj R., Lindequist U., Teuscher E., Preisner R.: [SuperToxic: a comprehensive database of toxic compounds](#). *Nucleic Acids Res* 37 (Database issue 2009): D255-9; [N45](#).

Predict compound toxicity

PROTOX enables uncomplicated predictions of oral rodent toxicities. To conduct a toxicity prediction, please click on the image.

Toxic doses and toxicity classes

Toxic doses are often given as LD50 values in mg/kg body weight. The LD50 is the median lethal dose meaning the dose at which 50% of test subjects die upon exposure to a compound.

Toxicity classes are defined according to the globally harmonized system of classification of labelling of chemicals (GHS).

- Class I: fatal if swallowed (LD50 ≤ 5 mg/kg)
- Class II: fatal if swallowed (5 < LD50 ≤ 50 mg/kg)
- Class III: toxic if swallowed (50 < LD50 ≤ 300 mg/kg)
- Class IV: harmful if swallowed (300 < LD50 ≤ 2000 mg/kg)
- Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000 mg/kg)
- Class VI: non-toxic (LD50 > 5000 mg/kg)

Toxicity targets




Toxicity targets are protein targets which have been associated with adverse drug reactions and toxic effects. Here, we predict possible binding to toxicity targets using a collection of protein-ligand-based pharmacophores. To see warning about possible toxicity targets of your input compound, please go to [Tox.Predict](#)

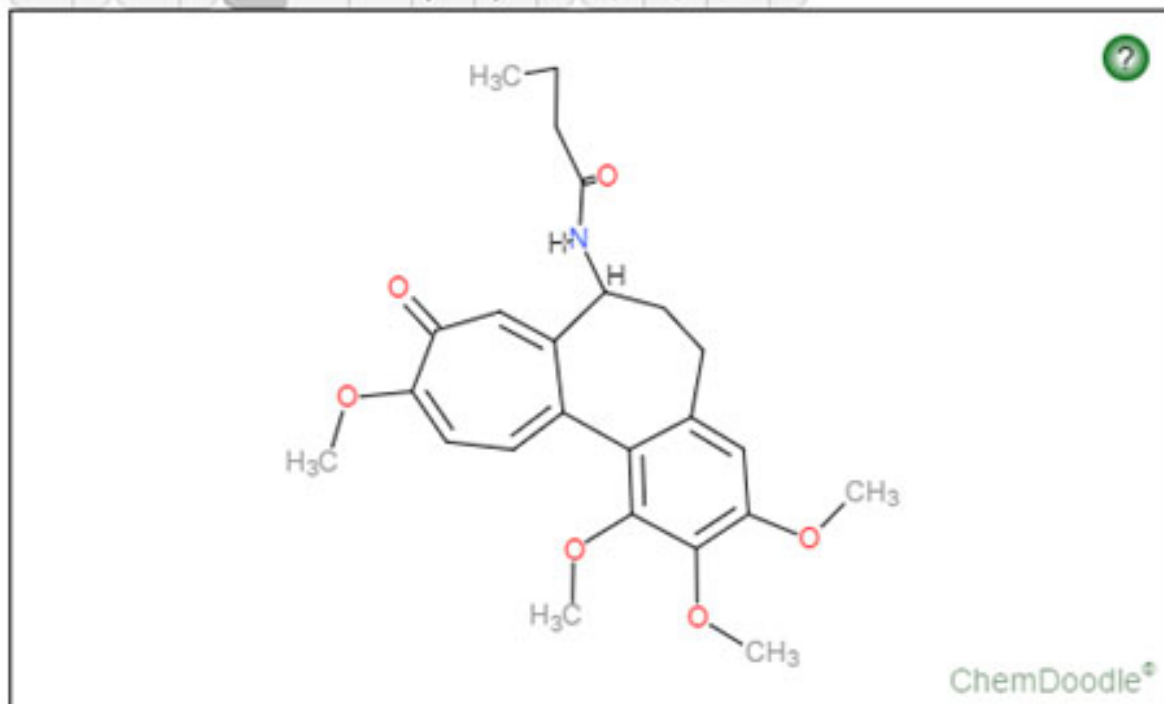
Chemical Input

As previously mentioned there are multiple ways you can enter a compound of interest. Depending on the available information you can enter the chemical's IUPAC name, common name, chemical formula, or draw out the chemical structure using the drawing tools provided on the PROTOX site. Using [PubChem](#) will generally be the quickest way to determine proper chemical names to use for input queries.




In this short example let's use the drawing tools to create the molecule phenol.

1. From the PROTOX homepage click on "TOX PREDICTION" at the top of the page. You will see chemical input webpage come up. There will be a molecule already in the drawing box that you can erase by clicking on the "water bottle" icon.

To load a molecule click . The  deletes clicked atoms and  deletes everything on the canvas.

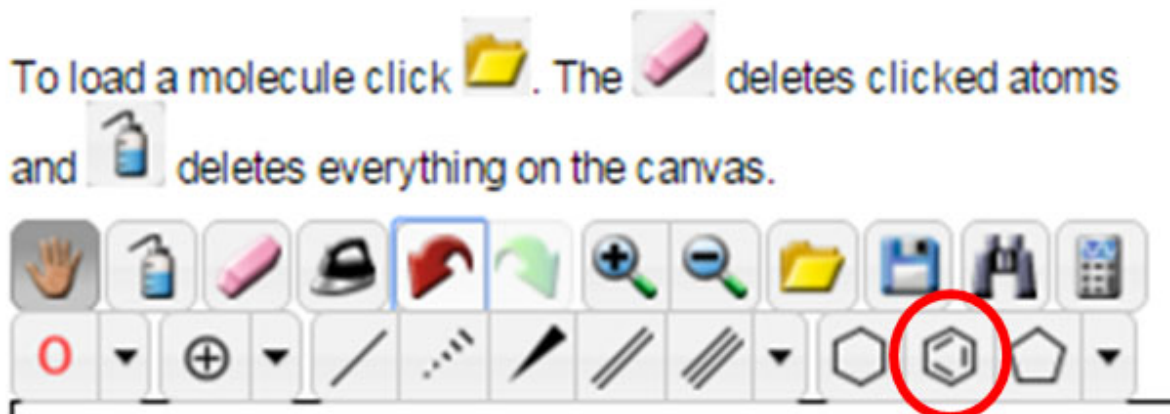


Start Tox-Prediction

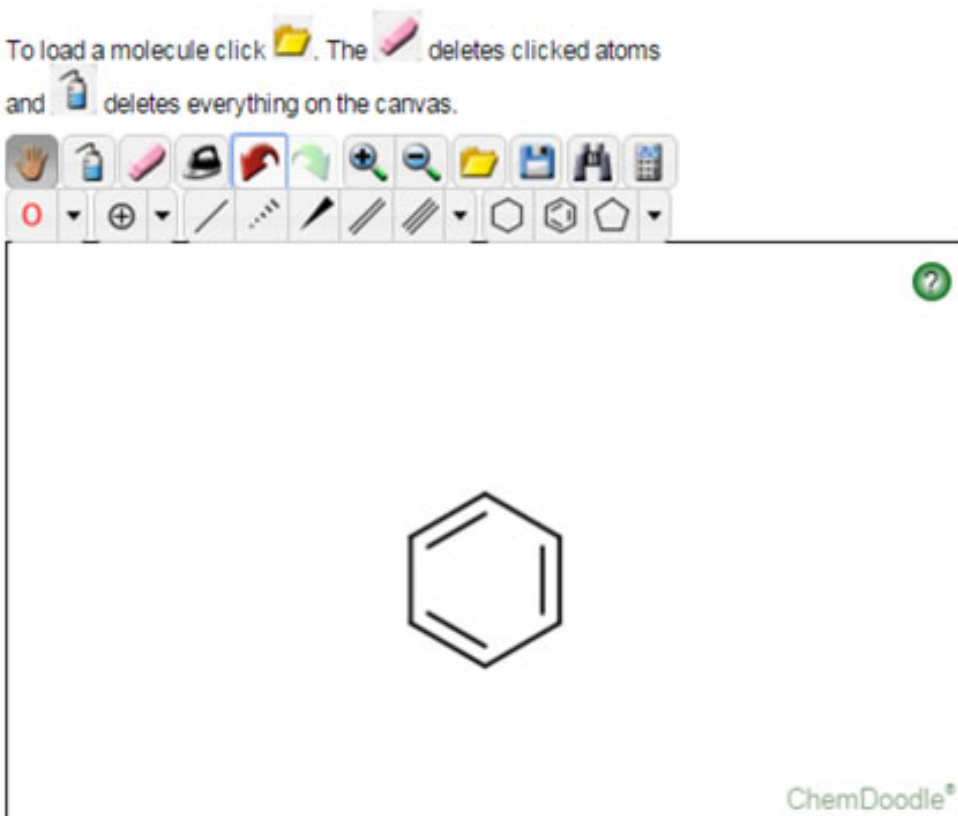
To load a molecule click . The  deletes clicked atoms and  deletes everything on the canvas.






2. Now that you have a clean slate to work with, draw out a phenol molecule. A quick search on PubChem lists the IUPAC name as phenol, also has the name hydroxybenzene, and the chemical formula C_6H_6O . Using the drawing tool bar click on the benzene ring button.





The following structure should now be in your drawing area.

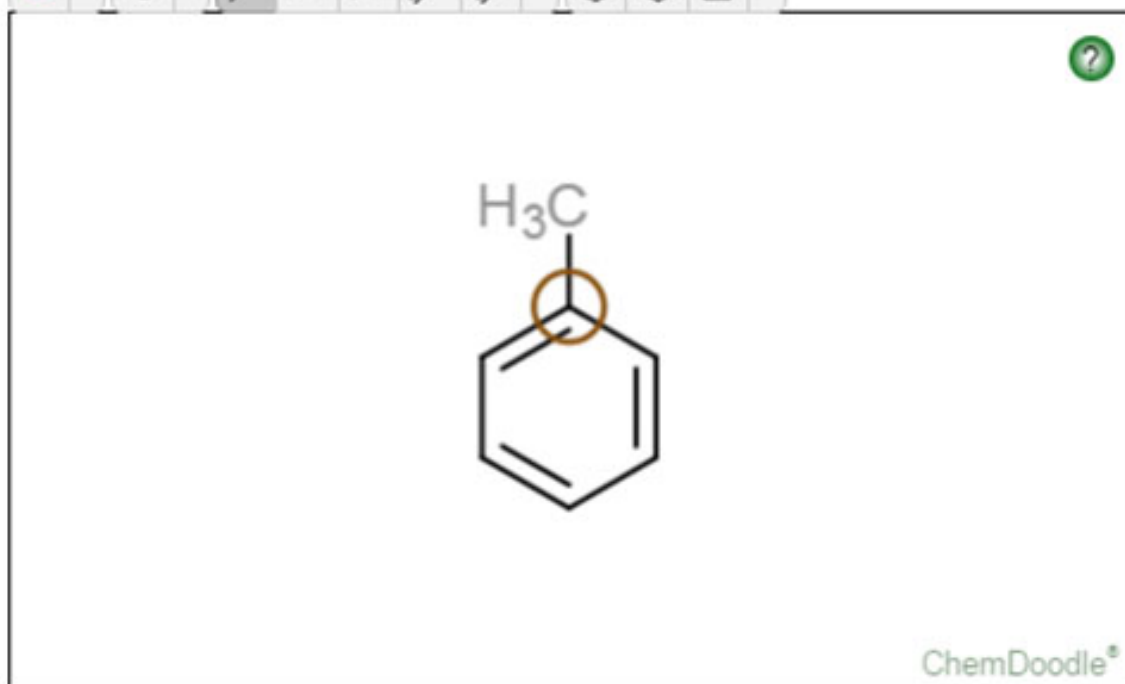


3. Now you will want to create a single bond off of any of the carbon atoms contained within benzene. Locate the "Single Bond" button and click once, then go to one of the carbons and click once more. Your structure should look like the one below. By default PROTOX will place a methyl group on the other end of that single bond.




To load a molecule click . The  deletes clicked atoms and  deletes everything on the canvas.

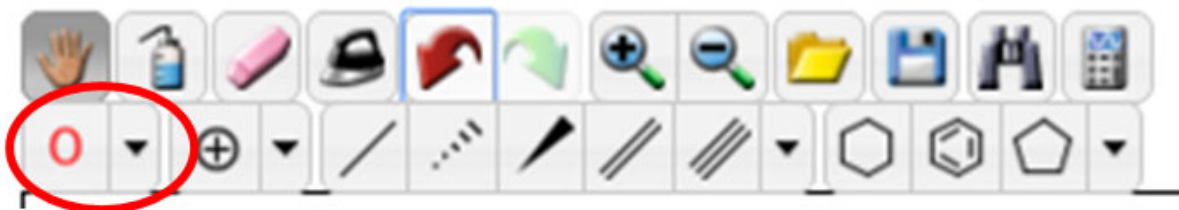


To load a molecule click . The  deletes clicked atoms and  deletes everything on the canvas.






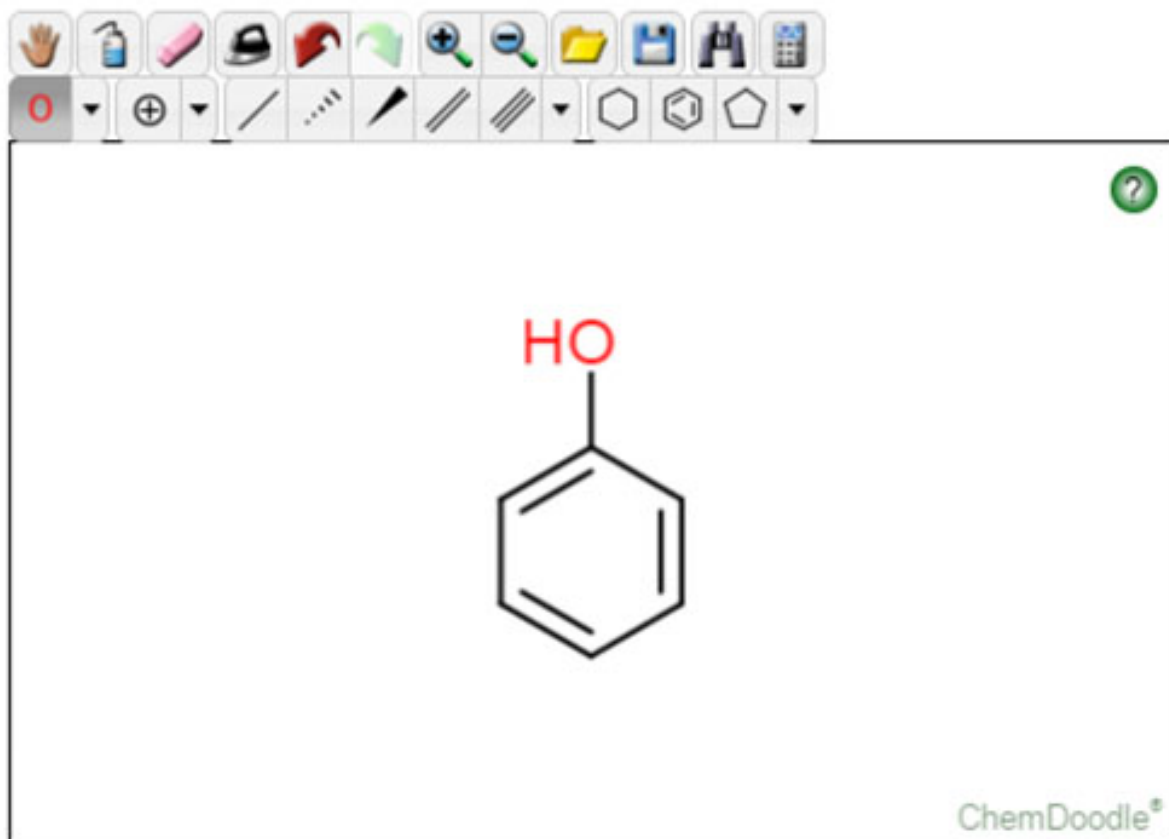
4. Lastly, because this chemical is phenol we need to change the methyl group (CH_3) to an alcohol functional group (OH). To accomplish this locate the element button on the lower part of the drawing toolbar. Initially it will have a capital C, however if you click the dropdown button to the right of the C it will allow you to select the red "O" in place of the carbon. You will notice that other elements can be selected from this dropdown menu. Click once on the red "O" and then click on the methyl group that is currently bonded to your benzene.

To load a molecule click . The  deletes clicked atoms and  deletes everything on the canvas.



This should replace the methyl group with an alcohol as seen in the structure below.

To load a molecule click . The  deletes clicked atoms and  deletes everything on the canvas.

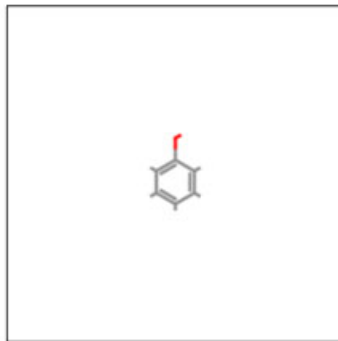


This is also a good time to familiarize yourself with the different input options for chemicals and to continue practicing with the drawing tools.

5. Once you have completed drawing the structure for phenol click on the "Start Tox-Prediction" button. After a short period of time PROTOX will display the results of your query. Part I of the results will display a variety of results some listed below:

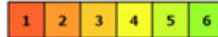
- Chemical structure, if you entered text based input
- Predicted LD50 value
- Predicted Toxicity Class (1 through 5)
- General compound information (top right)
- Where your compound lies within the comparison database relevant to molecular weight and LD50 value.

PART I: Oral toxicity prediction results for input compound



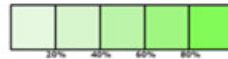
Predicted LD50: 270mg/kg

Predicted Toxicity Class: 3



Average similarity: 100%

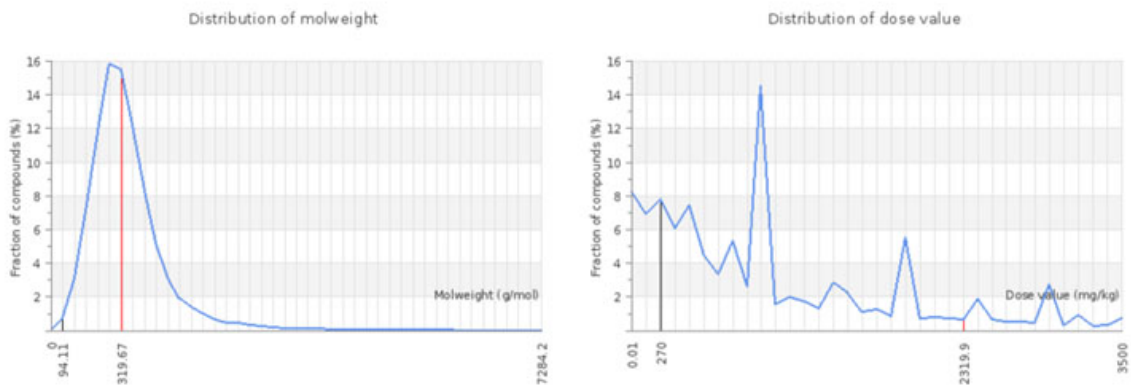
Prediction accuracy: 100%



Name	name:FAILED
Molweight	94.11
Number of hydrogen bond acceptors	1
Number of hydrogen bond donors	0
Number of atoms	7
Number of bonds	7
Number of rings	1
Number of rotatable bonds	0
Total charge	0
Molecular Polar Surface Area	20.23

Comparison of input compound with dataset compounds

Value of input compound
Mean value of dataset



Similar compounds located within the database and any toxic fragments that could be associated with your chemicals composition.

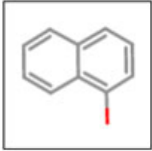
Similar compound 1

Formula	C6H6O	
molweight	94.11	
endpoint	LD50	
tox class, avg	4	
tox class, min	3	

Similar compound 2

Formula	C10H7O-	
molweight	143.16	
endpoint	LD50	
tox class, avg	4	
tox class, min	3	

Similar compound 3

Formula	C10H8O	
molweight	144.17	
endpoint	LD50	
tox class, avg	4	
tox class, min	3	

Toxic fragments

Fragments generation - Progress: 100 %

No tox related fragments in input structure found

Part 2 presents any potential biological targets that your compound may bind with relevant to oral exposures. It encompasses only 16 potential targets so there is limitation as to how many potential biological targets are assessed.

PART II: Toxicity targets

Possible binding to toxicity targets is shown below. For more information on the targets, please click on the individual abbreviations.



AA2AR	ADRB2	ANDR	AOFA	CRFR1	DRD3	ESR1	ESR2	GCR	HRH1	NR1I2	OPRK	OPRM	PDE4D	PGH1	PRGR
no binding	no binding	no binding	no binding	no binding	no binding	no binding	no binding	no binding	no binding	no binding	no binding	no binding	no binding	probable binding	no binding

Details about possible toxicity targets:

	Toxicity Target	Avg Pharmacophore Fit	Avg Similarity Known Ligands
	Prostaglandin G/H Synthase 1	0%	85.71%

Last updated: September 2015

Disclaimer: Compound structures submitted to the web server will not be released under any circumstances.

More detailed explanations regarding the data created from your search is covered in the [FAQ section of the PROTOX site](#).

Assignment:

Start again with Benzene as your starting base molecule. Run benzene through PROTOX's predictive modeling program. Make note of any relevant toxicology data produced from the program and keep record. Now add a methyl group to the benzene ring (Toluene) and record any relevant toxicology data.

Additional Information for Assignment:

If you performed any internet queries related to computational toxicology a variety of publications and government agencies should be compiled in the search results. This should convey the level of interest that the field of toxicology has engaged into newer research methodologies and paradigms. The U.S. Department of Health and Human Services National Toxicology Program is part of a collaborative effort with the following US regulatory agencies:

- the National Institute of Environmental Health Sciences/National Toxicology Program
- the National Institutes of Health/National Chemical Genomics Center (NCGC)
- the Environmental Protection Agency
- and most recently the Food and Drug Administration

The program is called TOX21 <http://ntp.niehs.nih.gov/results/hts/index.html>. The program is designed to streamline a more comprehensive approach to toxicology. Below are some of the goals that the program is attempting to achieve.

- "Research, develop, validate and translate innovative chemical testing methods that characterize toxicity pathways."
- "Research ways to use new tools to identify chemical induced biological activity mechanisms."
- "Prioritize which chemicals need more extensive toxicological evaluation."
- "Develop models that can be used to more effectively predict how chemicals will affect biological responses."

- “Identify chemicals, assays, informatic analyses, and targeted testing needed for the innovative testing methods.”
- “Complete construction in 2010 of a library of more than 10,000 chemicals for quantitative High Throughput Screening (qHTS) at the NCGC.”

If you noticed the fourth statement “Develop models that can be used to more effectively predict how chemicals will affect biological responses” you have already had some exposure to this type of computational model using PROTOX. This module will also expose you to other computational models available for researching the toxicity of your chemicals.

ChemSpider <http://www.chemspider.com/AboutUs.aspx> is a site owned by the Royal Chemical Society. It claims to encompass over 43 million chemical structures in its database. They also reference over 500 different databases for information on their listed chemicals including links back to many of the original references. By now you have used PubChem to look up chemical information. ChemSpider provides much of the same information with the addition of instrumental data, related articles and more. See the following screen shot for a search on toluene.

The screenshot displays the ChemSpider website interface for the chemical Toluene. The main content area shows the chemical structure of toluene (a benzene ring with a methyl group, CH_3) and its properties:

- Molecular Formula:** C₇H₈
- Average mass:** 92.138 Da
- Monoisotopic mass:** 92.062599 Da
- ChemSpider ID:** 1108

Below the structure, there are buttons for 'SD' and 'Q'. A 'More details' section is collapsed. A 'Names and identifiers' section lists various names and synonyms, including 'Benzene, methyl-' and 'Methane, phenyl-'. An advertisement for Agilent Technologies is visible on the right side of the page. The bottom of the screenshot shows the Windows taskbar with the time 12:51 PM on 2/18/2016.

The power behind a resource like ChemSpider is like an internet search engine. It is encompassing many databases to provide the most comprehensive set of information for your chemical. Click on the properties

tab and you will notice an extensive list of information that becomes available. Included is an option to access US EPA's EPISuite.

If you scroll farther down the page, you will see a dropdown for Toxicity (1). Clicking on that dropdown reveals LD50 values for both rats and mice. The first bit of information is ORAL LD50 values for rat toxicity data which is what the PROTOX model is based on.

Miscellaneous

1

Appearance:

^ Toxicity:

ORL-RAT LD50 636 mg kg-1 , IPR-RAT LD50 1332 mg kg-1 , IPR-MUS LD50 59 mg kg-1 Oxford University Chemical Safety Data (No longer updated)
[More details](#)

2

Safety:

First-Aid:

^ Exposure Routes:

inhalation, skin absorption, ingestion, skin and/or eye contact NIOSH [X55250000](#)

3

Symptoms:

^ Target Organs:

Eyes, skin, respiratory system, central nervous system, liver, kidneys NIOSH [X55250000](#)

4

Incompatibility:

Personal Protection:

^ Exposure Limits:

NIOSH REL : TWA 100 ppm (375 mg/m³) ST 150 ppm (560 mg/m³) OSHA PEL ? : TWA 200 ppm C 300 ppm 500 ppm (10-minute maximum peak) NIOSH [X55250000](#)

You should also notice ChemSpider provides information on exposure routes (2), target organs for toxicity (3), and workplace exposure limits (4) provided by reference to Federal regulatory agencies for workplace safety.

Resources:

- American Society for Cellular and Computational Toxicology. (2016). Homepage. Retrieved from <http://www.ascctox.org/>
- El-Masri, H. A., Mumtaz, M. M., Choudhary, G., Cibulas, W., & De Rosa, C. T. (2002). Applications of computational toxicology methods at the Agency for Toxic Substances and Disease Registry. *International journal of hygiene and environmental health*, 205(1), 63-69. Retrieved from http://www.atsdr.cdc.gov/mrls/articles/applications_of_computational_toxicology_methods_at_ATSDR.pdf
- Environmental Protection Agency. (2016). About the National Center for Computational Toxicology (NCCT). Retrieved from <http://www.epa.gov/aboutepa/about-national-center-computational-toxicology-ncct>

- National Institutes of Health. (2008). NIH collaborates with EPA to improve the safety testing of chemicals. Retrieved from <http://www.nih.gov/news-events/news-releases/nih-collaborates-epa-improve-safety-testing-chemicals>

This material is based upon work supported by the NSF Division of Chemistry and the Environmental Protection Agency under Grant No. 1339637.

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Module 8: ADME and Rational Chemical Design



Introduction: ADME characterizes drug and chemical uptake into a body and, therefore, needs to be considered before designing and creating new chemicals, or re-classifying use parameters for existing chemicals.

Learning Outcomes:

- Investigate how ADME properties should influence chemical design and creation.
- Associate different physicochemical properties with absorption.
-

Background Information: ADME stands for **A**bsorption, **D**istribution, **M**etabolism, and **E**xcretion. ADME is a term especially known in pharmacology and drug design, but that also has significant applications in both toxicology and green chemistry design. ADME “describes” the effectiveness of the drug by characterizing its toxicokinetics (uptake and fate of a chemical in the body). So before developing any chemical or drug, chemists should consider several metrics:

- i. the rate of the uptake of the molecule
- ii. how fast and where it is distributed
- iii. how fast is it metabolized
- iv. how fast can it be excreted from the body.

In the realm of pharmacology, knowing these rates helps medicinal chemists design drugs which not only are most effective, but more importantly, that target specific tissues. One of the classic examples of how understanding ADME led to an improved chemical design are allergy drugs. Allergy relief drugs, ‘aka’ antihistamines, are widely prescribed for seasonal and perennial allergies. The drug enters the brain, where it prevents histamine from binding to cells and blocks the allergy reaction mechanism. The first generation antihistamine drugs, however, were identified with a side effect. Because of their structure, antihistamines were very likely to accumulate in the brain and affect other receptors, which in turn lead to drowsiness. Understanding ADME allowed scientists to redesign the drug, which now enters the brain, reacts with the appropriate receptors on the cells, and is excreted before it affects other receptors.

Chemists and molecular designers have also used ADME to create guidelines for designing safer industrial chemicals. In this case, chemicals are designed such that they have a minimal effect on humans and environment. Ideally, chemicals shouldn’t bioabsorb (enter the body) in the first place.

However if they do, molecular designers want to reduce distribution, reduce bioactivation, and accelerate excretion. Many of the guidelines for chemists are derived from existing physicochemical properties and toxicity data. Examples of some guidelines are summarized in figures 1 & 2.

<h1>Molecular Weight [MW]</h1>		
MW>500 Da No lung absorption	MW>300 Da No GI tract absorption	MW>1000 Da No skin absorption

Figure 1. Desired molecular weight for decreased oral, respiratory, and dermal absorption. Molecular weight is given in Daltons.

<h1>Solubility</h1>	
log Pow<0 No GI absorption GI elimination	log Pow>6 Poor skin absorption

Figure 2. Desired solubility for decreased oral, respiratory and dermal absorption. Solubility is defined through log Pow and measures relative solubility of a chemical in water (a polar molecule) and octanol (a non-polar molecule). For details, refer to Aqueous and Lipid Solubility Module.

By considering physicochemical properties during the design phase of drug or chemical needs, we can reduce negative toxicological impacts and unintended side effects before the product makes it to market and living systems are exposed to a potentially dangerous chemical.

Assignment:

- Find a successful example on how understanding ADME led to a better chemical design. Summarize the issue and outcome in 1-2 paragraphs.
- Propose a new chemical (and, for fun, name the chemical!) that will be minimally toxic to humans based on its physicochemical properties. Detail out what properties you chose for this chemical to possess and how that has an impact on its toxicity using ADME properties to guide your decisions.
- There are several parameters which influence ADME. Changing these parameters from not preferred to desired can potentially impact bioavailability and absorption in organism. While molecular weight and solubility significantly contribute to increase or decrease in dermal, respiratory and digestive absorption, below you will find a list of parameters which also need to be considered while designing a molecule.

Parameter	Preferred	Not Preferred
Physical state	solid	liquid
LogP	Between 0 and 6	Less than 0 or more than 6
Molecular weight	More than 400 Da	Less than 400 Da
Ionization	Yes	no

Parameter	Preferred	Not Preferred
Particle size	More than 5nm	Less than 5nm
LogP	Less than 0 or greater than 5	Between 0 and 5
Molecular weight	More than 400 Da	Less than 400 Da
Vapor pressure	Less than 0.001 mmHg	More than 0.001 mmHg

Parameter	Preferred	Not Preferred
Particle size	More than 100nm	Less than 100nm
LogP	Less than 0 or greater than 5	Between 0 and 5
Phase	solid	liquid
H bonds (acceptors/donors)	More than 5/10	Less than 5/10
Molecular Weight	More than 500 Da	Less than 400 Da

Go to chemspider.com and put in following CAS numbers:

- Benzene **71-43-2**
- DMSO **67-68-5**
- Caffeine **58-08-2**

Using discussed parameters and their preferable and non preferable ranges, fill in the following tables to predict respiratory, dermal and digestive absorption of compounds.

DERMAL			
	71-43-2	67-68-5	58-08-2
LogP			
Molecular weight [g/mol]			
Phase			
Ionization [Y/N]			

Respiratory			
	71-43-2	67-68-5	58-08-2
LogP			
Molecular weight [g/mol]			
Vapor pressure [mmHg]			

Digestive			
	71-43-2	67-68-5	58-08-2
LogP			
Molecular weight [g/mol]			
Phase			
H bonds (acc/don)			

Assignment #1 answer found HERE: [ADME and Rational Chemical Design Answer #1 Key](#)

Assignment #2 answers will vary

Assignment #3 answer found HERE: [ADME and Rational Chemical Design Answer #3 Key](#)

Resources:

- Mulvihill, M., & Vulpe, C. (n.d.). Controlling ADME through chemical design. Retrieved from <http://bcgc.berkeley.edu/sites/default/files/Ch298010-2012%20Day%205.pdf>
- Di, L. & Kerns, E. H. (2010). Drug-like properties: Concepts, structure design and methods: From ADME to toxicity optimization. *eBook*. Retrieved from <http://www.acad.bg/ebook/cheminformatics/Drug-like%20Properties%20Concepts.%20Structure%20Design%20and%20Methods%20from%20ADME%20to%20Toxicity%20Optimization.pdf>

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Module 9: Model Organisms Zebrafish



Introduction: For decades, toxicology testing with animal models has been a crucial tool for studying the adverse health effects of chemicals and human disease. Vertebrate animal models are used because of the conservation that exists between humans and other vertebrates in the genetics, development, and metabolic pathways. However, it is worth noting that there are other models such as bacteria, yeast and fruit flies that have provided great insight into the biological functions of more complex organisms. When selecting a model for toxicology testing, it is helpful to have a good understanding of the toxicity endpoints, the mechanism of action if possible, and the extent to which various model organisms can approximate human responses to those endpoints or that mechanism.

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

- Recognize the benefits of using zebrafish as a model organism for toxicological research.
- Describe the hierarchy of testing models.
- Find examples of peer-reviewed research that uses zebrafish to study toxicology and disease.

Background and Information: Animal models are used with expectations that the discoveries made in the animal model will provide clearer insight into the biological functionality of other organisms; for example, humans. This becomes relevant when these studies can offer a unique insight into the biology of the disease and help to mitigate some of the harmful effects of drugs and chemicals. The reason that animal models can be used as a 'surrogate' system for humans is because of the great similarities in genes and pathways even between organisms that have been evolving independently for millions of years. For example, the GAPDH gene encodes glyceraldehyde 3-phosphate dehydrogenase enzyme, which helps to break down glucose for energy in organisms. The GAPDH gene in mouse and GAPDH gene in human share over 83% of identical sequence! This high sequence homology provides a foundation for extrapolating information collected from animals to humans. Figure 1 demonstrates the general hierarchy for testing models. Depending on what tier the research starts, the collected data can lead to inferences and rationale for moving to a more complex testing model. For example, during pharmaceutical drug development large amounts of data are collected from the bottom three tiers before moving to the top most tier (labeled epidemiology), which in this example would be human clinical trials. To date, there is a large database of animal model data that is available to use for predicting toxicity. [The scientific community as a whole, including government funded agencies like the National Institutes of Health \(NIH\), have fostered programs for education and training on the replacement, refinement and reduction of the use of animals in scientific research; commonly referred to as the 3 R's.](#)

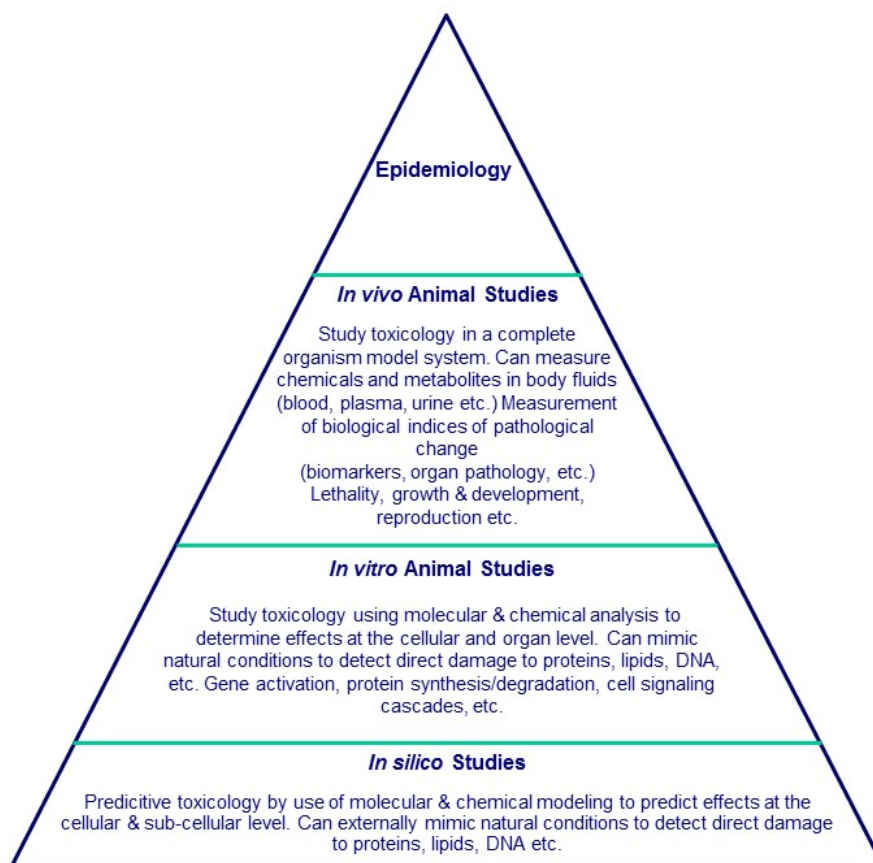


Figure 1: Hierarchy of testing models: Before a chemical is introduced to animal or human models, it undergoes rigorous in silico testing. During this stage chemical effect is modelled by sophisticated computer software. As we move up the pyramid, in vitro studies involve testing on cultured cells, rather than on live animals. Once approved, chemicals are tested on cellular and organ level and then on the whole organism. Only after a thorough in vitro and in vivo toxicological studies, chemicals (such as drugs) are introduced to a human population.

Why are scientists using a home aquarium fish to study human disease and chemical exposure? The answer is quite simply that zebrafish are an excellent vertebrate model organism and here are some reasons why:

- Small size and speedy growth: The same traits that make zebrafish popular pets also make them good model organisms. Adult zebrafish are small in size, they grow quickly, and they breed readily. They can reach sexual maturity in three months in the lab, and each adult female can produce hundreds of eggs a week. Conducting larger studies with higher numbers in your exposure groups is much easier with zebrafish than with mice.
- External fertilization and development: The eggs are fertilized and undergo development outside the mother's body, rather than the internal development of mammals or even the internal fertilization of birds, amphibians and reptiles. This allows researchers to examine and manipulate the embryos from the moment they are fertilized.
- See-through embryos: The optical clarity of zebrafish embryos and the chorions that surround them offers an amazing view of morphological development, organ development

and function. Rapid embryonic development: Zebrafish develop from fertilized eggs to something resembling tiny fish in 24 hours. This same period of development for mice takes up to 21 days. Check out this time lapsed video of a zebrafish embryo developing over a 24-hour period. The visual clarity and external development allow this incredible view of the developmental processes.

- Genetic manipulation: Zebrafish embryos can be easily manipulated, including making alterations to their genomes. This includes disrupting genes in the fish (either chemically or through gene-editing technologies) and then studying to see what happens when a gene's normal function is altered. This is often an effective way of identifying new genes or discovering novel functions of known genes. New genes can also be added, including genes for fluorescent proteins that allow researchers to follow specific cell types that are involved in disease in the live fish.
- Homology to humans: Zebrafish have many of the same genes, tissues, and organ systems as humans. Because zebrafish are more similar to humans than invertebrate models (such as the worm *Caenorhabditis elegans* and the fly *Drosophila melanogaster*), discoveries made in zebrafish are more likely to be directly applicable to humans than those made in invertebrate systems.

These attributes of zebrafish make them an excellent model for high-throughput screening of chemical compounds. Their size allows for the setup and maintenance of either individual fish or groups of fish in separated containers in which your compound of choice may be administered. This gives researchers a large amount of flexibility and statistical power when determining potential toxicities of chemicals. In addition to their relevance to human health, they are also a good model for other aquatic species: they can be exposed to contaminated water sources for a discipline of toxicology known as ecotoxicology. In these types of studies researchers can determine if pollution is affecting the local ecology.

Video: <https://www.youtube.com/watch?v=TramK44tujA>

Assignment:

As described above, zebrafish can be a powerful model for scientific research into human disease and development. Perform your own literature search on the zebrafish model being used for research into some form of human disease. <http://www.ncbi.nlm.nih.gov/pubmed> is a good starting point. Rather than focusing only on one unique protein or molecule, provide a big picture as to how the zebrafish anatomy and/or physiology is more similar to humans than is that of invertebrate or non-animal models for your chosen disease. Examples to focus on can be metabolic pathways, receptor mediated pathways, genes involved in the initiation and/or progression of the disease. There are numerous examples you can use to answer this question.

Resources:

- National Institutes of Health. (2012). New video highlights NIH investment in zebrafish research. Retrieved from <http://www.nih.gov/news-events/news-releases/new-video-highlights-nih-investment-zebrafish-research>
- The University of Sheffield. (2015). Fish for science homepage. Retrieved from <http://www.fishforscience.org>
- Zurlo, J., Rudacille, D. & Goldberg, A. M. (2010). Animals and alternatives in testing: History, science and ethics. Retrieved from http://caat.jhsph.edu/publications/animal_alternatives/chapter3.html

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