A Novel Model for the Cytotoxicity of Insoluble Metallic Nanoparticles

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Background

- Metallic engineered nanomaterial particles (MENAP) are used in many applications in industry.
- Nanomaetrial particles have a dimension less than 100 nm (0.1 μm).
- Insoluble (or relatively insoluble) MENAP can be oxides of iron, gold, zinc, silver, copper, and other metals.
- The particles could be inhaled, ingested, or enter the body via uptake through the skin (intact or wounded).
- MENAP exposure occurs in the workplace, environment, and in the home.

Background (continued)

- Because of their small size, MENAP have distinct properties compared with the bulk form of the same metals.
- Inhaled MENAP are efficiently deposited by diffusion-related mechanisms throughout the respiratory tract.
- Their small size facilitates uptake into cells and *transcytosis* across epithelial and endothelial cells into the blood and lymph circulation, potentially reaching the bone marrow, lymph nodes, spleen, heart, nervous system components and other sites.

Background (continued)

- Cellular and orgnelle uptake of MENAP is stochastic.
- Biological effects (e.g., cell killing, mutations, neoplastic transformation, cancer) based on organelle uptake are also stochastic, implicating the need for doubly-stochastic models.
- Serious health effects could arise from MENAP cytoxicity to critical cells such as neurons after prolonged exposure.
- This presentation introduces the stochastic microdose (STM) model for the cytotoxicity of insoluble MENAP.

Inhaled MENAP depositing in the nasal and tracheo-bronchial regions of the respiratory tract (Oberdörster 2005):

- Deposition efficiency depends on size.
- Can be taken up and translocated by sensory nerves.
- Can cross synapses, and may reach different CNS sites.
- Can localized in subcellular structures (e.g., mitchondria)
- Can induce inflammatory/oxidative stress and other responses.

Toxicity of MENAP

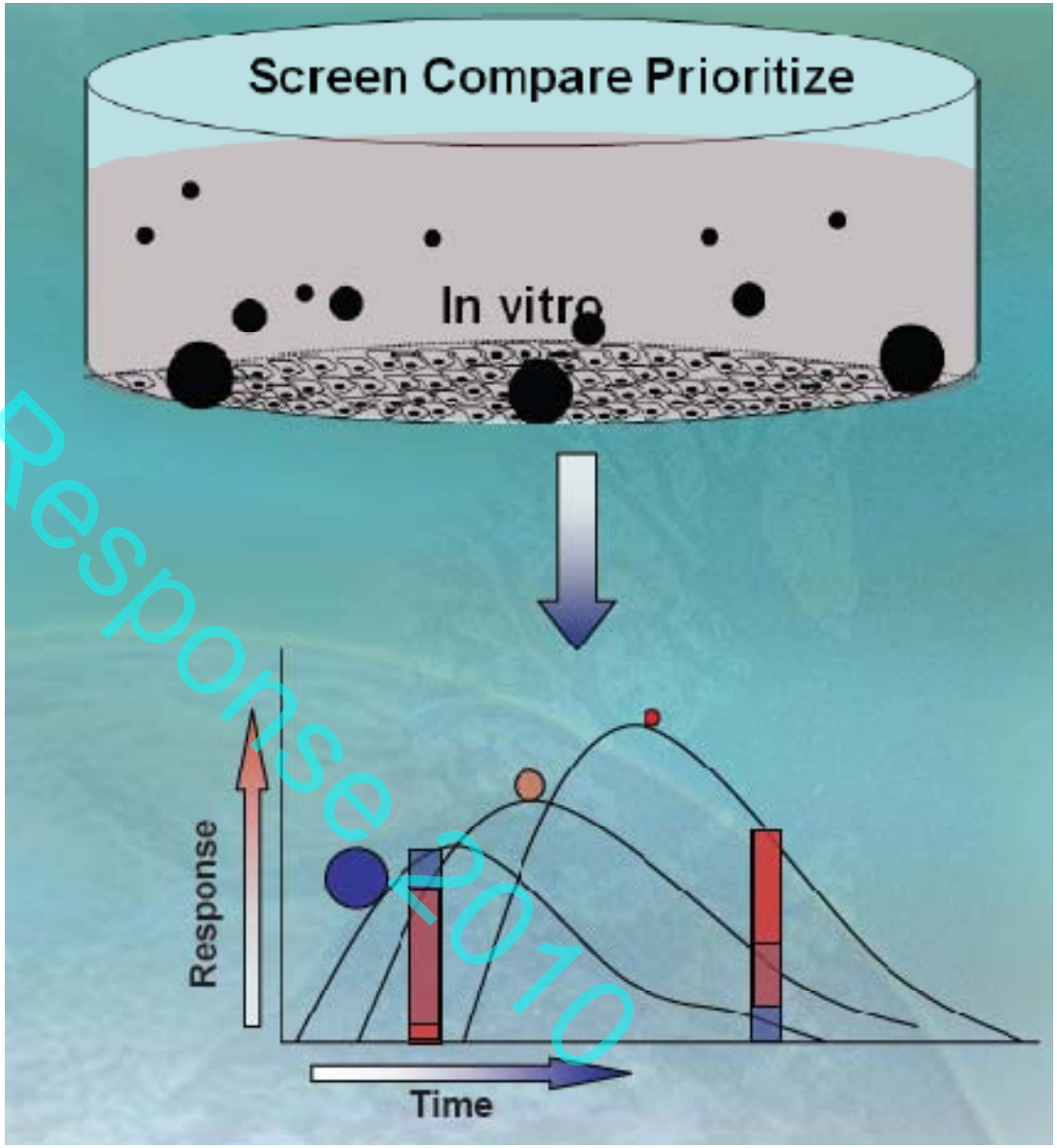
- Toxicity depends on physical and chemical characteristics of the particles, mode of intake, and their interaction with biological media (e.g., protein coronas).
- *In vitro* cytotoxicity studies are useful for comparing toxicity of different MENAP.
- *In vitro* cytotoxicity studies are also useful for screening and prioritizing needed *in vivo* research.

MENAP particokinetics *in vitro* (Teeguarden 2005)

Particle settling rate influences uptake by cells and timing of biological effects.

Particle settling depends on shape, density, etc.

Cell dose is not the same as media dose.



Modeling Approach

- The STM model (Scott 2010a,b) is used to characterize the *in vitro* cytotoxicity of copper nanoparticles.
- Hypothetical particokinetics employed for *in vitro* simulations.

STM Model Microdose Metrics

- Specific mitochondrion burden $B_m(t)$: organelle-specific MENAP count at exposure time t.
- Specific nuclear burden $B_n(t)$: nucleus-specific MENAP count.
- Mitochondria burden $M_m(t)$: expectation value for $B_m(t)$.
- Nuclear burden $M_n(t)$: expectation value for $B_n(t)$.

STM-Model-Related Assumptions: *In Vitro* Cytotoxicity Studies (Scott 2010a,b)

- Specific organelle burdens have exposure-time-dependent Poisson distributions.
- Cell killing by MENAP arises because of nuclear or mitochondrial damage.
- Lethal mitochondrial damage triggers the autophagic mode of cell death.
- Lethal nuclear damage triggers the apoptotic mode of cell death.
- The autophagic an apoptotic modes of death are independent competing risks.

Programmed Cell Death

- Apoptosis (type-I programmed cell death) is characterized by condensation of the cytoplasm and preservation of organelles, essentially without autophagic degradation.
- Autophagic cell death (type-II programmed cell death) exhibits extensive autophagic degradation of Golgi apparatus, polyribosomes and endoplasmic reticulum, which precedes nuclear destruction.

STM Model Related Assumptions (continued)

- Leathal damage via the autophagic mode is triggered when $B_m(t)$ exceeds a stochastic threshold N_m.
- Leathal damage via the apoptotic mode is triggered $B_n(t)$ exceeds a stochastic threshold N_n.
- $B_m(t)$, $B_n(t)$, N_m and N_n are assumed to have Poisson distributions.

Expectation Values for N_m and N_n

- The stochastic threshold N_m has expectation value μ_m (applies to Poisson distribution).
- The stochastic threshold N_n has expectation value μ_n (applies to Poisson distribution).
- When a Poisson distribution does not apply, the corresponding expectation values are μ_m^* and μ_n^* respectively (Scott 2010a).

MENAP Potency and Relative Potency for Cytotoxicity

Potency for autophagic mode of cell death = $1/\mu_m$, when N_m has Poisson distribution.

Potency for apoptotic mode of cell death = $1/\mu_n$, when N_n has Poisson distribution.

Relative potency = ratio of potencies for test particles to the potency for reference particles and is mode-of-death-specific (Scott 2010a).

Survival fraction (*SF*) for single replicate comprised of many cells for homogeneous population:

$$SF = \mathcal{P}(\text{Round}\{\mu_m\}|M_m(t)) \bullet \mathcal{P}(\text{Round}\{\mu_n\}|M_n(t))$$

$\mathcal{P}()$ is cumulative Poission probability given mean $M_j(t)$ and Round$\{\mu_j\}$ hits (particle uptakes).

Survival fraction averaged over multiple replicates (large number):

$E\{SF\} = \mathcal{P}(\text{Round}\{\mu_m\}|E\{M_m(t)\}) \bullet \mathcal{P}(\text{Round}\{\mu_n\}|E\{M_n(t)\})$

Experimental data for $E\{SF\}$ can be used to obtain estimates of μ_i and $E\{M_j(t)\}$ for fixed follow-up time t. $E\{M_j(t)\}$ estimates $M_j(t)$ for population.

Set $\mathcal{P}(\text{Round}\{\mu_n\}|E\{M_n(t)\})$ to 1 when adjusting for apoptotic mode.

Application of STM Model to Cell Killing *In Vitro* by Insoluble MENAP (Scott 2010a)

Hypothetical Example: MENAP are injected into cell culture media and associated particokinetics, model parameters, and measurement times are as follows:

$M_m(t)$ = 10(1-exp(-[ln(2)/20]*t);

M_n(t) = 4(1-exp(-[ln(2)/20]*t)

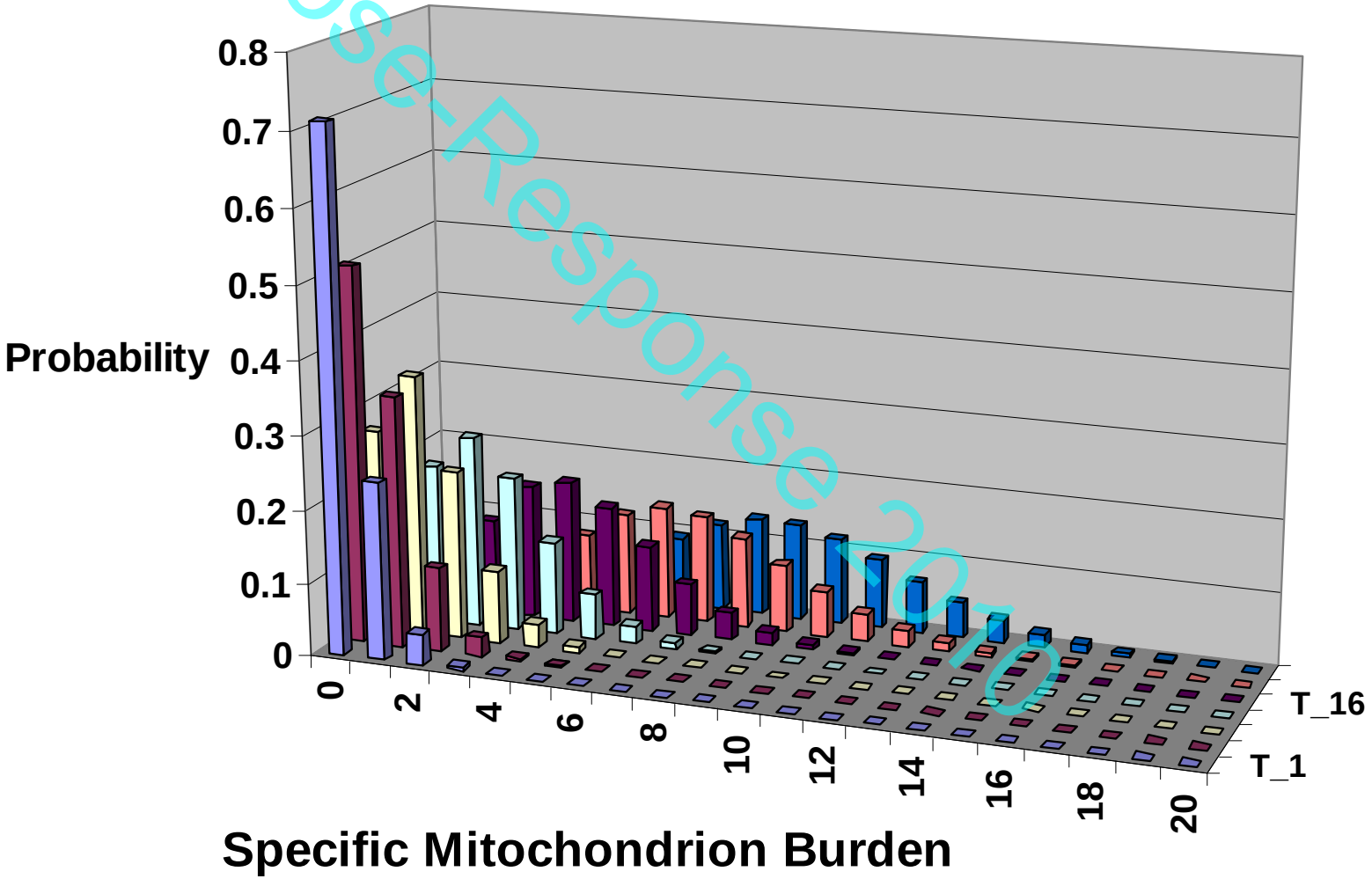
$T_{1/2}$: 20 minutes

N_m: Poisson distributed with mean μ_m = 5 MENAP.

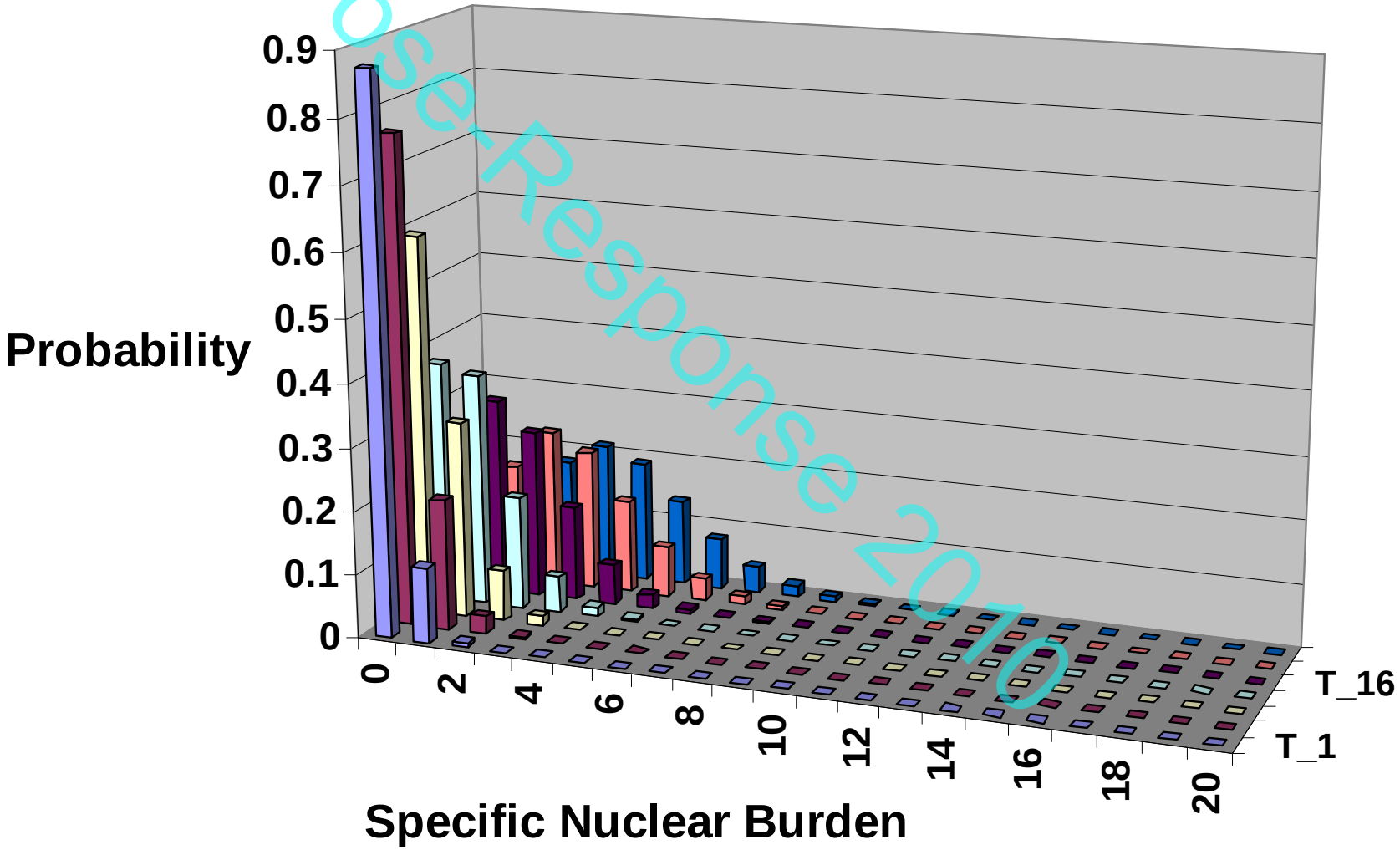
N_n: Poisson distributed with mean μ_m = 1 MENAP.

Follow-up times t: 1, 2, 4, 8, 16, 32, and 64 minutes.

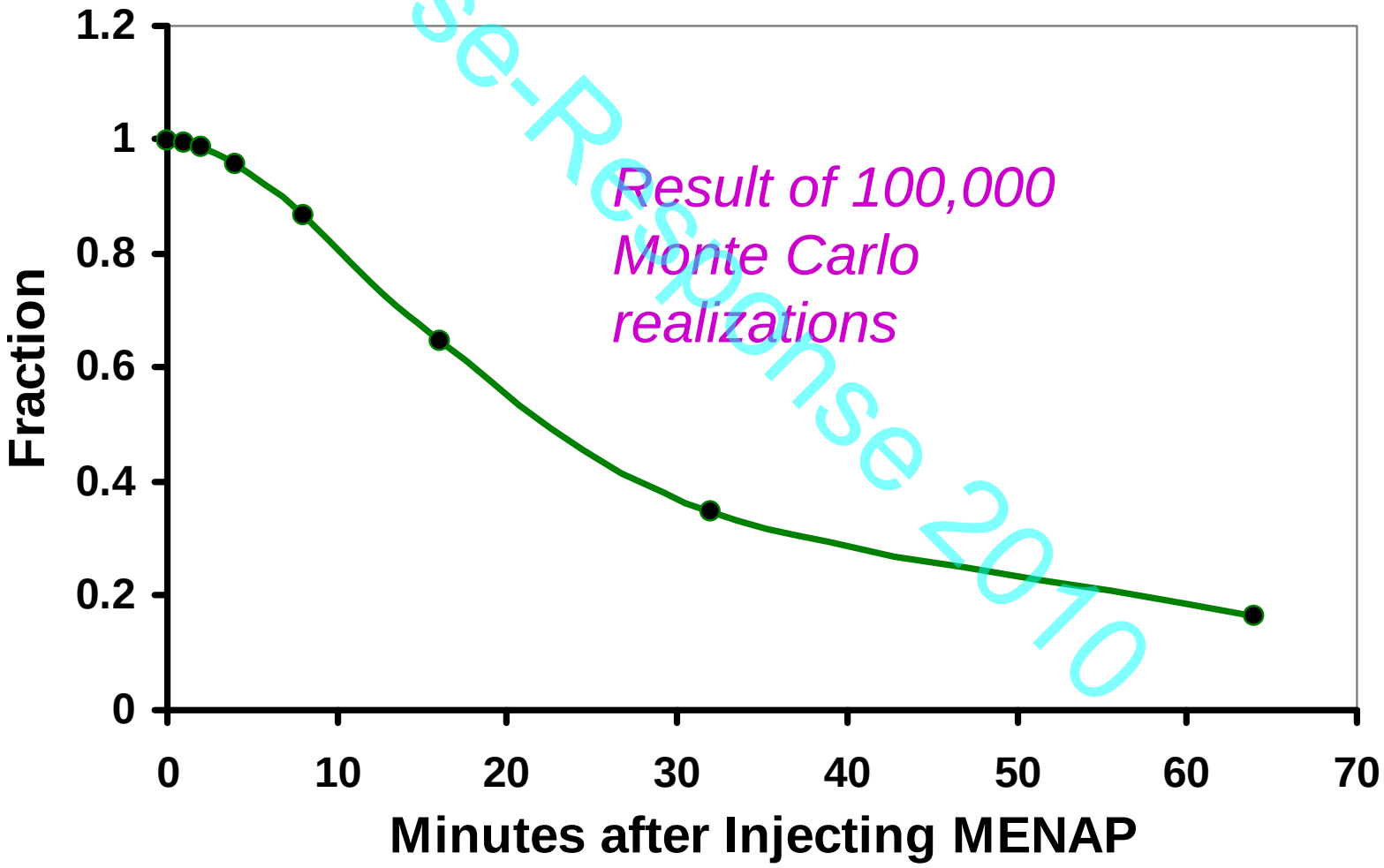
Simulated Poisson distributions for $B_m(t)$ at 1, 2, 4, 8, 16, 32, and 64 minutes after injecting insoluble MENAP



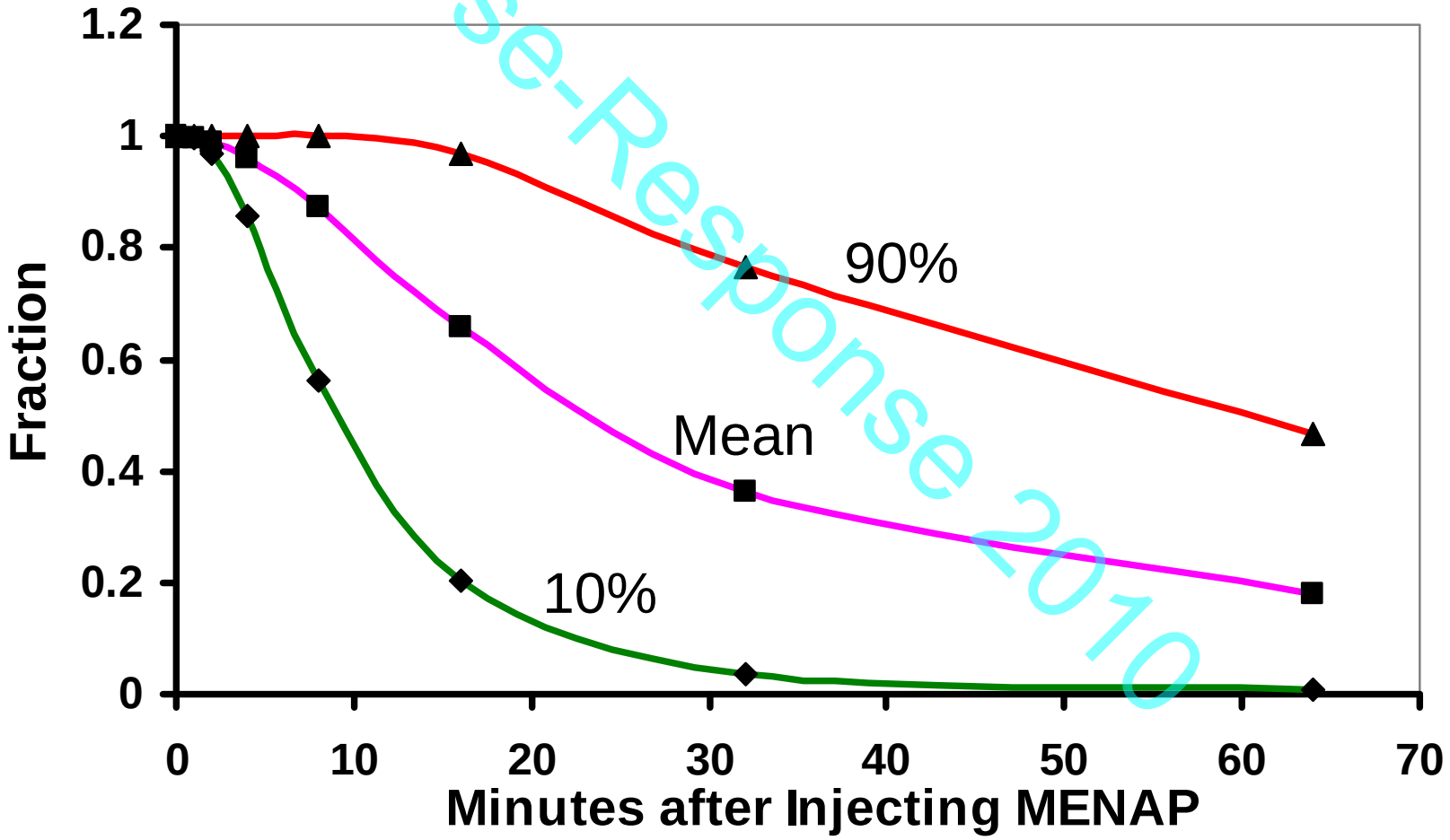
Simulated Poisson distributions for $B_n(t)$ at 1, 2, 4, 8, 16, 32, and 64 minutes after injecting insoluble MENAP



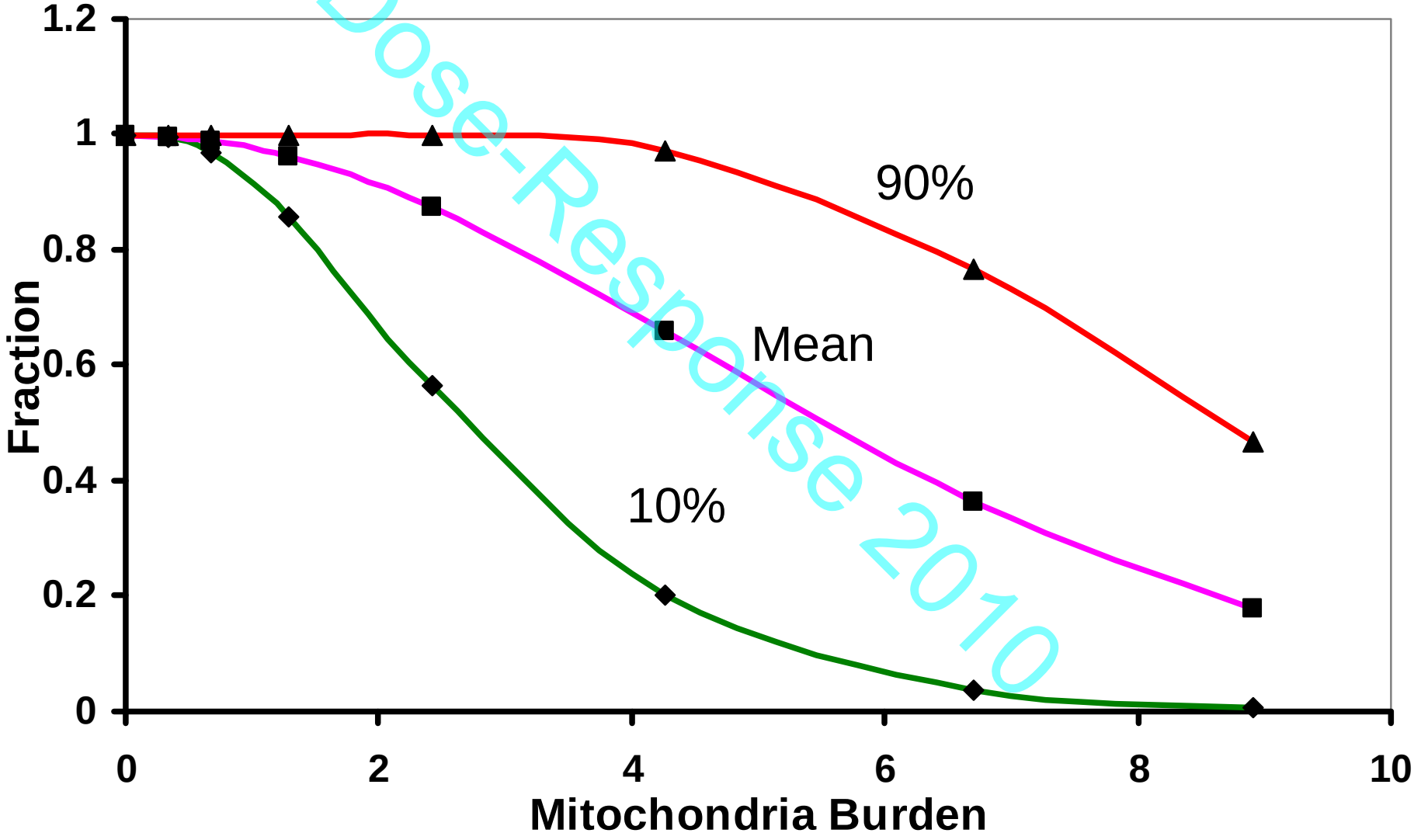
STM model simulated mean cell survival fraction *in vitro* when both apoptotic and autophagic modes of cell killing apply.



Monte Carlo results for mean survival fraction *in vitro*: Data adjusted to eliminate apoptotic mode of death



Autophatic-mode cell survival fraction plotted against the mitochondria burden



Application of STM Model to Published Data for Copper Nanoparticle Cytotoxicity *In Vitro* to Dorsal Root Ganglia (DRG) Neurons (Scott 2010b)

Single mode of cell death: autophagic

Source of the DRG Somatosensory Neuron Survival Data

Malathi Srivatsan (personal communications) based on publication:

Prabhu BM *et al.* 2009. Copper nanoparticles exert size and concentration dependent toxicity on somatosensory neurons of rat. Nanotoxicology 3(4):1-10.

DRG Somatosensory Neuron Data (Prabhu *et al.* 2009)

- Only data for 80 nm copper nanoparticles used.
- Cell viability was performed using MTS, a tetrazolium-dye-based assay.
- Viability was evaluated as a percentage of the control value.
- Copper nanoparticle considered to cause cell death via the autophagic model through damaging mitochondria.
- In applying STM model, the percentage of surviving cells was assumed to equal the reported percent viability.

Modeling Assumptions and Related Functions (Scott 2010b)

N_m (threshold) has a Poisson distribution with mean μ_m.

$M_m(t) = DCF_m * CT$ for concentration [C] time [T] product [CT].

C in micromolar

T in hours (24)

DCF_m, dose conversion factor (nanoparticles per micromolar-hour of exposure)

Bayesian inference methods employed via Markov chain Monte Carlo (MCMC) to fit STM model to real data.

Prior distributions assigned based on exploratory analysis of data.

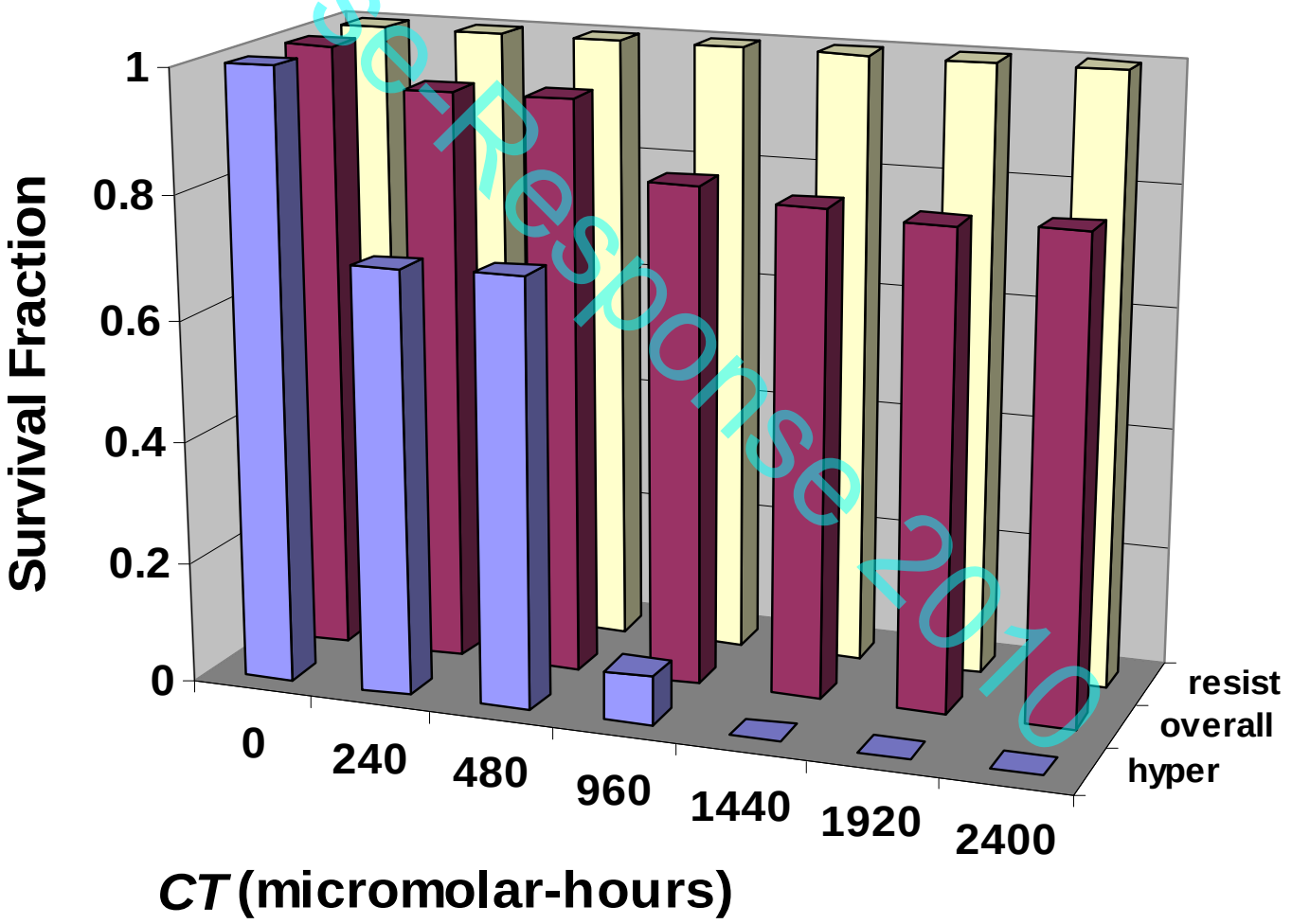
WinBUGS software used to carryout MCMC computations.

Exploratory analysis of data for survival of copper-nanoparticle-exposed DRG somatosensory neurons had a surprise:

The cell viability data suggested the presence of a hypersensitive subpopulation (fraction *f*) that were killed and resistant subpopulation that were not killed (about 80%) for the dose range studied.

The STM-model-related equations for homogeneous population were modified to allow for a mixed population with only the hypersensitive cells being killed.

Results of exploratory analysis of survival data for DRG neurons exposed to 80 nanometer copper nanoparticles *in vitro* (Scott 2010b).



Modified STM model equations for mixed population of cells and autophagic mode of cell death:

Averaging over cells in an individual replicate

$$SF = f \bullet \mathcal{P}(\text{Round}\{\mu_m\}|M_m(t)) + (1 - f)$$

$$M_m(t) = DCF_m \bullet CT$$

Averaging over multiple replicates

$$E\{SF\} = E\{f\} \bullet \mathcal{P}(\text{Round}\{\mu_m\}|E\{M_m(t)\}) + (1- E\{f\})$$

$$E\{M_m(t)\} = E\{DCF_m\} \bullet CT$$

Prior distributions used for parameters (Scott 2010b):

f, uniform from 0.0001 to 1 (0.2)

μ_m, uniform from 0 to 20 (1.0)

DCF_m, uniform from 0.001 to 0.01 (0.0043)

Values in purple are preliminary estimates based on exploratory analyses of the data.

MCMC iterations = 15,000 with 10,000 burn-in, single chain.

Posterior distribution means and standard deviations for $E\{f\}$, $E\{DCF\}$,and μ_m (Scott 2010b):

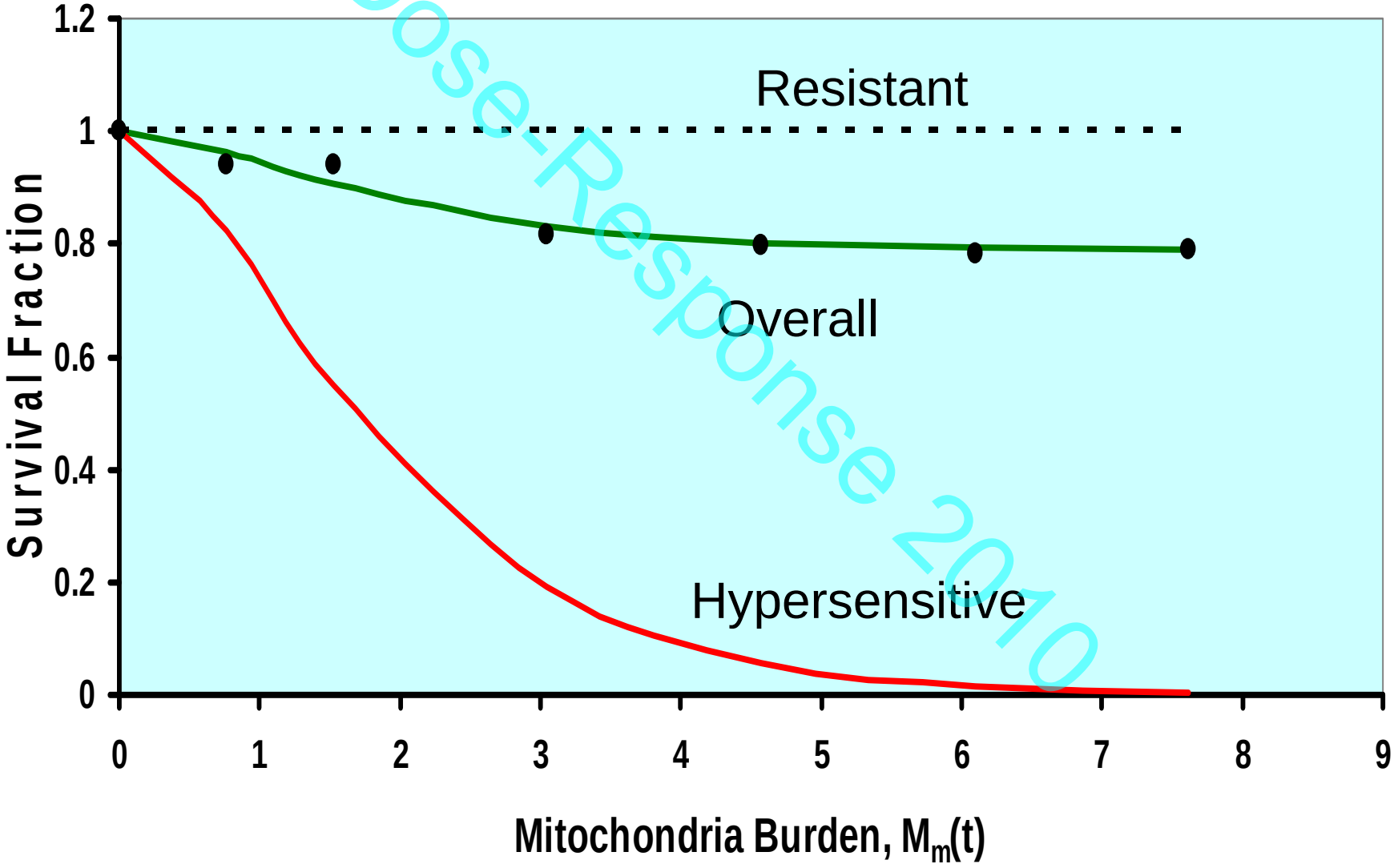
$E\{f\} = 0.215 \pm 0.013$

$E\{DCF_m\} = 0.0032 \pm 2.7E\text{-}04$

$\mu_m = 1 \pm 0.4$

MCMC error/parameter SD < 0.05

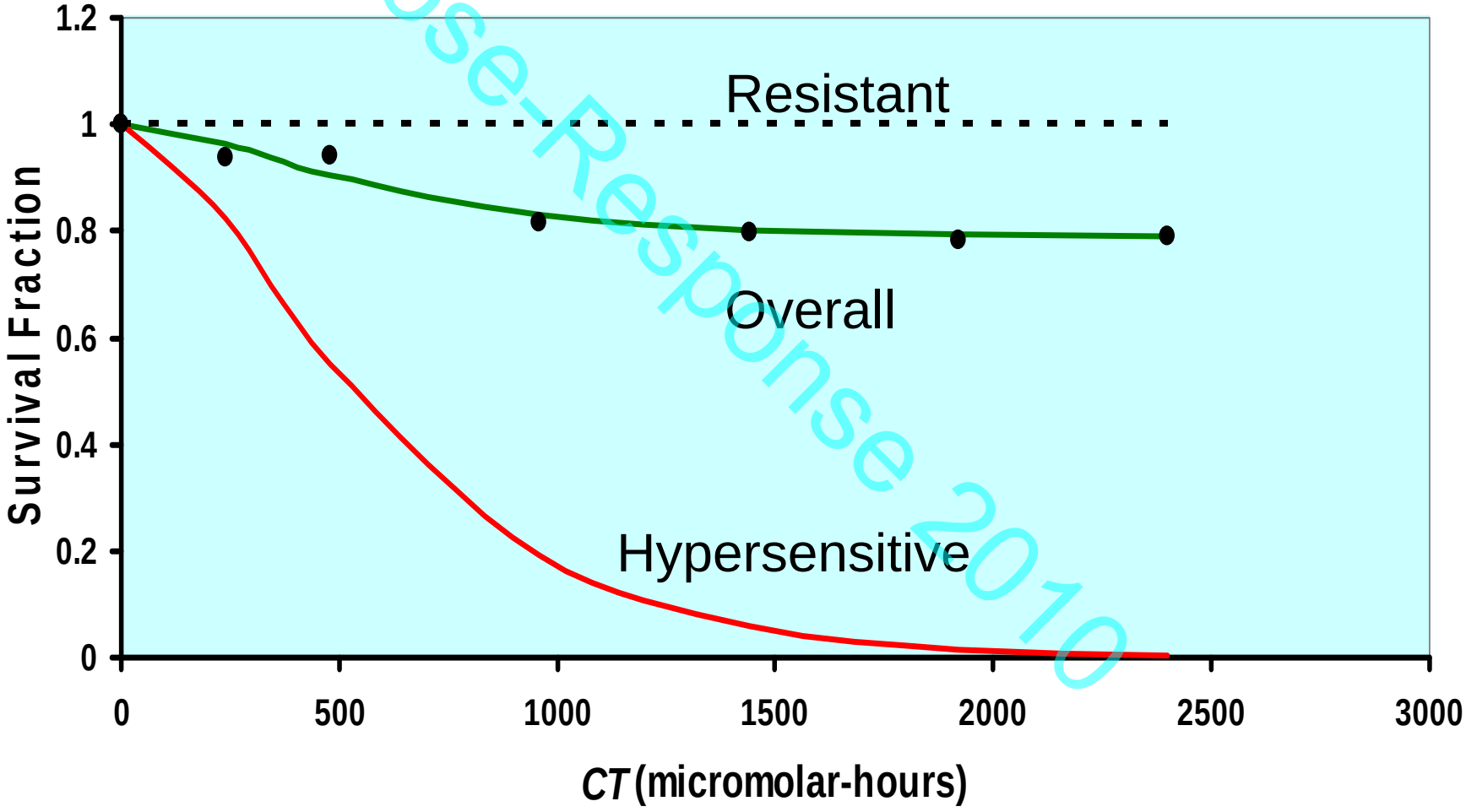
STM Model results: M_m(24h)=1.7 is expected to kill 50% of the hypersensitive neurons (Scott 2010b)



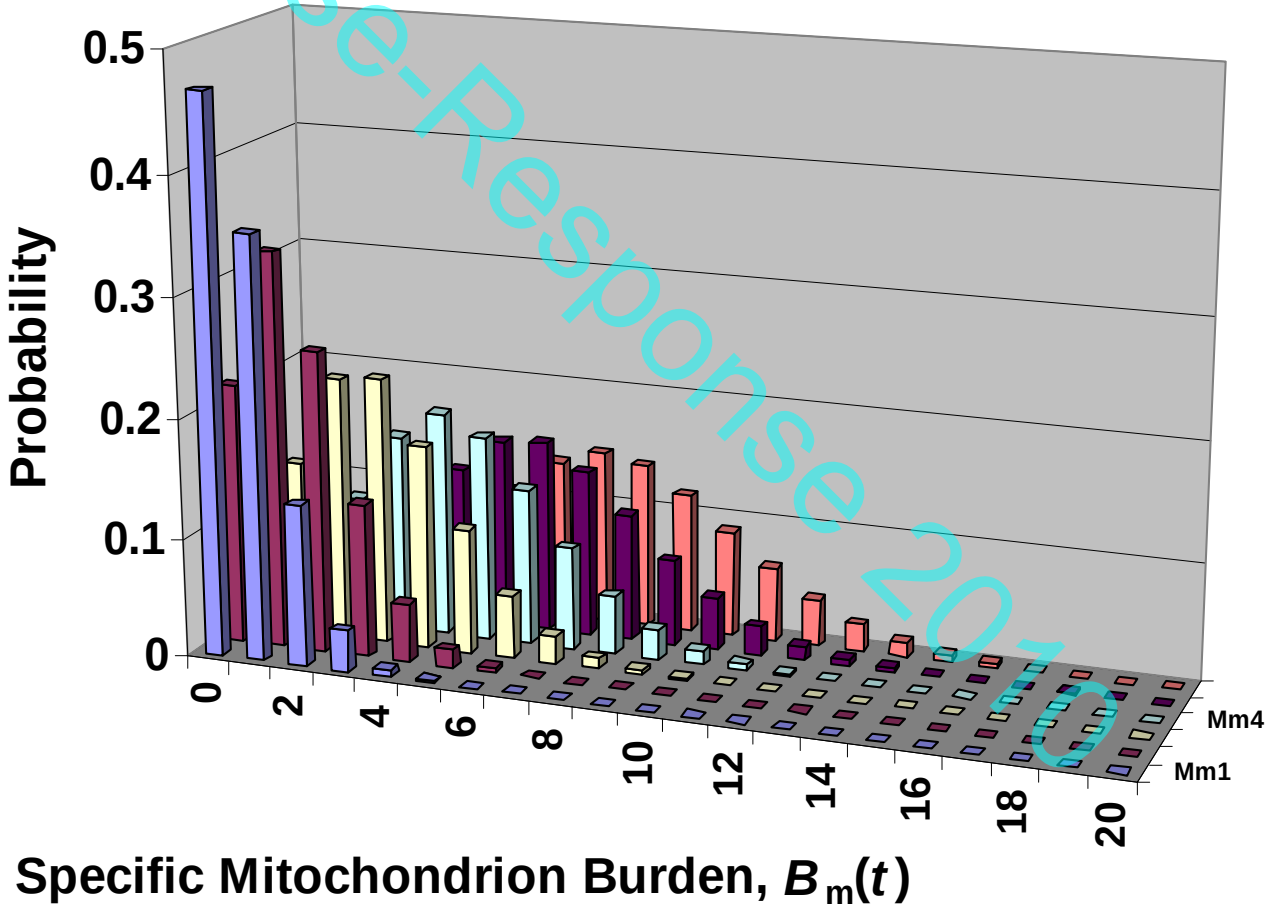
Comment

- For $M_m(t) = 1$, 63% of the mitochondria would be expected to contain at least 1 copper nanoparticle.
- For $M_m(t) = 2$, 86% of the mitochondria would be expected to contain at least 1 copper nanoparticle.

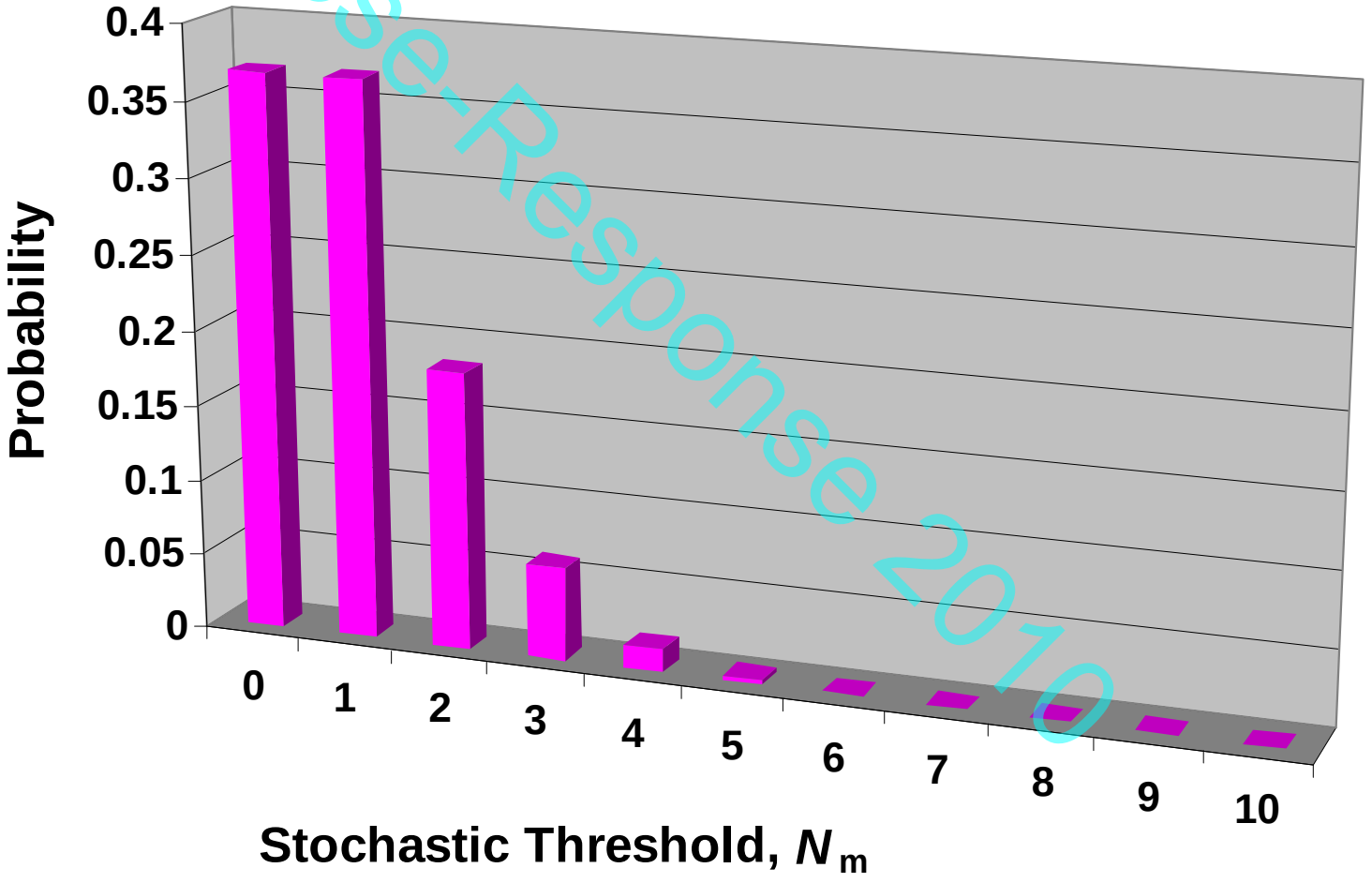
STM Model results: CT=530 μM-h would be expected to kill 50% of the hypersensitive neurons (Scott 2010b)



Biological microdosimetry: Central estimates of the distributions for $B_m(t)$ based on posterior distribution mean for DCF_m (2010b)



Central estimate of the stochastic threshold distribution of N_m based on posterior distribution mean for μ_m (Scott 2010b)



Conclusions

- The mixed population version of the STM model adequately characterized survival of DRG neurons exposed to 80 nm copper nanoparticles *in vitro*.
- The results obtained strongly suggest the presence of a hypersensitive subpopulation of neurons.
- If a hypersensitive subpopulation actually exists then new research is needed to investigate whether adverse neurological disorders could occur as a result of chronic exposure of humans to copper nanoparticles.

References

- Fissan H and Kuhlbusch T. 2nd International Symposium on Nanotechnology and Occupational Health. Minneapolis, Oct 3-6, 2005.
- Oberdörster G. 2nd International Symposium on Nanotechnology and Occupational Health. Minneapolis, Oct 3-6, 2005.
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- Scott BR. Dose-Response (2010a; in press)
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Acknowledgements

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Backups

Issue 1 Related to the STM Model Applications Presented

- Not certain that 80 nm copper nanoparticles are taken up by mitochondria; may cause lethal damage via interacting with mitochondrion membrane.
- Same modeling results would apply to mitrochondria membrane hits by multiple nanoparticles.

Issue 2 Related to the STM Model Application Presented

- Copper nanoparticles may not be insoluble.
- Similar results would apply if particle dissolution occurred after particle entry into the cytoplasm since the chemical dose to the mitochondria (or a different critical target) would be expected to be linearly related to the number of copper nanoparticles entering the cytoplasm.

Issue 3 Related to the STM Model Applications Presented

- Each cell and intracellular organelle is likely have a limited number of MENAP that can be taken up.
- This can be addressed in future research if nanoparticle uptake is the critical event for causing lethal damage.