Quantitative Systems Toxicology (DILIsym) Modeling of the Acetaminophen Cellular Pathways for Liver Toxicity Supports that Acetaminophen is Not a Carcinogenic Hazard in Humans

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ABSTRACT

BACKGROUND: Acetaminophen has a long history of safe use at therapeutic doses, but can cause liver injury at very high doses. The California Office of Environment Health Hazard Assessment (OEHHA) recently called for a scientific review of the carcinogenicity hazard potential of acetaminophen. This Quantitative Systems Toxicology work supported this review as part of a broader scientific weight-of-evidence assessment of the carcinogenicity hazard potential of acetaminophen.

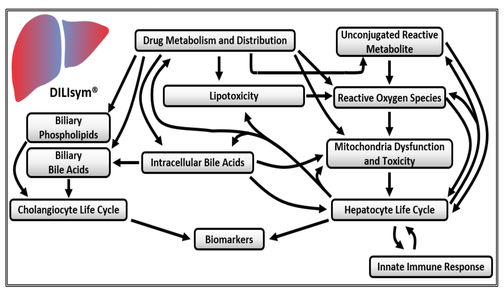
METHODS: DILIsym was applied to model acetaminophen drug exposure, metabolism and mechanisms of cell death. The model was qualified using experimental data and then used to simulate exposure to acetaminophen at therapeutic, supratherapeutic, and overdose conditions across representative populations with the goal of determining whether it is a carcinogenic hazard.

RESULTS: The model results provided the following important insights: a) acetaminophen effects are binary, with therapeutic doses showing no adverse effects, and high doses causing a burst of oxidative stress as a consequence of mitochondrial dysfunction, which results in cell death and precludes any lasting carcinogenic effects on DNA and b) simulated patients with representative variability in baseline Glutathione (GSH) levels across the population at therapeutic doses of acetaminophen are at minimal risk of production of oxidative stress in the liver and have excess buffer capacity.

CONCLUSION: Taken together, the simulation results demonstrate that the acetaminophen toxicity and mechanism of cell death at doses above the therapeutic range preclude it from being a carcinogenicity hazard at any dose level. The information from this study was submitted to OEHHA to support their evaluation.

METHODS

DILIsym Mechanism-Based Model



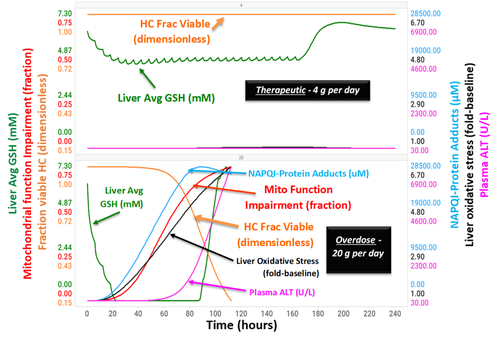
Summary of Experimental data that have been used to develop and validate the DILIsym simulations

Parameter/Endpoint	Experimental data supporting Kinetics	Experimental data supporting dose response	Model comparison to experimental data
Acetaminophen ADME and PK	√	√	Refs [1-3]
GSH depletion	√	√	Refs [1-3]
Hepatotoxicity	√	√	Refs [1-3]
Oxidative stress/ROS generation	√	√	Refs [1-3]
Mitochondrial inhibition/dysfunction	√	√	Refs [1-3]
Protein adduct formation	√	√	Refs [1-3]

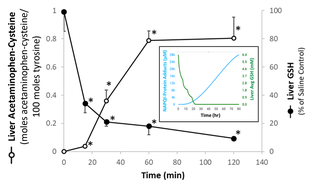
RESULTS

Sequence of Events in Acetaminophen Mechanism of Action (MOA) Pathway

Acetaminophen QID Dosing for 1 Week at Therapeutic or Overdose Levels in a Simulated Human



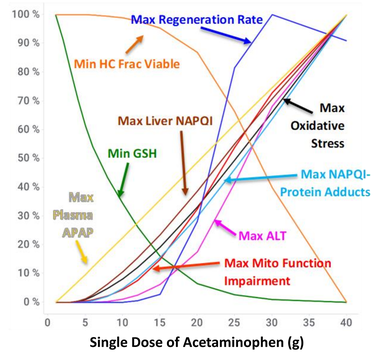
Mice Treated with Acetaminophen at a Hepatotoxic Dose of 300 mg/kg i.p. [4]



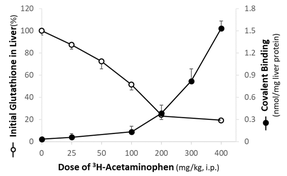
Kinetic view of acetaminophen effect in human liver under therapeutic dose and overdose

- At a therapeutic dose, there is sufficient capacity of hepatic GSH to bind up all of the NAPQI. As a result, mitochondrial function, liver oxidative stress, fraction viable hepatocytes (HC), and plasma alanine transaminase (ALT) do not change.
- Under overdose condition, GSH is depleted within a day, followed by an increase in NAPQI, impairment of mitochondrial function, and increase in liver oxidative stress. As a result of hepatotoxicity, the fraction of viable HC decrease and plasma ALT increases.
- <u>*Reactive metabolites and ROS produced during this sequence, in the event of toxicity, do not fall under the heading of key characteristics of carcinogens, since they only appear coincident with cell death.*</u>
- Kinetics of acetaminophen liver injury measured in mice support the simulated depiction of the event in humans [4].

Acetaminophen Single Dose Escalation in a Simulated Human



Hamsters Treated with Varying Doses of ^{3}H-Acetaminophen i.p. [5]

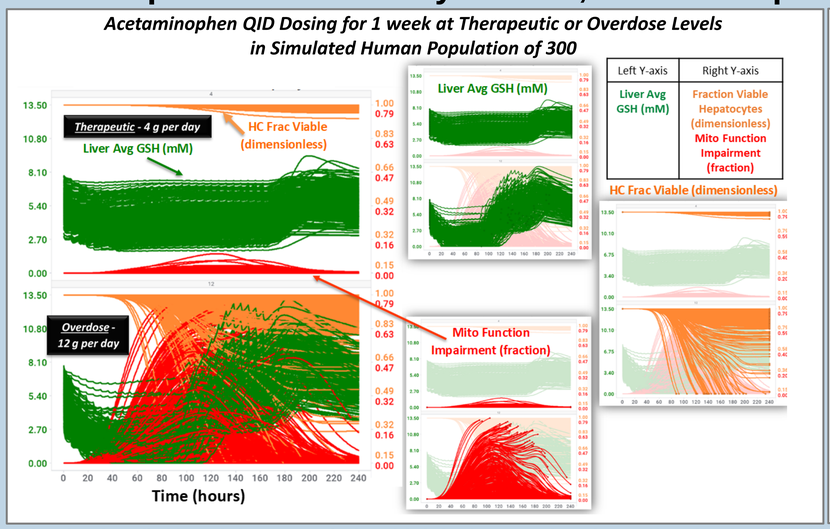


Dose escalation simulations of acetaminophen effect in human liver

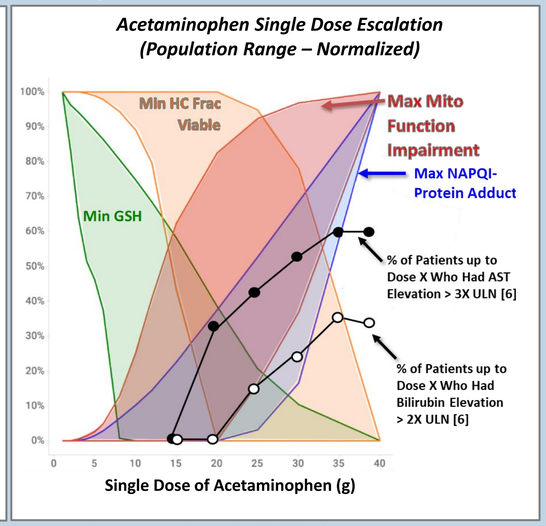
- Acetaminophen exposure at clinically relevant doses causes minimal GSH depletion, minimal cell death, and minimal mitochondrial injury and ROS.
- As acetaminophen exposure reaches approximately 10 g for the single dose case, there is substantial GSH depletion, increases in NAPQI protein adducts, impaired mitochondrial function resulting in ROS, cell death, and then rise in serum ALT. Subsequently, liver injury is repaired via hepatocellular regeneration.
- <u>*Events associated with acetaminophen toxicity either do not occur due to the GSH buffer or occur as a collective set of events associated with liver injury and cell death, precluding it from having any carcinogenic effects at higher doses.*</u>
- In hamsters treated with varying doses of ^{3}H-acetaminophen, NAPQI covalent binding increased sharply as liver GSH falls [5], consistent with trends shown in human simulations.

Population Variability in GSH, Acetaminophen PK, and ROS Clearance

Acetaminophen QID Dosing for 1 week at Therapeutic or Overdose Levels in Simulated Human Population of 300



Acetaminophen Single Dose Escalation (Population Range – Normalized)



Kinetic view (left) and dose escalation (right) of acetaminophen effect in simulated human SimPops of 300 individuals

- In simulated individuals, clinically relevant exposures of acetaminophen do not lead to meaningful cellular stress that would have any impact on carcinogenicity. At higher doses when cellular stress does occur, the buffering capacity is depleted and cell death also occurs.
- <u>*Simulations including population variability support that across a wide array of patient backgrounds, acetaminophen exposure only results in significant oxidative stress or DNA effects under conditions that cause cell death.*</u>
- Clinical data showed minimal damage to the liver below 10 g of acetaminophen, moderate cell death between 10 and 20 g, and severe liver injury beyond 20 g for most people [6], supporting the population level dose response simulations.

CONCLUSION

- Simulations results and mechanistic experimental data support that:
  - ➢ At therapeutic acetaminophen doses, cellular GSH deactivates the NAPQI metabolite and there is sufficient buffering capacity to prevent any meaningful protein adduct formation or oxidative stress.
  - ➢ Following overdose of acetaminophen, cell death occurs before any adverse conditions occur (e.g. oxidative stress or DNA damage) that could result in carcinogenicity.
- These results support that acetaminophen is not a carcinogenicity hazard to human health under any conditions, including at therapeutic (≤4g/day) and supratherapeutic doses (>4-12g/day) and an acute overdose (>15g).

ACKNOWLEDGEMENTS

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[1] Howell et al., J Pharmacokinet Pharmacodyn. 2012 Oct;39(5):527–41, [2] Howell et al., Toxicol Lett. 2014 Apr 21;226(2):163–72, [3] Woodhead et al., J Pharmacol Exp Ther. 2012 Aug;342(2):529–40,
[4] Muldrew et al., Drug Metab Dispos. 2002 Apr;30(4):446–51, [5] Potter et al., Pharmacology. 1974;12(3):129–43, [6] Davis et al., Q J Med. 1976 Apr;45(178):181–91