A systematic review of zebrafish embryo testing as a predictor of developmental toxicity in rats and rabbits: A pilot study

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Adapting Cochrane *Handbook for DTA Reviews*

http://srdta.cochrane.org/handbook-dta-reviews

"Systematic reviews of diagnostic test accuracy are very different from intervention reviews."

http://www.fraunhofer.de

http://www.nature.com

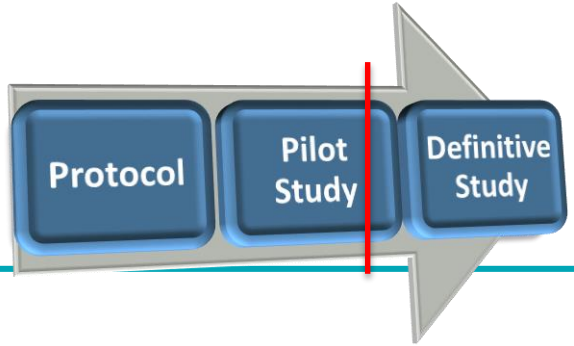
Medicine

Toxicology

Project Overview

- Project: *ZET as a Predictor of Mammalian Developmental Malformations*
- Aim: Retrospective assessment of test method performance
- Tool: DTA-type systematic review
- Who: EBTC Zebrafish Work Group:

- Francois Busquet (JHU)
- Tyna Dao (JHU)
- Dean Fergusson (OHRI)
- Burkhard Flick (BASF)
- Thomas Hartung (JHU)
- Sebastian Hoffmann (seh consulting)
- Manoj Lalu (OHRI)
- Alexandra Maertens (JHU)
- Michele Palopoli (JHU)
- Martin Stephens (JHU)
- Catherine Willett (HSUS)
- Hilda Witters (VITO)
- Robert Wright (JHU)



Context

- The routine test for pre-natal developmental toxicity is OECD Test Guideline 414
- Rats and rabbits are the most commonly used species
- Costly in terms of money, time & animals
- ZET: refinement and (partial) replacement
- Current ZET use: screening & prioritization

Question Framing

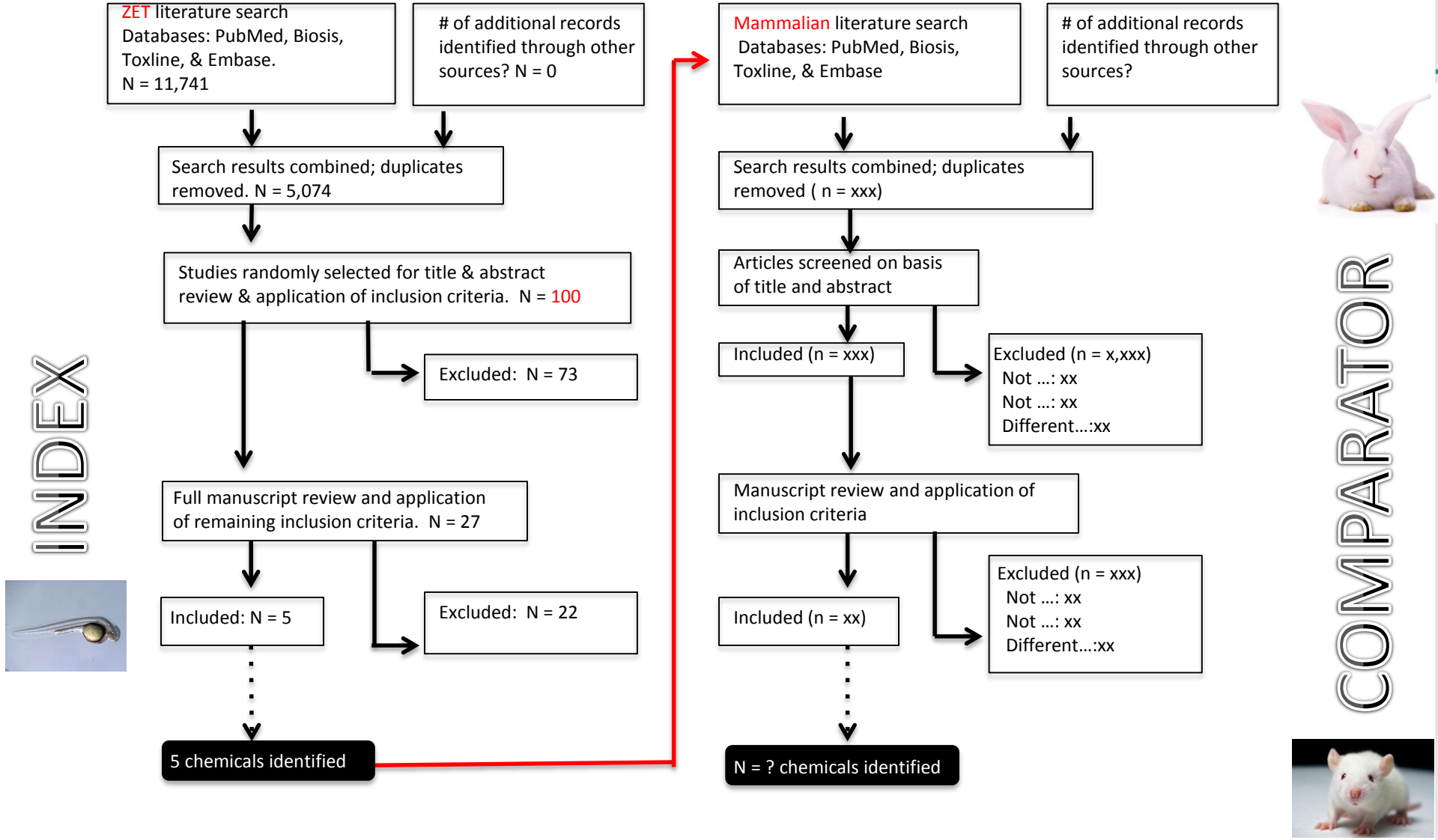
Index Test

How well does the ZET predict positive and negative outcomes from guidelines studies of pre-natal development toxicity in rats and rabbits (OECD TG 414 and equivalents)?

Comparator Test

Electronic Search Strategy

- Two phases:
  - Relevant studies & chemicals first identified in zebrafish
  - Then search for same chemicals in mammalian studies
- Search:
  - Zebrafish
    - Databases: BIOSIS, PubMed, Embase, Toxline
    - Performed April 24, 2014 → 5074 studies
    - Two independent researchers (TD, MP) + information specialist (RW)
  - Rats & rabbits: TBD



Flow of Studies (& Chemicals)

[Following PRISMA statement, where applicable]

Eligibility Criteria

Example: Inclusion Criteria for Mammalian Studies

- Studies investigating developmental toxicity endpoints
- Studies conducted on rats or rabbits in which the species' strain is reported
- Studies conducted on the same chemicals as those from zebrafish studies
- Studies reporting original data will be the preferred.
- Studies in which doses are administered orally via gavage or in food
- Studies in which the endpoints associated with positive findings are documented

http://www.psu.edu

http://www.scientificamerican.com

Data Extraction

Draft data extraction elements for zebrafish studies

Source	Chemicals assessed	# Zebrafish embryos per dose group	Age at first exposure (HPF)	Exposure duration (HPF)	Chemical concentrations used	Controls (-, +, solvent)	Water temp. (degrees C)	Water pH	Dechorionation?

2 independent reviewers (TD, MP) are examining studies & extracting data

Data Synthesis

		Rat Pre-Natal Developmental Toxicity Test (= comparator test)		
		Positive	Negative	
ZET Test (=index test)	Positive	True positives (TP)	False positives (FP)	Positive predictive value (TP/(TP+FP)
	Negative	False negatives (FN)	True negatives (TN)	Negative predictive value TN/(FN+TN)
		Sensitivity TP/(TP+FN)	Specificity TN/(FP+TN)	

Table: Performance measures of the ZET using the pre-natal developmental toxicity test in rats as the comparator test. Green indicates concordance; red, discordance.

Appraisal of Methodological Quality

- Random allocation of treatment
- Allocation concealment
- Blinding (of research personnel)
- Attrition rates low and similar across groups
- Blinding of outcome assessors
- Selection of appropriate control groups
- All measured outcomes reported
- Every animal accounted for
- Sample size calculation
- Statistical model explained
- Test animal details
- Optimal time window used
- Conflict of interest disclosed

Concluding Comments

- Limitations: no human data to serve as an independent standard
- Drafting protocol: gave us opportunity to translate assessment of DTA in medicine to test method performance in toxicology
- Pilot study: allowed us to operationalize the process
- Next steps: finish pilot, begin definitive study
- Application: to field of 'retrospective validation'
- Does not alter principles of validation; offers new tools and approaches
- Our focus on test method performance complements the focus on hazard ID & risk assessment in emerging SR literature in toxicology

Thank you!

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