



Alternative Approaches in Toxicology Research

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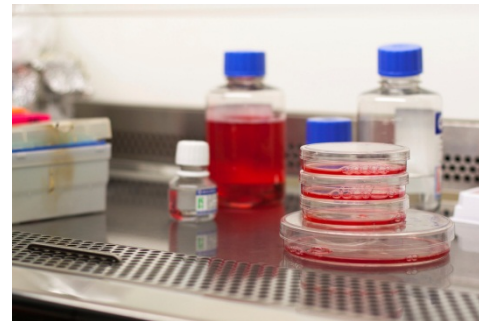
Views expressed in this presentation are those of the presenter and not necessarily those of the U.S. Food and Drug Administration

Outline

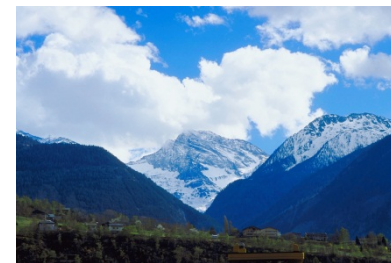
- State of the art



- Model systems



- Psychological challenges





FDA and Alternative Testing

“... There are still many areas where animal testing is necessary and non-animal testing is not yet a scientifically valid and available option. However, FDA has supported efforts to reduce animal testing. In addition, FDA has research and development efforts underway to reduce the need for animal testing and to work toward replacement of animal testing.”

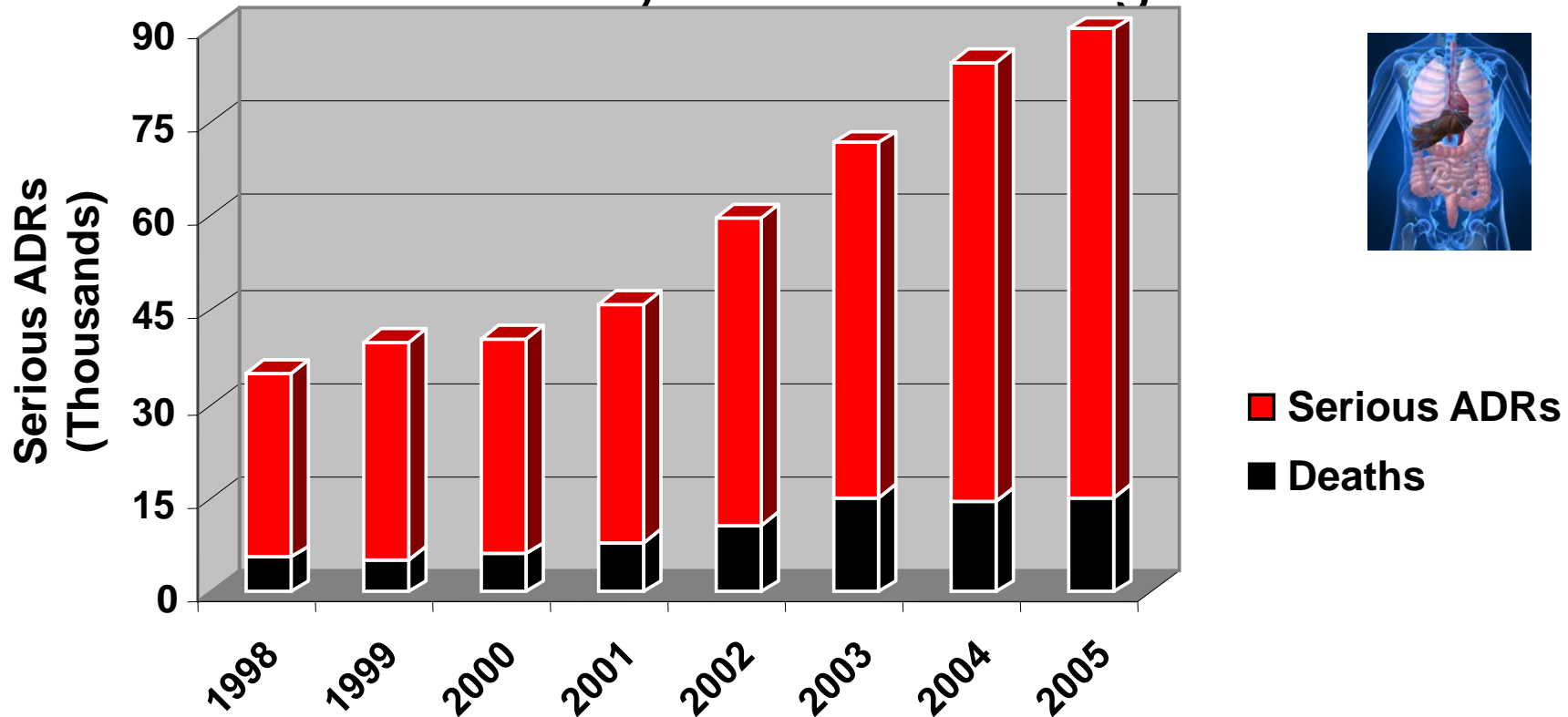
<http://www.fda.gov/AboutFDA/Transparency/Basics/ucm194932.htm>

Insufficient Predictivity of Current Methods

- <100% accuracy in predicting toxicity of even single compounds
 - Nonclinical test species identify many dangerous drugs/chemicals so are not tested in humans but...
 - Even when using multiple species of nonclinical animals, still miss ~30% of drug-induced adverse events seen in humans
 - Olson et al. Regul Toxicol Pharmacol. 32:56-67, 2000

- Individual patient susceptibilities (personalized medicine)
 - Relatively small numbers of humans (Phase I-III)

Serious Adverse Drug Reactions (ADRs) Caused by Marketed Drugs



Rate of ADRs and death growing faster than # of prescriptions

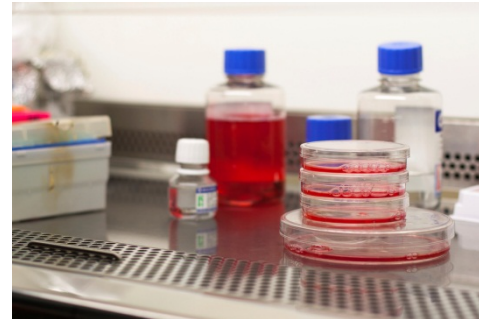
Moore et al., Arch Intern Med. 167:1752-1759, 2007

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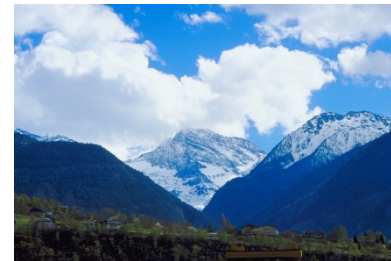
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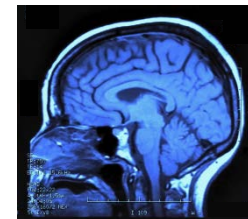
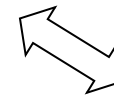
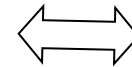
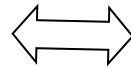
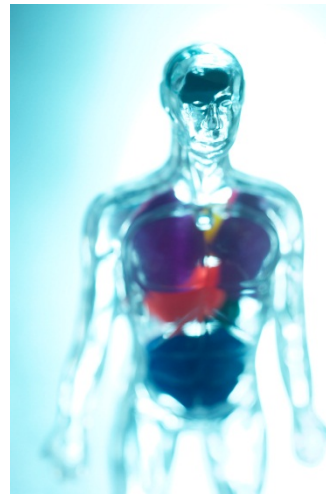
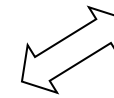
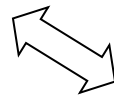
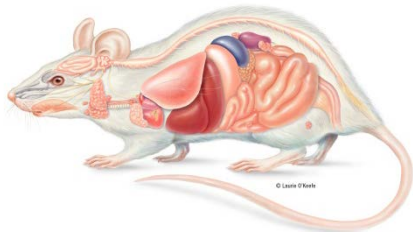
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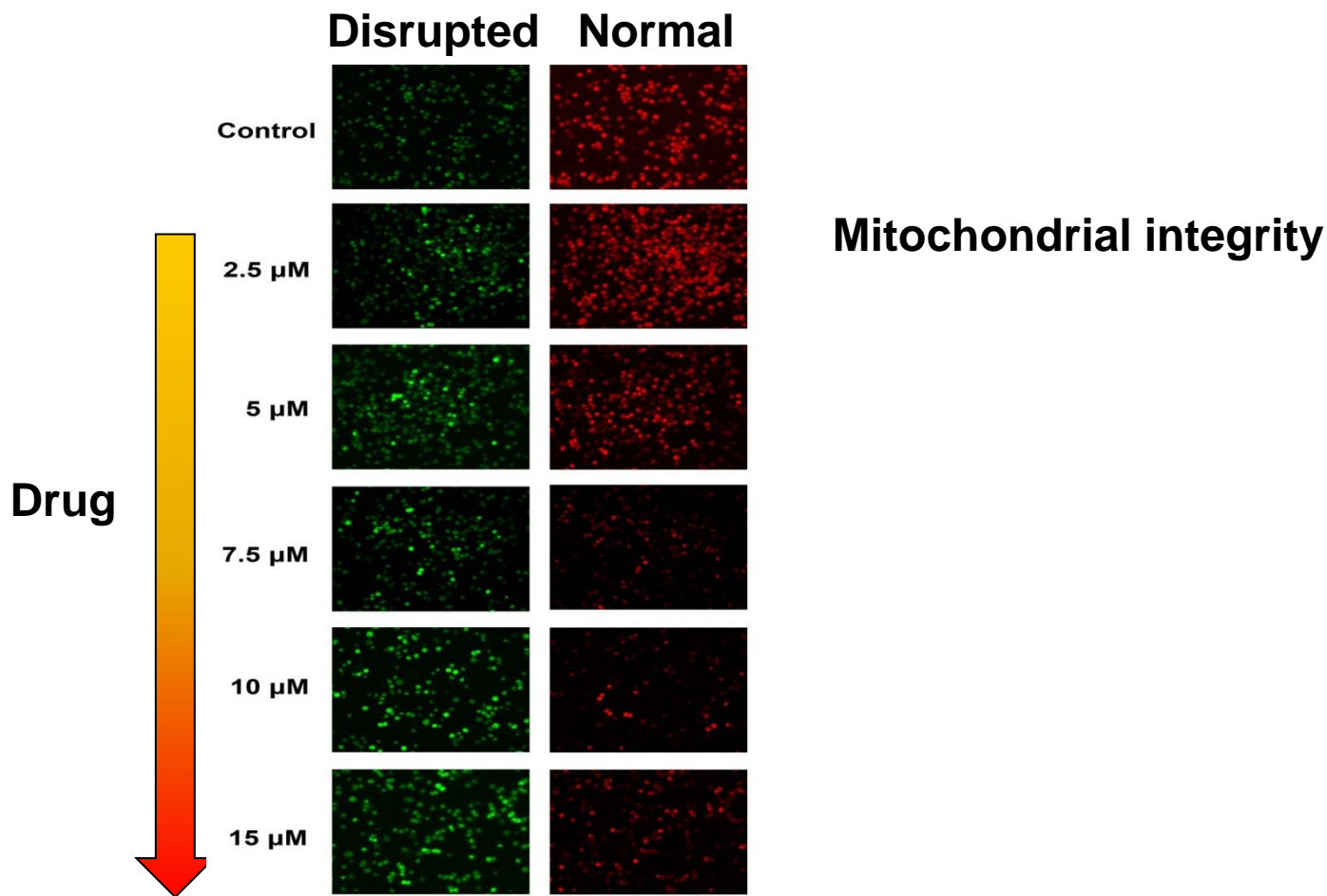
Translational Biomarkers



Organ Damage Induced by FDA-Regulated Products

- Areas of interest include
 - Liver
 - Heart
 - Lung
 - Brain
 - Developmental biology
 - Carcinogenicity
- Models systems include
 - Isolated mitochondria and microsomes, cell lines, stem cells (rodent and human), primary rodent and human cells, 3D cultures, human biofluids and tissues
 - Bio-imaging
 - Animal studies

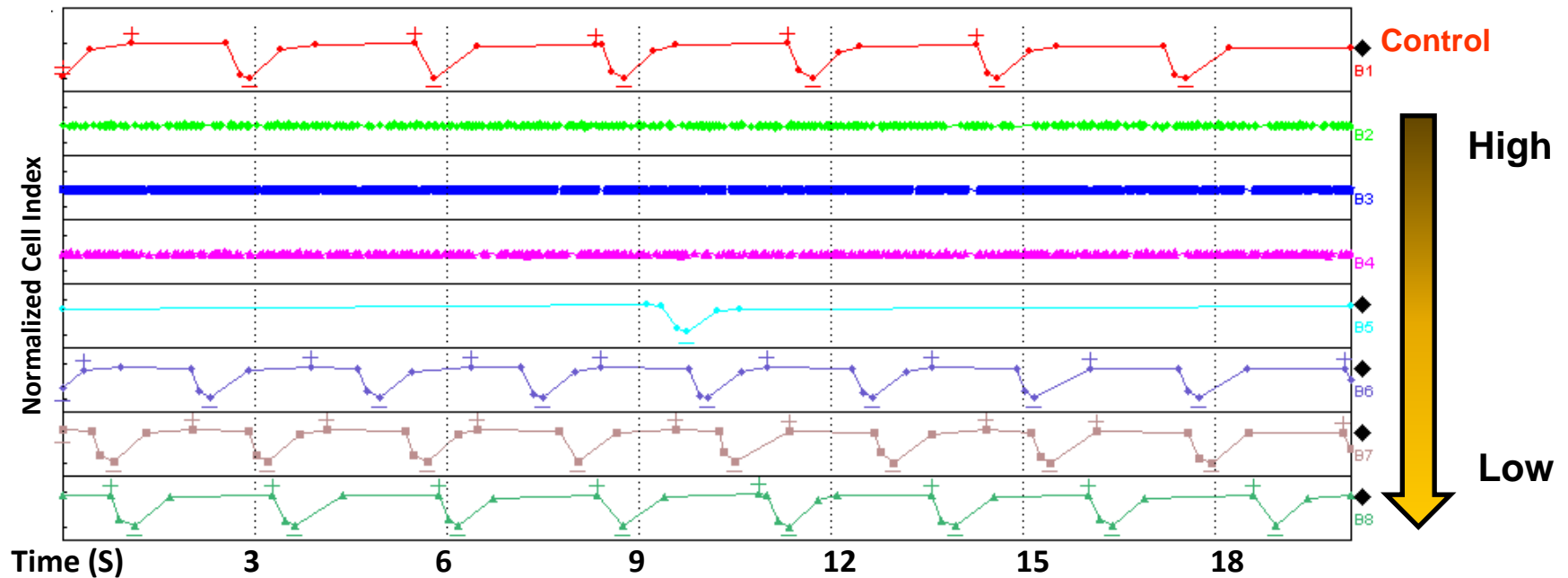
Primary Hepatocytes



Slide courtesy of Dr. Qiang Shi, FDA/NCTR

Real-Time Recording of iPS-Derived Human Cardiomyocytes

Viability and Contractility

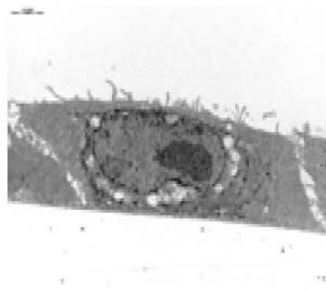


Differentiated Air Liquid Interface Human Airway Cultures

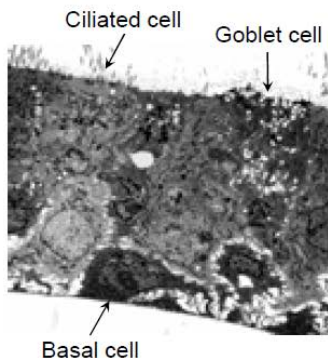


Normal human airway bronchial epithelium (tissue biopsy)

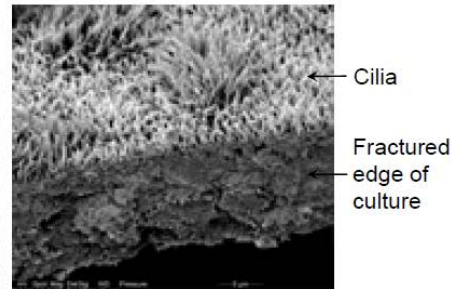
Day 1



Day 28



Day 28



Day 1- confluent monolayer

Day 28 –complex columnar epithelium with basal cells, goblet cells and ciliated cells



Developmental Toxicology

Examples: Stem cells and Zebrafish

ECVAM Validated mEST Assay

- Uses commercially available cell lines
- One endpoint is beating cardiomyocytes; not quantitative
- Cardiomyocyte is early marker of differentiation and may miss compounds that interfere with later development
- Expanding assay
 - To include new differentiation endpoints (osteoblasts) which are marker of later developmental stages
 - Expanding assay to include molecular markers

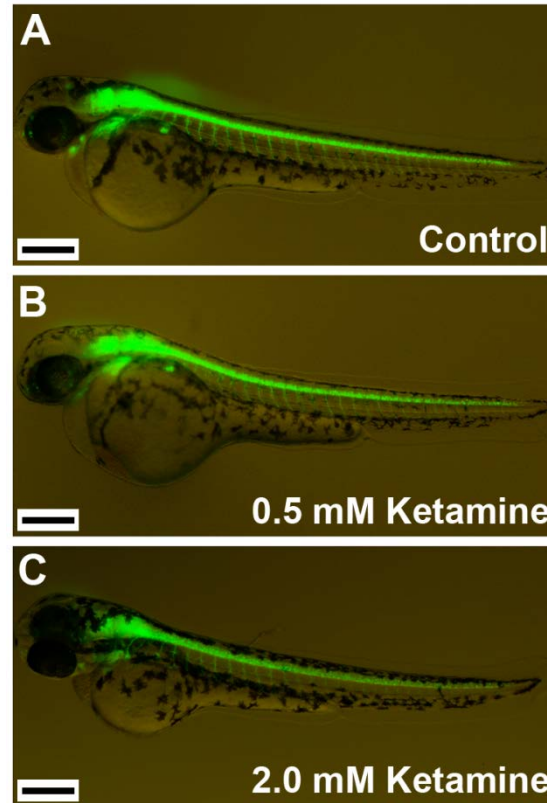


OECD Fish Embryo Acute Toxicity (FET) Test for Chemicals

- FET test is approved by the Organization for Economic Co-Operative Development (OECD) and uses zebrafish embryos
- Assessment of toxicity based on morphological (e. g., developmental arrest, formation of somite, etc.) and functional (presence/lack of heartbeat) endpoints
- Thorough controls and high fertilization/viability rates of the embryos and consistency of results are **REQUIRED** to ensure that observed toxic effects are in fact linked to the test compound

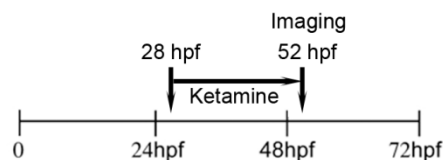
http://www.oecd-ilibrary.org/environment/test-no-236-fish-embryo-acute-toxicity-fet-test_9789264203709-en

Effect of Ketamine on Zebrafish Embryos

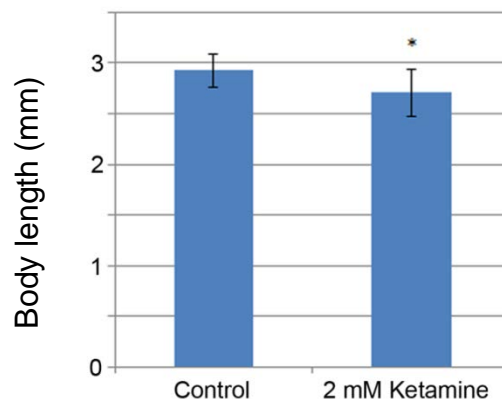
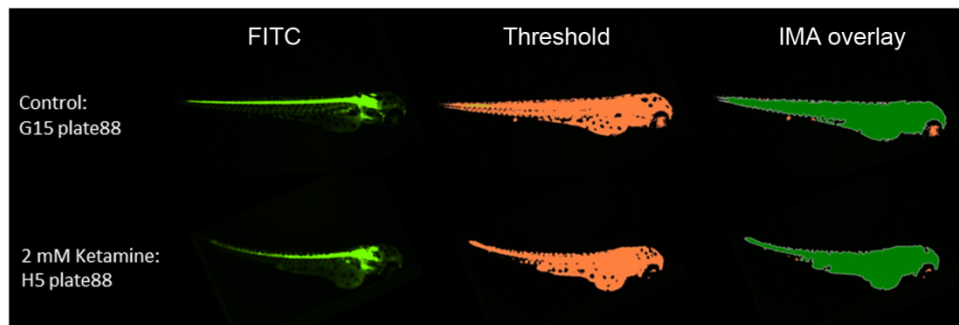


Slide courtesy of Dr. Jyotshna Kanungo, FDA/NCTR;
Kanungo et al., *J Appl Toxicol.* 33:410-417, 2013

Effect of Ketamine on Zebrafish Embryos



384 well format

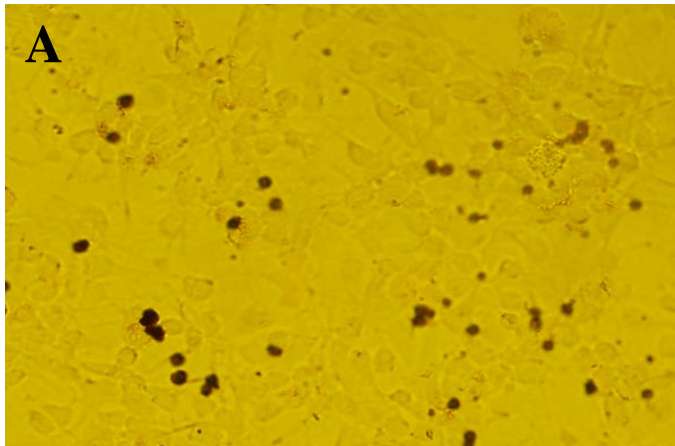
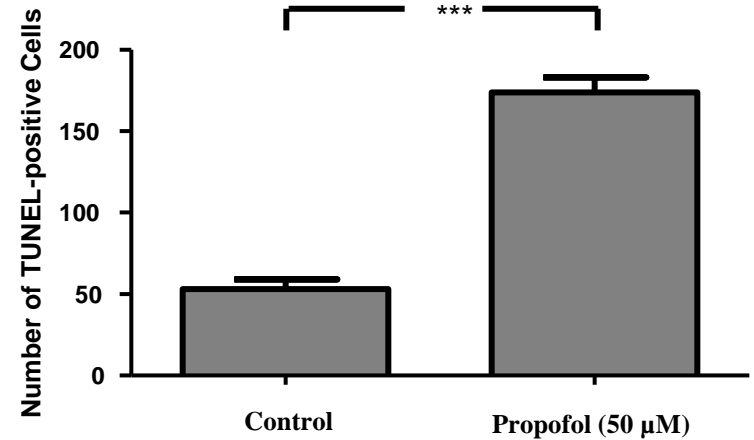


Neurotoxicity

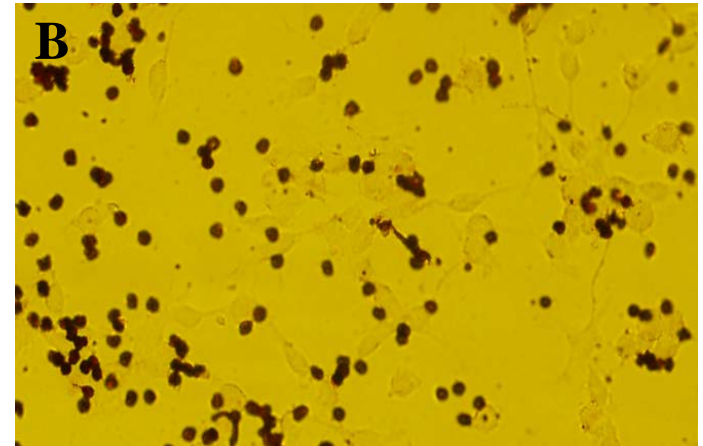
Examples: Stem cells and Bio-imaging

Anesthetic-Induced Neurotoxicity

Rat neural embryonic stem cells in growth medium; TUNEL staining



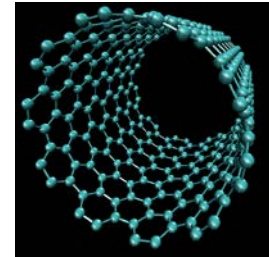
Control



Propofol

In Vivo Imaging Capabilities

- MRI
 - Anatomy
 - Blood flow
 - Edema (ADC, T_2)
 - Brain activity (fMRI)
 - Iron deposition (SWI)
 - Permeability (DCE)
 - Cell death (Na^+)
- MRS
 - ^1H : neurochemistry
 - ^{13}C : metabolic flux
 - ^{31}P : energy metabolism
- PET
 - ^{18}F label
 - FDG
 - Custom ligands



Nanotoxicology

Example: Cell-free and cell-based assays

FDA's Interest in Nanoparticles

Food



Veterinary



Biologics



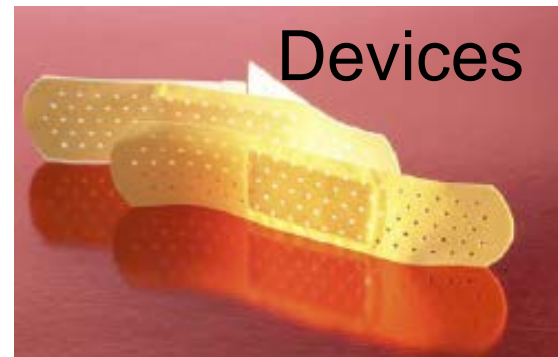
Drugs



Cosmetics



Devices



Tobacco



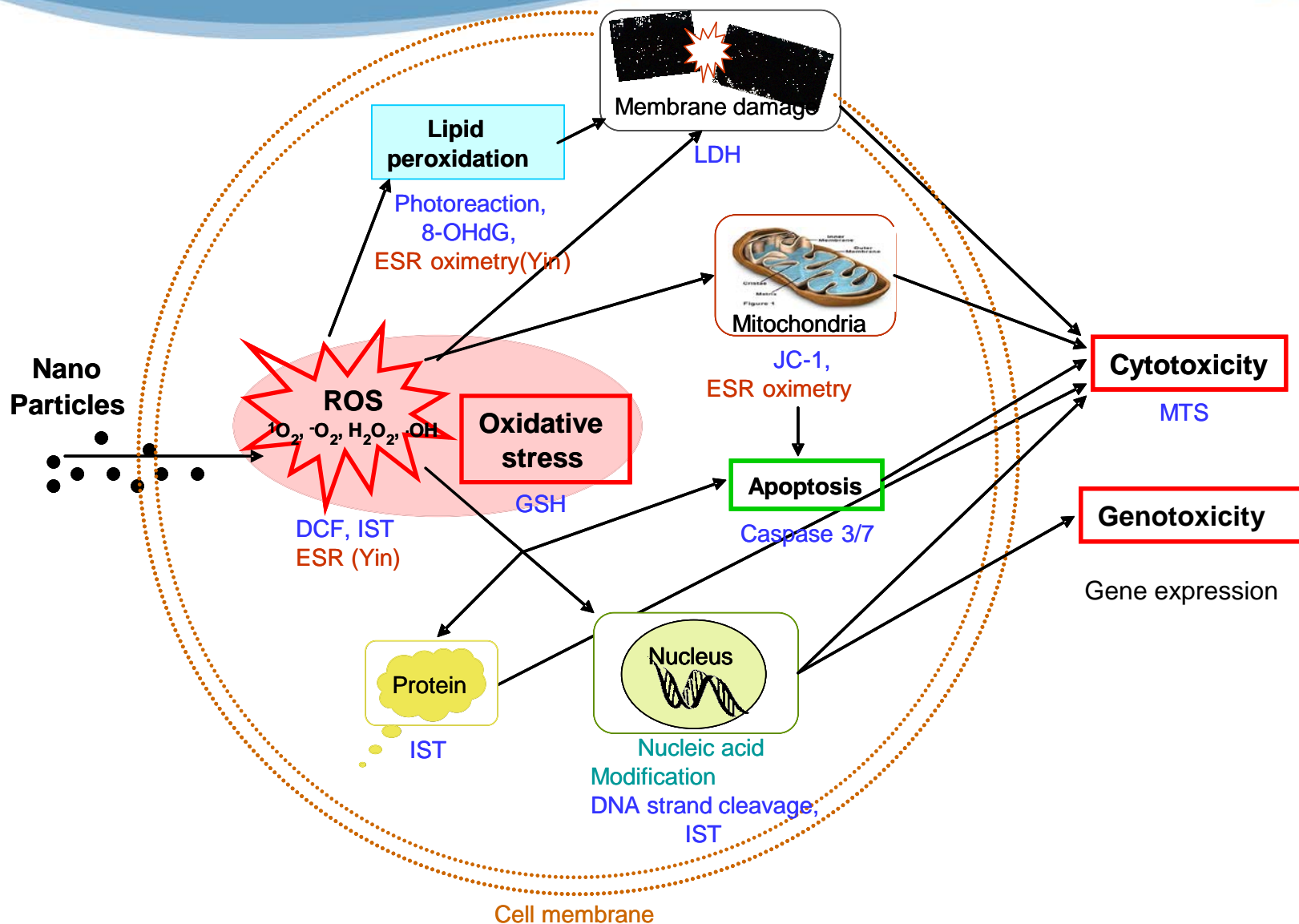
OTC Drugs

Induction of Oxidative Damage by Nanomaterials

Model systems to reflect target organs of exposure:

- HepG2 human liver carcinoma cells
- Human lung carcinoma cells
- Human keratinocytes

- Cell-free





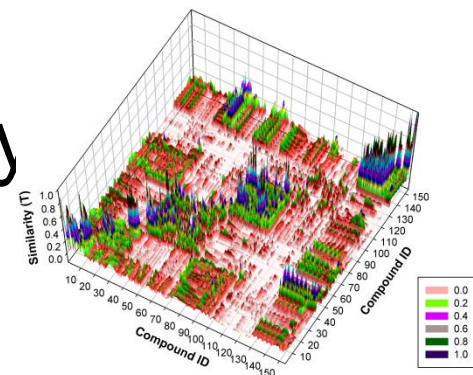
In Silico Modeling

QSAR

QSDAR

Computational Approaches

- Quantitative Spectral Data Activity Relationship (QSDAR)
 - Developed and patented at NCTR
 - Molecular quantum mechanical properties correlated to biological activity
 - To date has been used to predict endocrine disruptors, environmental toxicants, drug efficacy and drug toxicity



Computational Approaches

- Lock and key approach
 - Studying how drugs directly interact with HLA SNPs and cause immune reactions



Summary

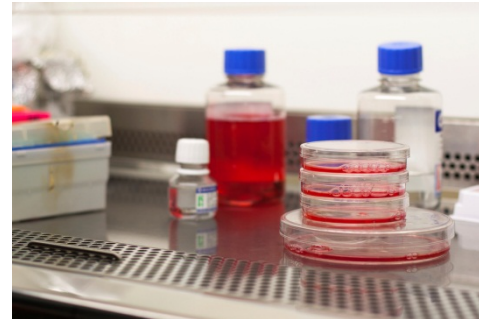
- These examples from NCTR demonstrate *in vitro* and *in silico* approaches to address safety issues
 - At this time, most such approaches do not replace animal testing
- Progress is being made but many hurdles remain

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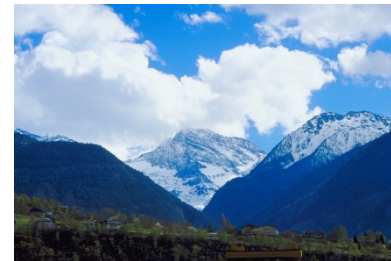
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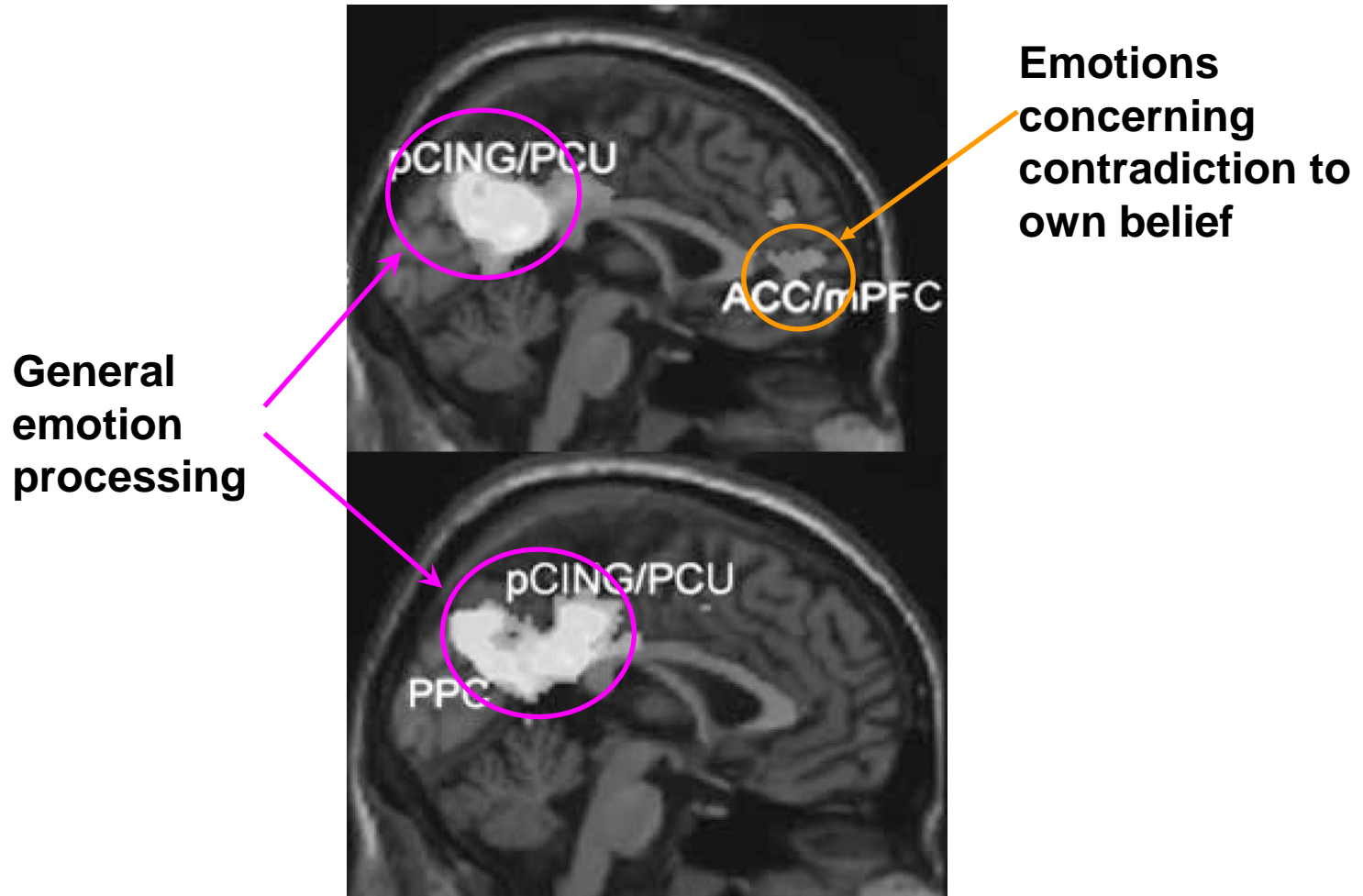
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If You Build It, They May Not Come

- Motivated reasoning
 - Prior belief effect
 - Pay more attention to information that reflects baseline belief
 - Confirmation bias
 - Seek out information that confirms prior belief
 - Disconfirmation bias
 - Spend more time reading contrary arguments to counter their conclusions

Biological Evidence



Conclusion from fMRI Study

- “As predicted, motivated reasoning was not associated with neural activity in regions previously linked to cold reasoning tasks and conscious (explicit) emotion regulation.”

Messages to Promote Childhood Vaccination

- Survey
- Respondents first completed questionnaire about health and vaccine attitudes
- Then approached with 1 of 4 messages
 1. MMR does not cause autism
 2. Textual information about disease danger
 3. Images of diseased children
 4. Dramatic narrative of infant almost dying

Conclusions

- None of these approaches increased intent to vaccinate a future child if parent was originally against vaccination
 - e.g., Correcting fear of vaccine-induced autism reduced future vaccination intent, suggesting parents brought up new issues to defend their attitudes
- Use of narrative and images of diseased children increased concern about MMR!
- Attempts to increase concerns about communicable diseases or correct false impressions about autism risk may be counterproductive

Solution?

- Learn that the generation and dissemination of evidence alone are insufficient
- Show how the new information will fit their beliefs/concerns vs. being threatening
- Present experts on both sides of the argument

Future

- Great strides being made but much left to accomplish
- Need to set reasonable expectations from the beginning
- Better communication tailored to audience

Acknowledgements

- NCTR
 - Rick Beger (Division of Systems Biology)
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