Alternatives for predictive toxicology in drug development: nice to have or added value?

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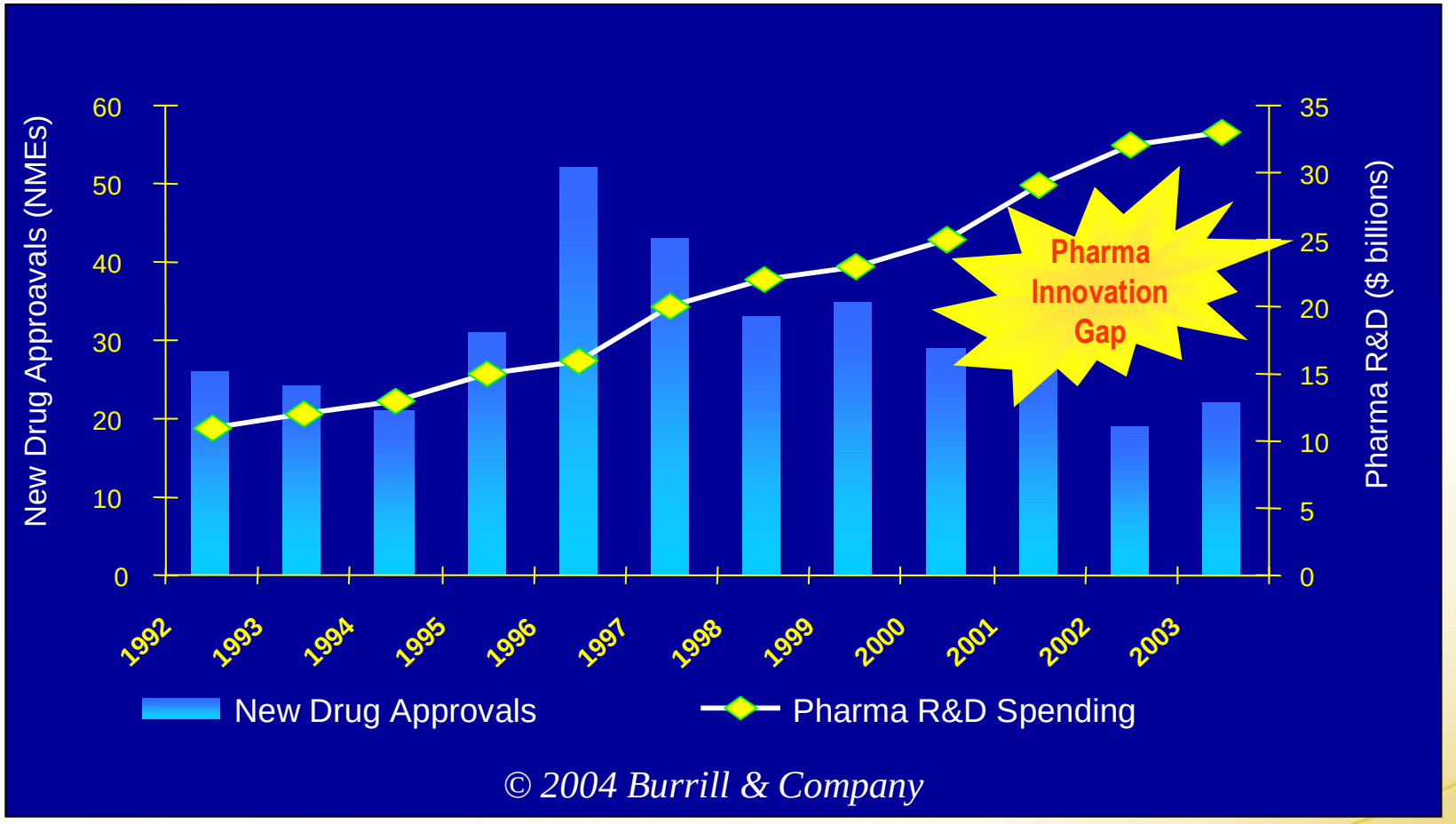
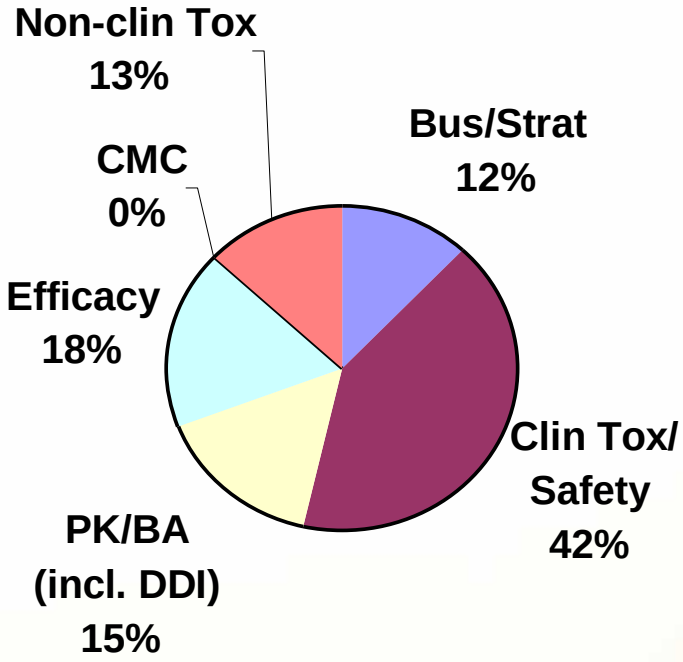
Introduction



New test models and lower attrition rate of drug candidates can help to address the "Pharma Innovation Gap"

Primary reasons for discontinuation in Phase I



PBF* Benchmark 2000-04
n=195

*Pharmaceutical Benchmarking Forum: Abbott Labs, Amgen, AstraZeneca, BMS, Lilly, GSK, J&J, Merck, Novartis, Pfizer, Roche, Schering-Plough

Need for better *in vitro* and *in vivo* liver toxicity test models

"Think out of the box"

"Discovery consists in
seeing what everyone else has seen
and
thinking what no one else has thought"
Albert Szent-Gyorgi (1893-1986)

Introduction

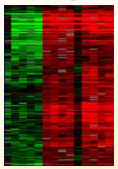
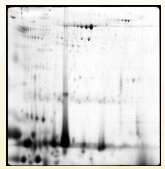
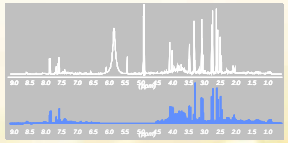
- Define new and better testing strategies: reconsider the testing paradigm for hepatototoxicity testing
- Make better use of existing regulatory test models

Try to get more with less animals

- Develop new in vitro and in vivo test models
- Implement HTS models for compound selection

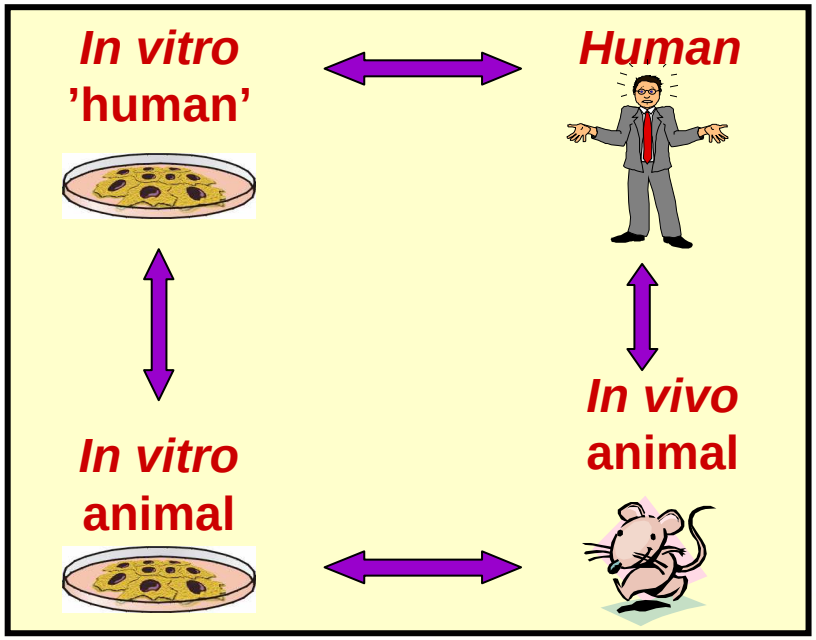
Know more earlier

- Integrate new techniques to define the mechanism of action

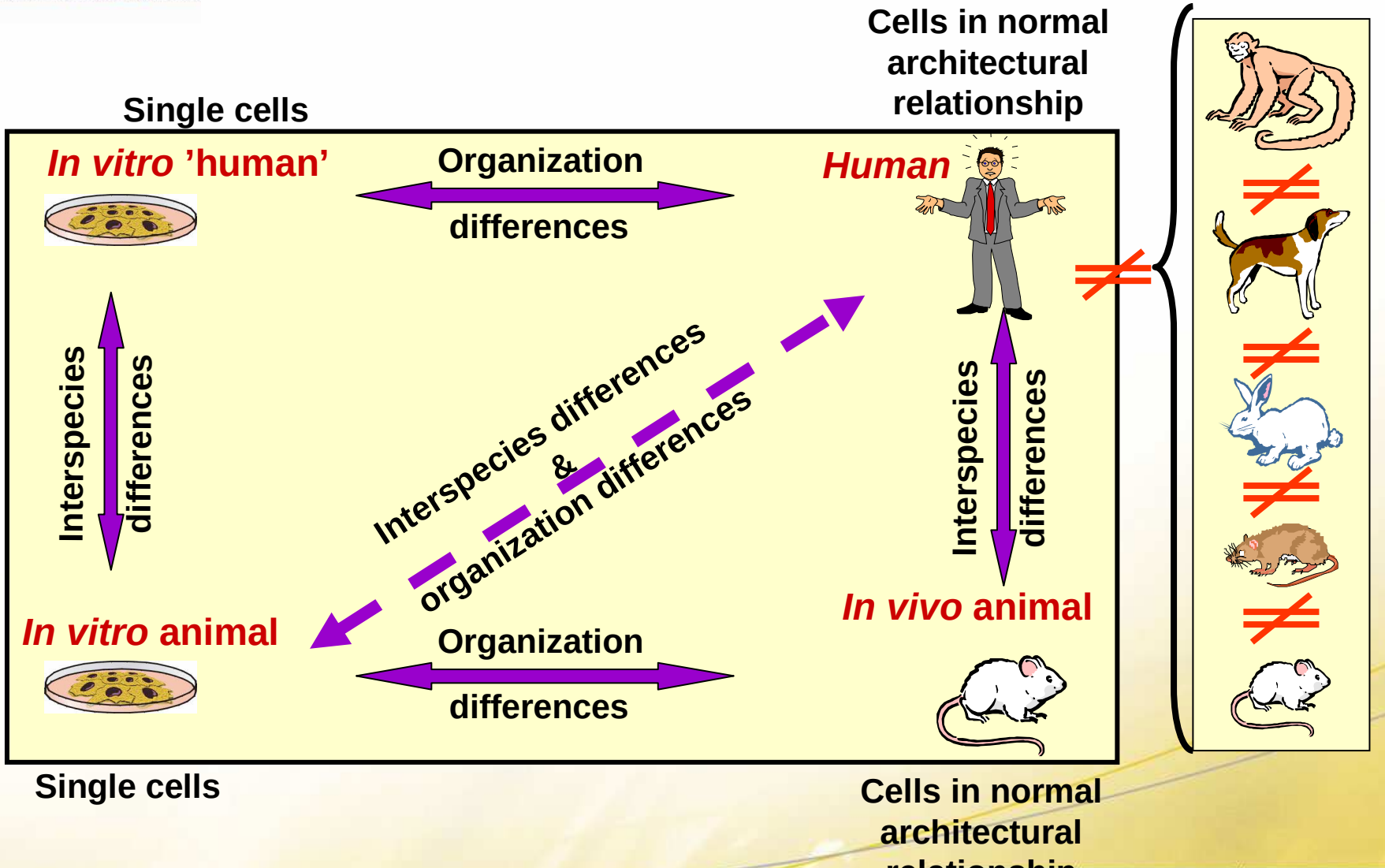

Testing paradigm for hepatotoxicity testing

Testing paradigm

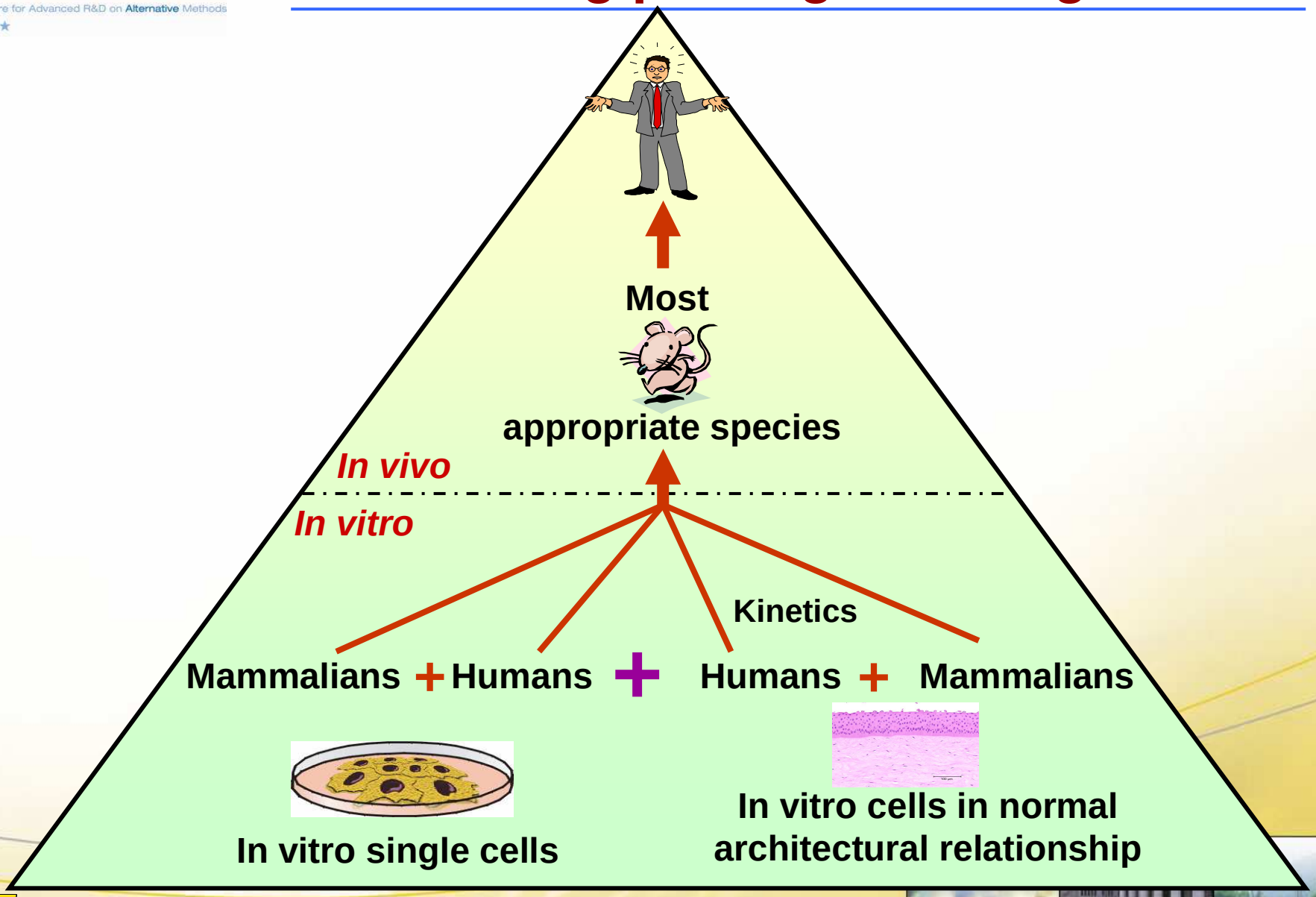


Current test models and testing strategies do not detect well human hepatotoxic compounds

Testing paradigm



Testing paradigm in drug research



Hepatotxicity testing: new models?

Most

• Chimeric mice?

appropriate species

• Lower organisms in vivo

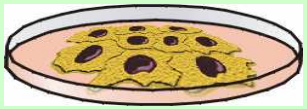
In vivo

In vitro

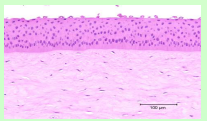
• Other cytotoxicity markers?
• Stem cells for hepatotoxicity?

Kinetics

Mammalians + Humans + Humans + Mammalians



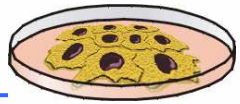
In vitro single cells



In vitro cells in normal architectural relationship

• Liver slices?

• Omics-techniques



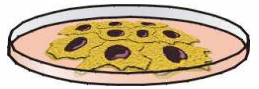
Cytotoxicity testing

Pfizer study [O`Brien et al. Arch. Toxicol, 2006; 80 (9): 580-604]

- **611 compounds tested in vitro on HepG2 cells (48h incubation; 7 parameters)**
  - **42 severely human hepatotoxic compounds**
  - **283 moderately human hepatotoxic compounds**
  - **286 non-toxic drugs**

		Sensitivity	Specificity
1	DNA synthesis	10	92
2	Protein synthesis	4	97
3	Gluthathione depletion	19	85
4	Superoxide induction	1	97
5	Caspase-3 induction	5	5
6	Membrane integrity	2	99
7	Cell viability	10	92
	Combination of above tests 1,3, 7	25	83

High need for improved cytotoxicity assays

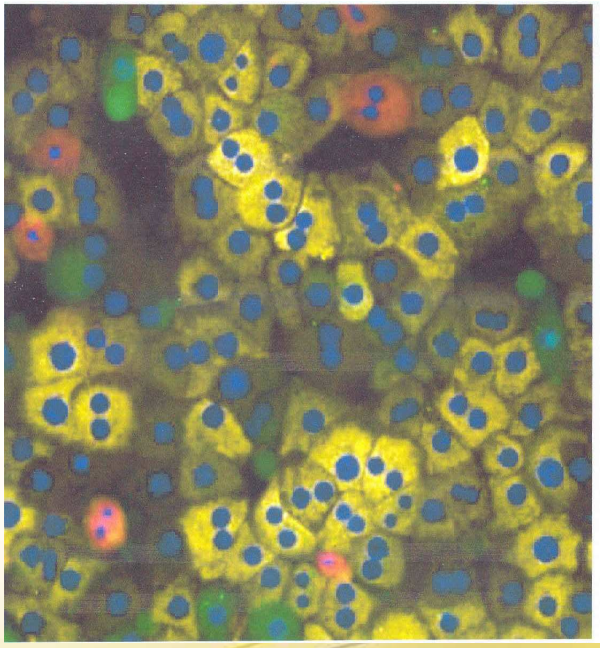


Pfizer study [O`Brien et al. Arch. Toxicol, 2006; 80 (9): 580-604]

Other cytotoxicity markers than the classical ones were tested in a high content screen

Tested 4 fluorophores in HepG2 for 3 days

- **Hoechst33342: nuclear size and cell number: nuclear shrinkage is hallmark of apoptosis. (late stage cytotox parameter).**
- **Fluo-4 AM: intracellular free calcium: early indicator of cell stress.**
- **TMRE: mitochondrial membrane potential: indicator of respiratory capacity of the cell (very early cytotox marker)**
- **TOTO3: plasma membrane permeability: late stage tox indicator (post mortem)**

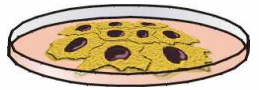


243 drugs

- **Sensitivity for human toxicity increased to 93%**
- **Specificity for human toxicity increased to 98%**

Results of a follow-up study by J&JPRD at CEREP

- The cytotoxicity assays with new parameters in HepG2 cells seem to be superior to classic cytotoxicity assays (LDH, ATP, neutral red, MTT, AlamarBlue, …)
- In most of calculated IC_{50s}, the mitochondrial membrane potential was the most sensitive parameter
- Lowest IC_{50s} with the new parameters are always lower than the in house IC_{50s} values with LDH, NR and ATP
- Another applied prediction model is more predictive than the IC_{50}



How predictive is this assay?

- **Currently database of 186 compounds (J&JPRD data, Cerep data, published Cerep data).**

	Pred. Mod 1	Pred. Mod 2	Pred. Mod 3	Pred. Mod 4	Pred. Mod 5	Pred. Mod 6
Severely hepatotoxic (42)	14+ 28- =33%+	24+ 18- =57%+	29+ 13- =69%+	31+ 11- = 74%+	36+ 6- = 86%+	42+ 0- =100%+
Moderately hepatotoxic (60)	26+ 34- =43%+	35+ 25- =58%+	48+ 12- =80%+	37+ 23- = 62%+	49+ 11- = 82%+	53+ 7- = 88%+
Toxic to other organs (48)	22+ 26- =46%+	29+ 19- =60%+	39+ 9- =81%+	27+ 21- = 56%+	37+ 11- = 77%+	42+ 6- = 88%+
Non-toxic drugs (36)	3+ 33- =8% false+	4+ 32- =11% false+	14+ 22- =39% false+	1+ 35- =3% false+	1+ 35- =3% false+	4+ 32- =11%false+

How predictive is this assay?

Prediction model 2

		Liver toxic in vivo	
		yes (*)	no
Cytotoxic in vitro	yes	59	FP 4
	no	43 FN	32

(*): Significant/Moderate Human Hepatotoxic

	%
Sensitivity	58
Specificity	89
Concordance	66

Prediction model 5

		Liver toxic in vivo	
		yes (*)	no
Cytotoxic in vitro	yes	85	FP 1
	no	17 FN	35

(*): Significant/Moderate Human Hepatotoxic

	%
Sensitivity	83
Specificity	97
Concordance	87

Pfizer study: sensitivity with conventional parameters was only 25%

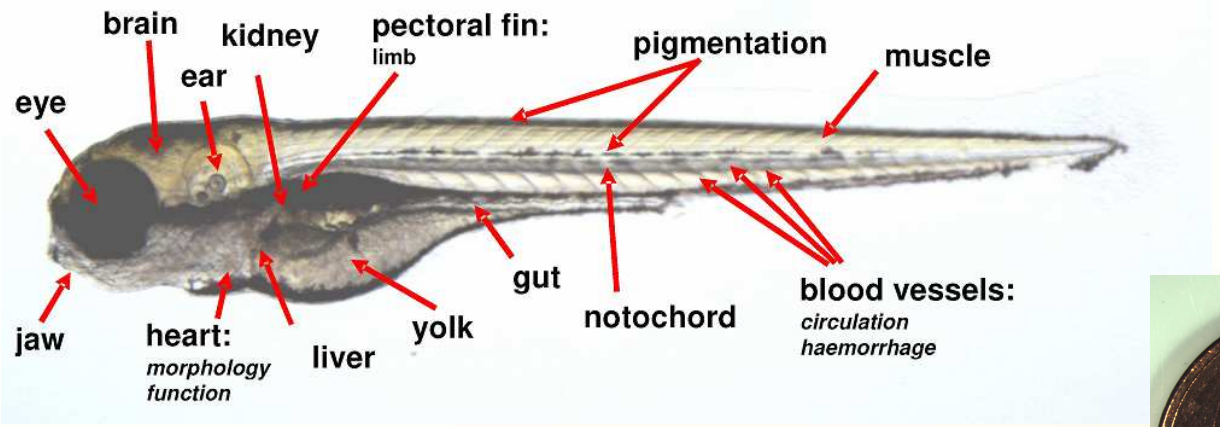
Conclusion on cytotoxicity testing

Sensitivity improved by using other parameters
and
by looking to the data in a different way.

Lower organisms:

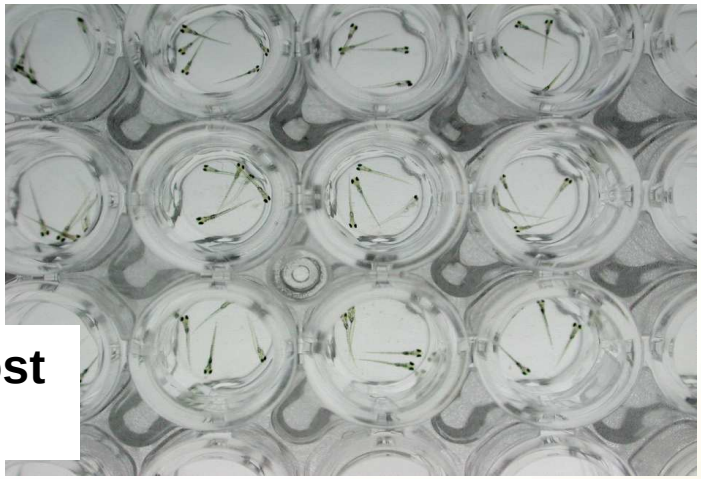
Why?

- **physiology and development parallels that of mammals**
- **optical transparency of the larvae makes real time observations of its internal organs simple**



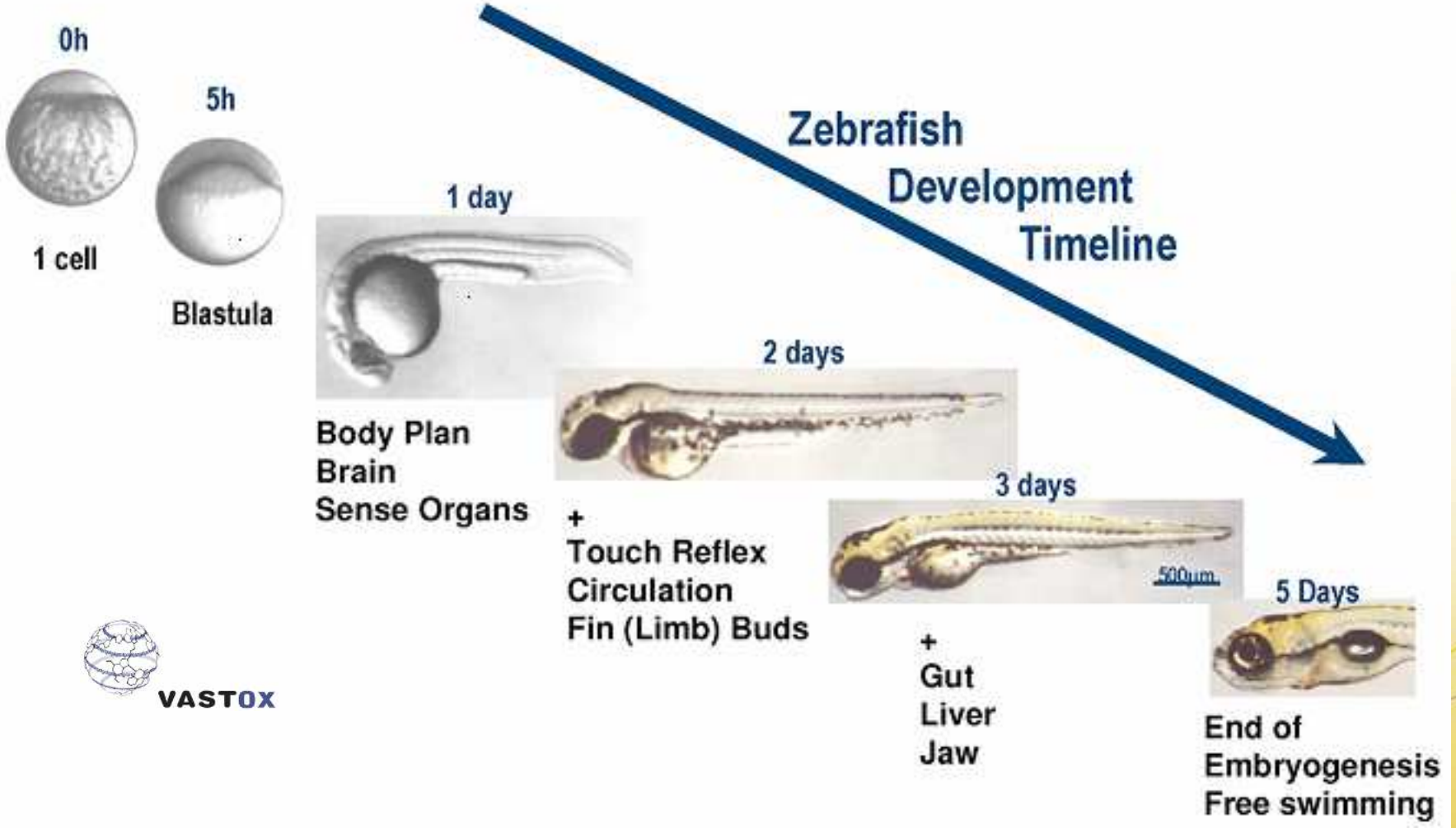
- **are small and inexpensive to maintain**

- larvae can live several days in a single well of standard 24, 96 or 384 well plates surviving on nutrients stored in their yolk sac



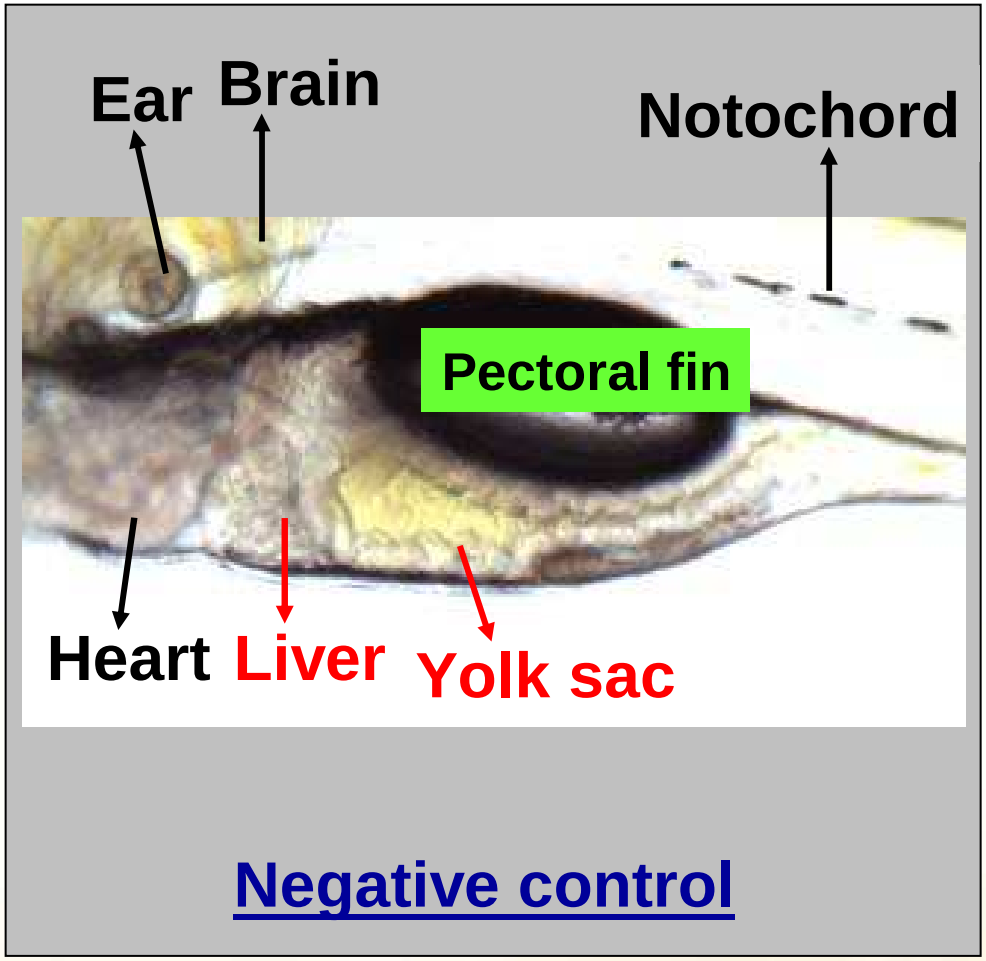
Zebrafish larvae at 6 days post fertilization in a 96-well plate

- easily bred in large numbers (a single pair of adults can routinely lay hundreds of fertilized eggs in a single morning)
- larvae absorb compounds in the surrounding water (Danieau's solution) through their skin and gills
- the liver constitutes 9% of the biomass
- compounds are solved in fish water or DMSO (tolerate up to 1.5% DMSO)

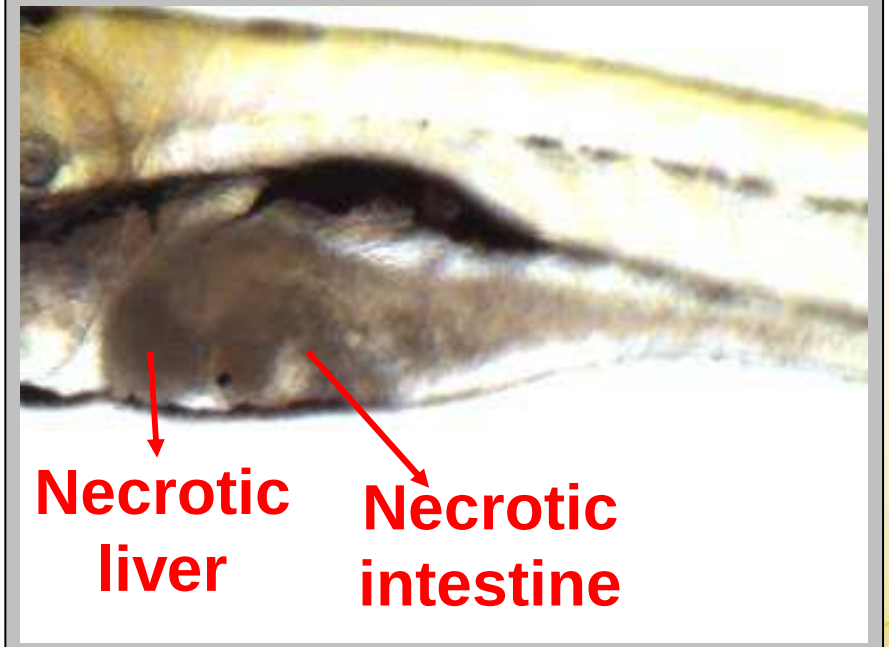


Test design: phenotypic screen for hepatotoxicity

- Concentrations: 0.5, 5, 10, 50, 100 and 500 µM
- 14 larvae per concentration
- Dosing takes place at 96 hpf (day 4) onwards at which time the liver is fully developed
- Assessment for liver toxicity at 144 hpf (day 6)
- Embryos are screened using a stereo dissecting microscope for the following endpoints:
  - Liver necrosis
  - Changes in size and shape of the liver
  - Yolk abnormality (yolk sac oedema)
  - Lethality



Negative control



Liver toxicity

CARDAM
Centre for Advanced R&D on Alternative Methods

Results

Name	Hepatotoxicity in mammalians	Hepatptotoxicity in the zebrafish		
		Phenotypic screen	Proteomic bio-marker screen	FINAL CONCLUSION
Lusaperidone	Monkey and human hepatotoxic			
Clofibrate	Rat and a few cases in humans			
Oxyphenisatin	Human hepatotoxic			
Ketoconazole	Rat hepatotoxic	(*)		
Itraconazole	Rat hepatotoxic			
Ridogrel	Rat hepatotoxic; not human hepatotoxic			
Acaftadine	Rat hepatotoxic	first study		
		second study		
J&J NCE 1	Rat hepatotoxic	ATN		ATN
J&J NCE 2	Monkey and human hepatotoxic; negative in rat and dog			
Amiodarone	Severely human hepatotoxic			
Danazol	Severely human hepatotoxic after metabolisation			
Valproate	Severely human hepatotoxic after metabolisation	(*)		
Furazolidone	Moderately human hepatotoxic			
Tamoxifen	Moderately human hepatotoxic after metabolisation (inhibition of taurocholate transport)			
Troglitazone	Human hepatotoxic (inhibition of taurocholate transport) (withdrawn from market)			
HP-Beta-CD				
J&J NCE 3				
Sucrose				
Gentamycin	Not hepatotoxic, nephrotoxic and ototoxic			
Praziquantel				
Biotine				
		Negative	Equivocal	Positive

ATN: Additional Testing Needed
(*): Bioanalysis needed to prove bioavailability

By courtesy of Johnson-Johnson PHARMACEUTICAL RESEARCH & DEVELOPMENT DIVISION OF JANSSEN PHARMACEUTICA N.V. and summit plc

Phenotypic screen

(20 compounds)

		Liver toxic in rodents and/or humans	
		yes	no
Liver toxic in zebrafish	yes	11	3 FP
	no	3 FN	3

	%
Sensitivity	79
Specificity	50
Concordance	70

By courtesy of Johnson-Johnson PHARMACEUTICAL RESEARCH & DEVELOPMENT and summit plc

Protein markers
(5 compounds)
+
Phenotypic screen
(20 compounds)

		Liver toxic in rodents and/or humans	
		yes	no
Liver toxic in zebrafish	yes	12	2 FP
	no	2 FN	4

	%
Sensitivity	86
Specificity	67
Concordance	80

By courtesy of and

Positive concentrations

Compound	Concentration (µM)															Conclusion
	0.5	1	5	7.5	10	20	25	30	50	75	100	200	300	500	1000	
Clofibrate																
Amiodarone											P			P		
Danazol																
Troglitazone																
Lusaperidone																
J&J NCE2																
Tamoxifen																
Itraconazole										L	L					
Furazolidone											L			L		
Gentamycin																
Praziquantel																
Ridogrel												L				
Acaftadine														L		
														L		
J&J NCE3																
Ketoconazole																
Oxyphenisatin																
HP-beta-CD																
Sucrose																
Valproate																
Biotine																
J&J NCE1																Additional testing

No effect	Liver necrosis	Weak liver toxic	P: Precipitation	Not tested	Lethal	L: Lethal

By courtesy of Johnson & Johnson Pharmaceutical Research & Development, Division of Janssen Pharmaceutica N.V. and summit plc

Conclusion on the zebrafish

- The zebrafish model is a suitable and promising model to screen for liver toxins. An acceptable sensitivity index was obtained.
- Human specific liver toxins were detected to be hepatotoxic to the zebrafish at low concentration levels.
- False positives were only obtained at high concentration levels.
- Phenotypic screen may be used as a first filter for liver toxicity.

General conclusion

- By considering test models which mimic more closely the human body, we may get more relevant information.
- By putting our test models in question, we may get more out of them.
- By exploring lower organisms, we may get very promising test models for screening purposes to deselect toxic compounds before they enter in animal and man.

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THANK YOU!