Peter Theunissen, PhD; Beltox/ETS 2024
09 February 2024, Antwerp

CBG MEB
MEDICINES EVALUATION BOARD

Qualification of NAMs approaches for detecting MEFL, under ICH S5(R3)

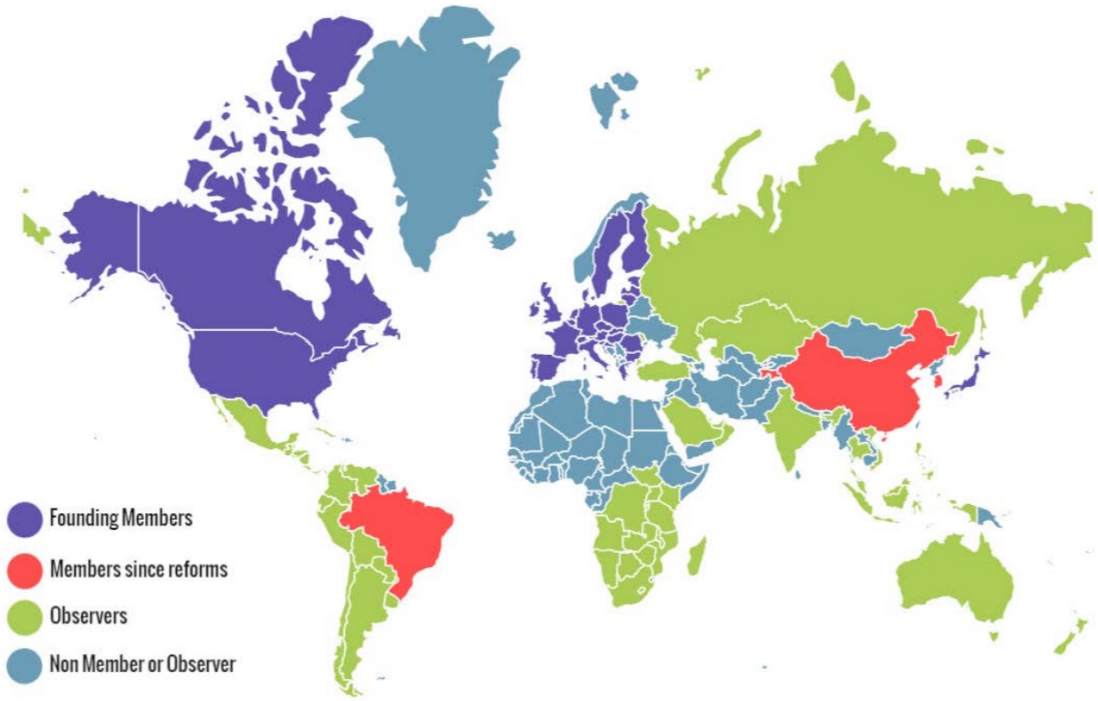
Learnings from the ICH experience

GOOD MEDICINES USED BETTER

Disclaimer

The views expressed in this presentation are my own and not necessarily those of the Medicines Evaluation Board (MEB), nor of the European Medicines Agency (EMA) or any of its working parties.

Current global DART guidance for Pharmaceuticals: ICH S5(R3)



ICH
harmonisation for better health

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

DETECTION OF REPRODUCTIVE AND DEVELOPMENTAL TOXICITY FOR HUMAN PHARMACEUTICALS

S5(R3)

Final version
Adopted on 18 February 2020

Globally Harmonize Test Guidelines

- Quality
- Efficacy
- **Safety**
- Multidisciplinary

Default Embryo Fetal Developmental (EFD) toxicity testing under ICHS5(R3)

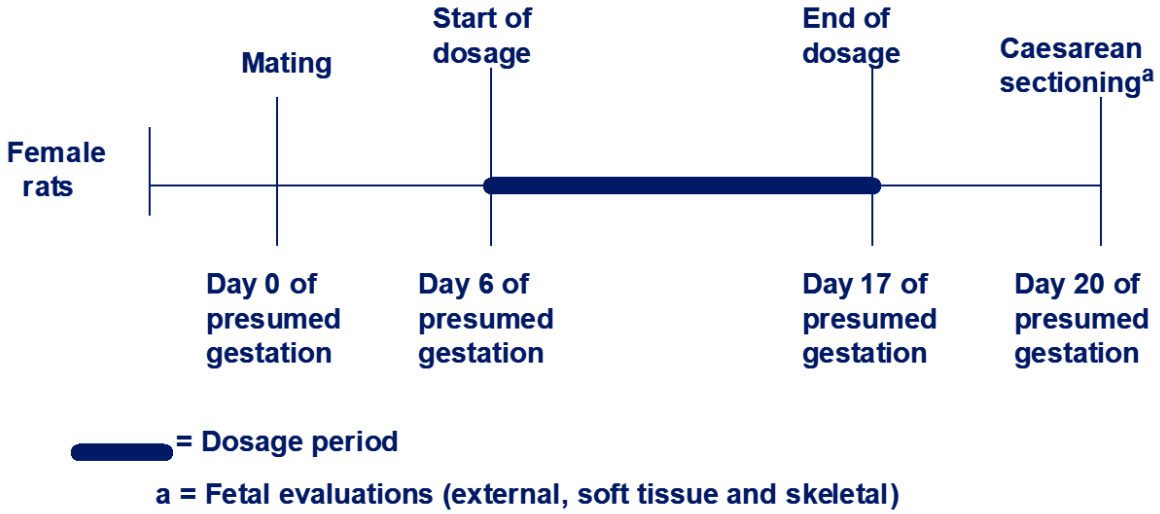
Effects of pharmaceuticals on reproductive cycle

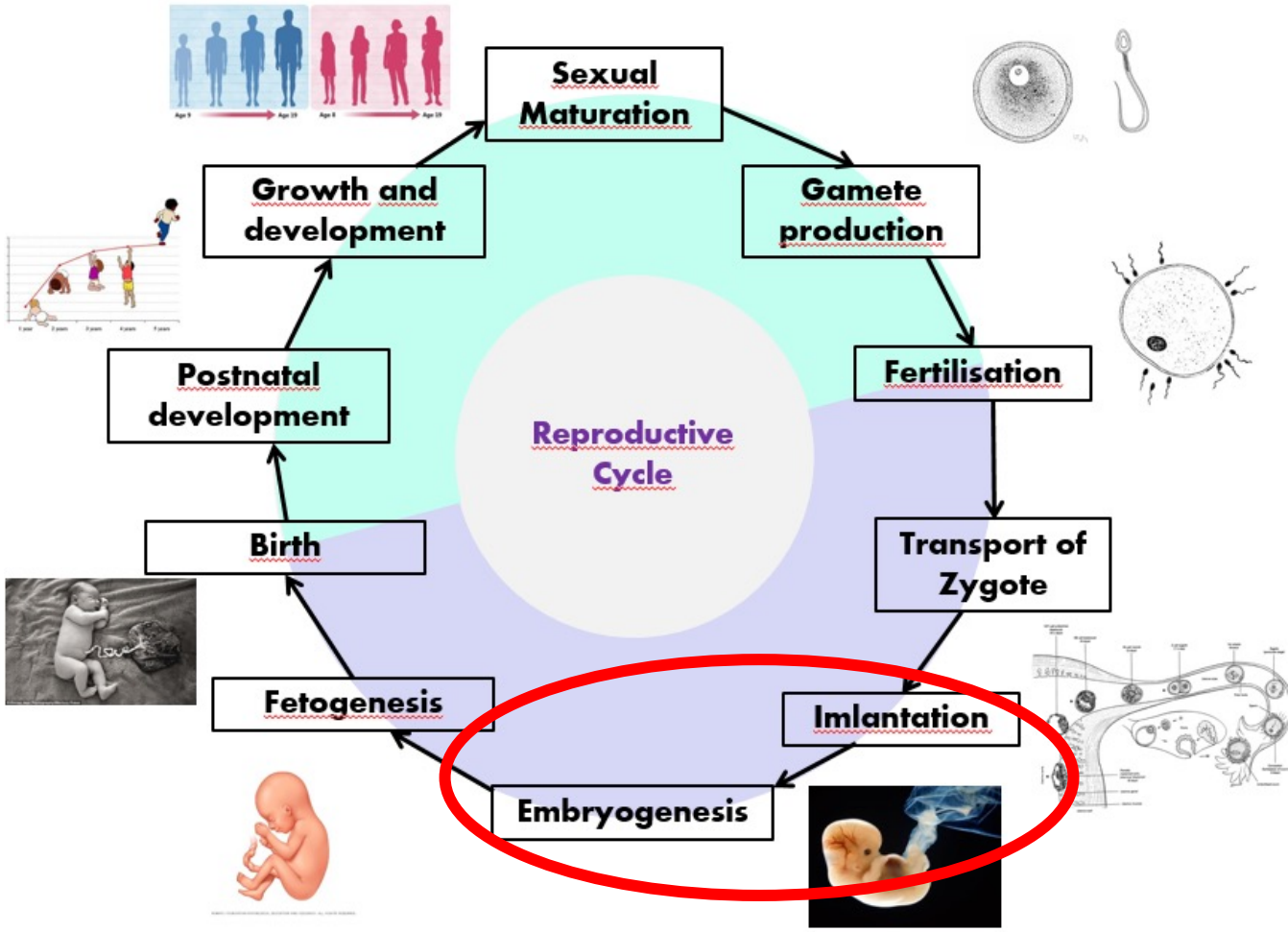
Embryo-fetal Developmental (EFD) Toxicity study

→ Implantation – closure of hard palate

→ Often preceded by pEFD/DRF (less animals)

→ Default 2 species (rodent and non-rodent)





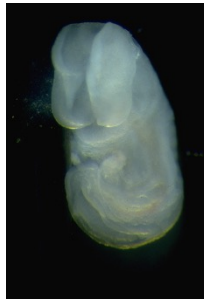
New Approach Methods (NAMs) for EFD toxicity testing (history)

Classic models

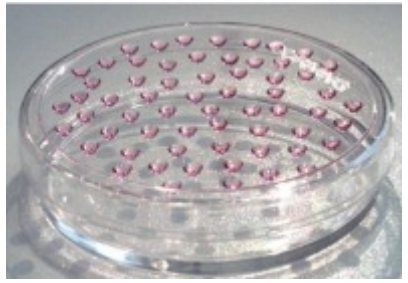
Whole Embryo Culture (WEC) (1970s)

Embryonic Stem Cell Test (EST) (1990s)

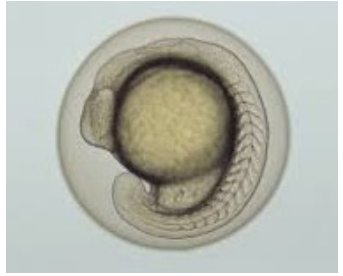
Zebrafish Embryo Toxicity Test (ZET) (2000s)



Verhoef, RIVM

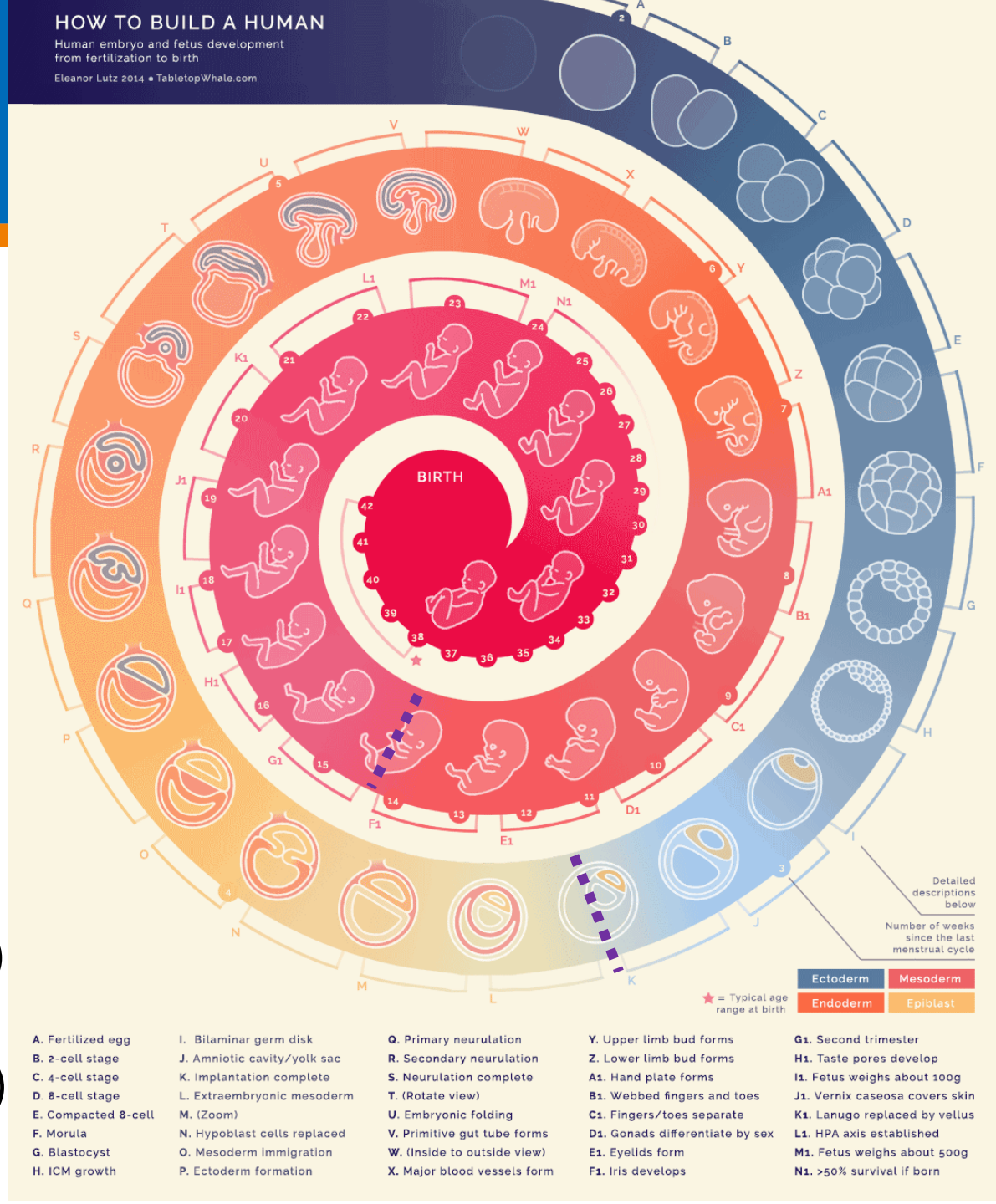


Van Dartel, RIVM



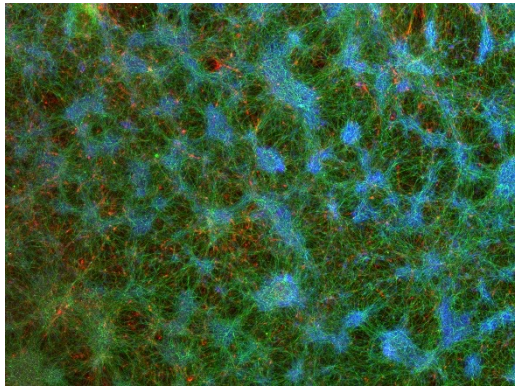
Hermsen, RIVM

- Investigate effects on development during window of implantation – closure hard palate (→ EFD window)
- Original endpoints based on morphology
- Validation effort by ECVAM WEC/cardiac EST (2004-2009)



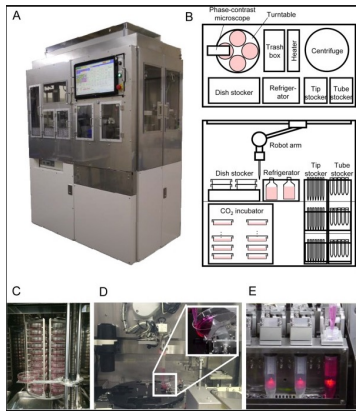
NAMs and innovation; Fast moving field!

Imaging



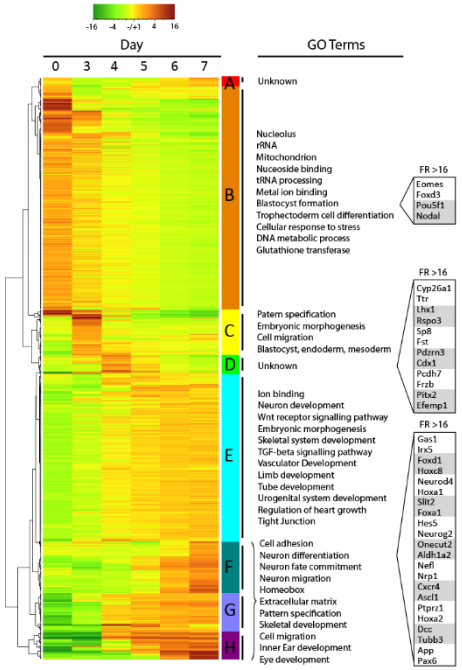
De Leeuw, 2020

Robotics



Konagaya, 2015

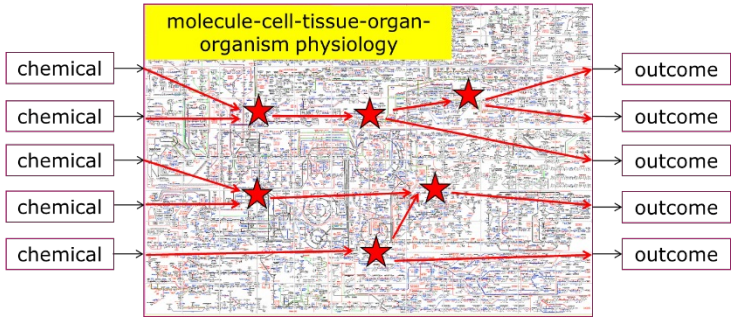
PCR / OMICS



Theunissen, 2011

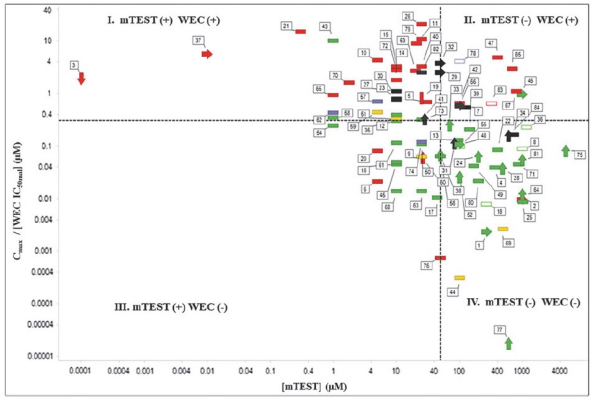
EPA

Adverse Outcome Pathways (AOPs)



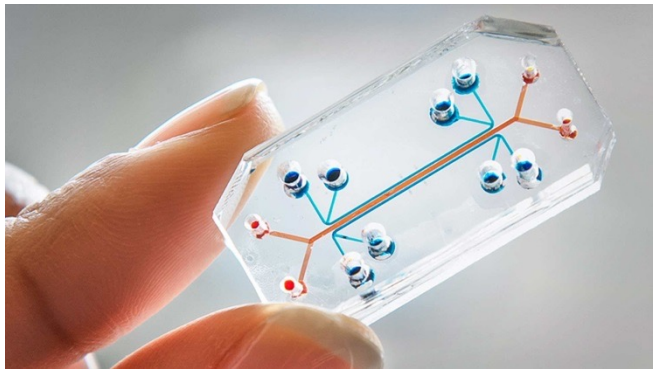
Piersma, RIVM

Tiered approach / Testing Batteries

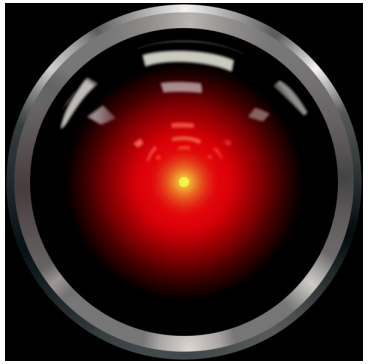


Green, 2018

Organ-on-Chip → embryo on chip???



Machine Learning / Artificial Intelligence



NAMs under ICHS5(R3) (2020-present)
Updating regulation takes some time… … …

2010 Start of preparatory process at ICH level

2015 Official start of Revision procedure

2019 Step 4 approval by ICH

2020 Step 5 regional implementation

First ICH guidance to include information on **use** and **qualification** of **NAMs** as alternative for EFD testing

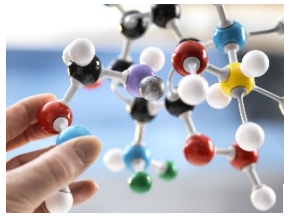
As science innovates quickly,
and changing regulation takes time →

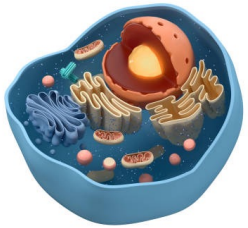
All information on NAMs and qualification in ANNEX 2: ICHS5(R4) **maintenance procedure**
→ Possibility for bi-annual changes to Annex

How to QUALIFY NAMs under ICHS5(R3): Context of Use

Under ICHS5(R3), NAMs approaches should:

- Provide a level of **confidence** for human safety assurance at least equivalent to that provided by the current testing paradigms.
- Be **qualified** within a **certain context-of-use (CoU)**, defined by
  - The **chemical applicability domain** of the assay, and
  - Characterization of the **biological mechanisms** covered by the assay.
  - **Regulatory** considerations:
    - What (non)-clinical endpoint should the assay predict (**MEFL**)?
    - What non-clinical (in vivo) assay could the OoC precede or complement (to inform study design)
    - What non-clinical (in vivo) assay could the OoC replace? (**rat, rabbit?**)
- In accordance with **GLP**
- However, if used for mode of action (**MoA**) exploration or as **supportive** for *in vivo* study:
  → no such rigorous qualification is required.





How to QUALIFY NAMs under ICHS5(R3): General Qualification Criteria

Qualification Criteria (ICHS5(R3), p36-37):

Description and **justification** of predictive model

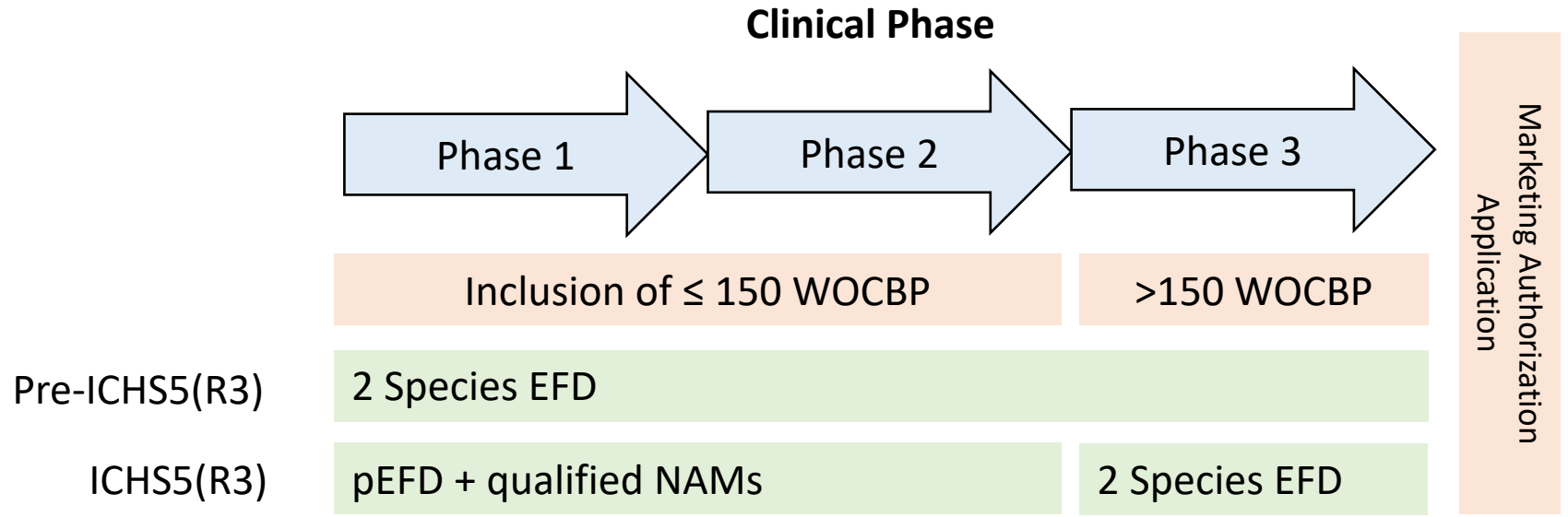
- Which species does it predict Malformations and Embryo-fetal lethality (**MEFL**) for?
- Evaluation of **biological plausibility** of the model,
  - **Mechanism** of embryonic development in the model + adverse effects
  - **Limitations** of the models (what is not covered by the NAMs approach)
  - Developmental **window** of prediction (compared to *in vivo* / human)
- Determination of **endpoints**, description of negative or positive (adaptive response?)
- **Statistical** evidence to predict MEFL in a species (accuracy, prediction, sensitivity, specificity etc.)
- **Historical data** of the test system
- **Reference** compounds
  - list of training sets / test sets, source of data
  - Description of chemical applicability domain

Scenario's for using (qualified) NAMs under ICHS5(R3)

- To support Phase I + II clinical trials (= saving animals by attrition)
- **Qualified** alternative assays predict **MEFL*** outcome in 'first' species)
- + pEFD *in vivo* study in 'second' species
- **Rodent** and **non-rodent** should be covered,
- Enable the limited inclusion of WOCBP (up to **150 WOCBP for up to 3 months**)

(EU and Japan regions only, US region no testing required).

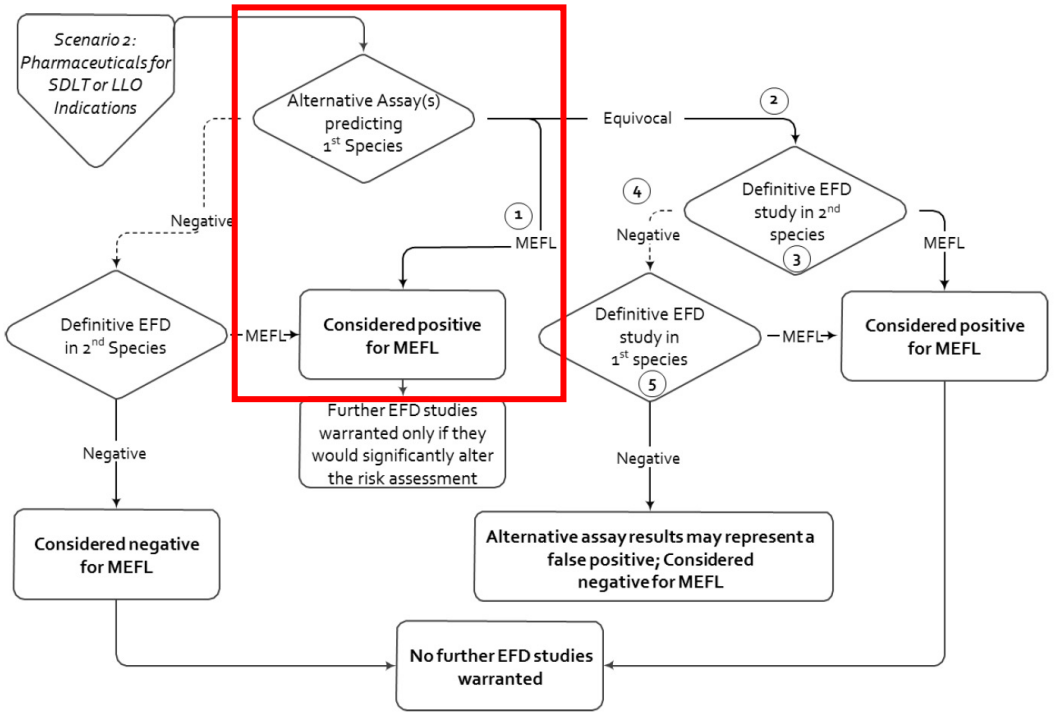
*MEFL = malformations and embryo-fetal lethality



Scenarios for using (qualified) NAMs under ICHS5(R3)

- To support Phase I + II clinical trials (=saving animals by attrition)
- **Qualified** alternative assays (predict **MEFL*** outcome in 'first' species) + pEFD in vivo study in 'second' species
- **Rodent** and **non-rodent** should be covered,
- Enable the limited inclusion of WOCBP (up to **150 WOCBP for up to 3 months**) (EU and Japan regions only, US region no testing required).

- Known **MoA** (class effects, known effect on developmental pathways) (ICHS5(R3)scheme figure 1 Annex 2, p39)
- No clinically relevant **exposure** possible in animals
- Support for WoE assessment when **equivocal** results in animal studies
- Indication for **severely debilitating** or **life-threatening diseases or late-life onset diseases**



ICHS5(R3) figure 2 Annex 2, p39

*MEFL = malformations and embryo-fetal lethality

Reference compound list for NAMs under ICHS5(R3)

29 example Reference Compounds are listed in Annex 2 and published by Andrews *et al.*, 2019.

- Known human teratogens
- Suspected human teratogens (but only sufficient animal data)
- Difficult to find (data on) true negative teratogens

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Analysis of exposure margins in developmental toxicity studies for detection of human teratogens

Paul A. Andrews[a,*], Diann Blanset[b], Priscila Lemos Costa[c], Martin Green[d], Maia L. Green[e,1], Abigail Jacobs[d], Rajkumar Kadaba[f], Jose A. Lebron[e], Britta Mattson[e], Mary Ellen McNerney[g], Daniel Minck[d], Luana de Castro Oliveira[c], Peter T. Theunissen[h], Joseph J. DeGeorge[e,2]

[a] Eisai Inc., Woodcliff Lake, NJ, USA
[b] Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, USA
[c] Agência Nacional de Vigilância Sanitária, Brasília, Brazil
[d] US Food and Drug Administration, Silver Spring, MD, USA
[e] Merck & Co, Inc., West Point, PA, USA
[f] Health Canada, Ottawa, Ontario, Canada
[g] Bristol-Myers Squibb, New Brunswick, NJ, USA
[h] CBG-MEB, Utrecht, the Netherlands

Positive Controls	Human Teratogen	Rat MEFL	Rabbit MEFL
Acitretin	X	X	X
Aspirin	X	X	
Bosentan		X	
Busulfan	X	X	X
Carbamazepine	X	X	X
Cisplatin		X	
Cyclophosphamide	X	X	X
Cytarabine	X	X	
Dabrafenib		X	
Dasatinib		X	
Fluconazole	X	X	X
5-Fluorouracil	X	X	X
Hydroxyurea	X	X	X
Ibrutinib		X	X
Ibuprofen	X	X	
Imatinib		X	
Isotretinoin (13-*cis*-retinoic acid)	X	X	X
Methotrexate	X	X	X
Pazopanib		X	X
Phenytoin (Diphenylhydantoin)	X	X	X
Pomalidomide	presumed	X	X
Ribavirin		X	X
Tacrolimus		X	X
Thalidomide	X	X	X
Topiramate	X	X	X
Tretinoin (all-*trans*-retinoic acid)	X	X	X
Trimethadione	X	X	
Valproic acid	X	X	X
Vismodegib	presumed	X	

Cyclophosphamide

CAS No.: 50-18-0

Rat NOAEL Dose C_{max} AUC	Rat LOAEL Dose C_{max} AUC	Rat Findings	Rabbit NOAEL Dose C_{max} AUC	Rabbit LOAEL Dose C_{max} AUC	Rabbit Findings	Human Dose C_{max} AUC	Margins NOAEL/Huma LOAEL/Human	Notes
NOAEL not identified (<2.5 mg/kg) [Chaube]	2.5 mg/kg IP GD9 [Chaube] Cytoxan C_{max} = 4.1 µg/mL[a] AUC = 3.65 µg·h/mL[a] PM C_{max} = 0.55 µg/mL[b] $AUC_{(0-24h)}$ = 2.13 µg·h/mL[b]	2.5 mg/kg GD9 [Chaube] embryolethal 5 mg/kg GD11 [von Kreybig, Mirkes] encephalocele exencephaly, microcephaly, limb defects (ie, syndactyly and ectrodactyly), defective facia development (cleft palate)	NOAEL not identified (<30 mg/kg)	30 mg/kg IV single doses on GD6-14 [Mirkes, Fritz] Cytoxan C_{max} = 151 µg/mL[c] $AUC_{(0-8h)}$ = 24.1 µg·h/mL[d] PM C_{max} = 0.07 µg/mL[e] $AUC_{(0-8h)}$ = 0.297 µg·h/mL[e]	embryo-fetal resportions, omphalocele, cleft lip/ palate, forelimb skeletal defects	1600 mg/m^2 (40 mg/kg) IV (highest dose, q 3 – 4 weeks)[f] Cytoxan C_{max} = 106 µg/mL[g] AUC = 798 µg·h/mL[g] PM C_{max} = 14.4 µg/mL[h] AUC = 352 µg·h/mL[h]	NOAEL: rat: NOAEL not identified, but LOAEL margins were <0.1 rabbit NOAEL not identified, but LOAEL margins were <1.5 LOAEL: rat C_{max}: 0.04 (4.1/106) AUC: 0.005 (3.65/798)	• MW CP = 261.086 • MW PM = 221.018 • Cytoxan is a prodrug, MEFL has been attributed to both phosphoramide mustard (PM) and acrolein metabolites

e. Extrapolated from reported value after 20 mg/kg cytoxan intravenous single dose in NZW rabbits (Anthony): C_{max} = 0.22 µM (0.049 µg/mL) from visual inspection of graph, $AUC_{(0\text{-}8h)}$ = 53.7 µmol·min/L (0.198 µg·h/mL).

f. From SmPC.

g. Extrapolated from reported value after 1000 mg/m² intravenous single dose cytoxan (Chan): C_0 = 254.4 µM (66.4 µg/mL), $AUC_{(0\text{-}inf)}$ = 1910 µM·h (499 µg·h/mL).

h. Extrapolated from reported value after 1000 mg/m² intravenous single dose cytoxan (Chan): C_0 = 40.5 µM (9.0 µg/mL), $AUC_{(0\text{-}inf)}$ = 996.3 µM·h (220 µg·h/mL).

References

Anthony LB, Long QC, Struck RF, Hande KR. The effect of cimetidine on cyclophosphamide metabolism in rabbits. Cancer Chemother Pharmacol. 1990;27:125-30.

Chan KK, Hong PS, Tutsch K, Trump DL. Clinical pharmacokinetics of cyclophosphamide and metabolites with and without SR-2508. Cancer Res. 1994;54:6421-9.

Chaube S, Kury G, Murphy ML: Teratogenic effects of cyclophosphamide (NSC-26271) in the rat. Cancer Chemother Rep 1967;51:363-76.

Fritz H, Hess R. Effects of cyclophosphamide on embryonic development in the rabbit. Agents Actions. 1971;2:83-6.

Holm KA, Kindberg CG, Stobaugh JF, Slavik M, Riley CM. Stereoselective pharmacokinetics and metabolism of the enantiomers of cyclophosphamide. Preliminary results in humans and rabbits. Biochem Pharmacol. 1990;39:1375-84.

Hong PS, Srigritsanapol A, Chan KK. Pharmacokinetics of 4-hydroxycyclophosphamide and metabolites in the rat. Drug Metab Dispos. 1991;19:1-7.

Mirkes PE. Cyclophosphamide teratogenesis: a review. Teratog Carcinog Mutagen. 1985;5:75-88.

von Kreybig T. Die teratogene wirkung cyclophosphamid wahrend der embryonalen entwicklungsphase bei der ratte. Naunyn-Schniedeb Arch Exp Pathol Pharmakol. 1965;252:173-95.

Additional References Evaluated

Claussen U, Hettwer H, Voelcker G, Krengel HG, Servos G. The embryotoxicity of cyclophosphamide in rabbits during the histiotrophic phase of nutrition. Teratog Carcinog Mutagen. 1985;5:89-100.

Regulatory application of NAMs preceding and after ICHS5(R3)

<u>Up to 2020:</u>

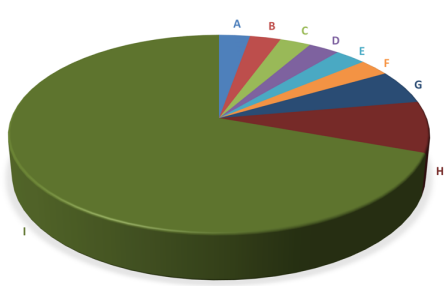
Companies do generate data in house

(60% of responders to IQ survey, not published)

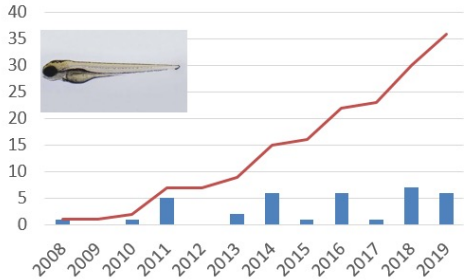
Sometimes shared through submissions

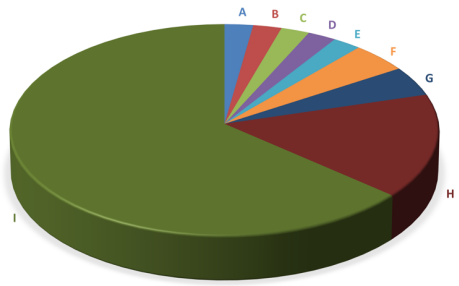
<u>Since implementation of ICHS5(R3):</u>

- No qualification exercises started at EMA
- One interested party at FDA, but not pursued further

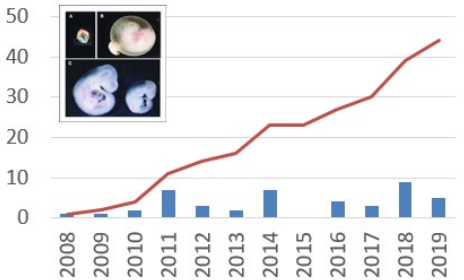


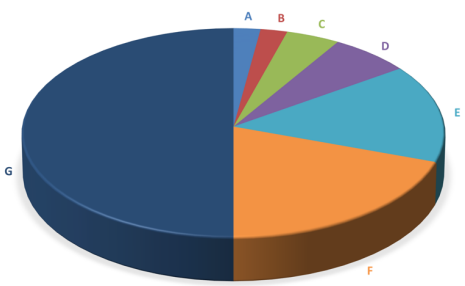
Zebrafish



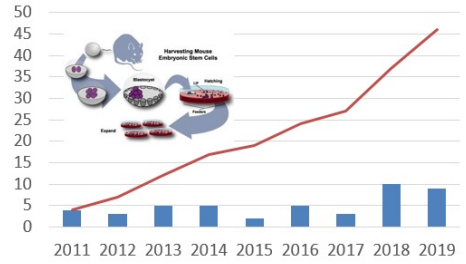


Whole Embryo Culture





Embryonic Stem Cells



Figures kindly provided by Ronald Wange, FDA

Companies are uncertain and reluctant to start formal qualification

Regulators need to get more experienced with NAMs for regulatory purposes

IMPASSE…

Some hurdles from a pharmaceutical industry perspective*

**HESI-DART, NAMs in DART testing for pharmaceuticals , publication in preparation*

Not clear what are the regulatory options and procedures.

- What exactly is **required** for qualification?
- How will qualfication be reflected accross different **global regions**?

Limitations of NAMs

Difficult to bridge the gap between hazard identification and risk assessment due to difficulty of **extrapolating exposure (QIVIVE)**.

To support WoCBP in phase I and II trials, 2x pEFD or 1x pEFD + NAMs are required

Generally, to support phase III trials, pivotal studies in 2 species are required

→ **Performing 2 pEFD will provide a higher probability of succes pEFD+NAMs**

Small- and medium-pharmaceutical companies do not have the opportunity to screen in-house.

→ **General qualification for a wide context of use by 3rd parties is required for more general application**

Lessons learned from ICH S5(R3)

Regarding implementation of NAMs:

Mismatch between…

What is intended in ICHS5(R3)

- Part of WoE (Battery of assays + literature)
- Context of Use

What is percieved by developers and end-users

- Single assay replaces an animal study
- One assay fits all

- Additional communication needed on the intentions of ICHS5(R3).
- Consult stakeholders at initiation of drafting a guidance.

General reluctancy to share data:

- Promote sharing screening data in paralel with pivotal EFD studies upon marketing application:

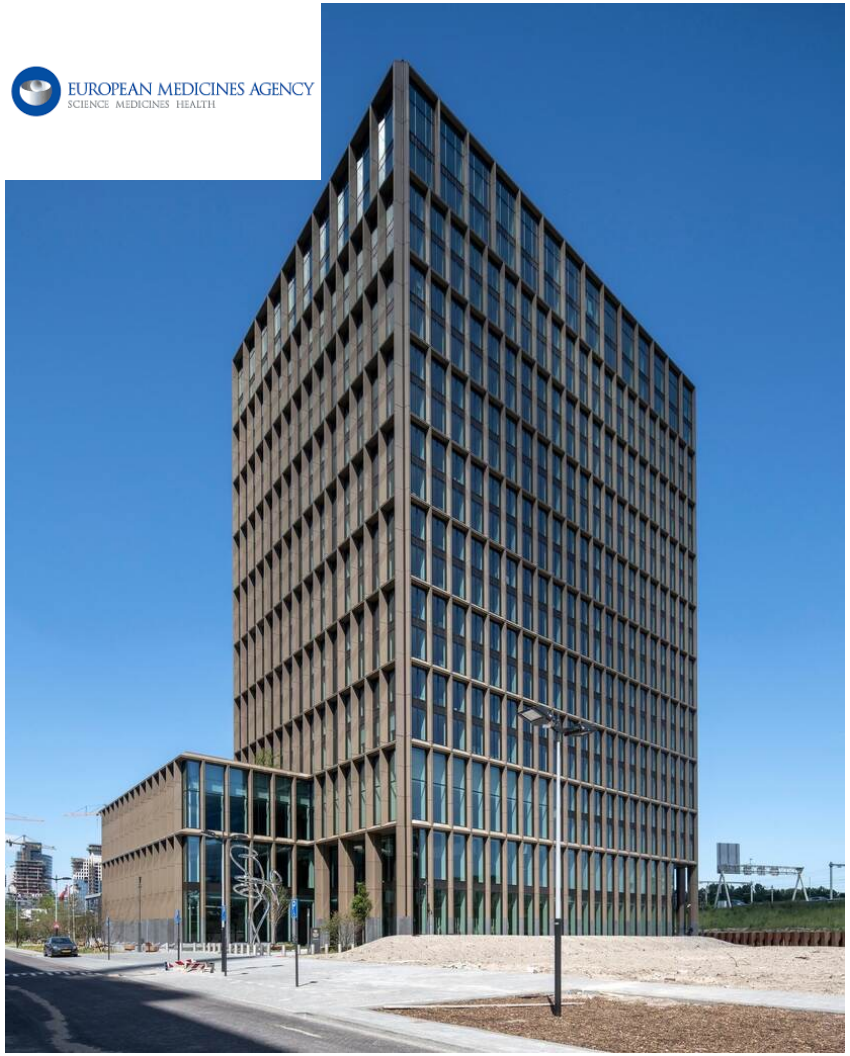
  → Voluntary Sharing of Data (*Safe Harbour*)
- Increase the (very) limited experience with NAMs for regulators

Regulatory perspective on future implementation of NAMs

EMA aims to increase knowledge on NAMs and accellerate Qualification of NAMs:

- 3Rs Working Party (3RsWP) re-started in 2022
- Early **dialogue** with assay developers through **ITF**
- Promote possibilities to share data through "**voluntairy data sharing**"
- **Guidance** on qualification
  - Update general 3Rs and Qualification guidance
  - Provide specific guidance for OoC/MPS
- **Support** for qualification applications (ITF/ScAd)
  - European Specialised Expert Community (**ESEC**) for NAMs

- Creation of a **worldwide cluster** of regulators for global alignment



Qualification at EMA: Innovative Task Force

Innovative Task Force (**ITF**)

- **Informal** exchange with EMA experts and ESEC members
  - **Present proof of concept** and discuss possibilities for qualification
  - Proactive identification & assessment of impact on current scientific, legal and regulatory requirements
  - Early identification of specialised expertise needs
  - Advice on eligibility to EMA procedures
  - Review of regulatory and scientific implications
  - Increasing awareness & learning at the EMA
- **Free** of charge
- https://www.ema.europa.eu/en/human-regulatory/research-development/innovation-medicines

Qualification at EMA: Qualification Advice

<u>Following steps after ITF:</u>

Formal talks with EMA before qualification (3RsWP / SAWP advice)

Formal Qualification (SAWP qualification advice / opinion)

Submission of proposal to the EMA in accordance with the procedure described in the *Guideline on Qualification of Novel Methodologies for Drug Development (EMA/CHMP/SAWP/72894/2008 Rev. 4)*

- Submission via qualification@ema.europa.eu
- Assessment by a “qualification team” (consisting of experts from member states)
- Possible involvement of other agencies such as FDA and/or PMDA

→ Formal EMA statement that NAMs can be used under a predefined context of use (Qualification Opinion)

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

10 November 2014
EMA/CHMP/SAWP/72894/2008
Revision 1: January 2012[1]
Revision 2: January 2014[2]
Revision 3: November 2014[3]
Revision 4: October 2020[4]
Scientific Advice Working Party of CHMP

Qualification of novel methodologies for drug development: guidance to applicants

Agreed by SAWP	27 February 2008
Adoption by CHMP for release for consultation	24 April 2008
End of consultation (deadline for comments)	30 June 2008
Final Agreed by CHMP	22 January 2009

Keywords	*EMA. CHMP. Novel methodology. Qualification. Scientific Advice. Biomarker.*

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

5 October 2020
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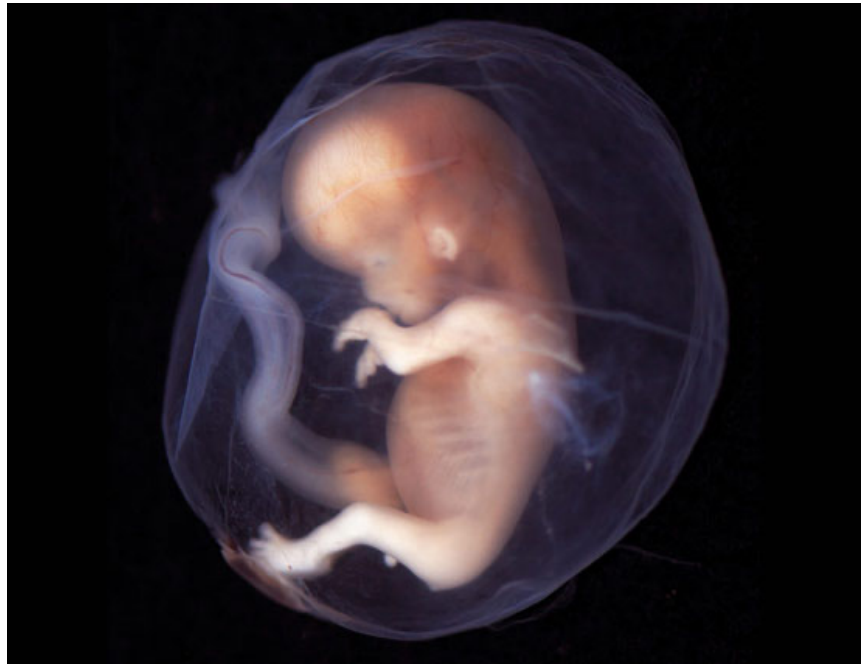
Veterinary Medicines Division

Guidance for applicants requesting scientific advice

Future of NAMs in DART testing

- Find common ground with stakeholders to start data sharing →
- Increased regulatory experience →
- Increase in Qualification applications →
- Increase in overall Context of Use →
- More scenarios in which NAMs can be used under ICHS5(R3) Annex II



Long term goals for NAMs in context of ICHS5(R3):

- Obtain more **human** relevant **mechanistic** data

- Decrease the use of test animals for DART testing

- **Increased relevance** of non-clinical data for **Labeling** for Pregnant women, Lactation and Fertility

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MEDICINES EVALUATION BOARD

GOOD MEDICINES USED BETTER