Acute/Chronic Ratios in Hazard Identification: Carboxylic Acid Induced Malformations

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Valproic acid (2-propylpentanoic acid)

- a known human teratogen
- causing neural tube and cephalic defects
- pharmacologically well studied
- teratogenic effects have been linked to inhibition of histone deacetylase

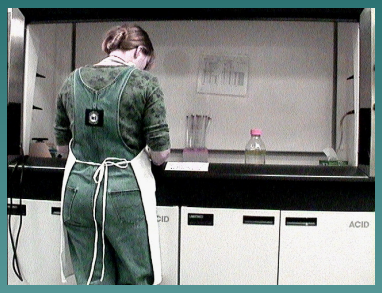
Structural Requirements

- Work in the 1990's with a mouse model in the laboratory of Heinz Nau revealed the structural requirements for teratogenesis
  - a carboxylic-group is necessary for activity
  - branching in the 2-position but only while maintaining an H-atom on the 2-postion C-atom enhances potency
  - there is a difference in potency for enantiomers

Frog Embryo Teratogensis Assay- Xenopus (FETAX)

- a static aquatic-based assay
- starts with early embryos and exposes them up to the tadpole (fetus stage)
- allows for the calculation of both a LC50 (lethality) and EC50 (malformation) value from concentration response data

Frog Embryo Teratogensis Assay- Xenopus (FETAX)



Frog Embryo Teratogensis Assay- Xenopus (FETAX)

FETAX: Developmental Toxicity of Carboxylic Acids

- tested 45 carboxylic acids and calculated
  - acute embryo toxicity (LC50)
  - malformation (EC50)
  - developmental hazard index (DHI) LC50/EC50
- DHI uses acute toxicity measured as lethality as a reference for malformation

QSAR modeling of Toxicity

$\log (LC50^{-1}) = 0.64 (\log P) - 2.70$;
$n = 45$, $r^2 = 0.819$

$\log (EC50^{-1}) = 0.66 (\log P) - 1.47$;
$n = 45$, $r^2 = 0.823$

no log P-dependent relationship
($r^2 = 0.001$) with DHI values

DHI for Saturated, Unbranched Carboxylic Acids

Acid	C-atoms	DHI
Acetic	2	1.4
Propionic	3	8.2
Butyric	4	8.4
Pentanoic	5	13.2
Hexanoic	6	12.4
Heptanoic	7	6.2
Octanoic	8	4.5
Decanoic	10	3.2
Undecanoic	11	1.8
Dodecanoic	12	1.5

DHI for Saturated, 2-Position Branched Carboxylic Acids

Acid	C-atoms	DHI
2-Methylpropionic	3:1	3.1
2-Methylbutyric	4:1	3.8
2-Ethylbutyric	4:2	3.7
2-Methylpentenoic	5:1	13.8
2-Propylpentenoic	5:3	27.9
2-Ethylhexanoic	6:2	13.6

Assessment of Reproductive Hazard

- reproductive hazard in this case is defined by malformation in reference to lethality
- acids with a 0.5 log unit difference in lethality and malformation potency have a DHI of about 3
- acids with 1.5 log unit difference in lethality and malformation potency have a DHI of about 30

DHI Assessment of Reproductive Hazard

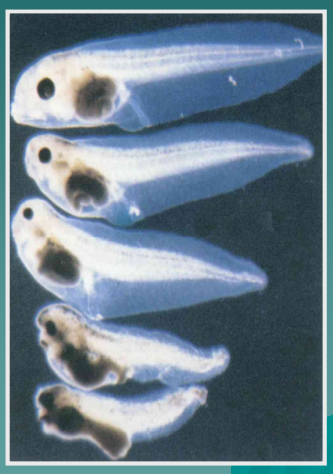
- acids such as acetic or decanoic with DHI of 2 or less are not reproductive hazards
- rather they elicit malformation at concentrations near lethal levels
- acids such as valproic acid (2-propylpentanoic acid) with DHI of 20+ are reproductive hazards

Characterization of Terata

- FETAX allows for anatomical characterization of defects
- the valproic acid syndrome, malformations include craniofacial defects, especially microcephaly
- non-specific malformations that only appear at or nearly at lethal concentrations include edema and axial skeleton defects

Characterization of Terata

- Control – stage 46
- Frog embryos with microcephaly
- Frog embryos with severe terata in most organ systems



Summary

- the DHI allows for the use of acute toxicity as a reference for more specific hazard
- teratogenic potency is highest for C5-C6 acids and decrease rapidly as the number of C-atoms increases or decreases
- teratogenic potency is higher for 2-position branched acids and highest for longer branched chains