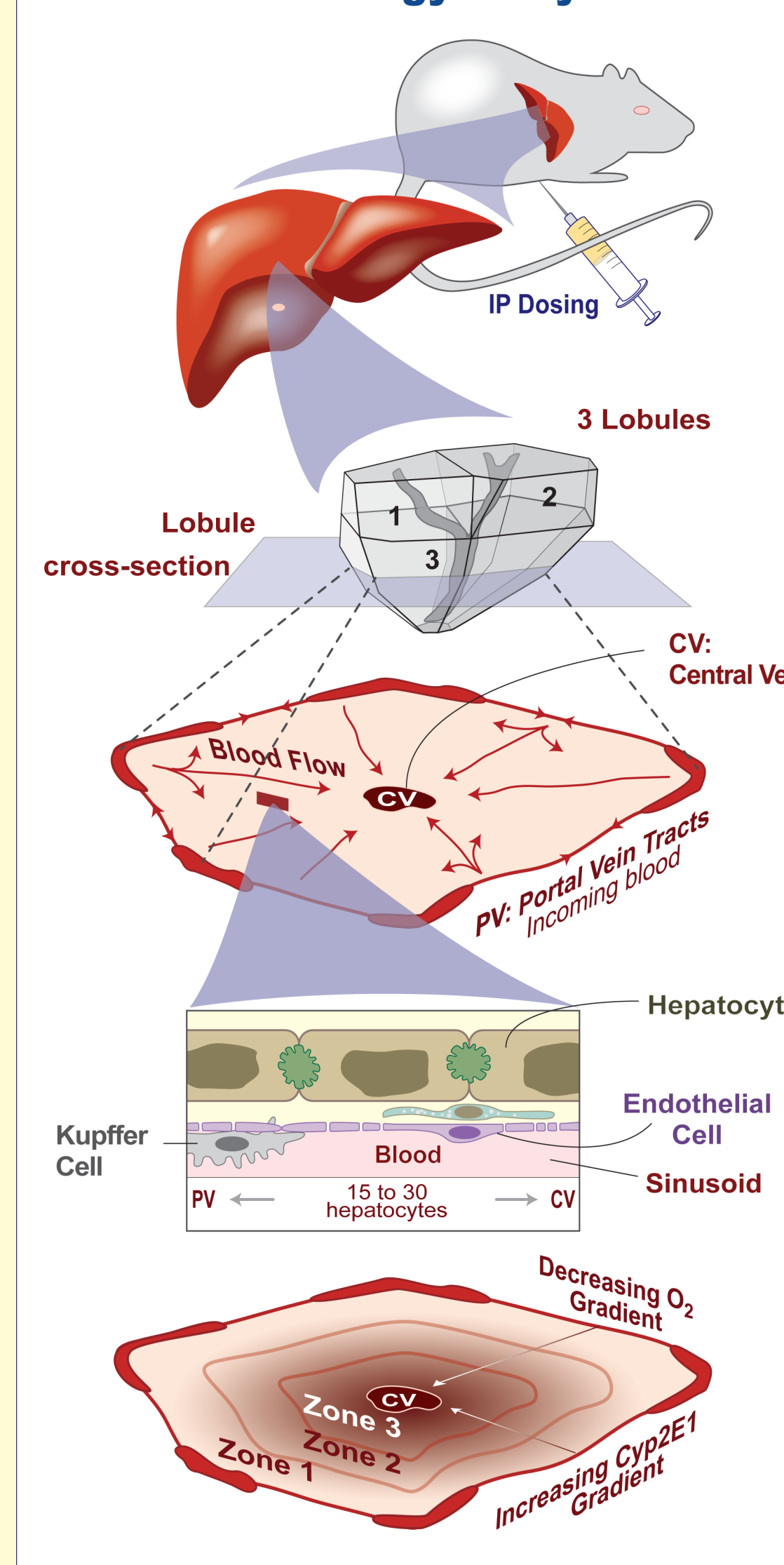


Multiscale Biology – Key Features



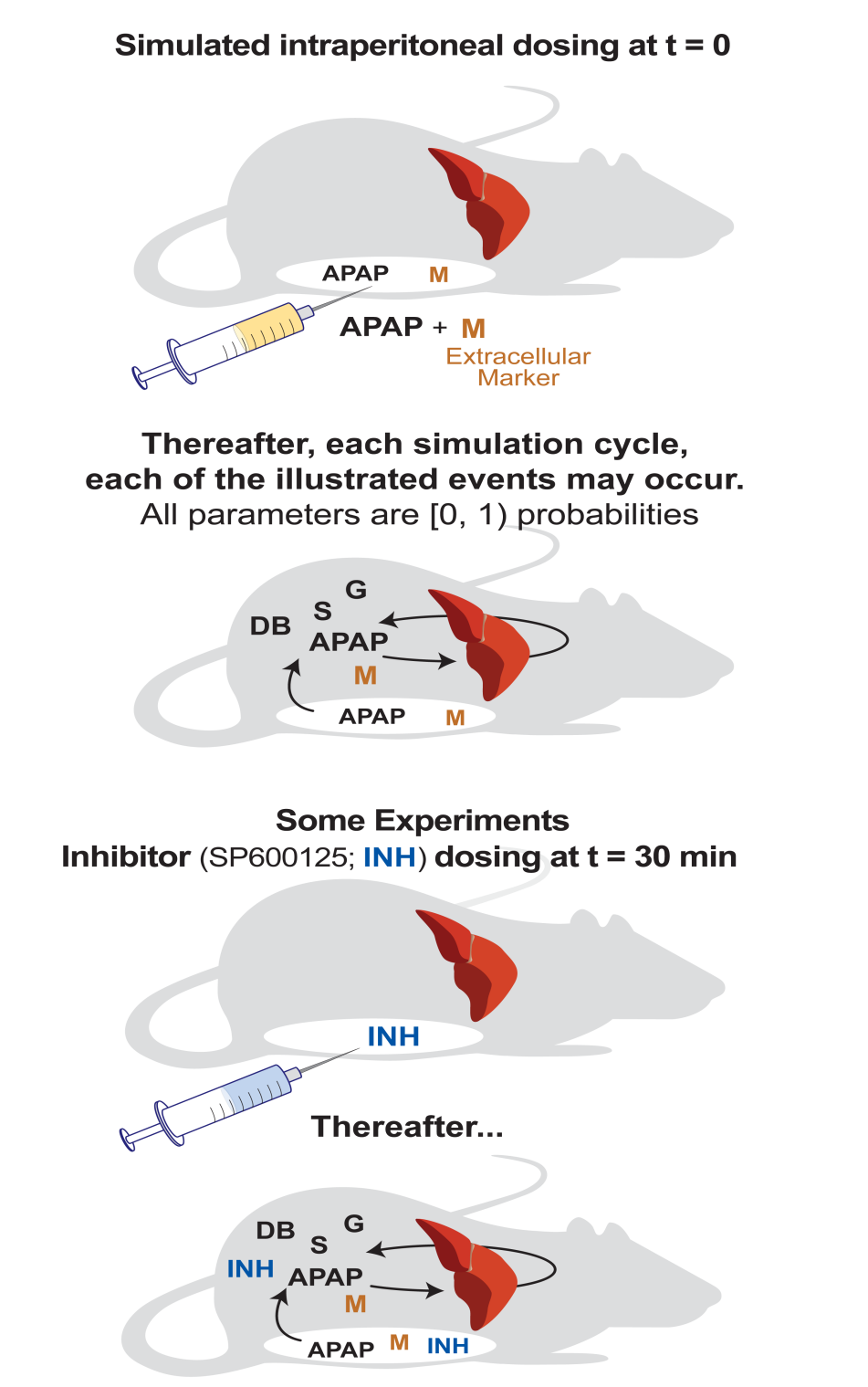
Objectives

Achieve two validation targets: They are uniquely characteristic of APAP toxicity. Do so without compromising validation targets already achieved.

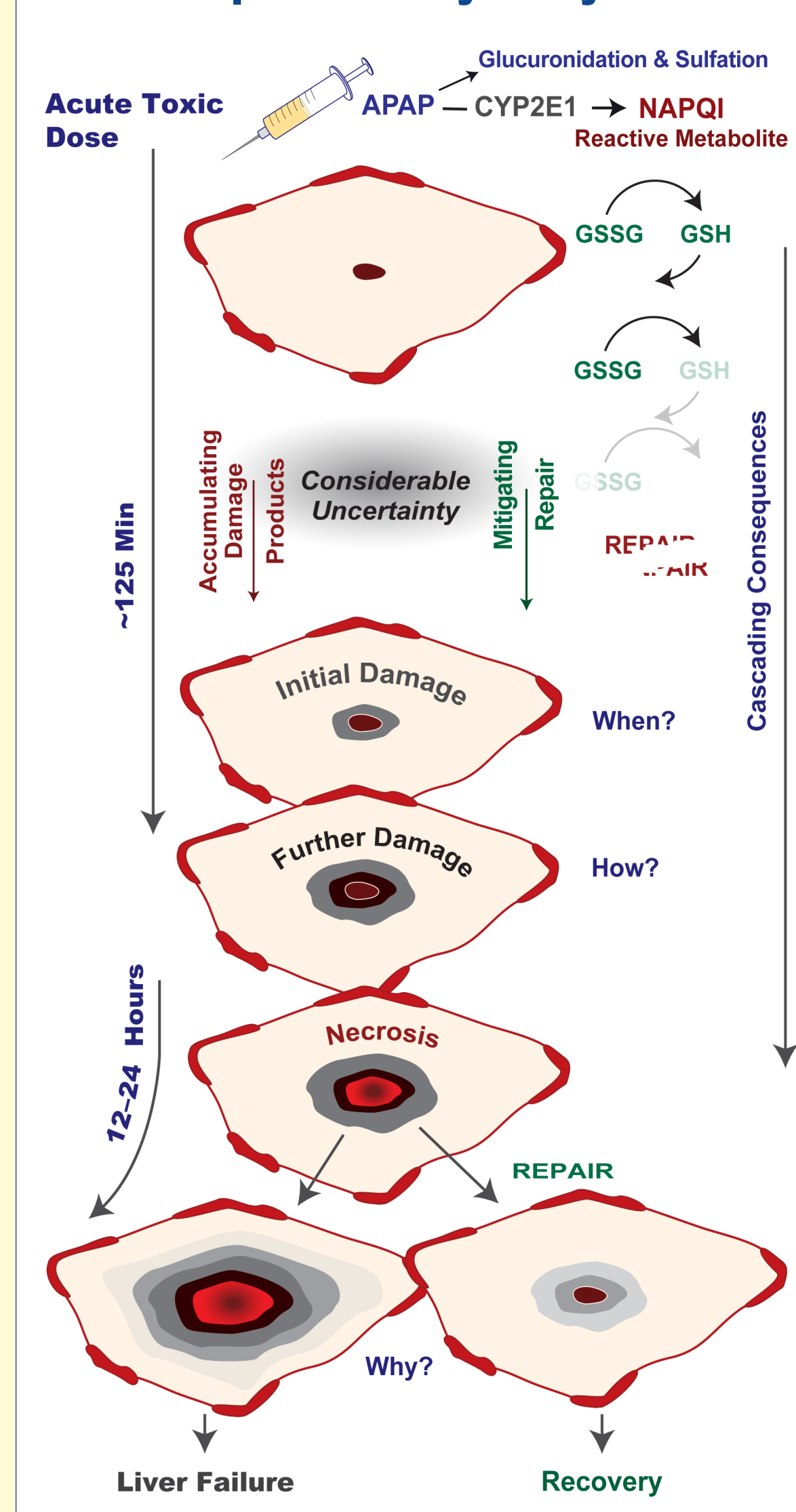
1. Necrosis occurs first in hepatocytes close to CV (Zone 3) and then progresses outward toward PV.
2. 30 min after APAP dose, an IP dose of SP600125 (INH; blocks Jun N-terminal kinase) significantly attenuates necrosis.

Challenge: Discover and improve, plausible analog mechanisms that stand as hypotheses that explain key features of hepatotoxicity and its inhibition in mice.

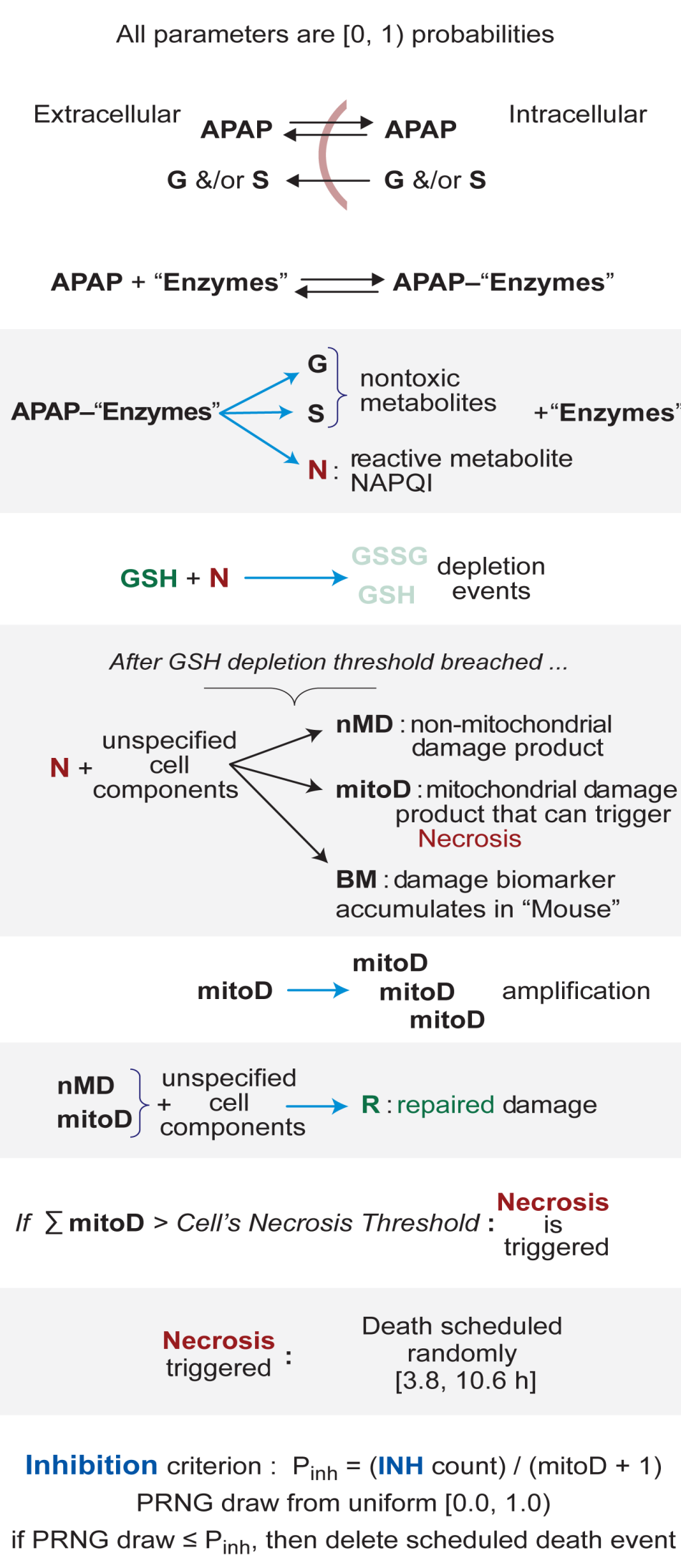
Key Mouse-level Events



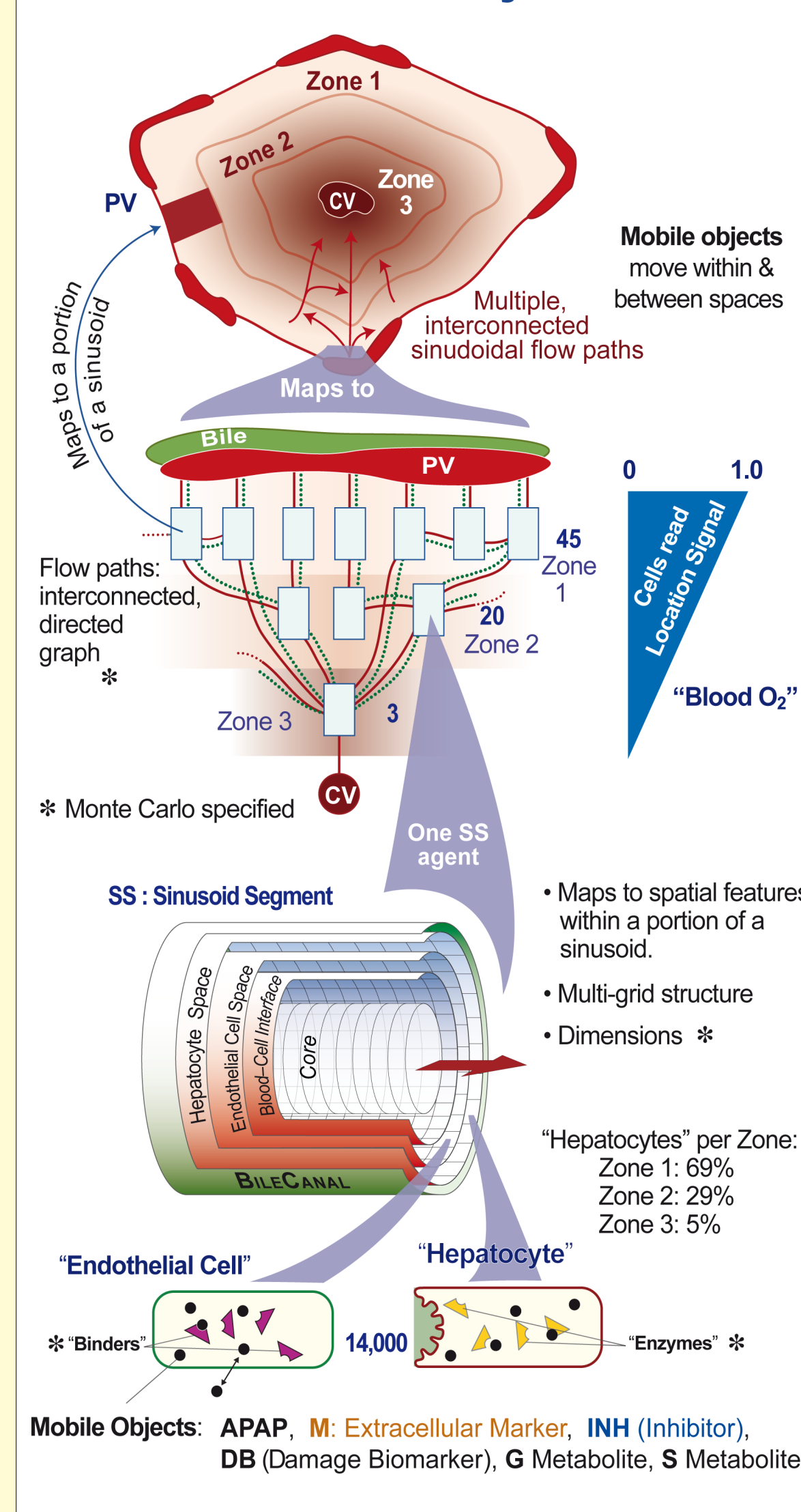
APAP Hepatotoxicity – Key Features



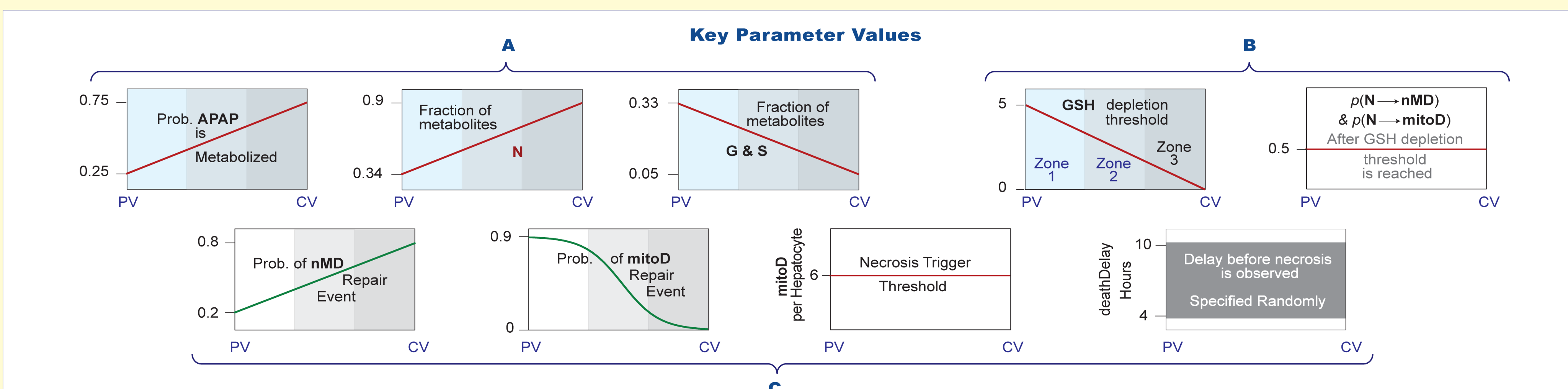
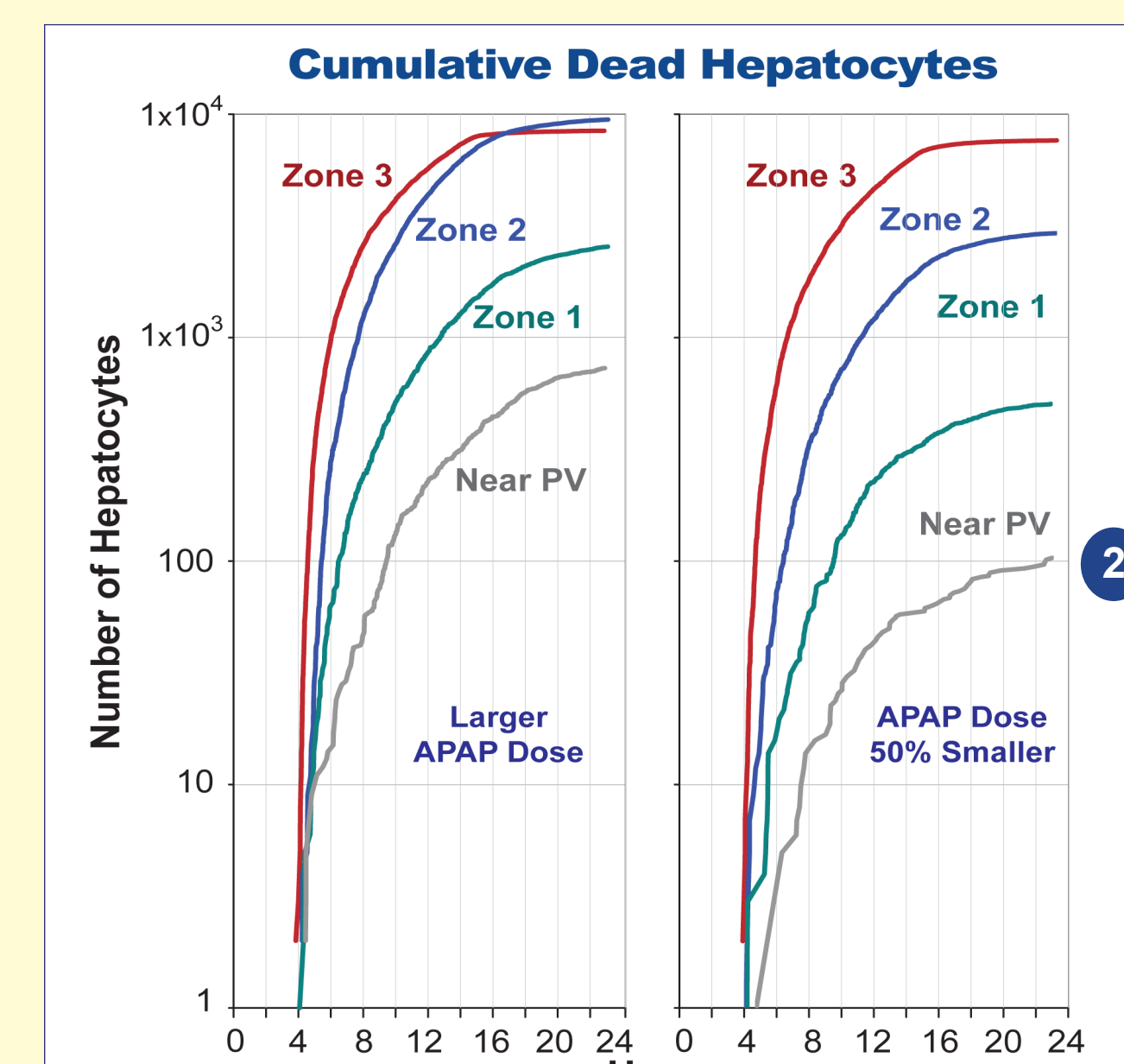
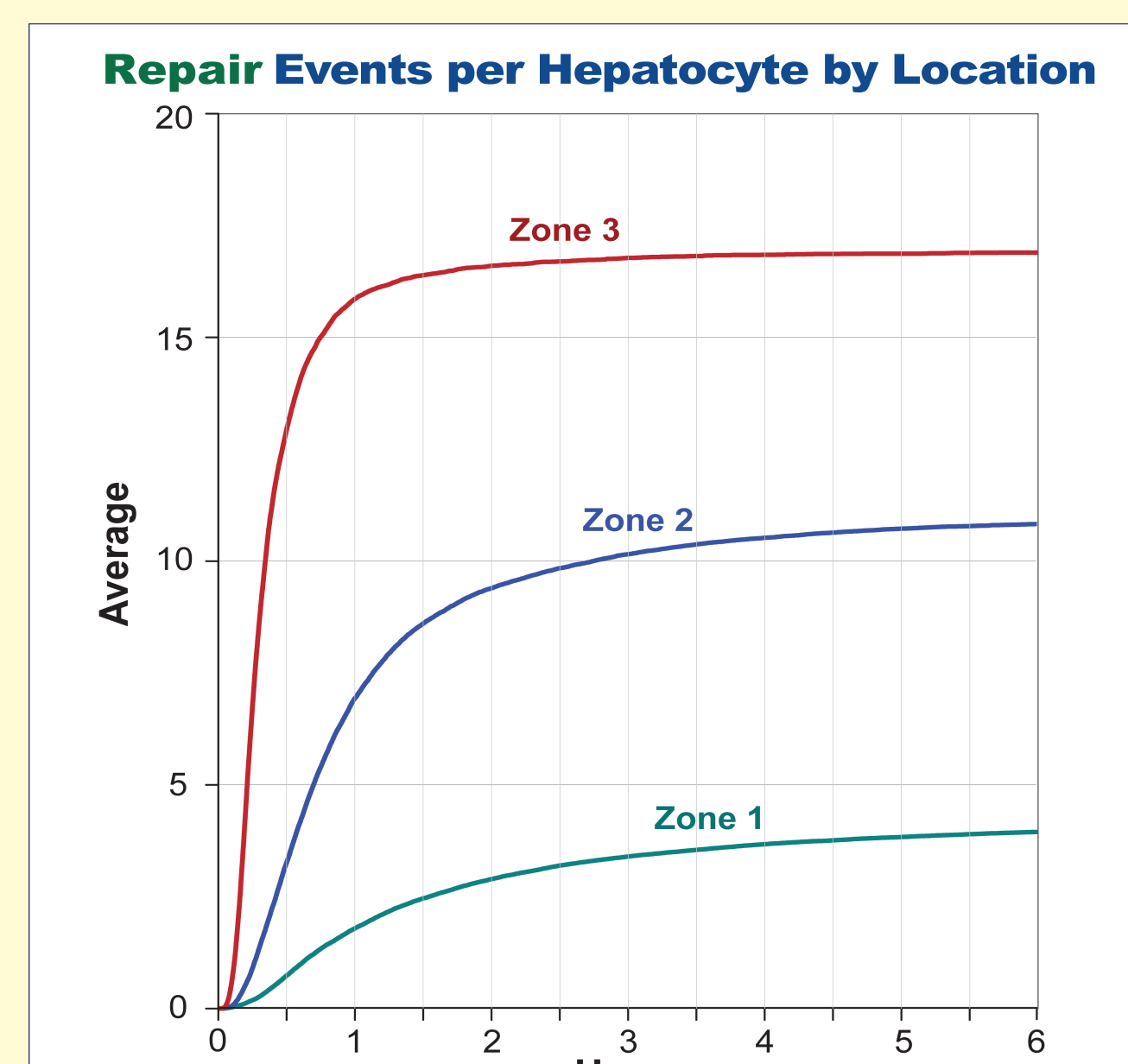
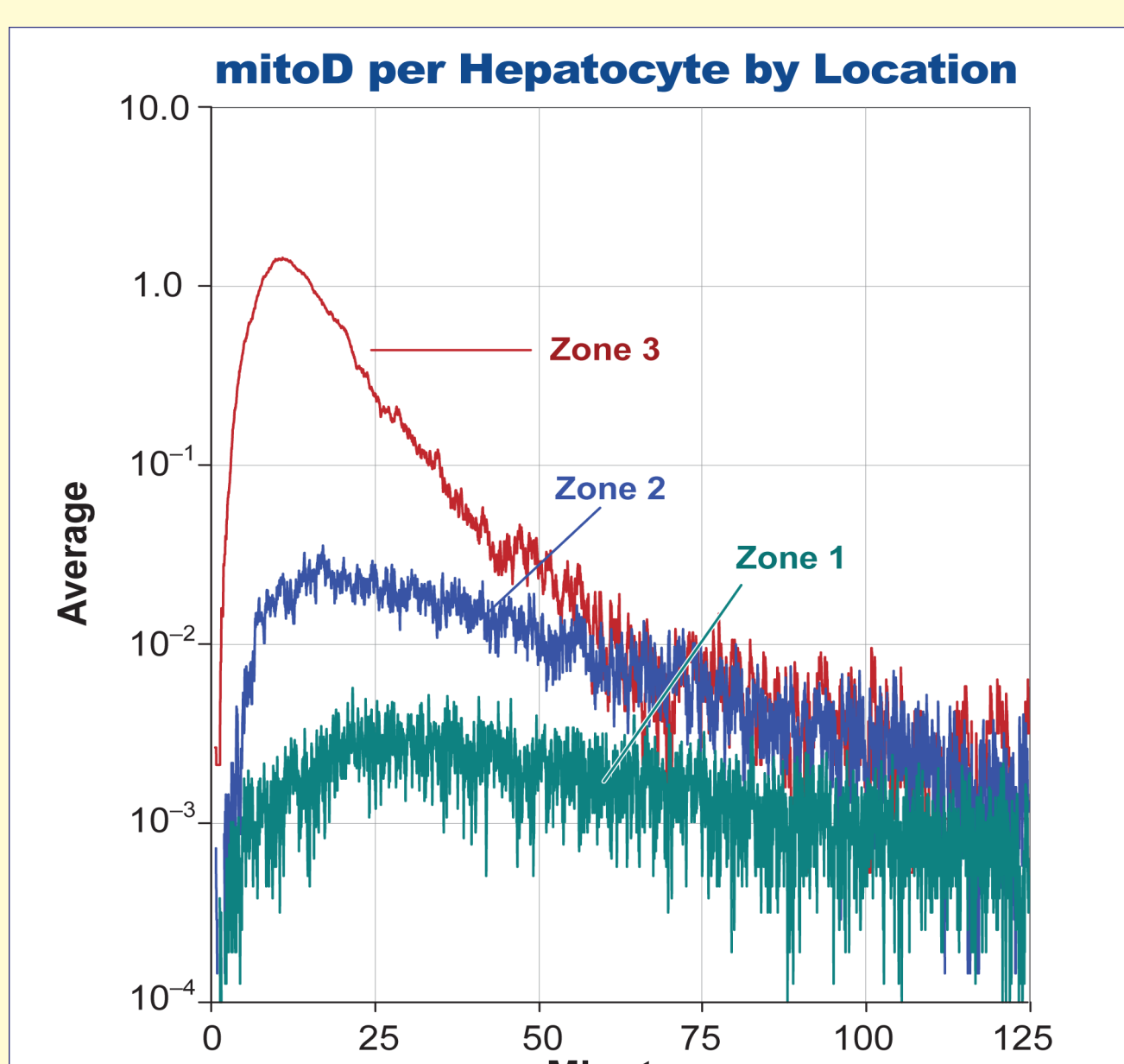
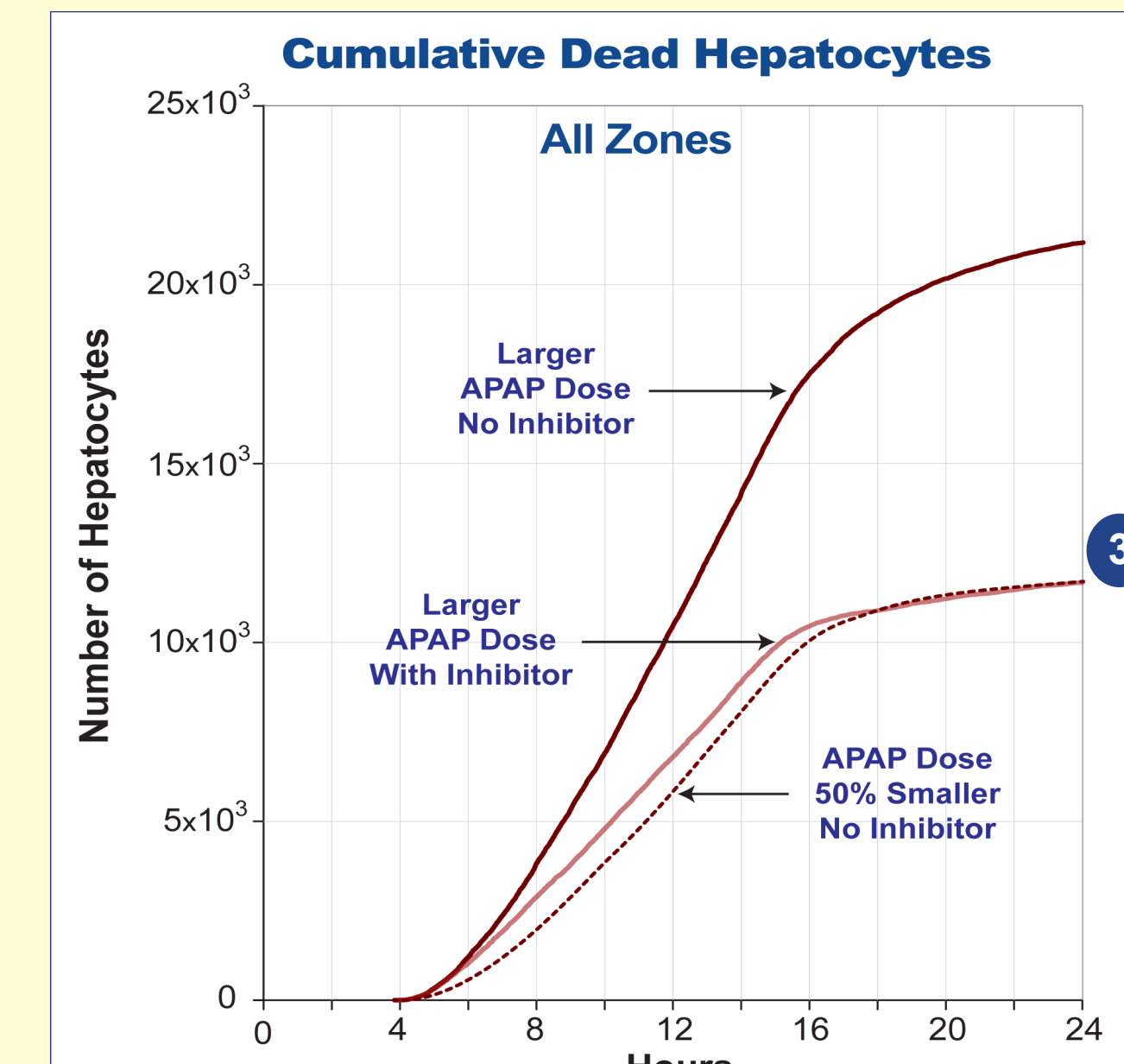
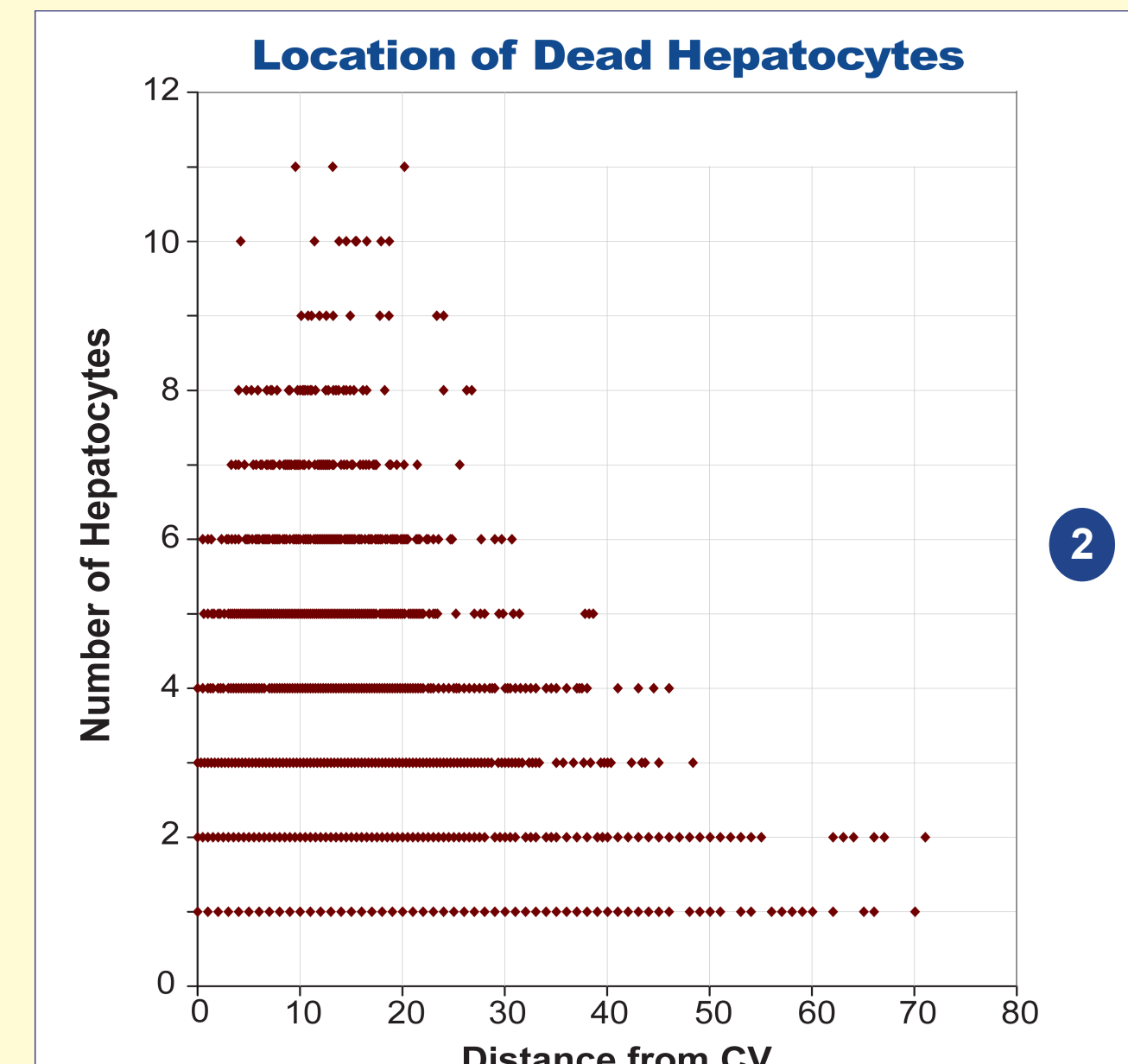
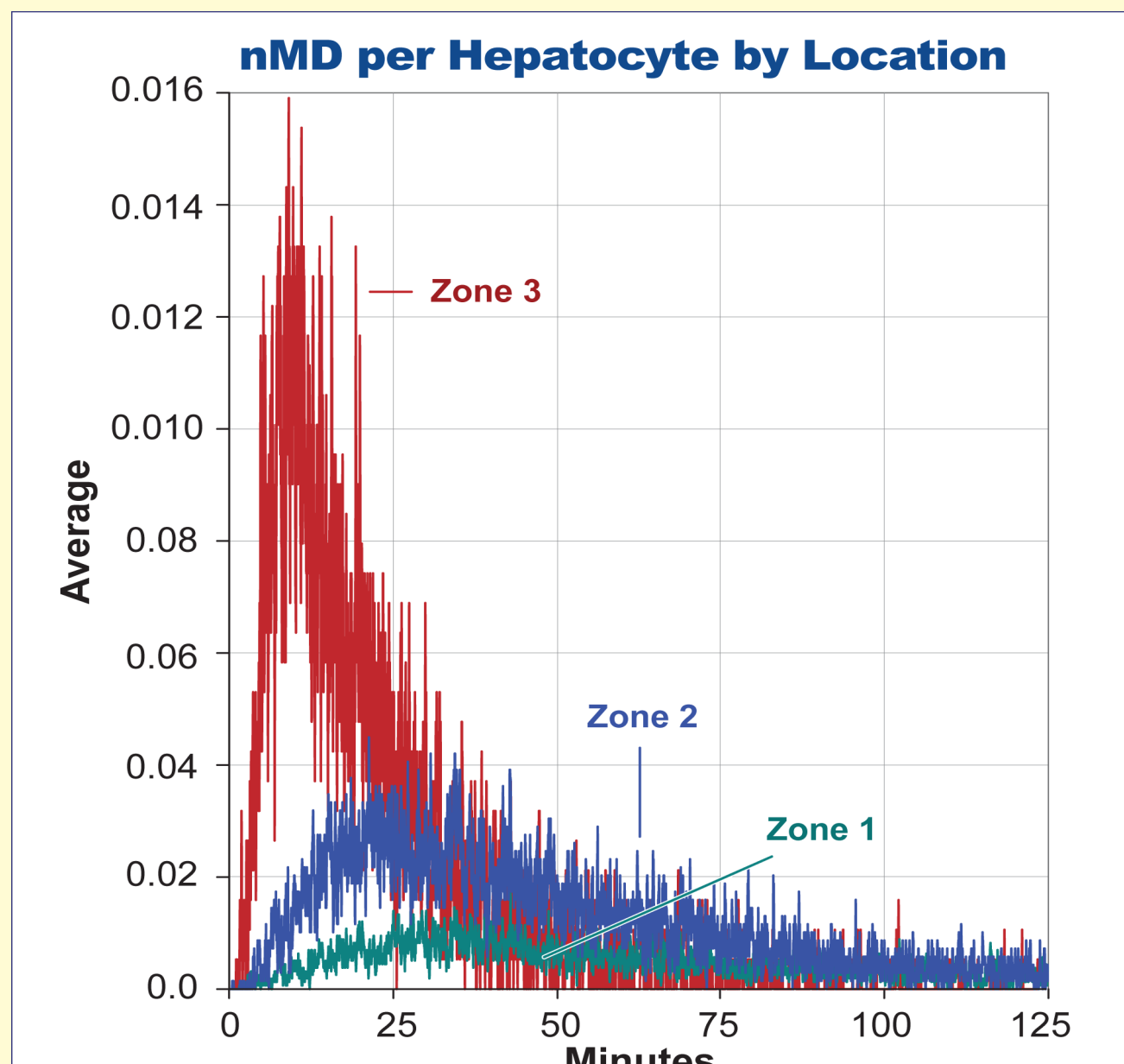
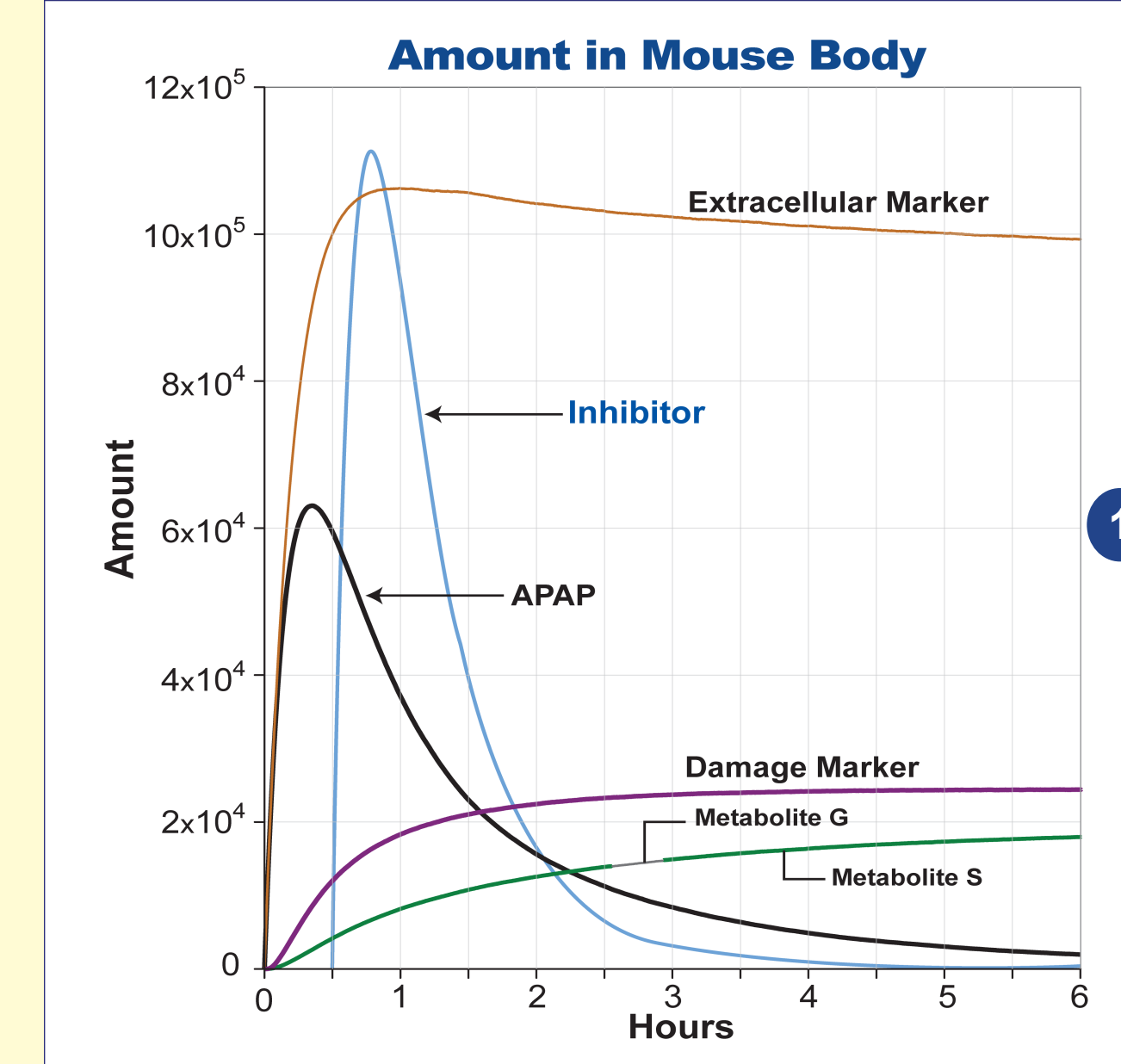
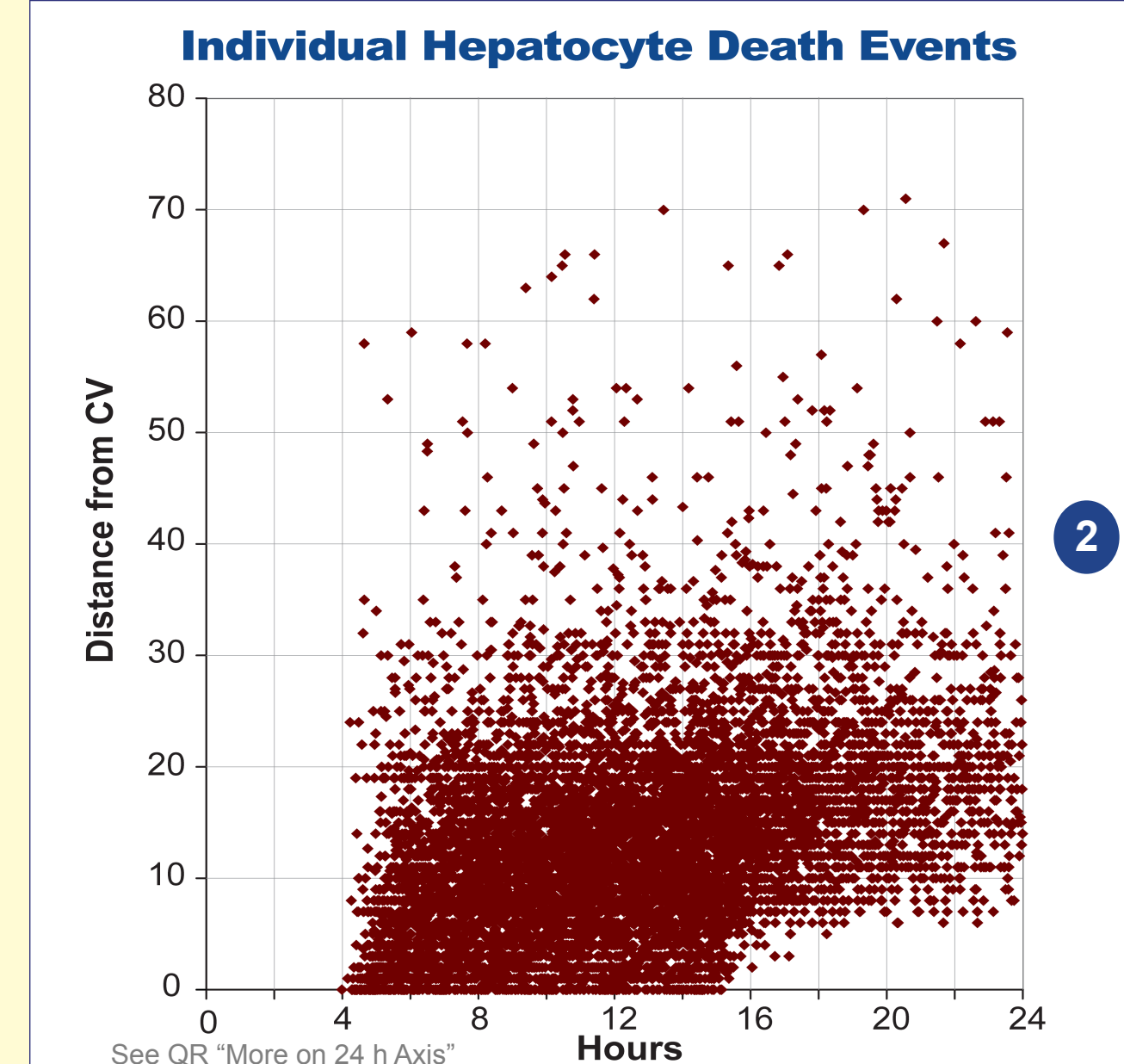
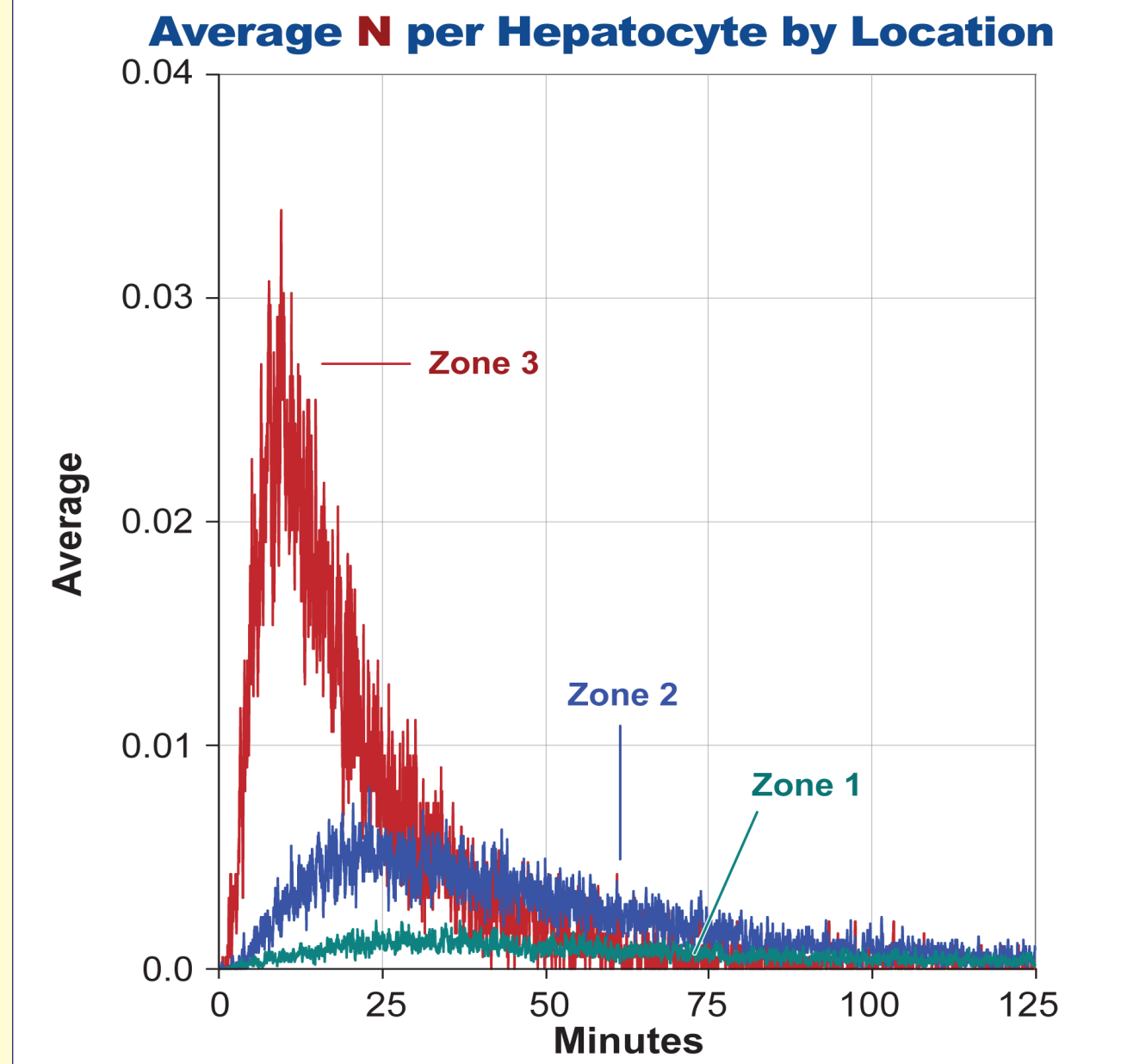
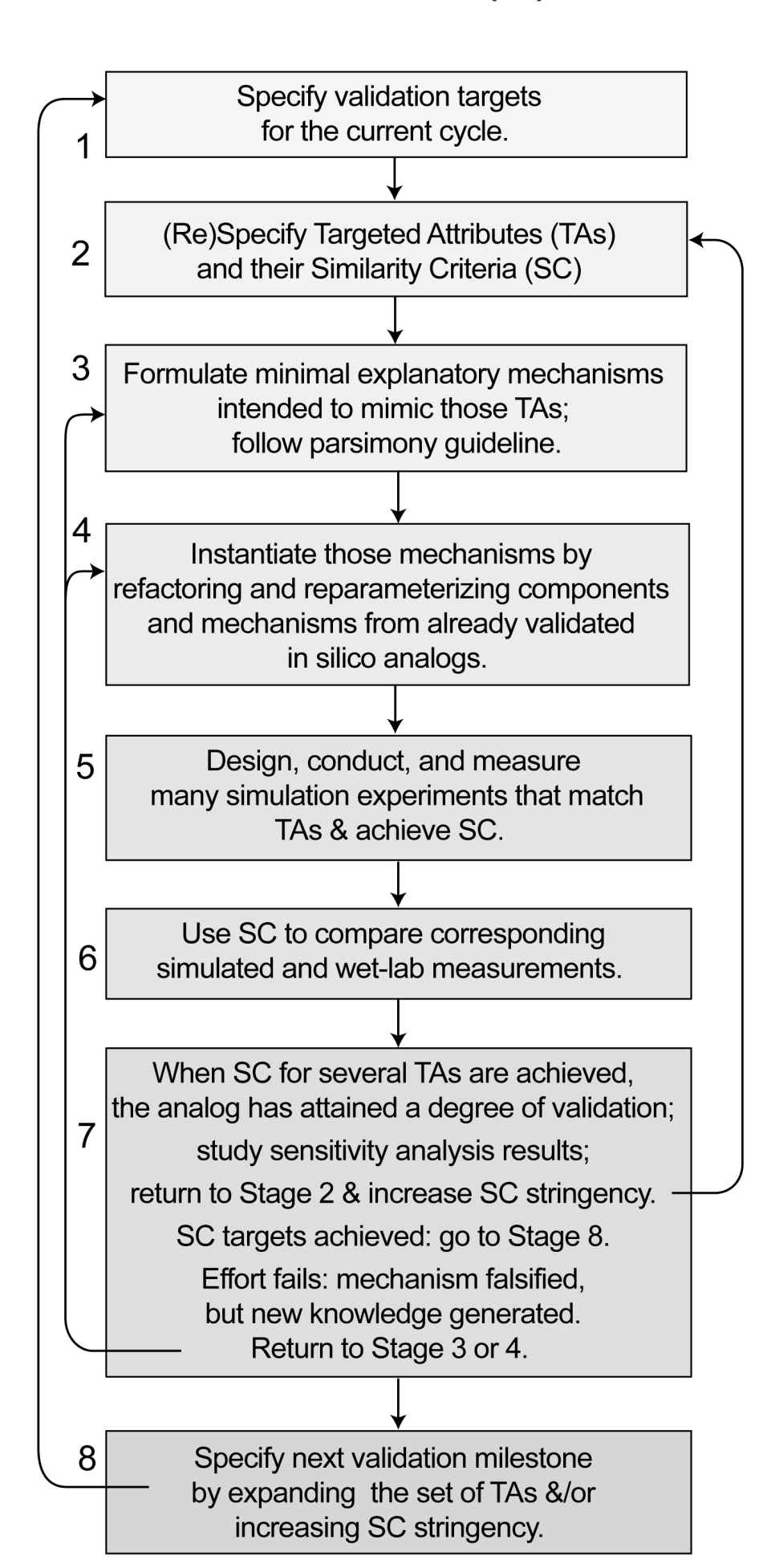
Within each Hepatocyte, each simulation cycle, each of the following mechanisms may occur



In Silico Liver – Key Features



Iterative Refinement (IR) Protocol



Validation Targets Already Achieved

- APAP pharmacokinetics, hepatic clearance and metabolite ratios. See QR "More on In Silico Liver"
- Single pass hepatic disposition profiles for eight compounds
- APAP PK measurements in mice following IP dosing
- Lobule zonation of metabolism (responsible enzymes exhibit different PV-to-CV patterns)
- NAPQI production increases PV-to-CV

Approach

Challenge plausible explanatory mechanistic hypotheses by experimenting on agent oriented In Silico Liver (ISL) analogs. See QR "More on Agents" and also QR "More about Analogs"

ISL mechanisms are concrete and biomimetic.

Mechanisms: comprised of nested modular spaces, modules, and components.

Prespecify and achieve key validation targets
A targeted attribute (TA) is phenomenon to be explained.
Validation targets are achieved when measurements of simulated and wet-lab phenomenon are quantitatively similar according to a **prespecified** similarity criteria (SC). For more, see QR "More on Qual/Quant Validation".

Achieve each validation target by cycling through the Iterative Refinement Protocol (IR Protocol).

Hypotheses:
Within each hepatocyte, there is a critical level in the accumulation of some macromolecular damage products: a **tipping point** . When reached, necrosis is triggered. The simplest tipping point scenario requires rapid accumulation of considerably more Damage Product in Zone 3.

Inhibitor reduces total cell deaths.



Methods

Agent-based & Agent Oriented Analogs

We started with an ISL that had already achieved several quantitative validation targets.

Core Method: the Iterative Refinement Protocol

When making mechanistic granularity decisions, we adhered tightly to a strong parsimony guideline.

Simulating inhibition required:

1. A separate set of INH objects that would percolate simultaneously with APAP objects through and interact with analog components
2. Giving analog components the ability to distinguish between APAP and INH yet avoid interaction; and
3. Achieving estimates of INH PK properties following IP dosing

If INH criterion is met, cancel Death event

All experiments 1 "APAP" = 8.33×10^{-4} dose infusion dose; 120,000 "APAP" object
One experiment: 24 Monte Carlo lobules. See QR Monte Carlo

Results

Parameter Values without INH

A. These three location-dependent parameter values enabled achieving multiple quantitative validation targets.

B. Because GSH decreases PV-CV, we specified that the GSH depletion threshold should do the same. $p(N \rightarrow \text{mitoD})$ was set arbitrarily at 0.5 and was not changed thereafter.

C. These parameterizations were arrived at following several IR Protocol mechanism falsifications. See QR "More on ISL" for details.

Map of APAP Mouse profile to APAP blood levels following an IP APAP dose achieved quantitative SC.

Key qualitative validation target achieved: more dose dependent Hepatocyte Death occurs first adjacent to CV. Thereafter, more Death occurs further from CV.

Following INH dosing, Hepatocyte Death was reduced. The reduction was comparable to that observed when APAP dose was reduced by 50%.

MSM Take Home Points

Using in silico experimentation *alone*, we:

- improved explanatory mechanistic insight by testing and falsifying many mechanistic hypotheses. To do so, it proved essential to adhere to the IR Protocol.
- discovered a plausible analog mechanism that explains how key multiscale features of hepatotoxicity may be generated and inhibited in mice. The mechanism is a concrete, coarse grain hypothesis. It is plausible because multiple validation targets were achieved.
- generated new knowledge by *falsifying* several dozen analog mechanisms that seemed possible initially because they achieved early validation targets.

The methods used make it straightforward to iteratively reuse and refine components to establish new, more explanatory mechanisms that achieve new validation targets.