1,2,3,4,7,8,-HEXACHLORODIBENZOFURAN

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Introduction

HCDF $C_{12}H_2Cl_6O$ (1,2,3,4,7,8,-hexachlorodibezofuran), known as congeners,has various harmful health and environmental effects. It also plays an important role in modulating the biological signaling pathways of the Aryl Hydrocarbon receptor.

AhR is the cytosolic transcription factor regulator followed by ligands (substrates, inhibitors, activators & translators). It binds to chemicals such as 1,2,3,4,7,8,-hexachlorodibezofuran, initiating chaperon dissociation and AhR translocation into the nucleus, with dimerization with ARNT (the AhR nuclear translocator), which leads to changes in the gene transcription.AhR also initiates protein-protein interaction with Estrogen Receptor 1 human-alpha, activated by the sex hormone estrogen.

ESR binds with several domains important for hormones. Chlorinated polycyclic aromatic hydrocarbon CIPAHs activateAhR,and bind with the AhR translocator protein to form a heterodimer. This causes transcriptional modulation of genes,leading to adverse cellular changes. Depending on the position of the chlorine atoms, HCDF $C_{12}H_2Cl_6O$compounds bind to the aryl hydrocarbon receptor at different strengths, and hence display different toxic activities.

Understanding the Molecular Dynamics of HCDF $C_{12}H_2Cl_6O$ and toxicity is an important step towards designing successful AhR inhibitors. It further leads to the investigationofAhR receptor behavioron unknown targets when the effects are not localized.

In this study, molecular modeling was performed using one of the leading isomer structures1,2,3,4,7,8,-hexachlorodibezofuran HCDF $C_{12}H_2Cl_6O$.

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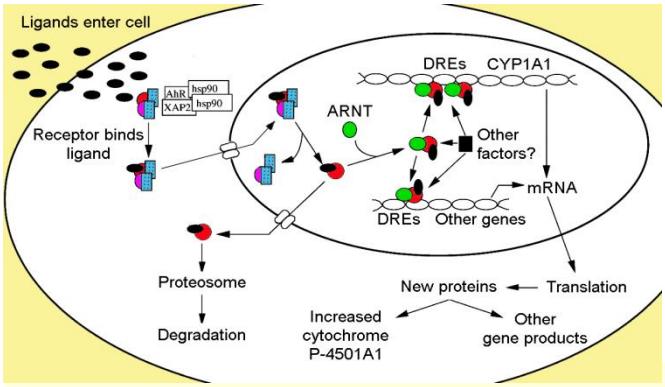


AhR Signaling Pathway (Denison and Nagy 2003)

Results

In silico tools make the following predictions for the compound:

ToxTree:

Contains elements other than C, H, O, N divalents S?

Yes (chlorine).

Estimation via Cramer rules: Toxic hazard:

High (Class III).

Lazar:

Relatively high confidence interval for carcinogenicity in rat and mouse (CI: 0.0542 &0.296).

Salmonella test predicted mutagenicity (CI: 0.134).

Hamster test predicted no carcinogenicity or mutagenicity.

admetSAR:

Good Human Intestinal Absorption positive (probability: 1.0000). Inhibitor of Cyp 1A2, Cyp 2C9 &Cyp 2C19 & non-inhibitor of P-gp substrate. No- AMES toxicity and non-carcinogenic. VirtualToxLab result shows compound is highly toxic with Toxic Potential: 0.464.

High affinity Targets:

ER-α is high Micro Molar range.

ADME:

Inhalation and Human Intestinal Absorption?

Yes, HIA + 1.0000.

In VirtualToxLab, the results for binding affinity found high AR (92.1 NM),compared with an experimental binding affinity of 230nM. This shows that the technology is able to predict

binding affinities close to the actual experimental values. It accurately predicts the experimental results.

At the same time, according to IARC classification, the molecule is not classifiable as to its carcinogenicity to humans, thus one has to test the compound on human subjects for several years.

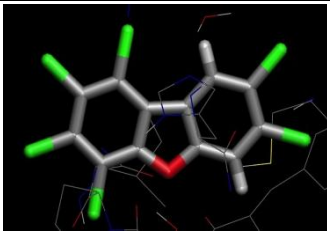
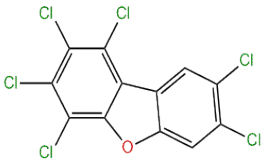
No information on the metabolism of dibenzofuran in mammalian organisms was found in the available literature. The compound is not present with sufficient references in the ranking for investigation in EPA (United States Environmental Protection Agency). There is a broad gap in the regulatory coverage. There are not many reliable references available.

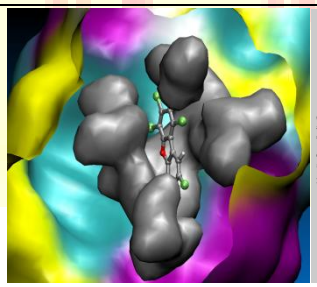
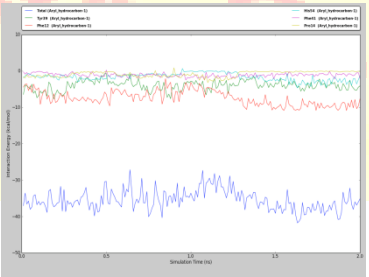
Aryl hydrocarbon receptor (AhR) is a crucial regulator in maintaining cellular communication both in skin and intestine. This leads also to the further investigation of AhR deficiency or the lack of AhR ligands.

Some limitations ofin silicolie in finding the regulatory dose calculations LOEL and NOEL, which are dispersed data without any centrally integrated database. This is also the case for FDA and the Environmental Protection Agency (EPA). There is a need to builda system that can efficiently handle the archival data, as well as advanced linguistic software to extract data. We also have difficulties in finding the views of both the molecule Pharmacologist and Toxicologist.

There is no clarity in predicting false negative or false positive results. It is not automatically the case that a highly toxic potential compound must be specified as dangerous, or that a safe compound can be considered as non-toxic, because adverse effects can be mediated through other unknown targets or though other mechanisms. Insilico suspected molecule 1,2,3,4,7,8,-hexachlorodibenzofuran cannot simply be used to predict the harmlessness of a chemical or drug in a body or organism because many other proteins present in the body could have adverse effects. The molecule is simply an indication of toxicity.

In silico toxicology – compound evaluation sheet

Compound: ***1,2,3,4,7,8,-hexachlorodibenzofuran*** ***Chlorinated dibenzofuran (CDFs)***	  1. **HCDF $C_{12}H_2Cl_6O$, aromatic hydrocarbon** 2. **Chlorinated dibenzofuran** 3. **1,2,3,4,7,8-hexachlorodibenzofuran** 4. **$C_1=C_2C(=CC(=C_1Cl)Cl)OC_3=C_2C(=C(C(=C_3Cl)Cl)Cl)$** 5. **Colorless crystals**
Known facts ***Mol. Wt. 374.87***	**Potential acute health effects**: Chlorine atoms at the **2,3,7,8-positions are harmful.** **Potential chronic health effects**: Can cause vomiting; diarrhea; anemia; more frequent lung infections; numbness and other effects on the nervous system; mild changes in the liver;skin and eye irritations; especially severe acne; darkened skin color; and swollen eyelids with discharge. **Carcinogenic, Teratogenicity Effects:** **IARC Classification:**3, not classifiable as to its carcinogenicity to humans. **ReproToxicity:** No information on the metabolism of dibenzofuran in mammalian organisms was found in the available literature.
ToxTree	**Class: High** **Q3. Contains elements other than C, H, O, N divalent S? Yes (Chlorine)**
Lazar *http://lazar.in-silico.de/predict*	DSSTox*Carcinogenic* Potency DBS **Hamster**: **non-carcinogen** (Confidence: 0.084) DSSTox*Carcinogenic* Potency DBS **Rat**: **carcinogen** (Confidence : 0.0542) *Mutagenicity***Salmonella**: **mutagenic**(Confidence : 0.134) DSSTox*Carcinogenic* Potency DBS **Mouse**:**carcinogen** (Confidence : 0.296)

<table>
<tr><td>admetSAR
http://www.admetexp.org</td><td>
Absorption
Blood-Brain Barrier BBB+ 0.9939
Human Intestinal Absorption HIA+ 1.0000
Excretion Toxicity
Human Ether-a-go-go-Related Gene Inhibition Weak inhibitor 0.7823
Non-inhibitor 0.8121
AMES Toxicity Non AMES toxic 0.5405
Carcinogens Non-Carcinogens 0.8040
Fish Toxicity High FHMT 0.9368
Tetrahymena Pyriformis Toxicity High TPT 0.9987
Honey Bee Toxicity High HBT 0.7402
Biodegradation Not ready biodegradable 0.9754
</td></tr>
<tr><td>VirtualToxLab</td><td>
<table>
<tr><td>AR: 4.9 µM</td><td>AhR: 92.1 nM</td><td>CYP450 1A2: 2.3 µM</td><td>CYP450 2C9: 101 µM</td></tr>
<tr><td>CYP450 2D6: 9.67 µM</td><td>CYP450 3A4: 12.5 µM</td><td>ERα: 14.4 µM</td><td>ERβ: 6.05µM</td></tr>
<tr><td>hERG: 12 µM</td><td>GR: 232 nM</td><td>LXR:>3.3 µM</td><td>MR: > 700 nM</td></tr>
<tr><td>PPARγ:> 2 µ M</td><td>PR: 57.1 nM</td><td>TRα: 36 uM</td><td>TRβ: 12 MM</td></tr>
<tr><td colspan="4">Toxic Potential: 0.464
High affinity Targets: ERα in high Micro molar range</td></tr>
</table>
</td></tr>
<tr><td>MD Simulation</td><td>


Binding mode(s):
The molecule stays inside the binding pockets, thus it is “stable”. The trouble in particular with this molecule is that it does not create any hydrogen bonds or any directed interactions. O_2 is below btw. Two aromatic rings are pulling electrons, as well as chlorine pulling the electron away, and this becomes O_2. There is no interaction with the protein. It has a stable constant energy and always binds to the target. TheAhR (Aryl hydrocarbon receptor) protein shows it does not go away. Virtual ToxLabdoes not predict the harmlessness of a chemical or a drug. It is just an indicator of toxicity in a body or organism. There is the possibility for other adverse effects caused by one of the many proteins in the body. (#H bond acceptors: 1. #H bond donors: 0. Average mass: 374.861694 Da. Water Solubility at 25 °C (mg/L): 0.0001138.)
Figure 1. Figure 2. -30-40 kcal/mol
</td></tr>
<tr><td>There sources: e.g. ToxPredict, Stitch,...</td><td>Ecotoxic effects >> Acute toxicity to fish (lethality)
Environmental fate parameters >> Persistence: Biodegradation
Q9.Two or more rings? Yes Class? Class 2 (persistent chemical)</td></tr>
</table>

ADME, Quick PK	**PercentHumanOralAbsorption** *>80% is high, <25% is poor* 100.000 **HumanOralAbsorption** *1: low, 2: medium, 3: high* 1
Conclusions	No information on the metabolism of dibenzofuran in mammalian organisms was found in the available literature. **Carcinogenic, Teratogenic Effects:IARC Classification:**3, not classifiable as to its carcinogenicity to humans. Results of VTL can be trusted. Lazar indicate its toxicity both **Carcinogenic, Teratogenic Effects:IARC Classification:**3, not classifiable as to its carcinogenicity to humans. **HCDF** is able to cross BBB and Intestine, and oral passages. Effects are caused only by strong affinity of HCDF to the Arylhydrocarbon receptors. Due to its hydrophobic nature and stability ADME properties favor the possibility of interaction. More investigations are required in vivo as well as in vitro, since there is no standard reference available yet.

Discussion

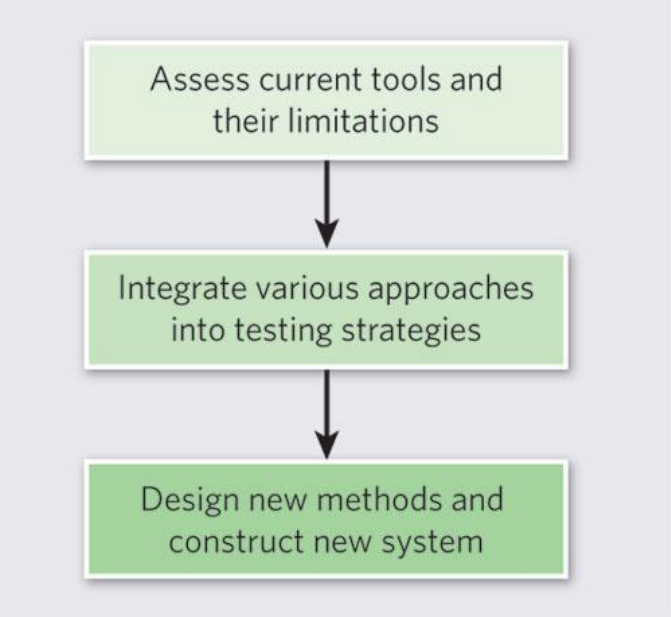
The conditions state that the above test cannot be the only proven method to determine whetherthe compound insilico-toxic. Investigating further, we find that the molecule HCDF $C_{12}H_2Cl_6O$ (1,2,3,4,7,8,-hexachlorodibezofuran) has similarities with Chlorinated Polycyclic Aromatic Hydrocarbon (CLPAH), which exhibits mutagenic activity towards Salmonella typhimurium in the AMEs assay.The presence of chlorine in aromatic benzene ring, aromatic halogenated, furthers its strong oxidizing nature due to highest electron affinity, consequently high electronegativity. Thecapability to takean electron onto itself is evidence in favor of being considered a toxic molecule.

The absolute number of interactions or lowest number of interactions has to do with the size of the molecule.The larger the molecule, the more contact with the protein. HCDF is a relatively large molecule with Molecular Weight 374.87.Further Molecular Dynamics confirm the VirtualToxLab, which shows that molecule 1,2,3,4,7,8,-hexachlorodibenzofuran stays inside the pockets and is therefore “stable” (Figure 1). The problem with this molecule is that it does not create any hydrogen bonds or any directed interactions. O_2resides in the molecule between 2 aromatic rings which are pulling electrons, as well as chlorine pulling the electron away,and this becomes O_2. The low water solubility shows that it has hydrophobic persistence. There is no interaction with the protein. It has stable constant energy and always binds to the target. The ARh (Aryl hydrocarbon receptor) protein shows it does not go away. The MD Simulation graph on compound sheet pageshows that the interaction developments over time were quite flat;it remains at the same level so is quite stable. Energy plot (Figure 2, page 4) shows avalue of -30-40 kcal/mol. It is merely an indication of the toxicity in the body or organism, since the high number of proteins present in the bodycan deliver undesirable effects. Its highly toxic nature is likely due to its aromatic halogen.

AdmetSAR shows that the molecule is not readily degradable.It is very stable and bounded.If it reaches fatty tissues, it can live there for 100 years. It binds to the Aryl Hydrocarbon receptor, indicating that it is hydrophobic (that is, not water soluble).

The compound is primarily a product of tar. It also occurs in radioactive waste, combustion product, vegetables and the environment. Because of its occurrence in nature, street workers can inhale it.It might be present in cigarette smoke, since it is a product of combustion. It might be

accidentally ingested from tap water.The important point here is that we inhale a very significant quantity. Thecompound is hydrophobic and dissolves into organic compounds.



Toxicology for the twenty-first century (Hartung 2009)

Modern cell culture techniques make it possible to study biological phenomena in vitro. This was not possible in early toxicological experiments, but in vitro studies are now viable and commonplace. In light of such developments,it is important to consider how regulatory toxicology can be improved. There is the potential for greater robustness, reliability, efficiency and affordability. The limitations ofthe current tools need to be objectively assessed. Various approaches need to be integrated into testing strategies, making the best use of existing methods by combining them strategically.

One of the largest remaining obstacles in cell culture has been the difficulty of procuring primary human cells, but the ability to isolate or generate stem cells—and to use these to produce most human cell types—has helped to overcome this obstacle.

Alternatives to animal testing

Promising alternatives to animal-based research include:

- bioinformatics
- biotechnology

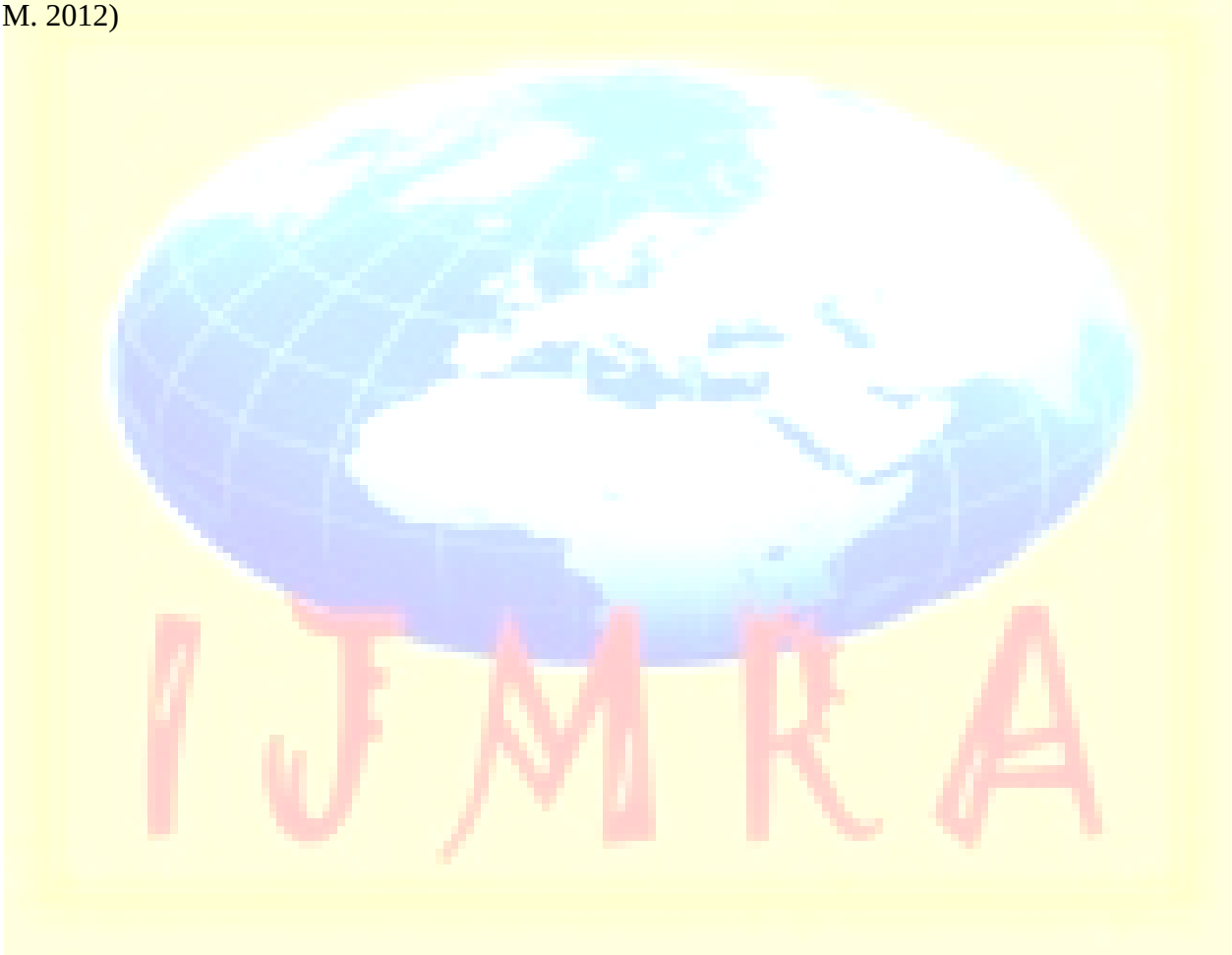
- metabolomics (the study of metabolic responses to environmental factors, drugs and diseases)*(Hartung 2009)*
- epidemiology (studying human populations)
- autopsies
- in vitro research (the use of tissue and cell cultures)
- computermodeling
- genomics (the study of an organism's chromosomes)
- proteomics (the study of proteins, especially their structures and functions)*(D. H. Conrad, J. Goyette, and P. S. Thomas 2008)*
- nanotechnology
- phage display (the rapid evaluationof many potentially useful antibodies and large-scale production of the selected antibodies)
- microfluidic chips

The United States Environmental Protection Agency's (EPA) ToxCast program is a good example of a toxicity testing strategy that pursues alternatives to animal testing. Another good example is the Human Genomic Project, which analyses the interactions between cells and molecules. It is important to consider how consistency and quality in and between new testing strategies and processes can be achieved.

Organs-on-Chips—which embed living cells in silico and plastic substrates to simulate biological systems—are becoming viable.Such research does not aim to create replacement organs for use in transplant surgery, but rather to replicate organs' functions in order to test substances for toxic and therapeutic effects. Human lung and intestine Organs-on-Chips have already been developed. Such technology has the potential to improve the speed at which new treatments can progress to testing on human subjects.

In an ABC (Australian Broadcasting Corporation) report on supercomputers, Professor Peter Taylor (Director of the Victorian Life Science Computation Initiative)reportedly said, "One could imagine that if our ability to model how, say, drugs interact with their targets and how they affect an organism as a whole, then we could basically avoid any sort of animal testing. We could do it all in silico, if you like, in the computer, which would certainly be a great benefit. I don't think there's anybody who would quarrel with trying to do that." (Tylor P. 2012; Merkes, M. 2012)

In the USA, it has been found that biomedical research involving chimpanzees is usually unnecessary, and new grants for such research have been suspended.The US National Research Council recommends the replacement of "animal-based tests with human cell-based assays, in silico (computer) models, and an increased emphasis on epidemiology."(NRC2007) Some pharmaceutical companies are moving away from animal research. While new medicines need to be safe and effective, animal testing has become unnecessary in light of the availability of other methods, such as those discussed in this paper. (Pharmaceutical-Technology.com, 2011, Merkes, M. 2012)



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